

By Edward E. Shea

THE LAST two decades have seen major changes in the law relating to product liability—the development of strict liability; long-arm jurisdiction; elimination or limitation of privity in most states; expansion of pretrial discovery; extension of design and warning duties to include foreseeable misuse; and interpretation of statutes of limitations to run from injury or from discoverability of injury or its cause rather than the time of sale of the product.

Recent court decisions adopting new theories of industry liability and expanding insurance coverage for latent injuries and diseases are making still more fundamental changes. The result is billions of dollars of potential liability exposure without clear answers where the liability may fall or what should be done to minimize the risk. As a result, there is growing interest in federal legislation to clarify the rights of claimants injured by products and the duties of product manufacturers and their insurers. This article discusses the court decisions and recent and pending legislation and gives reasons why adoption of national uniform

product liability legislation is timely.

A traditional requisite for proof of product liability based on negligence, breach of warranty, or strict liability is sufficient evidence to identify the manufacturer or seller of the product. In recent years, however, the courts have been presented with a major volume of cases involving latent diseases or injuries. Best known are the cases seeking to recover for asbestosis, mesothelioma, and bronchogenic carcinoma alleged to have resulted from exposure to asbestos and the cases seeking to recover for cancer suffered by daughters alleged to have resulted from their mothers' use of diethylstilbestrol during pregnancy. Under recent interpretations of statutes of limitations, these claims are not necessarily barred because the products were sold many years ago. Plaintiffs' counsel, however, found they were faced with a further barrier—the major difficulty or impossibility of proving the identity of the manufacturers and sellers of the asbestos and DES used.

To overcome this barrier, plaintiffs' counsel sought relief from the burden of manufacturer identification and sought to impose joint liability on manu-

facturers of asbestos and DES as industries. Two theories were argued initially: concerted action liability and alternate liability. Precedent for concerted action liability was found in *Hall v. E.I. du Pont de Nemours & Company*, 345 F.Supp. 353 (E.D. N.Y. 1972), in which the court ruled that manufacturers of products alleged to be essentially identical (blasting caps) could be held jointly liable for injuries if they engaged in concerted action by conspiracy or agreement to control the risks of the product, for example, through membership in an industry association. Alternate liability was argued on precedent found in *Summers v. Tice*, 199 P.2d 1 (Calif. 1948), in which the court ruled that two hunters who fired guns simultaneously could be held jointly liable for injury, although the plaintiff could not identify which hunter fired the shot causing injury.

An article, "DES and a Proposed Theory of Enterprise Liability," published in 46 *Fordham Law Review* 963 (1978), has been cited frequently in subsequent court decisions. The article pointed out that concerted action liability, as outlined in *Hall*, might not apply to a product manufactured by a large

Product

Liability:

Changing liability theories have produced a situation that cannot be measured by product manufacturers, sellers, and insurers.

A Continuing Process of Change

number of widespread manufacturers, only some of whom were named as defendants, and might require proof of a conspiracy, agreement, or understanding beyond mere parallel practices. The article noted that the events in Summers occurred simultaneously at one location and both hunters potentially responsible were defendants. The article proposed adoption in the pending DES cases of a hybrid theory called "enterprise liability." Under this proposal the burden of manufacturer identification would be shifted to manufacturers named as defendants on proof of several elements, the most important being proof that a generically similar defective product was manufactured by all the defendants, the joined defendants accounted for a high percentage of the defective products on the market at the time of the plaintiff's injury, and there existed an insufficient industry-wide standard of safety as to manufacture of the product.

The courts so far have been cautious about adopting industry liability and none has embraced "enterprise liability" as proposed in the law review article. In *Lyons v. Premo Pharmaceuticals Labs, Inc.*, 406 A. 2d 185 (1979), a New Jersey intermediate appellate court ruled that theories of industry liability do not apply when the manufacturer can be identified. In *Abel v. Eli Lilly & Company*, 289 N.W. 2d 20 (1979), a Michigan intermediate appellate court rejected enterprise liability. In a two-to-one decision, however, it found precedent in Michigan law to relieve the plaintiffs of the burden of manufacturer identification and allow them to proceed on theories of concert of action liability and alternate liability. In *Davis v. Yearwood*, 612 S.W. 2d 917 (1980), the Tennessee Court of Appeals refused to adopt theories of industry liability in a lawsuit against 102 defendants, including 59 manufacturers, resulting from a fire in a jail that began in a padded cell alleged to have contained a variety of materials including nylon, latex, polyvinyl chloride, and flexible polyurethane foam. Some lower courts have rejected all theories of industry liability in DES cases, and some have stated that adoption of enterprise liability would be proper only by a state supreme court or legislature.

In 1980 the Supreme Court of California announced a new theory of several industry liability. This theory was adopted in *Sindell v. Abbott Laboratories*, 607 P. 2d 924, in a DES case after finding lengthy allegations of par-

allel manufacturer activities insufficient to support concerted action liability, finding joint alternate liability unjustified because only five of 200 manufacturers were before the court, and rejecting enterprise liability because of the widespread decentralized nature of the industry and the fact that many standards followed in the industry are imposed by government regulation. The court formulated a new theory under which the action could proceed provided that manufacturers having a "substantial percentage" of the market are named as defendants with several liability allocated among them based on their market shares. This theory is now being called "market share liability," and it was followed in 1981 by a New Jersey court in *Ferrigno v. Eli Lilly & Company*, 420 A. 2d 1305.

New theories abound in product liability

The Appellate Division, First Department, New York in *Bichler v. Eli Lilly & Company*, 436 N.Y.S. 2d 625 (1981), upheld jury instructions that concerted action liability could be imposed on a DES manufacturer without proof of an actual conspiracy if the jury found either conscious parallelism or independent actions substantially aiding or encouraging failure to test the product. If upheld, this decision would considerably expand joint industry liability. A federal district court in Massachusetts, however, granted partial summary judgment in favor of manufacturers of DES after careful analysis showed that the evidence failed to demonstrate any actual agreement amounting to concert of action, aiding or abetting, or joint venture. *Payton v. Abbott Laboratories*, 512 F.Supp. 1031, (1981). As in *Sindell*, the court specifically rejected efforts to establish concerted action on the basis of alleged conscious parallelism.

Plaintiffs' counsel have moved quickly to employ the new theories of industry liability, and the result has been shock waves as manufacturers find themselves being named in large defendant groups alleged to be "industries."

The courts that adopted industry liability sought to remove a barrier to trial on the merits based on the allegations in the asbestos and DES cases. As a precedent, however, industry liability raises serious difficulties. Among the

problems are definition of industries and identification of their members; determination of the essential identity of products sold for different markets and uses or containing multiple components and raw materials; determination of market shares or other means of allocating liability; unavailability of defense evidence on the merits in older cases; and potential adverse effects on the activities of industry associations in developing product standards and test methods and acting as clearinghouses for product safety data.

In addition, there is a problem of duplication of defense cost in cases defended by multiple manufacturers. Each manufacturer retains its own counsel and wishes to defend its own product. Sharing of defense work and cost is possible within limits, but that co-operation tends to reinforce plaintiffs' theory that the defendants compose an industry group. If defendants responsible for vital portions of the investigation and defense should settle, the loss of continuity to other defendants can be a severe blow. Thus, there is considerable pressure on manufacturers to make cost-of-defense settlements regardless of the merits to avoid years of effort and expense.

Product liability insurance is part of the coverage commonly provided in comprehensive general liability policies insuring against liability for bodily injury and property damage. The coverage is accomplished by "products hazard" and "completed operations" provisions.

A general liability policy obligates the insurer with respect to bodily injury or property damage caused by an "occurrence" during the policy period, which is ordinarily one year. Limits of liability are stated per occurrence and in the aggregate. For example, a policy may limit liability of the insurer to \$500,000 for each occurrence and \$1,000,000 for all occurrences within the policy period, less a deductible amount of \$100,000 per occurrence. A fairly typical policy defines an "occurrence" as an accident, including continuous or repeated exposure to conditions, that results in bodily injury or property damage neither expected nor intended from the standpoint of the insured during the policy period. Some manufacturers carry "excess" policies with "drop down" provisions in the event that liability exceeds the limits of the primary policy, but many do not.

Most product accidents (such as vehicle collisions or pressure vessel ex-

* *Bichler* case has been upheld.

plosions) occur suddenly. The event alleged as the basis of the manufacturer's liability is clearly an "occurrence" under the policy for the year in which the accident happens. Thus, the coverage of that policy, including limitations and deductibles, applies.

Although the product is alleged to have been defective since manufacture, it has been accepted that an "occurrence" does not happen until the defect results in bodily injury or property damage — perhaps a number of years later. This method of coverage has important practical consequences. When it issues or renews a policy for a particular year, an insurer assumes the risk of sudden occurrences involving products sold in prior years when the manufacturer may have been self-insured or covered by another insurer.

Insurers found product liability coverage increasingly difficult to underwrite as the courts expanded product liability. A "crisis" developed in the middle 1970s. Premiums increased sharply and coverage was restricted or denied entirely to some manufacturers. This led the Department of Commerce to sponsor a study and propose legislation, to which I refer later.

As the 1970s progressed, the difficulties of product liability coverage again were magnified as litigation to recover for nonsudden injuries began to grow. Lawsuits were commenced against manufacturers of pharmaceuticals, chemicals, construction materials, and other products alleging that the products caused disease or injury either gradually over a period of years or caused harm that remained apparently dormant for years and then manifested itself as disease or injury. These cases claimed liability that far exceeded the customary limits afforded for "occurrences" within the annual policy coverage, including "excess" coverage, maintained even by major manufacturers. Because of the nature of the harm claimed, it was not clear when an "occurrence" had taken place.

Faced with enormous potential liability, manufacturers contended that coverage should be provided under policies issued in prior years. Insurers asserted claims against earlier insurers and against manufacturers with respect to earlier periods of self-insurance seeking to shift to them at least a proportionate share of liability for latent diseases and injuries. Two principal theories have been argued: "manifestation" and "exposure."

The manifestation theory was

adopted by the Supreme Court, New York County, in *American Motorists Insurance Company v. E.R. Squibb & Sons, Inc.*, 406 N.Y.S. 2d 658 (1978), and the insurer whose policies were in effect during the years when injuries from diethylstilbestrol manifested themselves was held liable. The exposure theory was adopted by a federal district court in Michigan in *Insurance Company of North America v. Forty-Eight Insulations, Inc.*, 451 F.Supp. 1230 (1980). The decision was affirmed on appeal, and the Supreme Court denied certiorari. The result was that insurers providing coverage during the years of worker exposure to asbestos insulation sold by the defendant manufacturers were held obligated for a proportionate share of the liability to claimants suffering from asbestosis, although the harm did not manifest itself until many years later. The exposure rule also was later applied by the court to claims resulting from mesothelioma and bronchogenic carcinoma.

What theory governs the asbestos cases?

In subsequent decisions the exposure theory was adopted by the Fifth Circuit in *Porter v. American Optical Corporation*, 641 F. 2d 1128 (1981), and by the U.S. District Court in the District of Columbia in *Keene Corporation v. Insurance Company of North America*, 513 F.Supp. 47 (1981). At that point, a potential trend seemed possible toward the exposure theory, which, the courts observed, tends to allocate liability widely among insurers and reduces the difficulty experienced by manufacturers, distributors, and retailers of asbestos and other products in obtaining current coverage.

Recent decisions, however, have not followed the potential trend and have opened possible new directions. In *Eagle-Picher Industries v. Liberty Mutual Insurance Company*, 523 F. Supp. 110 (D. Mass. 1981), the court adopted a manifestation theory. Placing emphasis on analysis of the policy language, Judge Rya Zobel held that injury occurs when exposure produces clinically evident diseases that can be diagnosed. On appeal of the district court's decision in *Keene Corporation*, the Court of Appeals for the District of Columbia Circuit held that insurers who had policies outstanding during periods of inhalation exposure, expo-

sure in residence, and manifestation, must each provide full coverage to the insured manufacturer (667 F. 2d 1034 (1981)). With one dissent, the court also held that the coverage must be provided by the insurers without proration for periods when the manufacturer was uninsured. The court stated that liability among the insurers should be allocated according to the "other insurance" provisions of their policies, which contain formulas for contribution by equal shares and by limits.

The only consistency now found in the courts' decisions is a tendency to decide in favor of theories that provide the greatest insurance coverage on the facts of the case before them. (Keene and *American Motorists* involved the relatively unusual situation in which the manifestation theory provided greater coverage than the exposure theory.) Resolution may have to await review by the Supreme Court, but its decision on March 8, 1982, to decline to review the Keene case may mean a lengthy wait.

Faced with growing exposure to liability and expense during the product liability crisis, manufacturers and insurers have sought legislative relief.

State legislatures have adopted a variety of reform laws. The most common restriction has been a statute of limitations requiring that any product liability action be commenced within a relatively short period (two to six years) after a product defect is discovered or became discoverable, but in no event later than a somewhat longer period (ten to 12 years) after the product was first delivered to its initial purchaser. Other common provisions clarify the availability of defenses based on (1) the state of the art at the time the product was made, (2) modification of the product subsequent to sale, and (3) use of a product beyond its reasonable anticipated life.

The Department of Commerce sponsored an interagency task force on product liability that included representatives of several government agencies supplemented by an advisory committee of representatives of industry, labor, law firms, insurers, and consumer organizations. The task force published its final report in 1977, and the department published a draft model code for adoption by state legislatures. After public comments, the Model Uniform Product Liability Act was published by the department in 44 *Federal Register* 62714 (1979), together with an analysis of its provisions. A statute con-

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THE TOXICITY OF FOODS OF NATURAL ORIGIN*

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(With 17 text figures)

A food is defined as a substance that is taken to replace physiological waste of tissue, to supply energy and heat, and to build up tissues.

Pharmaco-active substances of natural origin, and additives or contaminants of many different kinds introduced by man, occur in a large variety of foods. Many of them are generally not significant, but there are a few that may on occasion seriously affect a single individual, groups of individuals, and sometimes hundreds of animals or human beings, as for example in the case of poisoning with deadly mushrooms, mycotoxins, and pesticides. Even long-established foods may reveal unexpected dangers, as in the case of cheese, which is dangerous when the consumer is receiving treatment with antidepressant drugs of the type classified as mono amine oxidase inhibitors.

It has been known to mankind since the dawn of history that there are natural products which are acutely toxic, but it is only relatively recently that it has been recognized that chronic effects may follow the ingestion of certain materials which do not necessarily produce acute effects. Also, there is scanty information about the possible adverse effects produced by the ingestion of mixtures or combination of foods.

BACTERIAL FOOD POISONING

There are specific bacteria well known as causes of food poisoning; such infections are not considered here.

FOOD ALLERGY

Some persons react abnormally to specific foodstuffs that do not affect the vast majority of people; for example there is the well-recognized sensitivity of some persons to milk or eggs. Almost all allergic reactions, including the most unusual manifestations, can be evoked by foods.

It is possible also that attacks of migraine may be produced in certain susceptible individuals by tyramine and other amines present in foods such as cheese, fish, beans, milk, dairy products and chocolate, and in alcoholic beverages.

Individuals may occasionally show hypersensitivity to drugs present in food, as for example in the case of cow's milk containing penicillin used in the treatment of mastitis in the animal.

While there is evidence that food components can act as antigens and allergens there is a need for more investigation regarding the antigenicity and allergenicity of bacterial products and of additives and contaminants in food.

*Presidential Address (1973) delivered to the Royal Society of South Africa, in Cape Town and Pietermaritzburg.

BIOACTIVE SUBSTANCES

Foods may cause untoward effects because of their content of 'naturally-occurring' pharmacologically active substances. A review of many foods and the substances present as natural constituents or which they derive from contaminating sources will be considered later in this address.

Food additives and contaminants

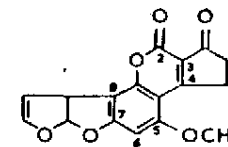
Food may be harmful because of additives or contaminants introduced by man. Food additives are foreign non-nutritive substances intentionally incorporated to enhance the taste, the structure, or the storage life of food. Food contaminants are harmful man-made foreign substances such as pesticides and radionuclides that have accidentally become included in food.

The requirements of our developed society have necessitated that the foods we eat are in many instances prepared and preserved by the addition of chemical substances which are regarded as harmless. Some additives have been used for many years and are generally regarded as safe, but these as well as newer chemicals used in foods need thorough evaluation, regarding their safety, through modern scientific methods of investigation. It is not possible to be completely certain that these additives will never produce harmful effects. The use of chemicals has nevertheless become a necessary part of modern food production and processing. The world is short of food, and the shortage grows worse as the population increases. Substances which prolong the shelf life of food or which diminish wastage are very important.

It is not the substances that produce severely acute toxic effects but rather those which have a slow or cumulative effect that are probably of greatest practical importance. It seems possible that with increasing recognition and knowledge of chemicals consumed by man and animal that the causes of some diseases may be revealed.

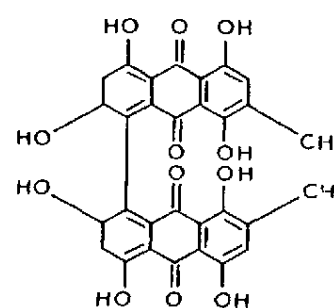
In addition to food additives, food contamination also is a subject of great importance. Antibiotics, for example, may come to be present in foods in a number of different ways. There has been some concern about the storage of pesticides in the fat of mammals, especially the chlorinated hydrocarbon insecticides such as DDT; residues of this persistent insecticide have been found, sometimes hundreds of kilometres from the nearest spraying operation, in the bodies of animals throughout the world. Such residues have accumulated to levels that are catastrophic for certain animal populations, especially carnivorous birds. Contamination of cow's milk and human milk and of hen's eggs with pesticides, especially with organochlorine residues is also well recognized.

Food contamination has recently come to the fore with the recognition of the production of aflatoxins and other mycotoxins by moulds such as *Aspergillus* growing in groundnut meal and other food material. The possibility of this type of carcinogen, and other fungal toxins, being toxic to man has caused concern about the use of certain foods, for example the use of groundnut meal as a milk substitute and an economical food in certain countries. The relationship of foods, feeds, food additives and

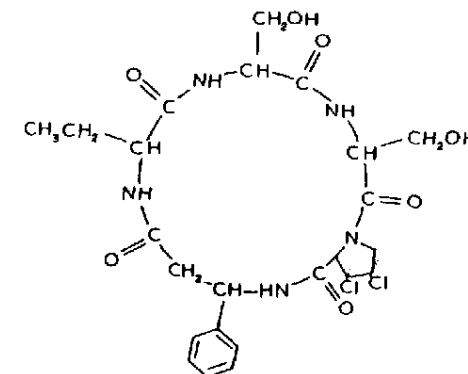
Aflatoxin B₁

contaminants and food degradation products to carcinogenesis has been much discussed in recent years.

In addition to the hepatocarcinogenic aflatoxins present in fungus-contaminated grain and peanuts and in the milk of cows given such contaminated food, other examples of potential carcinogens for man and animals include the following: the carcinogen responsible for hepatoma in rainbow trout; the hepatotoxic carcinogens luteoskyrin and islanditoxin which are present as a common contaminant of rice and rice products used by Asiatic peoples (these substances are produced by a mould



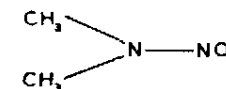
Luteoskyrin



Islanditoxin

Penicillium islandicum which infects rice, producing yellow rice or yellowsis, and may be a cause of primary liver carcinoma in Japan); the very potent carcinogen cycasin present as a natural ingredient, not as a contaminant, in unrefined cycad meal will be considered later.

The possible significance of N-nitrosamines as causal factors in human carcinogenesis is based on the widespread occurrence of nitrosamine precursors in biological



Dimethylnitrosamine

systems and the remarkable way in which various nitrosamines can affect different organs in the same species of experimental animal. This is important in the current

search for nitrosamines or other agents which may induce oesophageal and other cancers in man. These carcinogens can arise during the storage of fish meal preserved with nitrite. Distilled alcoholic drinks such as Kachasu in Zambia have been found to contain nitrosamines. It has been suggested that the high incidence of oesophageal carcinoma in certain African areas (Zambia; Natal Zulu; Xhosa) may be due to the presence of nitrosamines in certain foods and drink.

The presence of radionuclides in foods and the problem of safety levels are regularly studied in many countries. Everyone is now exposed not only to natural radiation but to some additional radiation such as that from strontium-90 present as a contaminant in milk and other foods.

ORDINARY FOODS HARMFUL IN CERTAIN INDIVIDUALS

Foods that are well known and widely used without causing harmful effects may cause disturbances in certain individuals who have basic disorders that may be aggravated or precipitated by an offending food substance. The following are examples.

(a) Allergic disorders, as already mentioned, such as urticaria, asthma and possibly migraine may result from the ingestion of certain foods by predisposed subjects.

(b) Coeliac disease is a gluten-induced enteropathy and the basis of treatment is a gluten-free diet plus certain supplements. The condition occurs in young children in whom there is interference with intestinal absorption of neutral fat (steatorrhoea), carbohydrate, vitamin D and other food factors.

Gluten is a mixture of the proteins gliadin and glutelin present, for example, in wheat flour and rye flour and many breakfast cereals, also in some proprietary preparations such as Ovaltine.

The normal intestinal mucosa is capable of hydrolysing polypeptides of gliadin, those with molecular weight under 2 000, whereas the coeliac mucosa is unable to do so, thus permitting the polypeptides (in some unexplained manner) to damage the mucous membrane of the proximal part of the intestine in these patients.

Many food manufacturers, for example in Britain, label packages of food prepared for use by patients with coeliac disease with a symbol (an ear of wheat with a line through it) to indicate the food is gluten-free.



(c) Patients who have hepatic cirrhosis with liver failure should not be given protein while the special treatment for this complication is being given. Other

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nitrogen-containing substances also should be excluded otherwise hepatic encephalopathy is likely to be produced.

(d) Maple syrup urine disease, so called from the characteristic odour of the urine, is an inborn error of the metabolism of branched-chain amino acids; the withdrawal of valine, leucine, isoleucine and methionine from the diet has produced some benefit.

(e) Favism is a haemolytic disorder often associated with haemoglobinuria, which is induced by the ingestion of the fava bean or by contact with the pollen of the plant. The red blood cells in these patients are deficient in the enzyme glucose-6-phosphate dehydrogenase (G-6PD) necessary for the continued integrity of these cells. The cells are also sensitive to a number of drugs, and to vitamin-K analogues.

(f) Phenylketonuria is an inborn metabolic error of amino acid metabolism. The basic defect is the absence of the hepatic enzyme, phenylalanine hydroxylase, which normally converts into tyrosine the dietary phenylalanine that is not used up in the synthesis of protein. Mental deficiency will occur unless a diet sufficiently low in phenylalanine content such as casein hydrolysate is started early. The brains of these infants are not damaged at birth and they will develop normally if a diet low in phenylalanine content is started early. There is a simple test for phenylpyruvic acid (one of the degradation products of phenylalanine) in the urine. Most states of America require a PKU test at birth.

(g) Galactosaemia is a rare inborn error of metabolism in which the body is unable to convert galactose to glucose. The accumulation of galactose-1-phosphate causes damage to the brain, liver, lens of the eye and the kidneys. In this case the manifestations of the disease may be prevented or reversed in many of the affected infants if the diagnosis is made early and a lactose-free diet given, for example, the elimination of milk products from the diet.

(h) Porphyria genetica (porphyria variegata), an important latent hereditary disease in South Africa, may be produced in certain individuals by alcohol and by drugs such as barbiturates and sulphonamides.

Hypervitaminosis

When pure potent vitamins, especially those of the fat-soluble type, are given in excess, they may act as poisons.

Vitamin A intoxication is recognized as two forms. There is an acute type due to the ingestion of a single large dose. The livers of the polar bear and certain other animals are toxic in this way because they are extremely rich in vitamin A, and potent vitamin A preparations have caused serious poisoning when given in the treatment of young infants and children. Chronic poisoning may result from the daily ingestion of moderately toxic amounts over a considerable period, for example in the treatment of certain diseases. The disorder may occur in food faddists.

Vitamin D administered to children in potent preparations has also caused serious ill-health.

Vitamin K analogues in large and repeated doses should not be given parentally to newborn infants as serious disturbances have been produced. Also, vitamin K

taken by the mother late in pregnancy can adversely affect the foetus.

Nicotinic acid ingested in large amounts may produce pharmacological and harmful effects. Poisoning has occurred from the consumption of meat in which nicotinic acid was an additive to prevent darkening of the meat. Taken in large doses for months this vitamin can aggravate diabetes mellitus.

CLASSIFICATION OF PHARMACO-ACTIVE FOODS

Pharmacologically active substances that may be present in foods may be considered under the following headings:

- Pharmaco-active substances in foods of plant origin
Pharmaco-active substances in foods of land animal origin
Pharmaco-active substances in foods of marine animal origin
Food additives and food contaminants introduced by man.

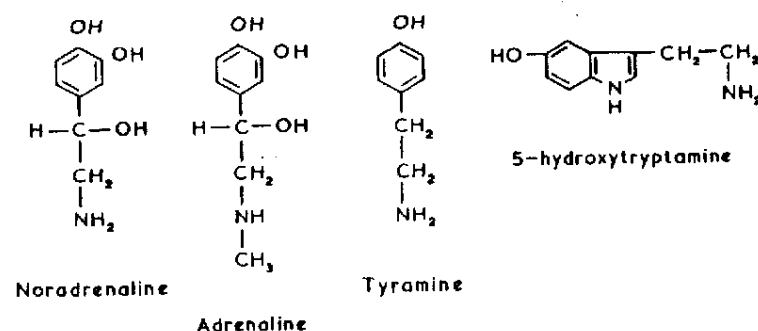
The limited time available for this address does not permit detailed consideration of the many active substances naturally present in foods of plant or animal origin. Attention will therefore be given to some of the more important examples in the above-mentioned groups and particularly with regard to those important for man.

FOODS OF PLANT ORIGIN

Many vegetable materials used as food contain potentially harmful substances such as the following:

Amines

Dopamine, noradrenaline, adrenaline, 5-hydroxytryptamine and tyramine are present in certain fruits and vegetables used as human food, for example in bananas and plantains, tomatoes, avocado and pineapples. Certain amines are also found in



cheese especially cheddar, Camembert, and Stilton cheese, in a number of other foods, and in certain wines. It has been demonstrated that arylalkylamines are more widespread in the plant kingdom than had previously been realized. The banana contains the largest amounts of these substances; banana peels contain enormous quantities.

AMOUNT EXPRESSED AS MG/100 ON FRESH FRUIT

	5-Hydroxytryptamine	Tyramine	Dopamine	Noradrenaline
Bananas (peel)	5-15	6,5	70	12,2
Bananas (pulp)	2,8	0,7	0,8	0,2
Plantain (pulp)	2-10	—	—	0,2
Pineapples (ripe)	2	—	—	—
Avocado	1	2,3	5	0
Tomato	1,2	0,4	0	0
Plum, red	1	0,6	0	+
Plum, blue	0	—	—	—
Eggplant	0,2	0,3	0	0
Potato	0	0,1	0	0,2
Orange	0	1,0	0	+

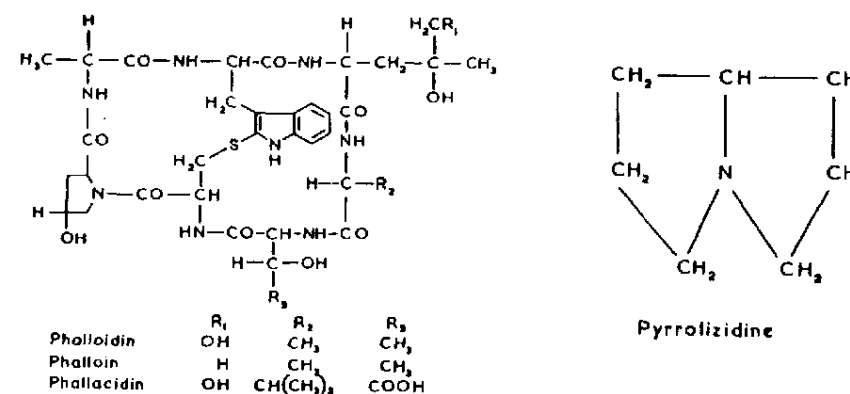
Cyanogens

Many plant foodstuffs contain cyanogenic glycosides. Such compounds have been found, for example, in maize, sorghum millet (kaffircorn), lima bean, kidney bean (haricot), sweet potato, manioc (cassava), almond, and in many other plant materials such as the seeds of apples, apricots, cherries, pears, plums, prunes and other fruit. Bruising of such foodstuffs causes the interaction of an enzyme and glycoside and the release of hydrocyanic acid. Poisoning is rarely produced by most of these foodstuffs because the cyanogens are present in small amount or in parts of the food (seeds, pips) which are not eaten. However, poisoning has not infrequently occurred from the ingestion of manioc and lima beans.

The seed of sorghum, the common sorghum or great millet known in South Africa as kaffircorn used in the preparation of Bantu beer, is edible, but the plant is toxic to stock due to the production of hydrocyanic acid from the cyanogenic glycosides.

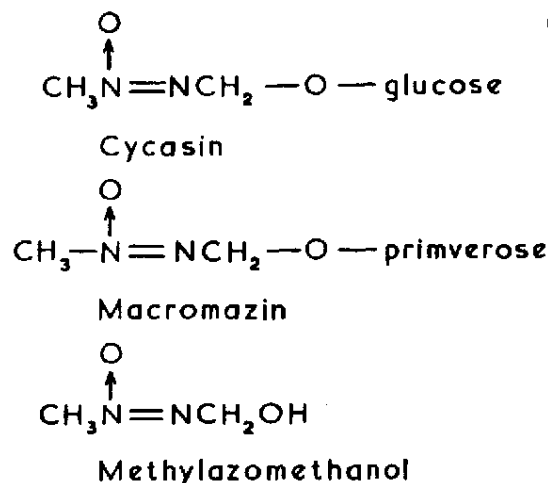
Hepatotoxins

A number of plant and fungal poisons can produce liver damage in animals and man. As already mentioned, *Aspergillus* aflatoxins and other mycotoxins may be present as contaminants in certain foods. The *Amanita* cyclopeptides in mushrooms



such as the Death Caps, *Amanita phalloides* and *Amanita capensis*, and the *Senecio* alkaloids (pyrrolizidines) are severe liver poisons. Many members of the *Senecio* genus of plants are eaten as spinach (imfino) or are used for medicinal purposes by the Bantu in some areas of South Africa. Alcoholic beverages are also important causes of pathological changes in the liver and other organs in man.

Cycad seeds or kernels, also called cycad nuts, are used as food especially in the Pacific area. Cycad flour prepared by soaking the seeds in water removes carcinogenic substances, but ground unwashed cycad seeds are extremely toxic; malignant tumours have been induced in the liver and kidneys of experimental animals and resemble those produced by nitrosamines. The toxic substances are azoxy glycosides such as cycasin and macrozamin in which the aglycone, methylazoxymethanol, is the



carcinogenic moiety; cycasin is decomposed to the aglycone by bacterial enzymatic action. Cycad meal may also be contaminated with fungi that produce mycotoxins like the aflatoxins so that carcinogens both from the meal and the fungi may be the cause of liver cancer and other malignancies in people who eat this food.

Metals

Vegetables and animal foods normally contain minute traces of many elements and at times metals such as copper, arsenic, and iron, usually in small amounts. Selenium poisoning occurs in certain areas in the United States and in other countries where fodder and grain are rich in selenium due to the unusual amount in the soil.

In South Africa numerous species of plants in the Karoo area have been found to contain dangerous amounts of selenium, and correlation has been claimed between selenium content of the vegetation and the incidence of geeldikkop and enzootic icterus, which are important ovine diseases in this country.

Nitrates

A number of plants growing under certain soil conditions have been found to contain dangerous amounts of nitrate (saltpetre). Some have caused serious losses in pigs and other animals from nitrate poisoning. The nitrate content of spinach has on occasion caused nitrate poisoning in infants who had eaten spinach. Nitrates are reduced to nitrite in the gastrointestinal tract and these convert haemoglobin to methaemoglobin.

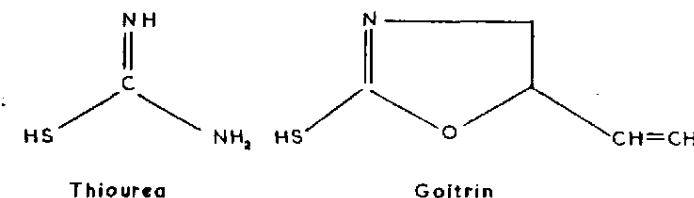
Oxalates

Certain plants, for example rhubarb, spinach, celery, and green plantain, contain oxalic acid usually in the form of acid potassium oxalate; they have occasionally given rise to fatal poisoning when used in cooking or for medicinal purposes. Normally 80 to 90 per cent of oxalate in the diet is not absorbed but is eliminated in the faeces as insoluble calcium salts. The oxalic acid content (mg per 100 gm fresh edible uncooked portion) of certain foods is shown in the following list.

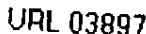
Rhubarb	..	..	..	100	-900
Spinach	..	..	..	320	-900
Celery (stalk)	..	..	..	620	
Almond	..	..	..	407	
Plantain (green)	..	..	..	517.5-524	
Plantain (ripe)	..	..	..	2,2	
Sorrel	..	..	..	100	-500
Cashew nut	..	..	..	318,4	
Parsley	..	..	..	100	-200
Beetroot	..	..	..	100	-200
Currants	..	..	..	25	-100
Prunes	..	..	..	25	-100
Beans	..	..	..	25	-100
Soyabean	..	..	..	77	

Goitrogens

A number of plants contain an antithyroid (goitrogenic) principle which has been identified as *l*-5-vinyl-thiooxazolidone, known as goitrin which is a cyclized thiocyanate. It has been found in high concentration in extracts from the seeds of



most *Brassicae*, for example cabbage seed and the edible parts of turnip. However, the goitrogens are present in such low concentrations, except in seeds, that it is doubtful whether human beings would eat enough for goitres to be produced; cooking destroys the goitrogenic activity. Cabbage also contains a thioglucoside, glucobrassicin, which



Lathyrus poisons

$$\text{HOOCCH(NH}_2\text{)CH}_2\text{CH}_2\text{CONHCH}_2\text{CH}_2\text{CN}$$

γ -glutamyl- β -aminonitrile

$$\begin{array}{ccc} \text{NH}_2\text{CH}_2\text{CH}_2\text{CH}(\text{NH}_2)\text{COOH} & & \text{N}\equiv\text{C}-\text{CH}_2-\text{CH}(\text{NH}_2)\text{COOH} \\ \text{Diaminobutyric acid} & & \beta\text{-cyanoalanine} \end{array}$$

Spices

Nutmeg contains myristicin, which may have psychotropic properties, and a number of other active principles. Myristicin is closely related in chemical structure to safrole, which is hepatotoxic and even hepatocarcinogenic in rats. (Safrole is a component of several essential oils. It was once extensively used in the U.S.A. as a



Myristicin

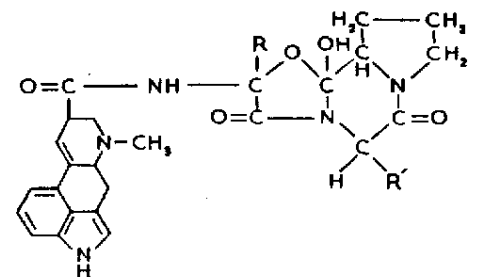


Safrole

Some other substances

[illegible]
$$\begin{array}{l} \text{H}_2\text{C} \begin{cases} \text{S} - \text{CH}_2 - \text{CHNH}_2 - \text{COOH} \\ \text{S} - \text{CH}_2 - \text{CHNH}_2 - \text{COOH} \end{cases} \\ \text{Dienkolic acid} \end{array}$$

Sanguinarine (in *Argemone mexicana*) which is one of a number of substances suggested as a causal factor in oesophageal carcinoma, and ergot alkaloids which can cause ergotism (St. Anthony's Fire), have occasionally been contaminants in bread.



Ergotamine, $R = -CH_3$, $R' = -CH_2-C_6H_5$

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Honey

This has on rare occasions been the cause of poisoning when it contained toxic material collected by the bee from plants such as rhododendrons, *Nerium oleander*, datura, and some other poisonous plants.

Honey and honeycombs may contain fumigants such as calcium cyanide, methyl bromide and paradichlorobenzene.

FOODS OF ANIMAL ORIGIN

Pharmacological activity of food of land animal origin may be considered from two points of view. Firstly, the intrinsic composition of the food may temporarily or permanently make it unsuitable and even unsafe for certain individuals. Secondly, the presence of additives and especially of contaminants may render it potentially or actually harmful for all persons who consume such food.

MEAT

The majority of mankind has a natural appetite for meat, and the flesh of more than one hundred different species of animal is regularly eaten by man.

Reference has already been made to the fact that protein in the diet, and other nitrogen-containing substances including a number of drugs, produce an abnormally increased concentration of ammonium ions in the blood, which must be avoided in patients who have cirrhosis of the liver. Otherwise, neurological symptoms may develop that progress to stupor, coma (and death); the condition is referred to as hepatic coma or portacaval encephalopathy and was previously known as meat poisoning or ptomaine poisoning.

In certain sensitive persons the ingestion of cooked crab meat has produced mild to severe anaphylactic shock.

Meat extracts, for example Bovril, contain amines that can enter the bloodstream when certain drugs, the monoamine oxidase inhibitors are taken by patients suffering from certain types of depression. A severe hypertensive reaction may arise, such as may occur when tyramine-containing cheese and certain other foods are ingested concurrently with these drugs.

Additives and contaminants

Nitrate or nitrites added to meat and meat products as preservatives have been the cause of poisoning (methaemoglobinaemia).

Sodium nicotinate or nicotinic acid used for the preservation of the red colour in meat, to prevent its darkening, has caused flushing and itching of the face and neck, nausea, abdominal cramps and sweating.

Cyclamates (sweetening agents) have been used in meats such as bacon and ham. In large doses they are carcinogenic in animals; the metabolite cyclohexylamine is an inducer of bladder cancer.

Meat grilled on skewers made from the wood of the ornamental shrub *Nerium oleander* L. (Ceylon rose) has caused death. All parts of this plant are poisonous due to the presence of digitalis principles.

Certain plants of the genus *Cotyledon* contain neurotoxic and cardiotoxic principles. Poisoning has occurred in dogs that have eaten the meat, blood, or entrails of poisoned animals. Human beings, for example Bushmen, have developed symptoms after eating raw or undercooked meat or the blood of such animal.

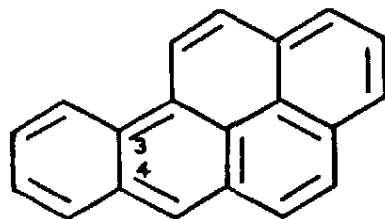
The meat of the quail, a bird related to the partridge and grouse, has on some occasions been poisonous. This has been attributed to the ingestion by the birds of toxic plants such as hemlock which does not affect them but which renders their meat toxic. During their travels in the desert the Hebrews ate manna and quail. The quail 'fell in great profusion around the camp of the Israelites' and the numerous deaths attributed to the eating of these birds is mentioned in the Old Testament (Numbers 11). In recent years some people on the island of Lesbos have developed an acute myoglobinuria shortly after eating quail, from sensitivity to some substance present in the quail. Muscular fatigue is regarded as important since the disorder rarely occurs in persons at rest. This observation has been suggested as an explanation for the deaths of the Hebrews. However, myoglobinuria also occurs occasionally in normal subjects, for example in military trainees after severe exertion, also in Haff disease (in Eastern Europe) possibly here due to an unidentified toxic substance present in contaminated fish, and in a variety of disorders of muscle.

The widespread use of pesticides and dissemination of radio-active materials over large areas has led to contamination of soil, water and plant and animal material. Meat and milk from cattle that have eaten this type of contaminated herbage present a danger to man that may become serious.

Broiled meat (steaks) and barbecued ribs, and smoked fish, contain complex polynuclear (polycyclic) hydrocarbons, some of which are carcinogens; these, however, are present in small quantities and are not regarded as dangerous to the consumers of such food. It has been demonstrated that polynuclear hydrocarbons are present in higher concentrations in steaks and barbecued ribs prepared in a restaurant, but the amounts present in these broiled foods are small and do not necessarily constitute a danger to the consumers of such foods.

In Iceland it is customary in rural areas to preserve mutton and fish by a heavy smoking process; these foods then contain among other compounds the carcinogenic

substance 3,4-benzpyrene. This is an example of contamination of a food by simple traditional processes. Gastric cancer is common among the Iceland population.



3,4-benzpyrene

Liver

Liver is a useful food containing naturally a variety of substances including certain vitamins and haematinic substances. However, there are circumstances where liver can be harmful and dangerous to life.

The livers of polar bears and certain other animals such as the Arctic fox, seal, whale and shark are extremely rich in vitamin A and acute intoxication (hypervitaminosis A) has occurred in man, for example in Arctic explorers and in their dogs, from the consumption of this material.

VITAMIN A (IU PER 100 GM WEIGHT, FRESH)

Cod-liver oil	200 000
Halibut-liver oil	4-6 million (+)
Shark-liver oil	3 million (+)
Polar bear liver	1,8 million
Seal liver	1,3 million
Tunny (tuna)	0,8-8 million
Tunny (tinned)	70-200
Sardines	4 800-54 000
Sardines (tinned)	136-290
Herring	9 000
Liver (sheep and ox)	4 000-45 000
Butter	2 400-4 000

Chicken liver eaten by patients taking the so-called monoamine oxidase inhibitor drugs for the treatment of depression may induce hypertension. As already mentioned, this may occur when certain other foods that contain amines are taken with these drugs.

Milk

Milk has abundantly been used in the management of gastric and duodenal ulcer. When ingested in large doses for months with much soluble absorbable alkali such as sodium bicarbonate, hypercalcemia, metastatic calcification in the arteries and kidneys (nephrocalcinosis) and other changes have been produced, a condition known as Burnett's syndrome.

Cow's milk may contain pesticides, and pesticide residues may be found in human milk. Also antibiotics, radionuclides, fungal toxins and active principles from certain plants may be present in cow's milk.

Penicillin in milk has caused skin and allergic reactions in persons sensitive to this antibiotic. Streptomycin and possibly chloramphenicol may also be incriminated in this respect.

Milk sickness (trembles; alkali disease) is a disorder of cattle in the United States, caused by eating white snake-root or rayless goldenrod. The poison in the milk (or meat) of animals has also caused illness in man. Several members of the Lincoln family, including the mother of Abraham Lincoln, and many early settlers in the United States died of this 'milk sickness'. The leaves and stem of the plants mentioned contain 'tremetol', which is a mixture of several compounds. The poison is not destroyed by pasteurization of contaminated milk or milk products.

Goitrogenic substances may be present in cow's milk, following the ingestion of cabbage, turnips, and certain other plants. Goitrogens (such as goitrin) in cow's milk have been suspected as the cause of goitres in Tasmania and Australia.

Death has occurred in an infant who drank milk from a cow that had eaten the foliage of *Nerium oleander* L.

Milk from cows fed very toxic aflatoxin-containing groundnut meal can induce liver lesions in ducklings.

Cheese

Various cheeses, for example cheddar, Camembert, and Stilton, have been found to contain tyramine. There may be enough amine present to produce a marked rise in blood pressure and other cardiovascular changes in patients receiving drugs that are mono-amine oxidase inhibitors. Sudden severe headache lasting for twenty minutes to several hours is associated with a marked rise in blood pressure. Death may occur from intracranial haemorrhage. An attack has followed the ingestion of as little as one ounce (27,5 gm) of cheddar cheese. The tyramine present in the cheese enters the general circulation and releases noradrenaline from local stores in nerve endings. It has been suggested that in certain susceptible individuals attacks of migraine are induced by tyramine and other amines present in cheese, milk, dairy products and certain other foods.

Moulds may be present in cheese, and many produce toxic substances from which the danger cannot on present knowledge be completely excluded.

Radionuclides may be present in cheese.

ATHEROSCLEROSIS

The possibility that certain dietary constituents such as animal fats, sugar and alcohol may be causal factors in the production of atherosclerotic changes has been much investigated for many years. More proof is still required that dietary factors and dietary measures influence atherogenesis in man. Other hypotheses have also been put forward regarding atherogenesis, for example that there is a deficiency of

essential fatty acids, that prostaglandins may play an important role in the effect of dietary fats. Drinking water has also been incriminated; some association has been found between softness of drinking water and increased death-rates due to cardiovascular diseases although the foods consumed may be more important in determining the ultimate effects than the type of drinking water.

FOODS OF MARINE ANIMAL ORIGIN

There are marine animals which regularly produce or which temporarily contain active principles that may cause poisoning when they are ingested by man.

SHELLFISH POISONING

Mussels may occasionally cause illness, and even death. The paralytic type of poisoning is most dangerous, caused by a potent toxin in the shellfish, which is known as clam toxin, mussel toxin, and saxitoxin (from its presence in the Alaskan butterclam *Saxidomus giganteus*).

Paralytic shellfish poisoning occurs when certain molluscs (mussels, clams, scallops) that have ingested toxic dinoflagellates are eaten by man. Under certain conditions 'blooms' of such toxic plankton may cause the phenomenon known as red water or red tide, visible in the daytime and luminescent at night; this heralds an outbreak of shellfish poisoning.

Toxic shellfish have been found sporadically in many parts of the world. Certain coastal regions of South Africa, for example the False Bay and St. Helena Bay areas, have been affected in recent years.

Poisonous mussels are indistinguishable from normal ones. They are themselves unaffected by the toxin which can only be detected by animal toxicity tests; an easy, quantitative chemical test for the poison is desirable.

The usual methods of cooking do not reliably remove the poison.

The clinical features of poisoning have been described by many authors. The pathognomonic symptoms arise within thirty minutes and include a tingling or burning sensation on the lips, gums, tongue and face, spreading to other parts of the body; this paraesthesia changes to numbness. In severe cases ataxia and interference with speech and swallowing occur. In the terminal stages there is motor weakness, and paralysis becomes progressively more severe. Death occurs from respiratory paralysis usually within twelve hours. Treatment is symptomatic as there is no specific antidote.

Paralytic shellfish toxin is one of the most lethal biological substances known. Man is very sensitive to it; the minimal lethal oral dose of purified toxin may be between 1 to 4 mg.

POISONOUS FISHES

Approximately five hundred species of marine fish are known to be toxic, and may be poisonous when eaten by man. Some are poisonous at all times, others only at certain periods or only in certain areas.

In conclusion it may be said that every meal very likely contains deliberate or accidental addition of many possibly injurious non-nutritive compounds such as additives and contaminants as well as pharmaco-active compounds in foods obtained from plants and from land and marine animals, and harmful synthetic substances in water, soft drinks and alcoholic beverages.

This review has been concerned mainly with naturally-occurring substances in foods that may cause mild but occasionally severe and sometimes fatal reactions in man or animals eating such foods.

Examples of toxic principles in certain natural foods

Of plant origin

Ackee fruit
Almond
Bananas, and some other fruits
Beans, broad
Beans, soya
Beans, Lima
Brassica seeds, and some other vegetables
Cycad seeds
Fungi
Jenghol seed
Lathyrus seeds (chick pea)
Legumes
Manioc (cassava)
Millet (Kaffircorn)
Nutmeg
Potato (green)
Rhubarb

Hypoglycins (unusual amino acids)
Cyanogenic glycoside
5-Hydroxytryptamine, noradrenaline, adrenaline
Dopa, dopamine
Trypsin inhibitor, haemagglutinins, ocatrogens
Cyanogenic glycoside
Thiooxazolidone, thiocyanates
Azoxy glycosides which yield methylazoxymethanol
Various active principles
Djenkolic acid (amino acid)
Beta aminopropionitrile
Haemagglutinins
Cyanogenic glycoside
Cyanogenic glycoside
Myristicin, and other active agents
Solanine
Oxalate

Of animal origin

Cheese
Fish
Liver, polar bear and some others
Meat, broiled
Meat extracts
Mussels
Quail

Tyramine
Various toxic principles
Vitamin A in large amount
Hydrocarbons (carcinogenic)
Amines (adrenergic)
Saxitoxin
Hemlock (?)

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LETTERS

Cancer and Diet

Bruce N. Ames' remarkable summary (23 Sept., p. 1256) of the evidence that cancer and cardiovascular and other degenerative diseases are of metabolic origin comes as welcome support for the hitherto-little-noticed contentions by Totter (1), Handler (2), and Fridovich (3) that oxygen radicals may be an important proximate cause of cancer.

The thrust of Ames' article would seem to be that cancer is essentially a natural aging process. No matter what we eat, the huge flood of oxygen radicals produced in many metabolic processes overwhelms all but the most heavy external carcinogens, such as tobacco in heavy smokers. To be sure, anticarcinogenic substances are of benefit, but to choose a noncarcinogenic diet would probably be equivalent to starving to death.

The implications of Ames' findings are broad and fall into three categories. First, our preoccupation with small effluents of carcinogens resulting from various industrial processes represents a serious misdirection of resources. This was revealed by Totter in 1980 (1), when he showed that overall cancer mortality in 19 countries, when corrected for completing risks, was not correlated with degree of industrialization, as measured by per capita energy use.

Second, the Delaney amendment, which seeks to eliminate the last trace of artificial carcinogen in food, seems to be targeting a tiny part of the carcinogenic burden and ignoring the major carcinogen, the ubiquitous oxygen radical. In short, the Delaney amendment may be based on wrong science and be wrong policy.

Finally, if we concede that cancer, like death itself, is "natural," then our primary focus in cancer research ought to shift far more toward early detection and extirpation of tumors. This, I believe, would require rethinking of the National Cancer Institute's underlying strategy, which at present seems to be dominated by the belief that cancer, unlike death itself, is a preventable disease.

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3. I. Fridovich, *Science* **201**, 875 (1978); subsequent personal communication.

On page 1260 of his article "Dietary carcinogens and anticarcinogens" Ames refers to "dietary selenium (usually selenite)." The term "selenite" could cause confusion because geologists know the mineral selenite, a variety of gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) containing no selenium. Ames must be referring to a compound of selenium when he uses the term "selenite." I doubt that the mineral selenite would inhibit tumor or counter the oxidative toxicity of mercuric salts.

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Perhaps the most provocative thing in Ames' provocative article is his placing of the figs in the family Umbelliferae. It is often said that disciplinary crossovers can reinvigorate static fields by bringing new insights unfettered by conventional wisdom. It remains to be seen if Ames' dietary pre- and proscriptions have as much impact on American life-styles as his creative taxonomy is likely to have on the family Moraceae.

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In his article "Dietary carcinogens and anticarcinogens," Ames states (p. 1258) that "high dietary fat is a promoter and a presumptive carcinogen," citing among his references the recent National Academy of Sciences (NAS) report *Diet, Nutrition, and Cancer* (1). The NAS report does state that "most of the data suggest that dietary fat has promoting activity"; however, this report does not refer to fat as "a presumptive carcinogen." Rather, the report notes that "there is not enough evidence to warrant the complete exclusion of an effect on initiation."

Ames writes that "the amount of ingested oxidized fat may be appreciable." Neither of the references he cites (2, 3), however, provides direct evidence in support of this statement. Shorland *et al.* (2) demonstrate that vitamin E supplementation to calves retarded lipid oxidation of some muscle tissue but not others during frozen storage. No estimates are provided regarding how much oxidized fat humans typically ingest. A perusal of *Autoxidation in Food and Biological Systems*, edited by Simic and Karel (3), reveals the same facts. There are no direct estimates of the amounts of oxidized fat ingested by humans, and it is well recognized that the unpalatable nature of rancid fats precludes their ingestion in significant quantities.

We strongly disagree with Philip H. Abelson's assertion that "the colon and digestive tract are exposed to many fat-derived carcinogens" (Editorial, 23 Sept., p. 1249). Unsaturated fatty acids in dietary fats are subject to chemical reactions (oxidation, polymerization, hydrolysis) that can occur to a limited extent during deep-fat frying. The extent of these reactions, however, depends largely on frying conditions, principally the temperature, aeration, and duration. Many of the studies used to support the implication that oxidation that can occur during cooking "form[s] mutagens, promoters, and carcinogens" were performed under exaggerated conditions that are unrealistic and not indicative of actual conditions. It is the usual practice of restaurants to discard frying fat when prolonged frying causes excessive foaming of the hot fat or when undesirable flavor or dark color develop. This being the case, Abelson's statements that "rancid fats are possible causative agents of colon and breast cancer in humans" and that "rancid fats should not be part of the diet" are unnecessarily alarming to prudent users of heated fat or other fat-containing products.

In support of the safety of fats heated under more realistic conditions, a 2-year animal feeding study by Nolen *et al.* (4) showed that animals consuming used frying fats as the sole source of fat in the diet throughout their life-span thrived as well as control animals consuming the same fat that had not been subject to frying conditions. Furthermore, if "the colon and digestive tract are [truly being] exposed to many fat-derived carcinogens," we should be seeing increasing colon and breast cancer mortality in the United States as a result of the marked increases in vegetable oil (much of it highly unsaturated) consumption since the early 1900's (5). In fact, however, data from the American Cancer Society indicate that age-adjusted mortality rates for both colon and breast cancer have remained essentially unchanged since 1940 (6).

Ames notes that "[s]ome fatty acids, such as $\text{C}_{22:1}$ and certain *trans* fatty acids, appear to cause peroxisomal proliferation because they are poorly oxidized in mitochondria and are preferentially oxidized in the peroxisomes." Data are not accumulating, however, to substantiate such a theory.

Citing the paper of Enig *et al.* (7), which has been criticized (8), Ames states that "Americans consume about 12 g of *trans* fatty acids a day and a similar amount of unnatural *cis* isomers, . . . mainly from hydrogenated vegetable fats." We believe these estimates of

consumption are excessive and are not supported by reliable data. A more reasonable estimate of consumption of "unusual" *cis* and *trans* positional isomers has been suggested by Emken to be around 9 grams per day (not 24 grams per day, as suggested by Enig *et al.*) or about 6 to 8 percent of total fatty acid intake (9). When one considers that the fatty acid composition of adipose tissue reflects that of the diet and that a range of from 2.0 to 5.8 percent *trans* fatty acids has been reported in human adipose tissue (10), an adult male consuming his recommended dietary allowance of 2700 calories per day (11) of a diet providing 40 percent of the calories as fat would ingest around 2.4 to 7 grams of *trans* fatty acids per day.

Contrary to the disputed hypotheses of Enig *et al.* (7), there are no reliable data relating *trans* fatty acids to tumor development. A study by Brown (12) not cited by Ames indicated no unusual incidence of tumors in mice treated with dimethylhydrazine (or with saline) and then fed a diet high in *trans* fatty acids for 17 months.

Finally, Ames mentions the disagreement between the NAS report (1) and a critique of this report (13) by the Council for Agricultural Science and Technology (CAST) on the appropriateness of recommending reduced fat consumption to the American public. In doing this, he indirectly quotes the CAST report as saying that, "until we know more . . . about which types of fats are dangerous, it is premature to recommend dietary changes" (13). However, the CAST report does not state that certain "types of fats are dangerous." We believe there are insufficient reliable data to justify the suggestion that certain fats in the current American diet represent a substantial cancer risk.

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We commend Ames for his review of natural dietary toxins, but not for concluding that, rather than reducing exposure to environmental and occupational carcinogens, "dietary practices are the most promising area to explore" for reducing cancer risks. Ames' article, moreover, is flawed by substantial errors, omission of relevant data, and reliance on tenuous hypotheses. These limitations are more significant in view of the major public health implications of Ames' article and the accompanying editorial by Abelson, press release, and publicity in the mass media.

Ames' position that there is no evidence for generalized recent increases in U.S. or U.K. cancer rates, other than for cancers attributed to tobacco, is based on epidemiological analyses that, with tenuous justification, exclude people over the age of 65 and also blacks of all ages and attribute a near exclusive tobacco etiology to cancers of various organs in addition to the lung (1). In fact, overall cancer rates have increased sharply since 1970 (2). Incidence and mortality rates in the United States, age standardized to 1970, have risen sharply since the late 1960's particularly for persons over 60, blacks of all ages, and a wide range of occupational subgroups (2-4). From 1969 to 1976, mortality rates increased for white and black males by 8 percent and 17 percent, respectively, and for white and black females by 4 percent and 6 percent, respectively. While this increase was pronounced for lung cancer—21 percent and 32 percent for white and black males, respectively, and 74 percent and 56 percent for white and black females, respectively—increases also occurred in other organs, including, for whites, the prostate (11 percent), male and female kidney (5 percent), and female breast (4 percent); sharper increases were noted for less common cancers, including those of brain, liver, esophagus, and multiple myeloma. Incidence rates rose more rapidly than mortality on an overall basis and for cancers of various organs, such as the colon, bladder, kidney, skin (melanoma), uterus, female breast, and prostate, besides lung (2); for whites, cancers of sites other than the lung accounted for approximately 70 percent of the increase. The most recent data show per-

sistence of these trends through 1980 (5). These trends are consistent with the theory that past exposure to industrial carcinogens, whose production have increased exponentially since the 1940's, are responsible for recently increasing cancer burdens (3, 4).

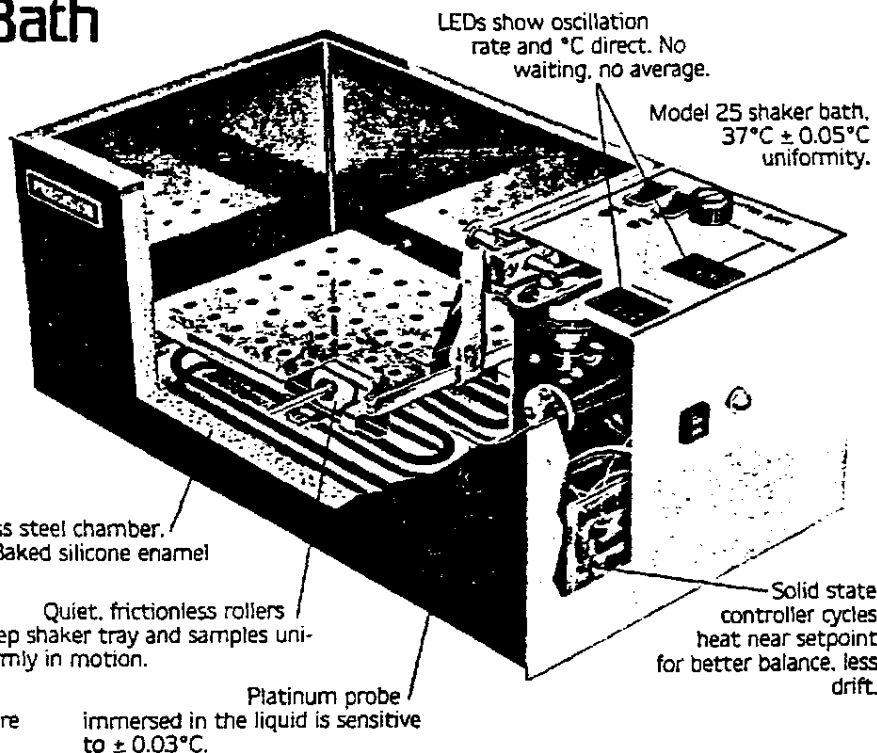
The assertion that smoking is responsible for essentially all lung cancer, and thus accounts for almost all recent increases in cancer rates, is negated by substantial evidence (3), including (i) the more than doubling of lung cancer rates among nonsmokers over the last two decades, with the proportion of these cancers in nonsmokers approaching 20 percent (3, 6); (ii) the sharply increasing incidence of adenocarcinoma of the lung, which is less closely related to smoking than are squamous and oat cell carcinomas (7); (iii) over the last three decades (8), the decline in the proportion of smoking males and the tar content of cigarettes, while lung cancer mortality increased at a rate that cannot be accounted for by cohort effects; (iv) the strong positive associations, largely independent of smoking habits, between lung cancer and exposure to a wide range of occupational carcinogens, including vinyl chloride, mustard gas and chloromethylmethylether, and carcinogenic processes, such as copper smelting and uranium, zinc, and lead mining (3, 4); (v) lung cancer rates in black men that are now about 40 percent higher and have been increasing more rapidly than in whites over the last 30 years, although blacks smoke less and start smoking later in life (4, 9); (vi) lung cancer rates that are almost equal in white and black women, although the proportion of whites smoking more than one pack a day is twice that of blacks (9); (vii) a threefold increase in lung cancer rates among women between 1950 and 1975, a steeper increase than could be accounted for by the modest rise in their smoking prevalence (8); (viii) the major geographic variations in mortality rates due to cancers of the lung (besides other organs) that have been associated with workplace and community air pollution (10) and are not explainable by differences in smoking patterns; (ix) the shift of the highest lung cancer rates from northeastern to southeastern and southcentral states after World War II industrialization of the South; and (x) the divergent trends and directions observed between cancers of the lung, on the one hand, and, on the other hand, of other organs, including the esophagus, buccal cavity, and pharynx (4), which have also been strongly associated with cigarette smoking (1). These considerations in no way

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detract from the critical importance of tobacco as a major cause of preventable disease and death.

In his statement that high-dose exposure to occupational carcinogens "might also turn out to be important for particular groups of people" [emphasis added], Ames does not acknowledge the substantial literature on occupational cancer. According to a 1978 federal estimate, occupational exposure just to asbestos and five other carcinogens could, on a worst case basis, account for 18 to 38 percent of all male cancers in coming decades (11). Even outspoken critics of these estimates, whose analyses Ames cites, concede that "the minimum proportion of all current cancer deaths attributable to occupation can hardly be less than 2% or 3%" (1), 4000 to 6000 male deaths per annum. Asbestos and coke plant workers both have lung cancer rates five to ten times those of appropriate controls (11). Some 10 million workers are now potentially exposed to 11 "high volume human carcinogens," and there are major excesses of cancers throughout a wide range of occupational groups, including oil refinery and petrochemical workers, rubber and tire workers, welders and metal-trades workers

(4), and atomic plant workers (12). These studies are all the more important as two- to fivefold excesses in cancer rates have generally been necessary before they could be detected by standard epidemiological techniques (13).

Contrary to Ames, substantive studies have documented the carcinogenic effects of urban air pollution or some related urban factor. Accordingly, the World Health Organization concluded that "it is probable that some urban atmospheric factor is involved [in the etiology of lung cancer], resulting from the air pollution from car exhausts, fumes from heating systems and industrial fumes" (14); automobile exhaust contains a wide range of carcinogens, many common to tobacco smoke. In addition, many epidemiological studies have documented large geographical variations in standardized cancer mortality rates, on an overall and organ-specific basis, with higher rates in communities located near smelters, petrochemical plants and facilities producing nuclear weapons, and in communities with high levels of atmospheric pollution (10, 15); definitive epidemiological evidence of carcinogenic and reproductive hazards from proximity of residence to hazardous waste land-

fills or industrial impoundments is not yet available, although preliminary data from sites such as Woburn, Massachusetts, are highly suggestive (16).

Ames dismisses the possibility that carcinogenic synthetic pesticides, marketed since the 1940's, may contribute substantially to cancer rates, as their dietary intake is claimed to be 10,000 times lower than that of age-old "nature's pesticides." There is, however, much evidence to the contrary. For example, a number of widely used chlorinated hydrocarbon pesticides have accumulated by many orders of magnitude in certain foods to levels comparable to those inducing cancer in small groups of experimental animals (17). Chub and trout in Lake Michigan have been found with aldrin and dieldrin residues above 0.3 part per million, and similar residues of chlordane and heptachlor have been found in the Great Lakes and in Long Island and New York City lakes; in 1983 Montana health officials warned against eating game contaminated with concentrations of heptachlor epoxide more than 100 times the Environmental Protection Agency's (EPA's) "acceptable intake level." Aldrin and dieldrin were found to be carcinogenic at dietary concentra-

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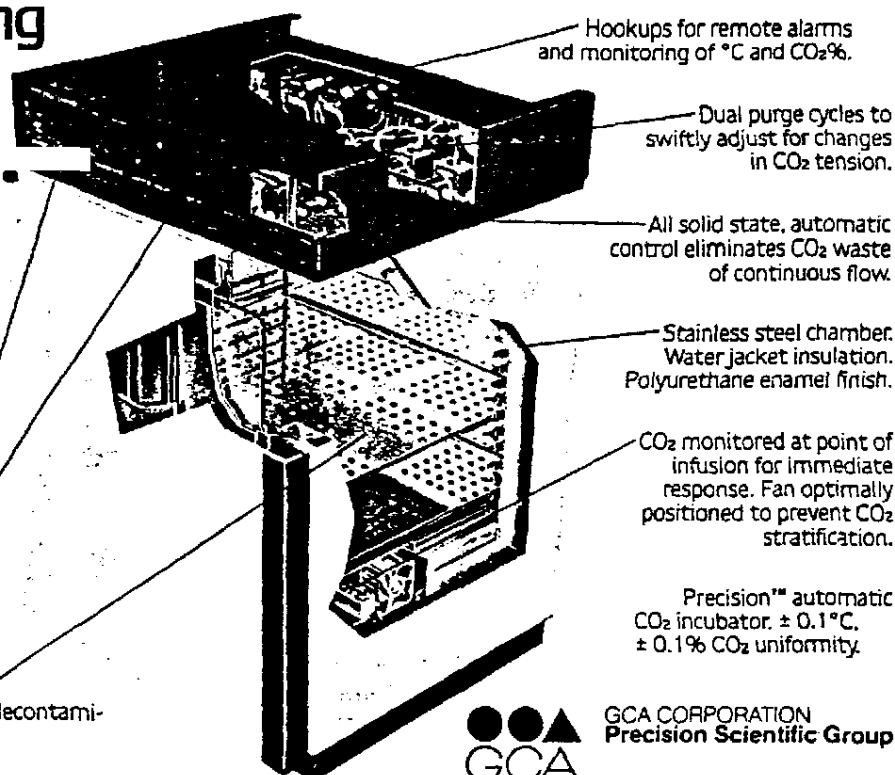
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tions of between 0.1 and 20 parts per million in five separate rodent bioassays, and residues of chlordane and heptachlor have been found in concentrations in human fat similar to those found in rats in whom carcinogenic effects had been induced by these pesticides (18). By all principles of extrapolation, such exposures would be expected to result in a significant excess of human cancers. The widespread use of chlordane and heptachlor for termite treatment represents additional major carcinogenic exposures. Indoor chlordane concentrations greater than an arbitrary interim guideline of 5 micrograms per cubic meter have led to the evacuation of more than 1500 contaminated homes at Air Force bases across the country (19) and to the petition by a New York State citizens' group, after the finding in April 1983 that 63 percent of 443 treated homes were contaminated, to ban the use of chlordane for termite treatment. Exposure to 5 micrograms per cubic meter of chlordane, approximately 50 micrograms per day for an average adult, according to EPA extrapolations that considerably underestimate risk for several reasons, including neglect of high-dose flattening, would be expected to increase lifetime

cancer risks by as much as 0.1 to 0.5 percent (20).

Ames' position on the significance of dietary burdens of carcinogenic synthetic pesticides is not supported by recent data on ethylene dibromide (EDB) residues, with concentrations up to 5000 parts per billion in flour and citrus pulp. EPA estimated, again using procedures that minimize risk, that lifetime exposures to "realistic worst case" dietary concentrations of 31 parts per billion of EDB would result in cancer risks of from 10^{-4} to 10^{-3} (21), about 300 to 3000 deaths per year; occupational risks were estimated to be as high as 40 percent. Ames has also objected to the regulation of EDB, saying that the "trace of the carcinogen EDB now allowed in food is insignificant" (22); this in spite of the fact that available noncarcinogenic alternatives include aluminum phosphide for grains and cold storage for fruits and vegetables.

The minimal references by Ames to problems of poorly regulated exposures to a wide range of environmental and occupational carcinogens are in contrast to his exaggerated emphasis of the roles of high-fat and low-fiber diets and of charred foods as "major risk factors,"

although evidence for such risks, where not negative, is generally inconclusive. A recent report concludes that "in the only human studies in which the total fiber consumption was quantified, no association was found between total fiber consumption and colon cancer" (23). The position that high fat consumption is a major cause of breast and colon cancer is based on experimental and epidemiological studies (1, 24). However, this evidence is weak and inconsistent (3, 25). There appear to be no data on the correlation between the proportion of fat in the diet, the critical variable examined in the animal experiments, and rates of colon and breast cancers on a nation-by-nation basis; while those rates are strongly correlated with absolute fat consumption, this correlation is equally good with other measures of industrialization, such as per capita energy production (3). Moreover, up to 20-fold increases in dietary fat were generally necessary to increase tumor yields in rodents after the administration of carcinogens, whereas between-country differences in total fat consumption are generally less than a factor of 2 (3). Finally, no evidence was found in two major case control studies of an associa-

tion between fat consumption and breast cancer rates (26). These considerations do not denigrate the importance of a prudent diet in the promotion of health nor the need for research in this area which could lead to future cancer prevention strategies; a low-fat and high-fiber diet not only decreases intake of fat-soluble synthetic carcinogenic contaminants but also reduces risks of cardiovascular disease and diverticulitis.

Evidence on the qualitative and quantitative significance in generalized diets of Ames' examples of "nature's pesticides" and on their carcinogenicity is unimpressive. For instance, conclusions about the carcinogenicity of pepper are based on the results of a single questionable study (27), and the inference that mushrooms are carcinogenic is based on the identification in certain mushroom extracts of unstable diazonium compounds that are carcinogenic in mice only after artificial in vitro stabilization.

The implicit identification of mutagens with carcinogens, the implication of an identity in their underlying mechanisms, the blurring of the distinction between different types of mutagens, the identification of quantitative mutagenicity with the results of Ames' bacterial assay, and the derivation of carcinogenic potency from quantitative mutagenicity data are all of questionable validity (28). Many mutagens are inactive in carcinogenesis tests, and many carcinogens are inactive in short-term tests for mutagenicity (29); glutathione is positive in the Ames test (30), although Ames recognizes it as an anticarcinogen and an antimutagen. Furthermore, recent evidence has suggested that gross mutagenic events, such as chromosome translocations, are more likely to be crucial in carcinogenesis than are the point mutations or deletions detected in the Ames assay (28). Moreover, while somatic mutations are likely to be involved in carcinogenesis, epigenetic events also appear critical.

Ames' discussion of free radicals and the potential anticarcinogenic effects of antioxidants is speculative and of dubious relevance. Even one of the authors cited in support of the thesis that carotenoid antioxidants are protective in smokers has admitted that various studies revealed only "a slightly lower than average incidence of cancer among people with above average intake of β -carotene" and that even this slim association may be artifactual (31). A recent large-scale case control study (32) produced no evidence "relating intake or serum levels of antioxidant vitamins to a reduced cancer risk."

Evidence for major carcinogenic ef-

fects of trace natural components of U.S. diets is speculative. Strategies based on this hypothesis offer little hope for cancer prevention, and the hypothesis affords no basis for Ames' trivializing the importance of reducing exposure to occupational and other environmental carcinogens. Understandably, such strategies are applauded by corporations resisting regulation of their carcinogenic products and processes and seeking, with others, to explain away cancer causation largely in terms of diet and faulty life-style (1). Strangely, Ames' current proposals appear at variance with his strongly argued recent positions (33). These include warnings that EDB is "a potent carcinogen" whose presence as an impurity in tris-BP [tris (2,3-dibromopropyl) phosphate] is one of the reasons why this flame retardant "should not be used"; that there are "enormous possible [carcinogenic] risks" from inadequately tested industrial chemicals, such as flame retardants; that a "steep increase in the human cancer rate from [industrial] chemicals may soon occur . . . as the 20- to 30-year lag time for chemical carcinogenesis in humans is almost over"; that "tens of thousands of man-made chemicals have been introduced into the environment in the last few decades—with widespread human exposure—to low but disturbing doses of these carcinogens" and that such chemicals should be tested for mutagenicity and carcinogenicity; and that priorities must be established to "minimize human exposure to these chemicals" (33). Clearly there is substantial evidence that, besides smoking, involuntary exposures to occupational and industrial environmental carcinogens are major and generally avoidable contributors to the burgeoning national cancer burden and to a wide range of other chronic diseases. Vigorous public health measures are essential to reduce such exposures.

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Weinberg and I are in agreement that cancer and heart disease appear to be in large part degenerative diseases of old age, that oxygen radicals generated during normal metabolism are likely to be major contributors to this aging process, and that it is unlikely we are going to eliminate them. I also agree that Fridovich, Totter, and Handler have made major contributions to the field.

We also agree that every meal contains natural carcinogens, and it is unlikely we are going to eliminate all of them. However, I do not think that this knowledge makes it any less important to work toward cancer prevention. By identifying smoking as a major cause of lung cancer and heart disease, we have furnished people with the knowledge that they can live 8 years longer on average by not smoking heavily. The incidence of stomach cancer is high in Japan and low in the United States, while colon and breast cancer incidence are high in the United States and low in Japan. This may be due to a limited number of dietary components, and if we could identify them, we might be able to prolong the life span of the people affected in both countries. Understanding some of the main causes of cancer may be the first step in preventing cancer, and although causes and mechanisms are complex, with more knowledge we should be able to sort out some of the major risks in our diet and intervene in many ways, both to minimize significant carcinogens and to maximize anticarcinogenic defenses. I also agree that the preoccupation with tiny amounts of man-made pollution has been blown up out of proportion.

Ingmanson rightly points out that selenite is another name for the crystalline form of the mineral gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$). The etymology is from the Greek for moonstone, "probably an allusion to the soft moon-like reflection of light from some of its faces" (1). The selenite I meant is the SeO_3^{2-} anion (analogous to sulfite and tellurite). The name of the element selenium is also derived from the Greek word for moon,

selenite. Selenite also means "a supposed inhabitant of the moon" (2); presumably there will not be any confusion with this last meaning.

Hunter says that "there are insufficient reliable data to justify the suggestion that certain fats in the current American diet represent a substantial cancer risk." The situation is confusing because there are so many types of fat and the evidence so far does not prove cause and effect. I referenced the considerable epidemiological literature associating high fat consumption with colon and breast cancer and the considerable body of animal experiments implicating high fat with cancer. The National Academy of Sciences committee (which also reviewed the field), and more recently the American Cancer Society, have advised the American public that it would be prudent to reduce their fat intake to lower cancer risk. It was important to point out the controversy as to where prudence begins and to mention the disagreements with this view. Recent reviews on nutrition and cancer also discuss fat (3). Most scientists would emphasize that the evidence linking fat to cancer is much less secure than that implicating cigarettes, alcohol, or asbestos with cancer. I had hoped that a discussion of plausible molecular mechanisms for a fat-cancer connection might provide some testable hypotheses. I discussed cyclopropanoid fatty acids, rancid fat, and peroxisome oxidation of certain fats. Newmark *et al.* (4) and Welsch and Aylsworth (5) have other explanations. All of these mechanisms are plausible, but we do not know which, if any, are correct.

Fat rancidity products in the diet still appear to be a possible source of mutagens and carcinogens that could contribute to colon and breast cancer. I listed in my article some of the carcinogenicity and mutagenicity data on the variety of hydroperoxides, enals, epoxides, and other reactive chemicals produced by the rancidity reaction. Appreciable amounts of lipid oxidation products may exist in palatable food. For example, Tsai *et al.* (6) have found significant amounts of cholesterol epoxide (a mixture of α and β) in commercial dried eggs, scrambled egg mix, and dried whole egg products containing additives, each averaging about 20 parts per million (ppm), although some samples reached eight times this. Cholesterol epoxide is a weak alkylating agent, induces sarcomas at the injection site in rats and mice, is positive in a sister chromatid exchange test, transforms hamster embryo cells,

induces chromosome damage in human fibroblast cultures, and is mutagenic in hamster cells (7, 8). Concentrations in human breast fluid, prostate secretions, or serum samples from particular people can be enormous (8, 9), although it is not clear whether the source is endogenous oxidation or the diet. We need more research on the extent of epoxide destruction by the acid in the stomach. In addition, lipid hydroperoxides are present in heated fat that is reused. A number of hydroperoxides have been shown to be mutagens and carcinogens, and others are likely to be, due to their generation of oxygen radicals. I discussed ionizing radiation as a mutagen-carcinogen that is active because it generates oxygen radicals, and I also referred to the carcinogenicity of hydrogen peroxide and fatty acid hydroperoxides. Even a small amount of oxidation (for example, a peroxide number as low as 2) which could be found commonly in cooking oil in restaurants and in fat (10), would represent a level of 1200 ppm (if it were a triglyceride hydroperoxide). Meat can also have a fair amount of rancidity. I mentioned Shorland's article (11) because it reviews some of the literature on rancidity in meat: "In contrast to fresh intact meat, cooked and uncooked ground meat becomes rancid within 48 hours at 4°C. . . . This phenomenon has been described . . . as 'warmed over' flavor. . . ." Rancidity products (as measured by malondialdehyde reaction) were found to be increased in ground meat stored in the refrigerator and in the urine of people who consumed the meat (12). I gave references to both sides of the trans fatty acid controversy, and I find Hunter's additional comments useful.

The letter from Epstein and his co-signatories implies that my inquiry into natural dietary carcinogens and anticarcinogens trivializes the importance of reducing exposure to the carcinogens of occupation and pollution and that, therefore, I am aiding the corporations, which Epstein *et al.* imply are the true causes of cancer. They also criticize me for changing my mind and seem to misunderstand the chief purposes of my article. One way in which biology advances is by the formulation of new hypotheses which can then be tested and either rejected, accepted, or (more commonly) modified and converted into the next generation of more specific and more testable hypotheses. It is through this process that scientists change their minds, which is, in fact, desirable. I was prompted to write the article in order

to draw together five areas of research:

1) The standard epidemiological view that dietary factors may be important in the etiology of certain types of cancer (3, 13, 14).

2) The awareness that increasing numbers of *natural* products are being identified as carcinogens in rodent studies and the realization that many of these compounds are present in very large amounts in the diet relative to the amounts of man-made carcinogens.

3) The finding in recent years of a large number of mutagens formed on cooking food and also among the group of natural pesticides present in plants and molds, many of which appear in the human diet in large amounts. A number of these mutagens have now been tested and shown to cause cancer in laboratory animals. These mutagenicity findings may be much more representative of the dietary hazards to which humans are exposed than the findings of animal cancer tests, in which very few chemicals are examined each year, almost all of which are man-made.

4) The relation between cancer and aging in animals of widely different life spans, such as rodents and humans, suggesting that cancer is a degenerative disease of old age. Of relevance to aging is the recent interest in the generation of oxygen radicals, a destructive process in normal metabolism, which leads to DNA damage and other damage in cells and could be a major force in both aging and cancer. Also relevant are the contributions of the radiobiologists who have demonstrated that the oxygen radicals produced by radiation appear to account for a good part of its mutagenicity and carcinogenicity.

5) The finding by many cancer researchers that a variety of nutritional factors can have a marked anticarcinogenic effect in rodents (for example, an azo dye gives 90 percent of the rats cancer, but only 14 percent of the animals get cancer if 4 ppm of a selenium-containing salt is added to the diet) (15). Many of the substances found to be anticarcinogenic in rodent experiments, such as selenium, β -carotene, vitamin E, and ascorbate, are components of our normal antioxidant defenses. Many epidemiological studies now implicate dietary factors as being possibly protective against cancer, with some evidence pointing toward antioxidants such as selenium and β -carotene.

I restricted my article to *dietary* carcinogens and anticarcinogens because, as I stated, "whether or not any recent changes in lifestyle or pollution in industrialized countries will substantially af-

fect future cancer risks, some important determinants of current risks remain to be discovered among long-established aspects of our way of life." In addition, I felt that there were certain unifying concepts in this area. I was not addressing future risks or that fraction of cancer today which might be caused by viruses, hormones, occupation, or pollution. I was not attempting to belittle these areas, as these and other causes are of concern (13, 14, 16). Doll and Peto (13, 16), for example, ascribe to occupational factors about 12 percent of all lung cancer deaths, plus a smaller percentage of other cancer deaths (totaling about 4 percent of all cancer deaths), and the emergence of such factors may have contributed to certain cancer trends—for example, over the last few decades the annual number of asbestos-induced U.S. lung cancer deaths has risen from perhaps a few hundred to perhaps a few thousand and is likely to continue to increase for some time yet. This increase is caused by the delayed effects of past heavy exposures and not the present asbestos levels, which are much reduced. Whether occupation causes about 4 percent of current cancer, as indicated by Doll and Peto and other leading epidemiologists (13, 16), or even double that, is irrelevant to my article. As indicated by Epstein *et al.*, I have previously advocated, and still do, vigilance in the area of man-made carcinogens and mutagens stemming from occupation and pollution (17).

Epstein and his cosigners state that a generalized increase in cancer not related to tobacco is in progress, even though the most distinguished epidemiologists who have studied the available data on national trends have come to exactly the opposite conclusion. The question of trends appears to have arisen because, in arguing that some important determinants of cancer are likely to await discovery among long-established aspects of the American way of life, I noted that "there is no convincing evidence of any generalized increase in U.S. (or U.K.) cancer rates other than what could plausibly be ascribed to the delayed effects of previous increases in tobacco usage," a conclusion that I drew after discussing the work of Doll and Peto (13) with many leading epidemiologists. If lung cancer is not included, the overall cancer death rates have declined, not only according to Doll and Peto (13, 16), who, incidentally, did evaluate blacks and people over 65 (13, p. 1272), contrary to Epstein's statement, but also according to both the American Cancer Society (18) and the recent thorough study by the

Environmental Protection Agency and the National Cancer Institute (NCI) of three decades of cancer (19). The recent NCI SEER data (20) also show no convincing evidence of any increases in the major cancers except for lung cancer, while liver, stomach, and uterine cervix cancer are declining. The argument by Epstein *et al.* for an increase is based on earlier and superseded SEER data.

The statements by Epstein *et al.* that 20 percent of lung cancer cases occur in nonsmokers and that lung cancer is increasing in this group both appear to be incorrect. Two recent representative studies of the very high lung cancer areas of South Louisiana and the petrochemical area near Houston show that only about 3 to 6 percent of lung cancer patients are people who have never smoked (21). The arguments by Epstein *et al.* about blacks and lung cancer are not supported by these studies; for example, for black males in South Louisiana, 97.6 percent of the lung cancer patients were smokers or ex-smokers, while the figure for white males was 97.7 percent. The study in Louisiana also showed a sizable modifying factor of diet: smokers who rarely ate fresh fruits and vegetables had a 30 percent higher risk from lung cancer for a given amount of smoking, in agreement with the results of Hirayama (22) in Japan on the influence of diet on cancer induced by cigarette smoking, which I quoted. There is no good evidence for any appreciable increase in lung cancer in nonsmokers (23): The conclusion by Epstein *et al.* is based on an earlier, flawed (13) study.

Thus, only one major cancer rate, that of lung cancer, is increasing, and this appears to be largely attributable to smoking; the rates of liver, stomach, and uterine cervix cancer are decreasing. Breast and colorectal cancer rates (both associated with high fat) have been fairly constant for decades. Some less common types of cancer are becoming more prevalent, but the causes still have to be determined. We cannot assume that these are due to occupation or pollution, although some may turn out to be. In the case of esophageal cancer in blacks, for example, an epidemiological investigation by NCI implicated high alcohol consumption as a major risk factor and a good diet as a protective factor (24).

The most authoritative and thorough study of causes of cancer in America (16) suggests that diet and life-style are major contributors to cancer and that the contribution of occupation and pollution are only a very small percentage of the cause of the major human cancers. This study

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discredits many of the arguments of Epstein *et al.* in detail. The estimates by Doll and Peto agree with those of other leading epidemiologists (13) and also are consistent with the analyses of cancer in different countries and immigrant groups (14), although everyone agrees there are uncertainties in the figures. The range of human intake of fat is more than Epstein *et al.* state, and the many animal tests showing an effect of fat were often over a comparable range (3). Breast cancer rates are not so closely correlated with industrial society. Modern industrial societies such as Japan and the United States have an ever-increasing life span and a lower overall cancer rate than many less industrialized societies, such as Finland and New Zealand (25). Czechoslovakia (after Luxembourg) has the highest cancer rate in the world (25) and does not have any corporations. The calculations that Epstein *et al.* refer to stating that 18 to 38 percent of the cancer in coming decades will be caused by asbestos and five other carcinogens appear to inappropriately assume that anyone with any exposure to asbestos would have the same risk as a person with massive exposure. These estimates have been shown to be erroneous in other ways as well (13, 15) and have been effectively criticized by a good number of the world's leading epidemiologists (26).

Epidemiologists studying urban air pollution have had a difficult time demonstrating any measurable effect on cancer because of the larger effects of small differences in smoking (27). In Contra Costa County, California, a possible connection between air pollution due to several refineries and excess cancer was widely publicized for several years. The connection evaporated when a more detailed epidemiological study showed that the excess cancer was explainable by the higher smoking rate in blue-collar workers, who made up a higher percentage of the population in the area (28). In this study, as in those discussed above, only 3 percent of the males with lung cancer had never smoked, and green vegetable consumption showed a marked protective effect. I pointed out in my article that one would have to breathe in Los Angeles smog for about a year to inhale an amount of burnt material equal to that inhaled by a smoker in one day. Therefore, one has to be suspicious of superficial associations with refineries, smelt-

ers, and so forth, until precise adjustments are made for both duration and amount of smoking. I also pointed out that the major human intake of browned and burnt material, even more than smoking, comes from cooked food, which is also full of mutagens and carcinogens, although it is ingested rather than inhaled, and we do not know the human risk from this.

Epstein *et al.* dismiss the whole area of dietary anticarcinogens, despite the fact that many leading scientists think it is one of the most promising areas in cancer research. There is a vast literature on oxygen radicals in pathology, and there is a large and striking literature on anticarcinogens in animal tests and on dietary protective factors in people. Although this is an inherently difficult area, the impact on prevention of cancer could be great. We hope our own work on noninvasive measurement of the high endogenous flux of oxidative DNA damage in individual humans (and the much higher rate in rats) may help to open up new approaches to measuring the effects of antioxidant modifying factors in humans and to the understanding of the contribution of the aging process to cancer (29).

Epstein *et al.* seem to have a double standard about carcinogens. They emphasize only industrial chemicals or pollution and belittle or ignore the evidence concerning the many natural carcinogens, mutagens, and teratogens discussed in my article, as evidenced by their comments on mushrooms, pepper, and fat. Toth, an expert on the carcinogenicity of man-made hydrazines, has also published numerous papers on the carcinogenicity of many mushroom hydrazines (30). Gyromitrin, a carcinogen in mice (31), is present in large amounts (500 ppm, dry weight) in the widely eaten false morel and has recently been shown to massively alkylate the DNA of rats (31). The mushroom contains several other carcinogenic hydrazines, including *N*-methyl *N*-formylhydrazine, which is both potent and stable (30). The common commercial mushroom *Agaricus bisporus* contains agaritine, a hydroxymethyl phenylhydrazine derivative (3000 ppm, 45 milligrams per mushroom (30)). A metabolic product of agaritine, a diazonium compound, is highly reactive and mutagenic and was found to cause stomach tumors in mice at low doses of both the sulfate and tetrafluoroborate salt (30). Agaritine is not appreciably destroyed by cooking and, when eaten, is distributed in tissues, where it is converted to the reactive mutagenic diazoni-

um metabolite by cytochrome P-450 (30). The diazonium metabolite is also present in the mushroom (about 2 ppm) as it is formed from agaritine by enzymes in the mushroom (30). Epstein *et al.* dismiss the mushroom work because the diazonium compound was found to be a carcinogen when tested as a salt, an irrelevant argument in view of all of the findings about agaritine and the fact that the diazonium ion would be the chloride salt in the stomach in any case. Toth has recently identified another carcinogen in *Agaricus*, *p*-hydrazinobenzoic acid (10 ppm, 150 micrograms per mushroom) (30). Gyromitrin and some other natural pesticides cause lung tumors when fed to mice (30). Lung tumors may conceivably be caused in humans by natural carcinogens as well as by smoking and by the occupational hazards listed by Epstein *et al.* The positive cancer test on pepper was done by skin painting and is not a very elegant test, but it is statistically significant for each of three sites: skin, lung, and liver. Piperine, the major natural pesticide in black pepper (present at 10 percent of its weight), is closely related in structure to the known natural carcinogens safrole, estragole, and methyleugenol, which are also widely distributed in spices and plant oils. The test should be repeated, although, unfortunately, no government agency seems very interested in doing cancer tests on natural products. Toth has also shown that capsaicin, the pungent material in hot pepper, is a mutagen, and he has some preliminary evidence for its carcinogenicity in mice (32).

Epstein *et al.* call all of these "trace natural components." My article was full of numbers showing that the amounts are not traces. Nature's pesticides are present at parts per hundred and parts per thousand, while man-made pesticides are present at parts per million and parts per billion. We are eating more than 10,000 times more of nature's pesticides than of man-made pesticides. The arguments of Epstein *et al.* about man-made pesticides do not invalidate this calculation. His calculations are based on rare, highly contaminated foods or people, while mine are based on Food and Drug Administration values for man-made pesticide residues.

Epstein *et al.* criticize my discussion of mutagens as potential carcinogens. There are 3000 laboratories using our test alone, and there are many other kinds of mutagenicity tests in which cells from mammals are used. Over the last 10 years, more than 5000 compounds, natural as well as man-made, have been

tested, and an amazing variety of mutagens in the natural world have surfaced. These include superoxide, hydrogen peroxide, and aldehydes generated by our normal metabolism; products of lipid rancidity; products of cooking food such as the brown color on our toast; many of nature's pesticides; and the aroma of butter (diacetyl). We cannot ignore this new information, as it is telling us something important about nature. There are good reasons for thinking that mutagens are potential carcinogens and that DNA damage is of concern in itself. Our test is successful at detecting carcinogens as mutagens (80+ percent) and has been improved since validation (33). In many cases its mutagens have later been found to be carcinogens in rodents (for example, natural metabolic products such as hydrogen peroxide and formaldehyde, natural pesticides such as allyl isothiocyanate from mustard, many pyrolysis products from cooking, nitropyrenes from diesel exhaust, and synthetic substances such as ethylene dibromide, hair dye components, and the flame retardant tris-BP). Recent evidence on oncogenes also supports a mutation-DNA damage hypothesis as one aspect of cancer causation (34). The identification of mutagens aids epidemiology in its search for hypotheses to test, serves as a bioassay for active principles in complex mixtures (for example, the mutagenic pyrolysis products from cooking, later shown to be carcinogens), and is a way of investigating active forms of carcinogens. Of course, it is far from a perfect guide. We need to understand promotion as well, although I pointed out recent evidence for an oxygen radical connection. We know that some chemical carcinogens are missed by mutagenicity tests, but the battery of short-term test systems agree with each other quite well and detect a remarkable percentage of the known carcinogens. Agents that are known to cause deletions, translocations, and chromosomal rearrangements, such as x-rays, are also detected by our test system (33). Whether there are many significant carcinogens that cause translocations and are not detected as mutagens in short-term tests remains to be seen. Glutathione, as pointed out by Epstein *et al.*, is converted to a mutagen by kidney homogenate (but not liver homogenate). This may be due to an enzyme specific for kidney that generates oxygen radicals from O₂ and glutathione (35), but this is not relevant to the role of glutathione as an antioxidant or the value of mutagenicity tests.

Epstein and his colleagues may be

drawing the wrong conclusion from the results coming out of animal cancer tests. The National Cancer Institute-National Toxicology Program animal cancer bioassay test program, which is the most thorough and extensive source of tests, is looking at only a small portion of the chemicals in the world. The current cost is more than \$500,000 per chemical, so it is difficult to test many chemicals. The tests are almost exclusively done on man-made chemicals and therefore, of course, find man-made carcinogens. Out of about 200 chemicals tested by NCI in 8 years, 60 percent were judged carcinogenic, 33 percent noncarcinogenic, and 7 percent inadequately tested (36). The high percentage of carcinogens found is somewhat disturbing, as the conventional wisdom is that carcinogens are very rare. This discrepancy could be accounted for by the fact that more suspicious chemicals are being tested. It also could be that carcinogens are more common than we think. We have no idea of what the true percentage of carcinogens is among chemicals in general (including natural ones) when tested at the maximum tolerated dose in rodents. Even if it is 10 percent, our current regulatory policies, which assume carcinogens are rare, are in trouble. I pointed out that we are ingesting enormously more in both number and amount of natural pesticides and other natural toxic molecules (and traditional mixtures such as cooked food) than we are of man-made substances. Plants have been devising nasty chemicals to kill off insects and animals throughout all of evolution.

There is no reason to think nature is any more benign than man. We already know about natural carcinogens, such as psoralens, aflatoxins, sterigmatocystins, pyrrolizidine alkaloids, safrole, asbestos, radioactive potassium in our body, radon coming up from the ground into our houses, mushroom hydrazines, hydrogen peroxide made during normal metabolism, and sunshine. The same detoxification and activation mechanisms appear to operate on both man-made and natural chemicals. Animal cancer testing will not be able to catch up with the large number of mutagens being uncovered. Therefore, it might be reasonable to plan on doing animal cancer testing on those natural mutagens that we eat in largest amounts to see how many are rodent carcinogens and to provide a benchmark of the natural hazard for setting priorities relative to man-made carcinogens.

Much fear of traces of man-made carcinogens is based on ignoring the natural background of carcinogens and using

"worst case" assumptions in extrapolating risk from the most sensitive rodent (when the chemical is given at the maximum tolerated dose) to low-dose human exposure. This quantitative extrapolation is viewed with great unease by much of the toxicological and epidemiological community because there is little scientific support underlying it (13). It is an extrapolation based on ideas of prudence, not on firm science (13, 37, 38). This is true, of course, for both natural and man-made carcinogens. We cannot validate these extrapolations. In a few cases, individual extrapolations from rats to man can be examined, although not very satisfactorily (38). In cases such as ethylene dibromide (EDB) (39), aflatoxin (38, 40), and vinyl chloride (38), the extrapolations appear to markedly overestimate the risk to man. We assume a linear response with dose, but we do not know if this is true. We have no basis for assuming metabolism in rodents and humans is the same. We do not know if we can extrapolate cancer risk from short-lived species such as rodents to long-lived species such as man because of the cancer-longevity connection (my article discusses this). Antioxidant defenses can differ markedly from rodents to humans (41). We know that some dietary changes make great differences in cancer incidence in rodent tests, but we do not understand which are the dietary protecting factors that appear to influence cancer risks so markedly in human epidemiology studies. We do not understand promotion or the interaction of carcinogens. Thus, it is time to do the same types of worst case risk calculations on natural chemicals as well as man-made chemicals before deciding what our priorities are. I emphasized that

To identify a substance, whether natural or man-made, as a mutagen or a carcinogen, is just a first step. Beyond this, it is necessary to . . . quantitate the approximate magnitude of the risk. . . . [T]he rapid progress of science and technology . . . should help to dispel confusion about how important health risks can be identified among the vast number of minor risks.

Epstein *et al.* distort my statement on EDB (42), which was in favor of the EPA standards and much more stringent occupational standards. Aluminum phosphide (phosphine gas), the suggested alternative of Epstein *et al.* to EDB for grain fumigation, is both extremely toxic to humans and flammable, and, despite their statement, it has not been tested for carcinogenicity (43).

Epstein and his cosigners offer sup-

port for the idea that cancer is basically a political problem, that it is "corporate cancer," and that the solutions are not scientific, since we already know what we need to know to solve the problem politically. Yet their fundamental hypotheses and beliefs are wrong or likely to be wrong. If we follow their advice, we will ignore the major area of diet as a source of both protective factors and risk factors for cancer. We will continue to be preoccupied instead only with industrial sources of cancer, trying to eliminate smaller and smaller risks even though the alternatives to these have unknown risks. Pollution is not being neglected when the budget of the EPA is equal to that of NCI, nor are occupational hazards when we have large government agencies such as the Occupational Safety and Health Administration and the National Institute of Occupational Safety and Health. Smoking, which causes 30 percent of cancer and 25 percent of heart disease, is largely neglected: we subsidize tobacco farmers. Understanding cancer mechanisms may turn out to be among the most cost-effective ways to reduce the burden of cancer that is not related to tobacco, and for the present the key questions are scientific, not political.

I would like to correct some errors and omissions in my article. I omitted a paper reviewing the carcinogenicity of the herbs comfrey and coltsfoot (44), which contain the very potent carcinogenic pyrrolizidine alkaloids that are widespread in plants. The use of comfrey is increasing markedly with the new interest in returning to natural herbs and the increase in health food stores. I overlooked the extensive work of Janzen and others (45) on the biological role of plant toxins and of Morton (46) on the connection between tannins in plants and esophageal cancer in a number of countries. I also overlooked a paper on the plasma levels in humans of the potato toxins in which the toxicology of these compounds was reviewed (47). Also overlooked were the natural mutagens in ginger (48) and in corn, rye, and wheat (49) and the work on the formation of mutagens by the reaction of sugars with amino acids (nonenzymatic browning or Maillard reaction) during the cooking of food (50). It was a single dose of 400 micrograms per gram, not 400 nanograms per gram, of the mushroom diazonium derivative that caused the appreciable cancer in mice. I would also like to call attention to the paper of Birnboim (51) on the role of oxygen radicals in strand breaks and promotion, which I overlooked, and to the book in which it

appears, which has many papers of relevance. I also apologize to Shapiro and my numerous botanist correspondents for misclassifying figs.

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Carcinogens Occurring Naturally in Food

by
Doris M. Hilker

Abstract

Carcinogens naturally occurring in food include metabolites of microorganisms which infect plants during harvesting and storage; products of the normal metabolism of plants and products of chemical changes occurring during processing, preservation, preparation or which are picked up from the environment, including contaminated water or soil. Some examples of these types of carcinogens are: fungal metabolites such as aflatoxins in peanuts stored under improper conditions; safrol from the oil of various plants; tannin in tea, grain and grapes; and polycyclic hydrocarbons including benzo(a)pyrene formed by smoking meat and fish. Epidemiological studies indicate that there is a high incidence of gastric cancer in the areas of the world where smoked fish are common in diets. Vitamin A may play a role in preventing the carcinogenic action of polycyclic hydrocarbons.

It is well established that certain chemical compounds have carcinogenic action in humans and in experimental animals. Recently, attention has focused on carcinogens in food as potential causes of human cancer. This attention has resulted largely from consumer concern about chemicals added to food during processing and preservation. However, much less attention has been paid to carcinogens occurring naturally in food material.

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This report will deal with the role of carcinogens as metabolites of microorganisms which infect plants during harvesting and storage; as products of the normal metabolism of plants; and as products of chemical changes occurring during processing, preservation or preparation, which are picked up from the environment through contaminated water or soil.

Carcinogens as Fungal Metabolites

Miller and Miller¹ have published several reviews on the subject of naturally occurring carcinogens in food (Table 1), some of the most potent carcinogens being fungal metabolites. Aflatoxin B₁, a product of certain strains of *Aspergillus* and *Penicillium*, can induce tumors in livers of rats fed Aflatoxin B₁ at 1 ppb. Other aflatoxins, B₂, G, M₁, Q₁, H₁ and P₁, are less active. The production of aflatoxins is greatest when crops such as peanuts are grown or stored under warm, humid conditions. Contamination of food with aflatoxin can be a serious health hazard in some areas of the world and has been related to a high incidence of cancer in certain parts of Asia and Africa.

Alcoholic beverages made from such products as grapes, maize, rice and barley may also become contaminated with aflatoxin by field or storage microfungi.^{4,5} In Kenya, epidemiological studies have implicated beer made from maize in the high incidence of esophageal cancer.¹⁰

The other fungal products, 4-ipomeanol, luteoskyrin, methylhydrazine and ethyl carbamate have been studied much less than the aflatoxins; however, it is known that they may occur in plants in amounts up to 5% of the dry weight. The pyrrolizidine alkaloids, which occur in many plant species, have been found in honey samples from the Pacific Northwest¹² in amounts up to 3.9 ppm. The alkaloids tend to concentrate in flowers,

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Table 1. Natural Carcinogens in Food³²

Carcinogen	Food source	Site of action
<u>Fungal origin</u>		
Aflatoxin B ₁	peanuts, grains	liver
4-Ipomeanol	sweet potatoes	liver
Luteoskyrin	rice	liver
Methylhydrazine	mushroom	liver, lung
Ethyl carbamate	fermented foods, beer, wine, yogurt, soy sauce	lung, lymphoid, skin, liver
<u>Plant products</u>		
Pyrrolizidine	herbs, honey	liver, skin, lungs
Alkaloids		
Safrol	sassafras, sesame oil, nutmeg, pepper	liver
Oil of Calamus	bitters, liqueurs	mesenchymal
Cycasin	cycad nuts	liver, kidney
Thiourea	turnip, cabbage	thyroid, liver
Tannin	tea, wine, betel nut	liver, buccal
<u>Polycyclic aromatic hydrocarbons</u>	smoked foods, plants, seafoods	gastrointestinal

constituting 0.15–0.3 percent of the dried flower material. Safrol and related compounds occur in the oils of a wide variety of plants that were used in the US as flavorings until 1960, when they were reported to cause hepatic tumors in rats and mice. Safrol is also present in the oil of sassafras, yet the bark of the sassafras tree is used for tea in certain parts of the US.

Carcinogenic Activity of Polyphenol Compounds

Tannins are polyphenol compounds present in many plants including tea, grains such as sorghum, and grapes. The betel nut also contains tannin, and the high incidence of buccal cancer in betel nut chewers in Asia has been attributed to this compound. Bhide and colleagues² induced gastrointestinal tumors in mice by giving them an aqueous extract⁴⁰ of betel nut quid. In addition, oral and gastric cancers were produced in the cheek pouch of hamsters by combinations of betel nut, lime and tobacco.⁴⁰

Bracken fern, which contains a high tannin content, is used as a food in some countries and is also consumed by animals. Neoplasms have resulted from feeding this material to rats and cows.³⁹ Experiments by Pamukcu and co-workers³⁸ involving fractionation of the bracken fern, however, indicated that tannin was not the carcinogenic factor. Other foods and beverages containing tannin (e.g., tea) may be potentially hazardous, but have been largely ignored. Several studies, however, have shown that tannin-containing

fractions of plants used for tea can induce cancer in rats given injections of the extract.^{25,38}

Plant Carcinogens

Oil of calamus contains compounds related to safrol which also have carcinogenic activity when fed to rats. Cycasin from the palmlike cycad tree is one of the more potent plant carcinogens. Nuts from this tree are consumed by people in the South Pacific Islands, but there appears to be no evidence of human cancer caused by consuming the cycad nut. Thiourea and related compounds fed to rats as 0.1 % of their diet have produced various types of tumors. Here again, we have no evidence that these compounds cause cancer in humans. More recently, a review by Weisburger³⁸ listed additional compounds from plant material with carcinogenic properties, including: β -asarone (found in *Aspris calamus*, or sweet flag), which was used as a flavoring in American vermouth before 1967 when it was banned by the FDA; estragole, a component of oil of tarragon which has been used as a flavoring agent in gourmet types of vinegar; and *N*-methyl-*N*-formylhydrazine, which is found in the wild mushroom *Gyromitra esculenta*. Another recent review by Brown⁸ gives extensive information on the genetic effects of naturally occurring flavanoids, anthroquinones and related compounds.

In recent years there has been a growing interest in the carcinogenic properties of *N*-nitrosamines. This interest has generally been concerned with nitrite-

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cured meats. However, nitrosamines have been reported in many other products, including cheese and fish.¹¹ Scanlan and co-workers⁴² analyzed commercially available bottled and canned beer for volatile nitrosamines and found N-nitrosodimethylamine (NDMA) in 23 of 25 samples with levels ranging from 0–14 ppb. Other workers⁴³ found NDMA in six or seven brands of Scotch whiskey, in addition to beer.

Mutagens in Meat

The development of the Ames method¹ for detecting mutagens has led to numerous studies on the mutagenic properties of various foods. Potent mutagenic activity that is distinguishable from benzo (a) pyrene (BP) activity has been found in cooked beef and beef extract.^{9,13} The charred surface of cooked fish has also been found by the Ames test to possess high mutagenic activity.³⁵ Attempts to define the mutagenic products in cooked meat have led to studies showing that pyrolysis products of certain amino acids, including phenylalanine and tryptophan, have mutagenic activity.^{40,51,52,59} Rappaport and colleagues⁴¹ reported that volatile mutagens from cooked meat exceeded those present in the cooked product prepared in an open container, but that this volatile material could remain on the meat cooked in a closed container. The subject of mutagens from cooked meat will be dealt with in more detail in another article in this volume. Coffee and tea,³⁴ as well as preserved fish,¹⁷ have also been shown by the Ames test to have mutagenic activity.

Polycyclic Hydrocarbons

The remainder of this report will be concerned with polycyclic aromatic hydrocarbons, particularly ben-

zo (a) pyrene (BP). There are at least 15 compounds of this class that are commonly found in food. For the sake of brevity this discussion will be limited to BP, even though several other compounds, including benzo (c) phenanthrene, 7,12,dimethyl benzo (a) athracene and 3 methylcholanthrene may be more carcinogenic and may be present in higher amounts in some foods. It should be mentioned that methods for analyzing the polycyclic aromatic hydrocarbons in foods differ from one laboratory to another with respect to extraction, clean up and detection procedures, so values may not be comparable.

Table 2 shows some of the reported values for BP analyzed in plant foods. In most cases, the values were below 1 ppb. The highest values were found in seaweed, but spinach, soy sauce and tea leaves also have levels above 1 ppb. Presumably, the major source of BP in plants is the soil. Fritz and Engst¹⁸ in East Germany analyzed soil at various distances from industry, and found that BP ranged from 0.8–60 µg/kg at the greatest distance from the emitter, 199–1100 µg/kg at medium distance and 3,000–1,500,000 µg/kg close to the emitter. Vegetables grown close to the emitter had ten times more BP than those grown at greater distances from the emitter. In a study in Hungary,³⁰ the BP content depended upon the depth at which the soil sample was taken. The highest content of BP was obtained from samples taken at 0.5 cm and the lowest content was from samples taken at 90–100 cm. The deeper soil sample contained 1.6–3.4 µg/kg.

Not all studies are consistent in showing that BP can be taken into plant tissue from the soil. Blum and Swarbrick³ investigated the translocation of ¹⁴C labeled BP into plants grown in a liquid nutrient medium saturated with this compound. The plants selected were green beans, cantaloupes and cotton, which have different growing times. This study indicated that the BP was not taken up by the plants. However, a Russian study⁷ in which oats were grown in sandy loam soil enriched with ³H labeled BP, showed that the label was taken into the plant tissue, with the

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Table 2. Benzo(a)Pyrene in Plant Foods

Food	Benzo(a)pyrene (ppb)	Source
Spinach	3.3	Shiraishi, et al., 1974
Seaweed	31.3	Shiraishi, et al., 1973
Carrots	0.7–1.9	Sidel and Happel, 1975
Head lettuce	0.2–1.3	Sidel and Happel, 1975
Tea leaves	6.8–7.6	Yamaguchi, et al., 1978
Soy Sauce		
Coffee	0.2–0.6	Strobel, 1974
Miso	0.95	Miyabe, et al., 1976
Bread	0.18–.26	Miyabe, et al., 1976
Rice	0.03–.08	Miyabe, et al., 1976
Potatoes	2.4–3.0	Miyabe, et al., 1976

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Table 3. Benzo(a)Pyrene in Seafoods		
Food	Benzo(a)pyrene (ppb)	Source
Lobster tail (fresh)	0.76	Dunn and Fee, 1979
Lobster tail (after impoundment)	281	Dunn and Fee, 1979
Fish and shellfish	0.8-9.85	Shiraishi, et al., 1975
Fresh fish, Estonia	0.11-5.96	Bogovskii, et al., 1978
Whitefish, smoked	4.3	Howard and Fazio, 1969
Whiting, smoked	6.9	Howard and Fazio, 1969
Cod, smoked	4.5	Howard and Fazio, 1969
Herring	1.0	Howard and Fazio, 1969
Smoked salmon	1.0	Lijinsky and Shubik, 1965
Smoked haddock	0.3	Lijinsky and Shubik, 1965
Katsubushi (bonito)	12-27	Masua and Kuratsune, 1971
Honobushi (bonito)	9	Masua and Kuratsune, 1971
Sababushi (mackerel of sardine)	7	Masua and Kuratsune, 1971
Urunebushi (mackerel of sardine)	2	Masua and Kuratsune, 1971

Table 4. Benzo(a)Pyrene in Meats		
Food	Benzo(a)pyrene (ppb)	Source
Grilled meat (electric)	0.18	Toth and Blaas, 1973
Grilled meat (charcoal)	0.5-50.5	Toth and Blaas, 1973
Grilled meat (fircones)	50-190	Toth and Blaas, 1973
Smoked ham	26	Potthast, 1977
Mutton sausage	0.07-.15	Fretheim, 1976
Bologna sausage	0.05-.08	Fretheim, 1976
Charcoal broiled steak	8	Lijinsky and Shubik, 1964
Barbecued ribs	10.5	Lijinsky and Shubik, 1964
Barbecued pork	5.0	Malanoski, et al., 1968
Liquid smoke flavor	0.1	Hirokado, et al., 1975

highest amounts taken into the stalk. The BP was not specifically identified in this study, and the measurements may have included metabolites or breakdown products of the BP. Blumer and Youngblood⁴ analyzed various types of New England soil, including continental, coastal, marsh and subtidal, in order to obtain information on the origin of polycyclic hydrocarbons. The investigators found a high degree of similarity in the molecular weight distribution among the various soil types and suggested that the hydrocarbons may have been formed in forest fires and transported by air. Thus, it would seem that industry and automobile exhaust may not be the only sources of carcinogenic hydrocarbons in plants.

The high incidence of gastric cancer in areas where smoked fish is common in diets and also in areas where fish are caught in contaminated waters has led to a number of studies of polycyclic hydrocarbons in seafood (Table 3). The amounts of BP in seafood are quite varied, but in general are higher than those in plant foods. Of particular interest is a study in which lobster was found to contain less than 1 ppb BP in

freshly caught samples but contained much higher levels after commercial impoundment in pens constructed with creosoted timber. In this study,¹⁸ shrimp, crab and salmon had no detectable BP, and the range of values for smoked fish was no higher than that for fresh fish.

The BP in meat products is shown in Table 4. Grilled, broiled and barbecued as well as smoked meats contain this compound well in excess of 1 ppb, which is considered to be the maximum level in commercially smoked meat in West Germany. Lijinsky and Shubik²⁰ state that the 8 µg/kg of BP in charcoal-broiled steak is approximately equivalent to the amount in the smoke of 600 cigarettes. In view of the rapid growth of "fast food" industries in the US as well as in other countries, with an increasing proportion of diets consisting of grilled, broiled and barbecued meat, the possibility that these foods may contribute to human cancer should be considered.

While there have been many studies on the content of carcinogenic polycyclic hydrocarbons in food and also on the possible mechanisms of carcinogenesis of

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these compounds, there have been few studies linking the consumption of foods containing these compounds to the development of cancer in experimental animals or humans. In 1946, Sulman and Sulman⁵³ studied the carcinogenic effects on rats and mice of wood soot from the chimney of a smoked sausage factory. They fed the smoked sausage to rats for two years *ad libitum*, but none of the rats developed tumors. Rats implanted with soot subcutaneously or injected with soot extracts did develop sarcomas. The authors gave no information on the quantity of sausage ingested or on other components of the diet. Dungai¹⁴ carried out a series of animal experiments in which rats were fed smoked mutton, smoked trout or salted codfish. Malignant tumors developed in some of the rats fed the smoked mutton and the smoked trout, but none of those fed the codfish developed tumors. Analyses of BP showed that the mutton, trout and codfish contained 1.3, 2.1 and 0.5 ppb, respectively. Dungai states that smoked mutton is consumed in considerable quantities, 1-2 lbs at a meal in Iceland. Codfish is smoked commercially for short periods of time, while the mutton and trout may hang in the smoke for weeks or months. On the basis of geographical distribution of cases of stomach cancer, Dungai states that "it is not only possible, but quite likely that the consumption of smoked meat, and eventually salted meat, may be of importance in this connection."¹⁴

In a review of carcinogens in the environment, Hueper²⁴ makes the point that animal experiments do not provide convincing evidence of the carcinogenic action of smoked foods since gastric cancer has not consistently been produced by feeding these foods to rats or mice, nor has it definitely been established that BP or other similar compounds are carcinogenic in man. Fark¹⁸ fed bacon to mice and obtained tumors in 8 to 20 of the animals but failed to confirm these findings in another study. In a recent study, Hect and colleagues²⁰ fed charcoal-broiled beef containing 52.7 µg/kg of BP to rats and humans and analyzed for BP in feces, using ¹⁴C labeled BP as the internal standard. Their study found that unchanged BP amounted to 11% of the total dose in the feces of rats but was not detected in the human feces. The fate of BP in the human was uncertain, since the urine was not analyzed. Michels and Einbrodt³¹ did analyze human urine for BP and found an average of 0.546 µg/l in urine from inhabitants of an industrial area and 0.416 µg/l in urine from an uncontaminated area. It was not stated whether these differences were statistically significant.

It has been reported that vitamin A and certain of its analogues can prevent the carcinogenic activity of BP and some other polycyclic aromatic hydrocarbons. Chu and Malmgren⁸ showed that 0.5% vitamin A plus BP or 7,12 dimethylbenzanthrene added to diets of hamsters prevented the development of gastrointestinal carcinoma which developed without the vitamin A. The work of Hill and Shin²¹ showed that BP is me-

tabolized to a product (BP-X) in the presence of microsomal mixed function oxidases from liver and lung tissue from rats and hamsters. The formation of this product is strongly inhibited by retinol and some of its derivatives. Vitamin A becomes more important in relation to gastric cancer when one considers that the diets of people in areas that have a high incidence of that cancer are very low in vitamin A.⁵⁷ The interrelated effects of vitamin A-poor diets, consumption of carcinogenic hydrocarbons and the incidence of cancer need further study.

In summary, carcinogenic chemicals are present in many, if not most, of the foods we eat. Epidemiological, animal and chemical studies provide some evidence that the consumption of foods containing these compounds may be related to human cancer. Much research, however, is needed to improve our ability to predict the relative toxicity of these chemicals. Information is also needed on the amount of carcinogenic chemicals an individual must consume in order to show carcinogenesis and on the interrelated effects of other dietary constituents.

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Nutrition and the cancer patient

2. Food and the aetiology of cancer

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It has been suggested that about 90 per cent of human cancers are caused by chemical substances, the remainder being caused by viruses and radiation. There are three possible routes by which exogenous chemical carcinogens may enter the body. Thus, they may be absorbed through the skin, from the lungs, or from the gastro-intestinal tract. It is this latter route that we are concerned with here. Of potentially carcinogenic compounds that may enter the body in food, some are intended additives such as colourings or flavourings, while others are accidental contaminants derived, for instance, from packaging materials or from oils used to lubricate machinery. Still other chemical carcinogens that may be found in food are mycotoxins, such as aflatoxin, which result from the contamination of food by micro-organisms. A further possibility is that carcinogenic compounds may be formed in the intestine from otherwise innocuous constituents of food by the action of intestinal flora.

Chemical carcinogens that enter the body in food, or are formed from compounds that do so, may be involved in the aetiology of cancer in only certain organs, "the target organs". Thus, they may be involved in cancer of the gastro-intestinal tract, the bladder, or the liver. A consideration of the incidence of cancer in different parts of the gastro-intestinal tract (Table 1) shows that the incidence is higher in those parts of the tract in which the luminal contents are likely to remain for a longer time in contact with the epithelial lining. In the intestine the incidence is highest in the shortest part of the tract, the rectum, where the contents are moved only at the time of evacuation. A longer contact time also accounts for the higher incidence of cancer of the stomach in the prepyloric region, and of cancer of the urogenital tract at the base of the bladder. Primary cancer of the liver may also result from its function as a storage organ.

Cancer of the stomach

Epidemiological studies have shown that cancer of the stomach is about twice as common in men as it is in women. The incidence in the general population varies considerably from one part of the world to another. For instance in 1958-59 it was about 70 per 100,000 of the population in Japanese men whereas the comparable value for this country was about 30; among the white population in the United States it was about 15. Such variations in geographical distribution suggest that an exogenous environmental factor is involved, and a dietary one has been sought. In some countries the dietary habits may be so complex as to make it impossible retrospectively to identify a dietary factor. Japan, however, with its generally high incidence of cancer of the stomach together with an uneven distribution in the country appears more promising. One study in Japan, in fact, has suggested that the high incidence of carcinoma of the stomach in certain areas is associated with the consumption of larger amounts of salty food and smaller amounts of milk. The occurrence of a carcinogenic poly-

cyclic hydrocarbon, 3,4-benzpyrene, on and in smoked and roasted foodstuffs such as fish or coffee, has also attracted attention. Similar compounds may also be present in fried food.

3,4-benzpyrene was isolated from pitch in 1931 at the Royal Cancer Hospital. About 60 years before this, in 1875, von Volkmann at Halle in Germany, had described occupational skin cancers among workers in the tar and paraffin industry and studies on experimental animals had shown that repeated application of coal tar to the skin caused cancer. It is interesting to note that this same compound is also present in tobacco and in the polluted atmosphere of many of our cities. Its presence in the air and particularly in cigarette smoke is thought to be responsible for cancer of the lung. Thus, the same carcinogen, 3,4-benzpyrene, may enter the body by two different routes and cause cancer in two different sites, the stomach and the lungs.

Food additives

(a) *Intentional*: A wide range of compounds are intentionally added to foods for various purposes such as colouring, flavouring, sweetening, and as preservatives and emulsifiers. These compounds are submitted to very stringent tests to make reasonably certain that none of them is potentially carcinogenic. It is interesting to note that one compound that might have been used as a colouring agent for butter, "butter yellow" (4-dimethylamino-azobenzene) was shown in 1936 to be a very effective liver carcinogen in rats!

Sodium nitrate may be used as a preservative for fish and meat. Some years ago, an outbreak of severe liver disease in sheep in Norway was traced to the consumption of fish meal preserved with sodium nitrite. This compound had reacted with one called dimethylamine, which had been formed as the result of slight decomposition of the fish, to form dimethyl-nitrosamine, which is a potent liver toxin that eventually produces liver cancer.

The use of cyclamates as artificial sweeteners in foods was banned in 1969. This ban followed a demonstration that it is changed to cyclohexylamine in some individuals and excreted as such in the urine. Other work had shown

TABLE 1.
Distribution of cancers of the gastro-intestinal tract
(Adapted from Huser and Conway, 1964)

	Percentage of cancers	
Stomach — prepyloric region	60%	of all gastric cancers
Small intestine	2.5-10%	of intestinal cancers
Duodenum	2.0-5%	of intestinal cancers
Jejunum-ileum	0.5-7%	of intestinal cancers
Large intestine	90.0-97.3%	of intestinal cancers
Caecum colon	30.0-35.0%	of intestinal cancers
Sigmoid	20.0-25.0%	of intestinal cancers
Rectum	40.0-45.0%	of intestinal cancers

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Use of skin-fold calipers to measure subcutaneous fat

that cyclohexylamine produced cancer of the bladder in the rat. It should be emphasised, however, that there has been no evidence whatever that cyclamates have caused cancer in humans.

(b) *Unintentional:* A variety of potential carcinogens such as herbicides, insecticides, fertilisers, antibiotics, detergents, metals, besides products derived from fungi and moulds, may contaminate human food. One of the most widely used insecticides, DDT, was shown to produce hepatomas after prolonged feeding to rats in amounts greatly in excess of those likely to be consumed by man. However, DDT and other chlorinated pesticides pass into milk and therefore even infants are likely to have some degree of exposure to these compounds. It is also known that these compounds accumulate in various tissues, particularly fat tissue.

Both natural and synthetic oestrogens are known to be potent carcinogens in a variety of species, and to cause cancer in a variety of organs such as the breast, uterus, etc. In women, cancer of the breast often regresses after ovariectomy, adrenalectomy or hypophysectomy. It is therefore evident that hormones can stimulate the growth of a breast cancer, even if they cannot actually induce it. It is possible that women may receive exogenous hormones of the same type in food, for they are some times implanted in domestic animals as growth promoters. It is not inconceivable, therefore, that in such circumstances food may play a role in the growth of human cancer.

In 1960 a large number of turkeys died as the result of a liver disease called Turkey X. The cause of this disease was traced to the Brazilian groundnuts that had been used as mash for feeding the birds. The nuts were contaminated with a mould, *Aspergillus flavus*, and subsequent work showed that this mould produces a group of extremely toxic substances, which were called aflatoxins. Many investigations have now been done on these substances, and they have

been shown to be carcinogenic in several animal species. There is no direct evidence that they are carcinogenic in man, although indirect evidence would suggest that they are. Thus, in comparison to the peoples of the Western world, the incidence of primary hepatoma is high among the negriform races, and particularly those in Africa. It is not inconceivable that at least some of this higher incidence is the result of the ingestion of aflatoxins. These substances have been reported, for instance, as a contaminant of maize meal in Ethiopia. The incidence of hepatoma in Indonesia is among the highest in the world, and in that country fermented peanut press cake, a popular food and a good source of dietary protein, has been found to contain aflatoxins.

It is important to realise that both genetic and nutritional factors may interact with mycotoxins, or other toxins of plant origin. In developing countries where people have been living on a low protein diet, an increase in the protein intake may act as a kind of catalyst for the induction of cancer in a precancerous dormant liver. The problem may be still further complicated by the fact that the additional protein ingested may have come from cereals or other products that are readily contaminated with mycotoxins.

There is evidence, too, from animal studies, that hepatotoxins such as aflatoxin may show their maximal carcinogenicity when given with a diet that is deficient of pyridoxin (vitamin B6). Thus, it could be reasoned that the high incidence of primary liver cancer in African groups implies that the diets are deficient in pyridoxin, or alternatively, contain antagonists to pyridoxin.

Intraluminal formation of carcinogens

Recently, attention has been focused on the possibility that carcinogens may be formed in the intestine as the

result of ingesting dietary constituents that are themselves innocuous.

An epidemiological study of environmental factors that may be responsible for cancer of the colon and rectum in Japanese living in Japan has shown that cancer of the colon is significantly less common in Japan than in the United States, whereas the incidence of cancer of the rectum is similar in both countries. Moreover, Japanese patients with cancer of the colon were found to come from a higher socio-economic group of the population than those with rectal cancer, and this higher socio-economic background was associated with the eating of a more Western-type diet. These workers suggested that the proportion of fat in the diet might affect the pathogenesis of cancer of the colon through the action of the bacterial flora. A study of the effect of vegan diets on bacterial flora and faecal steroid concentration has led Dr Frey Ellis and his collaborators to postulate a similar correlation between diet in Western countries and the relatively high incidence of cancer of the colon.

The incidence of cancer of the breast is higher in North America and North-West Europe than it is in Africa, Asia and South America, and within a country is highest, like that of cancer of the colon, in people of the higher socio-economic classes. This led Wynder to postulate that there is a relationship between dietary fat and breast cancer. He suggested that dietary fat may affect hormone production or the retention of hormones by adipose tissue. The effect may, however, be mediated by the action of the gut flora on bile steroids resulting in the formulation of oestrogens. This work can only be considered at the moment as suggestive for we would like to know, among other things, the potency of the particular oestrogen produced by the bacteria as compared with the normally occurring oestrogens.

It may be that the type of fat in the diet is also important. Dietary saturated fats have been incriminated in the aetiology of atherosclerosis, and the results have been reported of an eight-year controlled clinical trial in which men had been assigned randomly to a conventional diet or to one similar in all respects except for a substitution of vegetable oils, rich in polyunsaturated fats, for saturated fat. Fatal heart disease was found to be more common in the men having conventional diet, whereas cancer was more common in the group having vegetable oils. Further work is obviously needed before too much is made of these results, but taken in conjunction with what has been said about the possible relationship of dietary fat to cancer of the colon and breast, it may not be too early to advise certain patients to reduce their fat intake and particularly the intake of polyunsaturated fats.

Attention has recently been drawn to the similarity in the epidemiology of diverticulitis and carcinoma of the colon. The incidence of diverticulitis has apparently increased over the past 100 years or so, and it has been suggested that this increase is associated with the ingestion of a diet rich in refined carbohydrate and consequently with a low roughage content.

It is always said that "prevention is better than cure". Preventive measures have greatly reduced the incidence of infectious diseases, including tuberculosis. It may be that at least a part of the answer to the cancer problem lies in more strenuous measures to reduce our dietary intake of potentially carcinogenic chemicals. The comparatively recently appreciated possibility that even the "normal" constituents of our diet in certain circumstances may play a role in carcinogenesis, is one which opens up a whole new area for investigation.

Next week: Metabolic effects of cancer

Hormone treatment instead of prostatectomy?

Benign prostatic hypertrophy or prostate enlargement is a common condition in males over 50.

In one in five, the prostate enlargement is severe enough to obstruct the urine passageway, resulting in inability to empty the bladder. The most common treatment for this condition is prostatectomy, a simple but costly surgical procedure for removal of the prostate. Thousands of these operations are performed every year.

A new treatment, still in the experimental stage — the result of which may play a significant role in the development of a new, non-surgical treatment of the prostate — may replace the now common surgery. A female hormone, medrogestone, given orally or by injection, shrinks the diseased prostate, making an operation unnecessary.

Research urologists in the USA believe that if clinical trials demonstrate its effectiveness, medrogestone may become the treatment of choice for this disease in the future. This particular hormone appears to be free of the undesirable side-effects associated with female hormone treatment.

Before recommending hormone treatment for the prostate on a routine basis, however, the research urologists want to know if

medrogestone actually has any effect on the antibacterial factor found in prostatic and seminal fluids. This is important, to avoid compromising the patient's natural defences against bacterial infections.

Earlier research found that seminal and prostatic fluids contain a potent antibiotic substance which kills bacteria responsible for urinary tract infections. This discovery not only accounted for the absence of urinary tract infection in healthy males, but explained how the body fought off bacteria which caused such infections.

If further study confirms that medrogestone is effective in humans and has no effect on the antibacterial factor in prostatic fluid, prostate surgery for this benign disease may become a thing of the past.

Additionally, the US research urologists plan to study the biochemical factor in prostatic and seminal fluids, and its clinical importance as a defence mechanism against urinary tract infections; they also intend to investigate the possibility that the peri-urethral glands of the female may secrete a similar substance, and that this agent is responsible for the absence of harmful bacteria on the urethra and external vagina of normal females.

The mutagens that cooking produces

John S. Wishnok

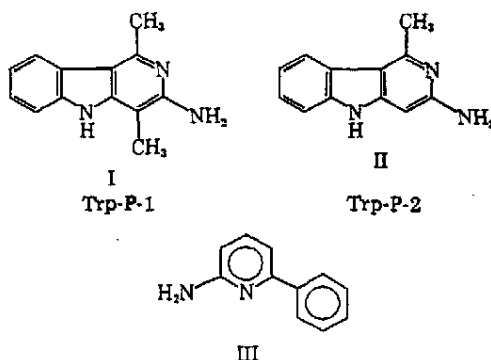
Can carcinogenic substances be formed during the cooking of food? The answer is yes. Experimentally, activity is found in simple extracts of surface char or smoke condensates (1). However, the greatest specific activity is usually found in the extract of a basic fraction of the char or smoke condensate. Substantially lower mutagenic activities are observed if NaOH is used in the work-up rather than NH_4OH (2). The origin of this effect has not yet been elucidated, although it may be due to the formation of mutagenic nitrogen heterocycles via condensation reactions of ammonia with carbonyl-containing pyrolysis products (3, 4). Much of the early work was done with NH_4OH , and in some cases the base was not specified. Thus the quantitative aspects of all these reports remain questionable.

Soon after Ames developed mutagenicity assays via bacterial tester strains, reports began to appear noting mutagenic activity in crude extracts of smoke condensates or charred surfaces obtained from broiled fish or meat (5), and these early reports have been followed by a steady flow of related publications. Much of this research can be loosely divided into three general areas:

- screening of various types of cooked food for mutagens or for compounds that alter mutagenic activity
- chemical characterization of the mutagens
- biochemistry of the food mutagens (cancer initiation, metabolism, adduct formation, etc.).

Following the initial experiments with cooked whole fish and meat, several food components or model systems were studied. Smoke condensates from the pyrolysis of lysozyme, histone, DNA, RNA, starch, and vegetable oil were tested. The greatest activity was found in the protein pyrolysate following microsomal activation (6).

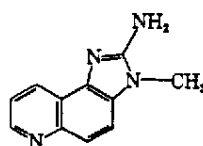
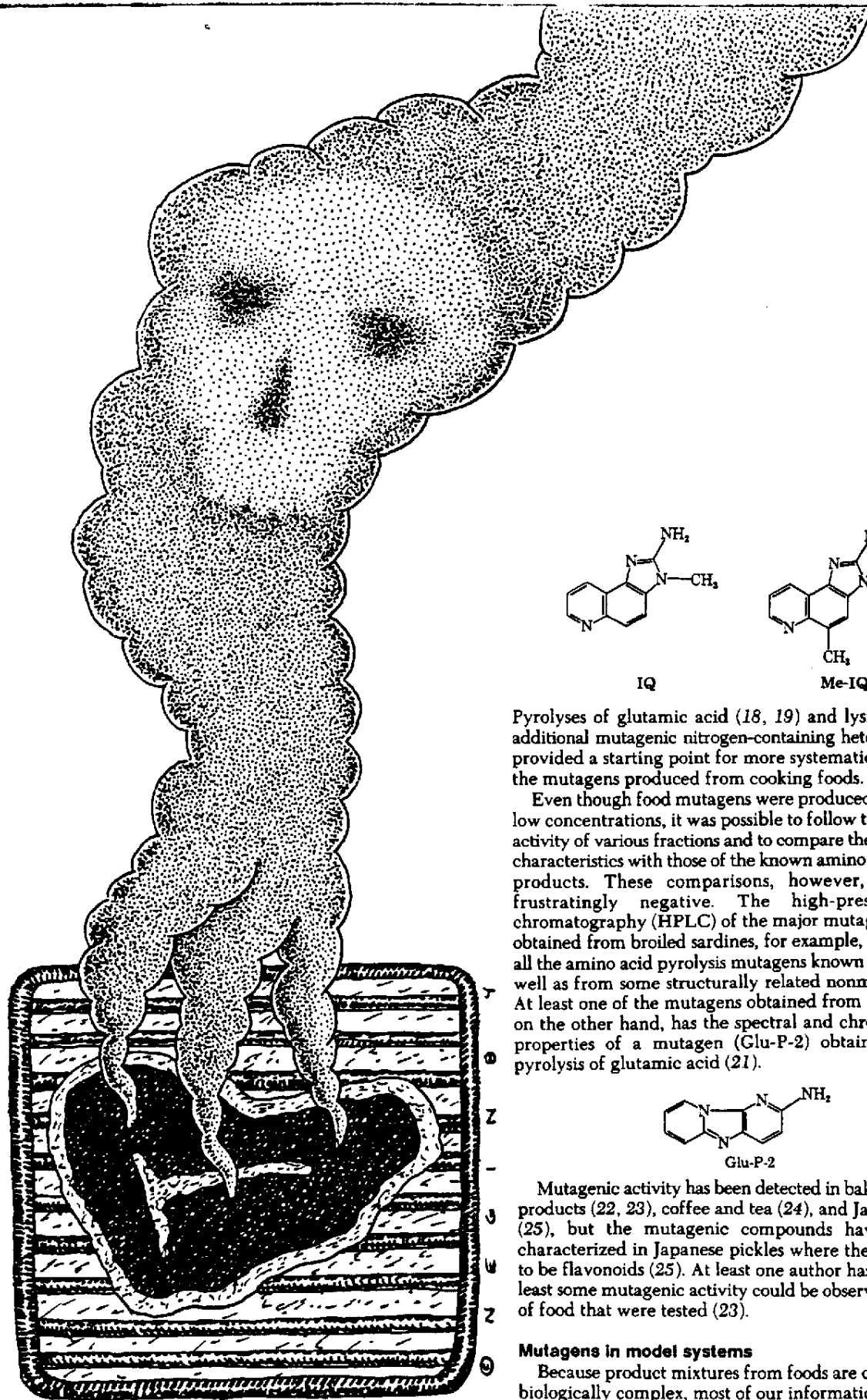
These observations, along with solubility and chromatographic behavior, indicated that the mutagens were organic bases. This in turn suggested that proteins might be major precursors of the mutagens, and a series of amino acids were then pyrolyzed. The smoke condensate from tryptophan showed high Ames test activity. Two mutagens (I and II) arising from tryptophan and one from phenylalanine (III) have been reported (7).



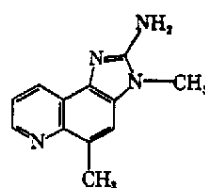
To date, a wide variety of foods have been tested for mutagenic activity, but beef has received the most attention, followed by fish, with noticeably less attention having been paid to other foods. Benzo(a)pyrene is formed during the grilling of beef, pork, lamb, and turkey (8). Most of the recent research, however, is focused on compounds other than benzo(a)pyrene, which are formed under generally milder cooking procedures.

Such mutagens have been observed, for example, in beef extract after refluxing for several days or from frying ground beef at surface temperatures of less than 250°C (9-13). When beef is cooked isothermally, there appears to be an induction period prior to which there is relatively low mutagenic activity (13). A sharp rise in activity occurs between 140°C and 180°C (12). When beef patties are fried between 140°C and 250°C as much as 99% of the mutagenic activity appears in the cooking vapors rather than on the meat surface (13). Interestingly, the total mutagenic activity obtained from the broiling of ground beef is increased by adding proline to the meat before cooking. Of 20 common amino acids tested, only proline had this effect (14).

To date, only one of the beef mutagens, Trp-P-1, has been unequivocally characterized (15). The homologous mutagens IQ and methyl-IQ have been isolated in broiled (16) and roasted (17) fish.



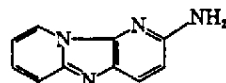
IQ



Me-IQ

Pyrolyses of glutamic acid (18, 19) and lysine (19) gave additional mutagenic nitrogen-containing heterocycles, and provided a starting point for more systematic screening of the mutagens produced from cooking foods.

Even though food mutagens were produced in extremely low concentrations, it was possible to follow the mutagenic activity of various fractions and to compare the fractionation characteristics with those of the known amino acid pyrolysis products. These comparisons, however, were often frustratingly negative. The high-pressure liquid chromatography (HPLC) of the major mutagenic fraction obtained from broiled sardines, for example, differed from all the amino acid pyrolysis mutagens known at the time, as well as from some structurally related nonmutagens (20). At least one of the mutagens obtained from broiled squid, on the other hand, has the spectral and chromatographic properties of a mutagen (Glu-P-2) obtained from the pyrolysis of glutamic acid (21).



Glu-P-2

Mutagenic activity has been detected in bakery and cereal products (22, 23), coffee and tea (24), and Japanese pickles (25), but the mutagenic compounds have only been characterized in Japanese pickles where they were shown to be flavonoids (25). At least one author has stated that at least some mutagenic activity could be observed in all types of food that were tested (23).

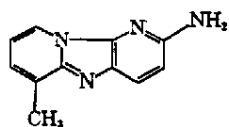
Mutagens in model systems

Because product mixtures from foods are chemically and biologically complex, most of our information comes from

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studies of various model systems. Tryptophan pyrolysate was the most mutagenically active, but 11 of 20 pyrolysates of common amino acids gave positive Ames tests (18). The mutagens of interest in all cases were distinct from benzo(a)pyrene, although many amino acids do yield benzo(a)pyrene upon pyrolysis, usually at somewhat higher temperatures (650–850 °C) than would be used for cooking (26).

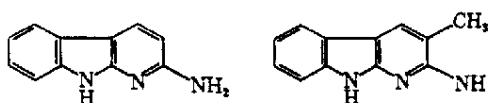
The next highest mutagenic activity among common amino acids, following pyrolysis, occurred with glutamic acid. Again, two mutagenic components were isolated from the pyrolysate and characterized as Glu-P-1 and Glu-P-2.



Glu-P-1

In most cases in which mutagens have been identified, the compounds are isolated in pairs differing only by a methyl group.

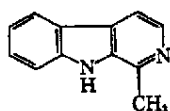
Pyrolysis of proteins also yields mutagens. There is reasonably persuasive evidence for the presence of Glu-P-2 in casein pyrolysates, but only about 10% of the mutagenic activity of the pyrolysate could be attributed to Glu-P-1 and Glu-P-2 (27). The amount of Trp-P-2 in various protein pyrolysates roughly corresponds to the percent of tryptophan in the protein (28). Soy globulin also produced two additional mutagens, which have also been shown to be present in the tryptophan pyrolysate (28–30):



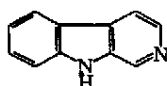
These compounds are much weaker mutagens than Trp-P-1 and Trp-P-2. Note that they too constitute a methylated–nonmethylated pair.

Mutagenically significant non-mutagens

All of the mutagenic compounds that have been characterized are nitrogen-containing aromatic heterocycles with an exocyclic α -amino group. Some structurally related pyrolysis products (e.g., harman and norharman) without the exocyclic amino group have also been observed (31).



Harman



Norharman

Neither harman nor norharman is mutagenic, but they enhance the activity of several mutagens (32–34). Norharman increases the mutagenicity of 4-dimethylaminoazobenzene 40-fold, while harman has no effect. Harman does, however, enhance the mutagenicity of 2-aminoanthracene, 2-aminofluorene, and 2-acetylaminofluorene (34) while it lowers the mutagenicity of several polycyclic aromatic hydrocarbons such as 7,2-dimethylbenzanthrene, benzo(a)pyrene, chrysene, and dibenzo(a)anthracene (33).

The complexity of this picture is increased by the observation that mixtures of norharman and aniline or norharman and *o*-toluidine are mutagenic even though none of these compounds are mutagenic when tested alone. *m*-Toluidine and *p*-toluidine remain nonmutagenic in the presence of norharman (35). It's not known how norharman and harman elicit these effects, although it has been shown that these compounds interact with DNA. Harman apparently interacts more strongly with DNA, yet norharman is the more effective co-mutagen (36).

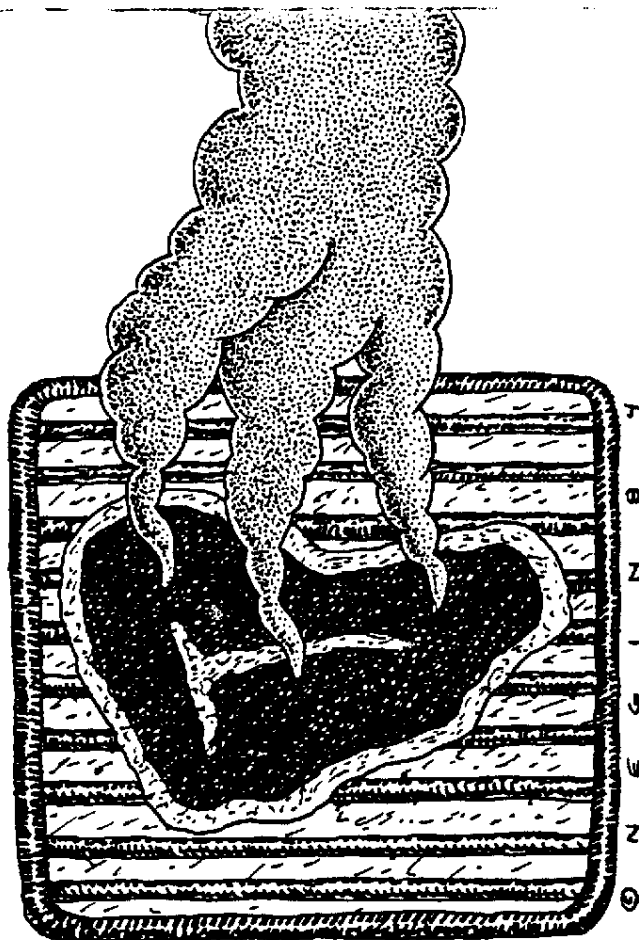
There is evidence that mutagens can influence each other's behavior (27–29, 37). For example, various mixtures of four mutagens obtained from tryptophan pyrolyses exhibit synergistic activity (35). We found that the mutagenic activity of the basic fraction from boiled beef extract with added IQ is essentially the sum of the two activities (38a), while the addition of Trp-P-2 to the basic fraction depresses mutagenicity (38b).

Metabolism and structure—activity variations

Despite the complexity, patterns of metabolism and of some aspects of structural effects of mutagenic activities are beginning to emerge. It was shown fairly early, for example, that the exocyclic α -amino group is necessary for mutagenic activity, and that three-ring heterocyclic systems are more mutagenic than two-ring systems. The fusion position also has a pronounced effect on the relative potency (39).

The presence or absence, as well as the position, of methyl groups also affects the mutagenicity of the pyrolysis mutagens. Glu-P-1 is a stronger mutagen than its six methylated isomers or the nonmethylated Glu-P-2 (40). Trp-P-2 is a stronger mutagen than the dimethyl Trp-P-1, or the monomethyl isomers (41–43). Mutagenic activity roughly parallels the degree of noncovalent interactions with DNA as measured by fluorescence quenching and equilibrium dialysis (41, 42, 44). Similar results were obtained for Glu-P-1 and Glu-P-2 (45).

In these experiments, as well as a variety of others, activation by induced liver microsomal fractions is required (46). For Trp-P-2, binding to DNA occurs following treatment with either microsomes or cytosol; NADPH and ATP are required. The degree of binding depends on the nature of the inducer (47–49). Both Trp-P-2 and Glu-P-1



form C₈-guanine derivatives when treated with microsomes in the presence of DNA, apparently via the *N*-hydroxyl derivatives (43, 50-52). In the case of Trp-P-2, four metabolites, two of which are direct-acting mutagens, can be observed via HPLC. The major mutagenic metabolite from Trp-P-2 was isolated and characterized as the 3-amino hydroxy derivative.

Carcinogenicity of food mutagens

Some of these mutagenic food pyrolysis products also have carcinogenic activity in laboratory animals, although their potency is much less than that of 3-methylcholanthrene (53-55). Both Trp-P-1 and Trp-P-2 produce chromosome aberrations, with Trp-P-1 being 5-10 times more effective than Trp-P-2 (57, 58).

Because of the complex and unpredictable synergism among these compounds and the fact that human exposure to them will invariably be part of a complex food matrix, it is probably premature to attempt to assess the potential importance of these findings to human cancer epidemiology.

Summary

Research on food mutagens is still developing and many current results are contradictory or confusing. Nonetheless, several conclusions are emerging from this work:

- Strongly mutagenic compounds can be formed from most types of food during normal cooking procedures.
- The mutagens that have been characterized all appear to be of the same structural type, i.e., 3-ring nitrogen-containing heterocycles with an exocyclic amino group on a carbon adjacent to a ring nitrogen. In model

systems, these compounds appear as homologous pairs differing only by the presence of one methyl group.

- The relative mutagenic potency within a series of these mutagens is strongly affected by substitution, but no general rational pattern of structure-activity relationships has been developed.
- The mutagenicity of mixtures of these compounds often differs from that expected on the basis of individual potencies, but no rational pattern for this behavior is evident.
- Nonmutagenic compounds, which enhance the activity of mutagens, are also formed during cooking procedures.
- All of the cooking-related mutagens that have been studied require microsomal activation in order to exert their mutagenicity.
- This microsomal activation also requires induction of the liver microsomal systems. The most effective compounds are known inducers of mixed-function oxidases.
- The active metabolites are apparently the *N*-hydroxylated derivatives at the exocyclic amine. Adducts at the C₈ position of guanine have been characterized.
- Some of these compounds have been individually shown to be carcinogenic in laboratory animals but it is not clear how they may behave when administered in combination.

Acknowledgments

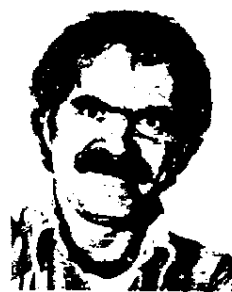
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THE ACADEMIC HIERARCHY

THE PRESIDENT:

Leaps tall buildings at a single bound,
Is more powerful than a locomotive,
Is faster than a speeding bullet,
Walks on water,
Gives policy to God.

The ACADEMIC VICE-PRESIDENT:

Leaps short buildings at a single bound,
Is more powerful than a switch engine,
Is just as fast as a speeding bullet,
Walks on water if sea is calm,
Talks with God.

PROFESSOR:

Leaps short buildings with a running start and favorable winds,
Is almost as powerful as a switch engine,
Can fire a speeding bullet,
Walks on water in an indoor swimming pool,
Talks with God if special request is approved.

ASSOCIATE PROFESSOR:

Barely clears a quonset hut,
Loses tug of war with locomotive,
Misfires frequently,
Swims well,
Is occasionally addressed by God.

ASSISTANT PROFESSOR:

Makes high marks on walls when trying to leap tall

buildings,

Is run over by locomotive,
Can sometimes handle a gun without inflicting self-injury,
Dog paddles,
Talks to animals.

GRADUATE STUDENT:

Runs into buildings,
Recognizes locomotives two out of three times,
Is not issued ammunition,
Can stay afloat with life jacket,
Talks to walls.

UNDERGRADUATE STUDENT:

Falls over doorstep when trying to enter building,
Says, "Look at the choo-choo,"
Wets himself with a water pistol,
Plays in mud puddles,
Mumbles to himself.

DEPARTMENT SECRETARY:

Lifts tall buildings and walks under them,
Kicks locomotives off tracks,
Catches speeding bullets in her teeth and eats them,
Freezes water with a single glance,
Is God.

CHEMTECH Book Review Editor Gil Mains of Oklahoma State University sent us this from "Pople's Gaussian 80 Q.M."

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Diet and the Chemical Environment as Modifiers of Carcinogenesis

A. E. M. McLEAN¹

We are faced with the problem that, on the one hand, chemical carcinogens such as benzo [a] pyrene, and also possibly oncogenic viruses, such as the Epstein-Barr virus, are ubiquitous, and that, on the other hand, each culture, social group and region of the world has its different characteristic pattern of tumour incidence (Doll, 1967; Lijinsky & Epstein, 1970; Ringertz, 1971; Shabad *et al.*, 1971).

The studies of migrant peoples suggest that the differing patterns of cancer incidence are mostly determined by environmental rather than genetic factors (Haenszel & Kurihara, 1968). The environment has social, biological, chemical and physical components. It seems to me most likely that the differing patterns of cancer incidence have their origin largely in differing chemical environments.

It is difficult to imagine mechanisms whereby social or interpersonal factors, *per se*, could modify carcinogenesis, though no doubt they might do so by altering endocrine states, as through anxiety or pregnancy. Again, there seems little evidence of differences in bacterial, parasitic or viral status for most of the groups that differ in cancer incidence. Genital herpes and endemic malaria could be exceptions. In a country like England, practically all social classes share the same respiratory virus infections, intestinal parasites are rare and seem unlikely to be involved in tumorigenesis, but social gradients of cancer still exist. The physical environment, temperature, radiation and the like can account for varia-

tions in a few instances, as with skin cancers, but the bulk of the variation in cancer incidence must be attributed to variation in the chemical input to the members of the populations concerned.

The chemical environment of man, that is all the molecules that impinge upon him, can be arbitrarily divided up into the following groups:

- (1) air, and inhaled substances, such as smoke and dust;
- (2) water;
- (3) food and drink;
- (4) substances ingested for social or therapeutic purposes, or for pleasure (medications, herbs, drugs, betel chewing);
- (5) substances concerned in surface applications — cosmetics, soaps, oils, etc., applied either by individual choice or through industrial exposure.

The chemical environment is indivisible and, from the point of view of a liver cell, it does not matter whether a molecule arrives via an anal suppository or an industrial smoke.

We breathe about 11 kg of air per day, which is the carrier of important contaminants in cigarette smoke and industrial effluents. Drinking-water is consumed in variable amounts, and the 1–3 litres that we consume every day may well modify carcinogenesis, as it seems to modify coronary heart disease, by virtue of its variable mineral content. Little is known about this factor (Crawford, Gardner & Morris, 1971).

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The bulk of the molecules that come into contact with cells come as food. We eat 1—3 kg of food per day, and food is inextricably a part of man's culture. It has great symbolic significance, and food habits merge gradually into social customs, such as dancing, drinking, the use of herbs and spices, washing or cosmetics, making it difficult to isolate single factors in food.

The components of the diet can be looked at from three points of view:

- (1) general nutritional adequacy;
- (2) contamination with carcinogenic substances; and
- (3) factors in the food that alter metabolic networks, and so alter response to carcinogens.

NUTRITIONAL ADEQUACY AND CARCINOGENESIS

The nutritional adequacy of a diet is usually measured by its ability to support growth and reproduction, without producing signs of deficiency disease. It can be correlated with, and summarized by, its total nutrient content (i. e., carbohydrate, amino-acids, essential fatty acids, vitamins, minerals, etc.). The exact means by which an adequate nutrient intake is achieved seems to matter very little. For instance, in rats the best growth rates are achieved with about 20% of the calories as fat, but whether this is olive oil, corn oil, or herring oil matters very little.

In a number of experimental studies, it has been found that a reduction in calorie intake and of protein intake tends to reduce the incidence of cancers in laboratory animals (White, 1961; Tannenbaum & Silverstone, 1953; Ross & Bras, 1971). But human populations vary more in the kinds of foods that are taken in than in the values found when these foods are reduced to "calories" or "protein".

In some laboratory instances, specific dietary deficiencies lead to sensitivity to chemical carcinogens. For instance, riboflavine deficiency leads to a marked sensitivity to azo dye carcinogenesis, associated with low levels of one pathway of metabolism of the carcinogen (Miller *et al.*, 1948). However, human deficiency diseases are not in general associated

with carcinogenesis. For instance, Jamaica has a high incidence of childhood malnutrition, but no marked excess of cancer of the liver or other organs.

In Africa, the areas of high oesophageal or liver cancer incidence are areas in which malnutrition is prevalent, but equally malnourished areas without these cancers are found nearby (Oettle, 1964).

CARCINOGENS IN FOOD

The problem about carcinogenic molecules in food is that they seem to be a universal background; just as cosmic radiation falls on us all, so do carcinogenic molecules enter us. This means that, while they may play a part in carcinogenesis, they cannot be held responsible for the differences between individuals, and often provide no explanation for the differences between populations. Benzo [α] pyrene can be found wherever there is fire and industry. Low levels of nitrosamines can be detected in all kinds of foods, and it seems possible that they are synthesized *in vivo* (Lijinsky & Greenblatt, 1972; du Plessis, Nunn & Roach, 1969).

There are some instances where carcinogenic substances are found in foods in amounts that approach the carcinogenic levels for animals. Thus, aflatoxin contamination of market produce is widespread in Africa and other tropical countries (Lopez & Crawford, 1967). The brackens eaten in Japan seem to be carcinogenic (Hirono *et al.*, 1972), and smoked foods in Iceland may be related to a high stomach cancer incidence.

ENZYME INDUCTION AND FOOD

Diets that are nutritionally adequate can be made up in an immense variety of ways. The protein content can vary widely. The foodstuffs that make up the diet can be purely vegetarian or almost exclusively animal products. Fat content can range from 10% of calories in Japan to 42% in Britain.

Such varied diets can support life and growth because the enzyme composition of the body is matched to the chemical input by the process of induction of enzyme synthesis.

Thus the alanine amino transferase (glutamine pyruvic transaminase — GPT) of the liver is raised whenever there is a rapid flow of amino-acids through the liver. This occurs either when high protein diets are fed, or in starvation, when muscle protein is broken down for gluconeogenesis. When a low-protein high-calorie diet is fed, the levels of GPT in the liver fall, both in rats and in man (Schimke, 1962; McLean, 1966) (see Table 1).

Table 1. Effect of low protein diet on enzyme activity in human and rat liver^a

Diet	Alanine transaminase ^b (μ M/mg protein per hour)	Malate dehydrogenase ^b (μ M/mg protein per hour)
Adults: hospital diet	12.5 $\pm$ 2.5	14.2 $\pm$ 2.9
Children: 3 g protein/kg per day	11.8 $\pm$ 2.2	16.2 $\pm$ 2.2
Children: 1 g protein/kg per day (isocalorie)	6.5 $\pm$ 1.2	20.4 $\pm$ 1.1
Rats: 30% casein	12.5 $\pm$ 3.5	30 $\pm$ 6
Rats: no protein (2—3 weeks)	1.2 $\pm$ 0.8	40 $\pm$ 4

^a Source: McLean (1966) and unpublished data.
^b Mean $\pm$ standard deviation.

THE TRIPLE SYSTEM OF DETOXICATION

One of the biochemical requirements of any animal is a method for the disposal of the absorbed molecules from food that are either toxic or inert and do not fit easily into metabolic pathways. Steroids, for example, are readily absorbed but poorly excreted. To prevent the accumulation of such compounds, both exogenous and endogenous in origin, a triple system of disposal has evolved. In the liver, it consists first of proteins specialized for the uptake of molecules such as bilirubin and steroids, the Y and Z proteins of Arias (1971). This leads on to the microsomal hydroxylation system, centred on cytochrome P 450, which is capable of hydroxylating an immense variety of substrates. Drugs, such as phenothiazines, bar-

biturates and many others, are attacked, while steroids, polycyclic hydrocarbons, and fatty acids are also drawn into this system. Some compounds, such as nitrosodimethylamine and carbon tetrachloride, are converted into highly reactive metabolites by the system (McLean, 1971), but in the majority of instances hydroxylation leads either to excretion of water-soluble metabolites, or else to immediate conjugation with chemical groups, such as glucuronides. The conjugation step is almost invariably an inactivation that destroys pharmacological activity, and as a result, the triple system is, as a rule, an effective detoxication mechanism.

Poisons are substances that escape this system, often because they form reactive metabolites that may even attack distant sites. For instance, tetraethyl tin and lead are dealkylated to triethyl compounds by liver microsomes, and the triethyl compound then attacks brain mitochondria, of which it is a potent inhibitor (Cremer, 1959).

The hydroxylation system is found not only in the liver, but also, to a lesser extent, in the lung, kidney, gastro-intestinal tract, and skin.

Hepatic synthesis of the hydroxylation system and its central cytochrome P 450 is stimulated about six-fold by phenobarbitone and other inducers. As a result, the ability of animals to withstand compounds that are either detoxicated or activated by the hydroxylation system of one or other tissue depends very greatly on the previous exposure to inducing drugs.

HYDROXYLATION AND CARCINOGENESIS

Compounds that are toxic or carcinogenic have to react with cell components in order to exert their effects. This means that a highly reactive molecule, such as a free radical or epoxide, is usually the effective agent. Such reactive molecules cannot live long in a natural environment, so that the reactive molecules must be generated in the body from nonreactive precursors. The most usual enzyme system for generating such reactive molecules is the hydroxylation system.

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Microsomal hydroxylation seems to be responsible for the activation of polycyclic hydrocarbons, carcinogenic amines, pyrrolizidine alkaloids, nitrosoamines, and aflatoxin (Miller, 1970; E. K. McLean, 1970; Garner *et al.*, 1971; Butler, Mattocks & Barnes, 1970).

In many instances, the interaction of carcinogen and microsomal enzymes is complex, since multiple hydroxylations may inactivate, as will further conjugation. So while a certain level of hydroxylation generates a reactive molecule from, say aflatoxin, higher levels protect animals from the toxic and carcinogenic action of aflatoxin *in vivo* (McLean & A. K. Marshall, 1971).

This means that the interaction between carcinogen and target animal will depend on the exact level of hydroxylation enzymes in the animal. The hydroxylation activity of the different tissues, is, in turn, determined by the diet.

The effect of increasing microsomal activity is often an increased resistance by the animal to carcinogenesis. The induction of the entire triple system of detoxication is a more effective mechanism of detoxication than one would predict if only hydroxylation were increased. The protection given by induction is not absolute, but rather a shift of the dose-response curve so that a smaller proportion of the exposed population develops tumours (Miller *et al.*, 1958).

DETERMINANTS OF HYDROXYLATION

The activity of hydroxylation enzymes in animals and man is determined by three main factors:

- (1) genetic background;
- (2) dietary protein and fat intake; and
- (3) intake of non-nutrient enzyme inducers.

Studies in various strains of animals and human studies of twins have shown that hydroxylation activity and cytochrome P 450 levels in the liver are under strong genetic influence. The genetic factors determine not only the basal level of hydroxylation, but also the response to inducers like phenobarbitone

(Vesell & Page, 1969). For mice, we know that the genetic factor influences the activity of the enzymes in liver, kidney and intestine (Nebert & Gelboin, 1969).

In rats, we have shown that dietary protein and the dietary fat content exert a predominant influence on cytochrome P 450 levels and microsomal hydroxylation activity. These effects are found not only in the liver (Kato, Oshima & Tomiyawa, 1968; McLean & McLean, 1966, 1972), but also the kidney and lung (Paine¹).

Table 2. Effect of diet and phenobarbitone on cytochrome P 450 levels in rat liver microsomes^a

Diet	Cytochrome P 450 level ^b (n M/g) in liver, with:	
	Tap water	Phenobarbitone in drinking-water
No protein, no fat	8 ± 1	24 ± 6
No protein + 15% herring oil	13 ± 1	53 ± 17
20% casein, no fat	18 ± 3	60 ± 7
20% casein + 15% herring oil	24 ± 7	123 ± 19
Stock pellets	30 ± 3	124 ± 7

^a Source: Marshall & McLean (1971).

^b Mean ± standard deviation.

Table 2 shows that both protein and a source of unsaturated fat (in this case, herring oil) are required for synthesis of basal and induced levels of cytochrome P 450 in liver. In previous work, (McLean & McLean, 1966) we showed that 6% protein in the diet reduced drug metabolism to about half maximum and that, as protein levels became adequate for growth, so they supported synthesis of drug metabolizing enzymes.

This suggests that in all those parts of the world where protein supplies are rate limiting for growth, they are also rate limiting for hydroxylation.

¹ Unpublished data.

Similarly, the experiments showed that maximal cytochrome P 450 levels are achieved only if a source of poly-unsaturated fatty acid is incorporated in the diet.

Many substances are present in natural diets that are either inducers or synergists of inducers. Wattenberg (1971) and Wattenberg, Page & Leong (1968) have shown such inducing activity for liver, lung, intestine and kidney by flavones, and by some factor present in cabbages and many other vegetable foods.

We have found that benzo[a]pyrene hydroxylation in kidney responds markedly to the fat content of the diet, but not to protein. In contrast, the lung hydroxylase responds to protein and not to fat (see Table 3).

In this respect, the benzo[a]pyrene hydroxylase differs from the mass of cytochrome P 450 in the liver, which depends on both fat and protein (Marshall & McLean 1969, 1971).

The hydroxylation system consists of a number of elements. Coon showed that it could be split into a reductase (NADPH-P450 reductase), a lipid component, and a terminal haemoprotein (Coon, Autor & Strobel, 1971). The terminal haemoprotein portion gives the substrate specificity. The hydroxylase system from different organs, or after different dietary or induction treatments, has different substrate specificity.

After induction with methylcholanthrene, the haemoprotein that is synthesized has an absorption peak at 488 nm (i.e., P 448) and is highly active in benzo[a]pyrene

hydroxylation, while phenobarbitone induces the P 450 haemoprotein, which has a wider substrate range and a lower activity towards benzo[a]pyrene (Kuntzman *et al.*, 1971).

NUTRITION AND NITROSODIMETHYLAMINE

Nitrosodimethylamine (DMNA) is metabolized by liver microsomes (Magee & Vandekar, 1958), and there is good evidence that metabolism is required for it to exert its toxic and carcinogenic effects (Magee & Swann, 1969; Lijinsky, Loo & Ross, 1968; McLean, 1971).

The toxic and carcinogenic effects of DMNA are profoundly altered by changes of diet. When a low-protein, highly purified diet is fed, the acute hepatotoxic effects of DMNA are greatly reduced (McLean & Verschuuren, 1969), while the renal carcinogenic effect is enhanced (McLean & Magee, 1970).

Metabolism of DMNA by the liver of rats fed low-protein diets is reduced to about one half that found when stock pellets are fed. As a result, DMNA persists in the blood for a long time after a single dose. Renal metabolism of DMNA is not affected by the diet, and renal exposure to DMNA is effectively increased (Swann & McLean, 1971).

We have recently been measuring the metabolism of DMNA to formaldehyde by liver microsomes and postmitochondrial fractions. (One mole of formaldehyde is produced per mole of DMNA metabolized).

We find that rats fed purified low-protein diets have low levels of DMNA metabolism,

Table 3. Effect of diet on benzo[a]pyrene hydroxylation in liver, kidney and lung^a

Diet	Benzo[a]pyrene hydroxylation ^b (pM/mg protein per 30 min)		
	Liver	Kidney	Lung
Low protein, no fat	1163 ± 214	75 ± 26	36 ± 19
Low protein, 5% fat ^c	1461 ± 131	152 ± 54	40 ± 7
20% casein, no fat	2755 ± 573	101 ± 11	65 ± 32
20% casein, 5% fat ^c	3353 ± 1218	221 ± 100	71 ± 19

^a Source: Paine & McLean (1972) (unpublished data).

^b Mean ± standard deviation.

^c Olive oil.

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but this can be restored to the levels found in rats fed stock pellets by giving phenobarbitone. Benzo[*a*] pyrene has very much less effect on the rate of DMNA metabolism. However, phenobarbitone has no effect on DMNA toxicity, while benzo[*a*]pyrene greatly increases the hepatotoxic effect (Table 4).

It seems likely that there are several possible pathways of DMNA metabolism, only some of which lead to toxic effects; we do not know whether carcinogenic effects are similarly affected.

Arcos and his co-workers suggest that inducers, like 3-methylcholanthrene or phenobarbitone, inhibit DMNA metabolism in their rats; in fact, what they show is a reduced specific activity (DMNA metabolism/mg microsomal protein), when inducers cause a large increase in microsomal protein, while DMNA metabolism per 100 g rat remains relatively constant (Venkatesan, Argus & Arcos, 1970).

Stock rat diet seems to contain factors that stimulate DMNA metabolism in the maximally "toxic" direction. It would seem of importance to know more about these alternative pathways of DMNA metabolism and the dietary factors controlling them.

THE SIGNIFICANCE OF DIETARY INDUCERS OF HYDROXYLATION ACTIVITY

We now have a series of loosely linked observations:

(1) carcinogenic chemicals are widespread in the environment;

(2) populations differ greatly in their patterns of cancer incidence;

(3) microsomal hydroxylation seems necessary for the activation of carcinogens, but if taken far enough can then inactivate these compounds;

(4) dietary factors alter microsomal hydroxylation activity in many tissues in experimental animals; and

(5) populations differ widely in their dietary habits in ways that we would predict should alter their microsomal hydroxylation activity.

These observations seem to me to lead to two conclusions.

First, we badly need to develop methods to enable us to measure microsomal hydroxylation in large numbers of people in order to see whether patterns of hydroxylation correlate with cancer patterns, and whether diet influences microsomal hydroxylation in man.

Second, we should perhaps look at diets made up to resemble those used by human populations, to see whether the diets alter hydroxylation and whether they confer resistance or susceptibility to carcinogens, when given to experimental animals.

Table 4. Effect of diet on metabolism of, and liver injury from nitrosodimethylamine

Diet	DMNA metabolism ^b (μ M/g liver per hour)	Indices of liver injury ^a	
		Plasma isocitrate dehydrogenase (nM/ml min)	Plasma bilirubin (mg/100 ml)
Stock pellets	1.41 $\pm$ 0.2	1000	1.54
3% Casein, 5% olive oil	0.62 $\pm$ 0.2	135	0.32
3% Casein, 5% olive oil + phenobarbitone	1.31 $\pm$ 0.1	180	0.36
3% Casein, 5% olive oil + benzo[<i>a</i>]pyrene	0.75	851	1.0

^a Measured 24 hours after i. p. administration of DMNA (90 mg/kg).

^b Mean $\pm$ standard deviation.

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Toxicities of Natural Foods in Man: A Survey

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Foods are considered to be substances that nourish cells and enable them to grow, multiply and repair themselves. Many foods may produce bodily harm, however, by some intrinsic toxicity or by excess consumption, contamination, preparation, inherited inability to process them, hypersensitivity of the individual, or other.

The literature is scattered and voluminous and the early reports are untrustworthy. In the present discussion, man rather than animals is emphasized as well as food toxicity encountered chiefly in the United States. We have not included any commentary on federal policies of increasing toleration of food practices dangerously leading to toxicities (see Marshall, 1982).

FOODS CONTAINING CARCINOGENS

Saccharin

Saccharin is the most widely used sugar substitute. In large doses it incites bladder tumors in laboratory animals and the FDA has placed it on a regulated list.

Tea

The black teas from Ceylon and India contain considerable *tannin* and *gallic acid*. These are controversially suspect as causative factors in esophageal cancer (Lewis and Elvin-Lewis, 1977, p. 119).

CONTAMINANTS FAVORING CARCINOGENICITY

Fungus Contaminants (Mycotoxins)

Some strains of *Aspergillus*, a mold, produce *aflatoxins*. The one found most often in food is aflatoxin B₁. It inhibits protein synthesis, causing the death of liver cells particularly. B₁ is one of the most potent hepatocarcinogens known. It is found chiefly in figs, peanuts and other nuts, and cereals (Osborne, 1982).

There is a toxic substance called *patulin* which can occur in apple juice due to contamination by molds. The substance is suspected of being carcinogenic (Crosby, 1979).

Toxins can be present in food that is not visibly moldy and they can persist even if the food is cooked or otherwise processed.

Polycyclic Aromatic Hydrocarbons (PCHs)

PCHs are an important class of carcinogens because they are being widely dispersed in the atmosphere, soil and water from the smoke and smog of industrial cities and some have tumorigenic potency. Dangerous levels of PCHs are now detected in many foods, particularly leafy vegetables (Harvey, 1982).

CONTAMINATION OF DRINKING WATER

Water is a food. Contamination by industrial wastes possibly leading to carcinogenicity is a huge problem and will not be considered here. There is atmospheric contamination of an incidental nature, however, nitrates posing a problem. It is contended that a high nitrate concentration in water supplies provides a risk of gastric cancer (Walters and Smith, 1981).

A concern about nitrates in water peripheral to the cancer issue centers on their reduction to nitrites by bacteria which can live in an infant's stomach due to low acidity therein. Hemoglobin, especially the persisting fetal type in the infant, is susceptible to oxidation by nitrites and methemoglobin is formed. Oxygen delivery is impaired. Cyanosis in infants who are given water from wells is a manifestation of nitrate toxicity (Gleason et al., 1969, p. 100).

Water per se can produce ill effects. Massive overdrinking (which has been used in torture) produces the syndrome of "water intoxication". Cerebral edema, convulsions and coma can follow.

Water causes gastric distress in many persons because it can excessively stimulate gastric juice, which irritates the stomach lining. That is why "club soda", which contains sodium bicarbonate, may produce no such symptoms.

FOODS DEVELOPING CARCINOGENIC PROPERTIES BECAUSE OF PREPARATION

Charcoal broiling can result in the formation of carcinogenic hydrocarbons. Smoke imparts a flavor derived from phenolic materials produced when wood is burned in conditions of oxygen deficiency. While the phenolic flavors are formed, polynuclear aromatic hydrocarbons are also produced. Some of these, especially 3, 4-benzpyrene and benzanthrane, are carcinogenic. These occur in smoked fish, caramelized sugar and roasted coffee (Crosby, 1979). Benzpyrene is the ubiquitous carcinogen in city smog. Its metabolites, particularly epoxides, are active in producing malignancy in animals and possibly in man.

Fats and oils subjected to prolonged overheating by frying are controversially thought to produce carcinogens. The toxicity is claimed to increase if an oil is used repeatedly and if a shallow pan allowing oxidation is used.

Yannai (1980) presents a succinct review of toxicity induced by food processing. Thus, a cured meat such as bacon contains trace amounts of *N*-nitrosodimethylamine (NDMA) and on frying bacon *N*-nitrosopyrrolidine is formed. Both substances are claimed to be carcinogenic (Gough et al., 1976). Fortunately, both nitrosamines get lost to a considerable degree in the vapor during cooking, although some remain in the rasher and in the cooked-out fat (Holmes and Woodburn, 1981). Wasserman et al. (1978) state that frying bacon at low or medium

heat for less than 10 minutes at 350°F. Benzene et

temperature at 350°F. The nitrite in *Clostridium* produce nitrosamines in animals (Piers et al., 1978). Agents (Widdowson et al., 1978).

In 1978 it was found that the nitrite levels and that the nitrite levels are markedly reduced.

Mutagens are produced in the distribution in the food chain. Mutagenic activity is observed in the food chain. There are correlations between the levels of mutagens and the levels of carcinogens in the food chain.

Although it is a claim that the nitrite levels are reduced and that the nitrite levels are reduced.

Charred meat and glutamic acid are produced in the food chain. Decisions arise from the food chain. The food chain destroys pathogens and has a high level of contamination.

There is a claim that the food chain is a source of carcinogens. The food chain is a source of carcinogens.

The amino acid, amygdalin, is a source of cyanide. The amino acid, amygdalin, is a source of cyanide. The amino acid, amygdalin, is a source of cyanide.

Apricot pits should be avoided as a cancer risk.

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heat for less than 10 minutes can result in the least nitrosamine levels, although Pensabene et al. (1974) report that N-nitrosamine formation is dependent on temperature and not time.

The nitrite in bacon processing imparts flavor and color and prevents growth of *Clostridium botulinum*. Since the nitrites react with amines in the body to produce nitrosamines which are inconclusively implicated in cancer of laboratory animals (Pierson and Smoot, 1982), there are proposed substitutes or sparing agents (Widdus and Busta, 1982).

In 1978 it became known that NDMA was present in domestic and imported beers and that barley malt was the source (Havery et al., 1981). FDA regulations to modify the malting process have reduced the present nitrosamine levels markedly.

FOODS CONTAINING MUTAGENS

Mutagens are agents that produce genetic mutations and they have a wide distribution in the diet. There are *mutagenic flavonoids* in many vegetables and mutagenic activity is said to exist in extracts from coffee and tea (Maugh, 1982). There are controversial opinions as to whether mutagens in foods contribute markedly to the risk of cancer (Committee, 1982).

A. Caffeine

Although there is no evidence that caffeine is involved in carcinogenesis, there is a claim that it induces chromatid breakage (genetic damage) in human cells *in vitro* and that it could prove to be a dangerous mutagen (Kahlmann et al., 1968).

B. Charring of Meat and Fish

Charred meat and fish can develop mutagenic potential. Tryptophan, serine and glutamic acid may produce mutagens at high temperature pyrolysis. Difficult decisions arise. Although cooking of meat and fish may produce mutagens, it also destroys pathogenic organisms. Further, some vegetables that contain mutagens have a high nutritive value.

FOODS CONTAINING TOXICANTS (MAINLY CYANOGENIC GLYCOSIDES)

A. Fruits

Apple (*Malus* spp.)

There is a toxic part of apples, mainly in the seeds. Lewis and Elvin-Lewis (1977) cite a case where one cup of seeds was eaten and caused death, from cyanide poisoning.

The amino acid phenylalanine in the seed converts to the cyanogenic glycoside *amygdalin*. This hydrolyzes on digestion to hydrocyanic acid. The cyanide poisons oxygen carriage by cytochrome oxidase. Victims may die within minutes of exposure. The lesson here is (1) that all parts of a food are not harmless, and (2) that children, especially, must learn this early.

Apricot (*Prunus armeniaca*)

Apricot plants contain amygdalin in the seeds, bark and leaves and ingestion should be avoided (Lewis and Elvin-Lewis, 1977, p. 41). Laetrile, legal in Mexico as a cancer cure, is an extract of apricot pits. The theory is that the extract yields

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cyanide, which preferentially attacks tumor cells. Cyanide is released by beta-glucuronidase common to tumor cells.

Elderberry (*Sambucus* spp.)

The fresh leaves, stems and roots contain a poisonous alkaloid, and a cyanogenic glycoside called *sambunigrin* causing cyanide poisoning. Red elderberries are very toxic.

The fully ripe fruit is harmless and is used in pies, wine, jelly and pancakes. Other parts of the plant are used as drugs.

Children using peashooters made of elderberry stems have been poisoned (Lewis and Elvin-Lewis, 1977, p. 50).

Cherries (*Prunus* spp.)

Seeds and leaves contain the cyanogenic glycoside *amygdalin*. The seeds are the most toxic. The fleshy part of sweet and of sour cherries is edible. That of the laurel cherry (*P. laurocerasus*) contains amygdalin.

Pear (*Pyrus communis*)

Pear seeds contain amygdalin and are potential poisons.

Peach (*Prunus persica*)

All parts of the peach contain amygdalin, concentrated mainly in the bark, leaves and seeds. Poisoning has followed ingestion of peach kernels (Liener, 1980, p. 152).

Plum (common is *Prunus domestica*; wild is *P. americana*)

The plum is the least toxic member of the genus *Prunus*, but the leaves and seeds contain cyanogenic glycosides that convert to hydrocyanic acid.

Prune (*Prunus domestica*)

The prune is the partially dried fruit of a plum. The laxative effect of prunes is well known. Prunes contain derivatives of *hydroxyphenylisatin* which acts as a gastrointestinal irritant.

Almond (*Prunus amygdalus*)

Almonds are the fruit of the almond tree. The seeds of the sweet variety are edible. It is difficult to separate the edible sweet almond from the bitter almond (*Prunus amygdalus* var. *amara*). The seeds of the bitter plant contain high yields of amygdalin (Abrol, 1967). Lewis and Elvin-Lewis (1977, p. 41) state that 50 to 70 bitter almond nuts are fatal for adults and 7 to 10 for children.

FOODS CONTAINING TOXICANTS

B. Vegetables

Onion (*Allium cepa*)

A toxic principle thought to be an alkaloid may cause an anemia in man if onions are eaten in "large" amounts for one or more weeks (Schmutz et al., 1979, p. 155). The bulb contains the toxic material. Other related suspected foods are chives, garlic, leeks and shallots.

Onions and other plants in the genus *Allium* contain sulfur compounds called *glucosinolates*. These give rise to thiocyanates, isothiocyanates, nitriles, goitrin and progoitrin. Glucosinolates substitute for or displace iodine, preventing its uptake or accumulation in the thyroid gland. Heat reduces the direct action of these substances. Normal patterns of consumption cause no harm.

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Eggplant (Solanum melongena)

Eggplant is a fruit eaten as a vegetable. Botanically, it is a berry. Its leaves, stems and fruit contain solanaceous alkaloids that are claimed, without solid evidence, to be poisonous. The plant belongs to a nightshade family long suspected as being potentially toxic. Glycosides of *solasodine* are common in eggplant and solasodine is teratogenic in hamster fetuses (Kingham, 1979, p. 75).

Fava Bean (Vicia faba)

Fava (broad) beans may produce acute febrile hemolytic anemia. The condition is called favism. Crises due to dry or cooked beans are of moderate intensity but severe attacks follow ingestion of fresh, raw fava seeds.

Sensitivity is hereditary and involves a deficiency of glucose-6-phosphate dehydrogenase. Causative toxic substances may be *divicine* and *isouramil* (Liener, 1980, p. 283).

The plant is widely cultivated in parts of the United States. Most cases result from eating fresh beans and thus peak incidence occurs at harvest.

Lima Beans (Phaseolus lunatus)

Lima beans contain a lectin (protein hemagglutinin) called *phaseolunatin* or *linamarin* (Lewis and Elvin-Lewis, 1977, p. 44). This hydrolyzes in part to hydrocyanic acid. Thorough cooking reduces the danger of poisoning, but may not fully destroy the toxic principle. Preliminary soaking before cooking helps eliminate the toxic lectin.

Kidney (Navy) Beans (Phaseolus vulgaris)

The kidney bean contains lectins (hemagglutinins) associated with human gastrointestinal disturbances. The toxin is completely destroyed by boiling (Bender and Reaidi, 1982). Of interest, the bean also contains inhibitors of alpha-amylase from the saliva and pancreas. The active inhibitor, called *phaseolamin*, when taken orally blocks starch digestion and this is the principle behind starch-blockers for weight control (Bo-Linn et al., 1982). The FDA is now in litigation as to whether these agents are drugs or foods.

Wheat (Triticum aestivum)

Wheat also contains the alpha-amylase inhibitor. This may persist through bread baking and infants or adults having impaired digestive enzymes may not destroy the inhibitor. A similar problem also arises in some wheat-based breakfast cereals (Liener, 1980, p. 450).

Bread cannot be made without the gluten of wheat and some uncertain fraction in the gluten exacerbates the symptoms of patients with celiac disease (Goodhart and Shils, 1973, p. 423). Wheat is also a very common food allergen, due in part to albumin and gliadin.

Spinach (Spinacia oleracea)

The leaves of spinach contain salts of *oxalic acid* that could become precursors of calcium oxalate crystals. Excesses have been controversially claimed to cause precipitates in the kidney. Spinach also lowers blood sugar (Lewis and Elvin-Lewis, 1977, p. 218). The concentrations of oxalates or hypoglycemic factors are not ordinarily toxic.

Between 1959 and 1965 in Germany, 15 cases of *nitrite* poisoning followed ingestion of spinach by babies (Simon, 1966). Poisoning occurs only if spinach contains much nitrate, which bacteria of the stomach convert to nitrite. Infants less than three months of age should not be given spinach because of bacterial action.

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Arena (1979, p. 718) cites the death of a five-year old girl who ate a large number of raw spinach leaves. Epstein (1969) states that vegetables in highly fertilized soil contain 1,000 to 5,000 ppm of sodium nitrate, well exceeding the maximum permissible amount in foods.

Nitrates can convert to nitrite in cans and the nitrite reacts with the metal to produce nitrogen which swells the can. Metal cans have caused toxicity for other reasons, essentially because of dissolved tin or the lead solder used and, for that matter, even plastic containers contain toxic polymers (Liener, 1980, p. 417).

Potato (*Solanum tuberosum*)

The "Irish" potato is edible so long as it is not very green. Since it is a modified stem, a potato exposed to light for a period of time becomes green and it may accumulate toxic amounts of a glycoside alkaloid called *solanine*. The toxic parts of the potato include green areas under the potato skin and the sprouts. The skin is harmless when eaten in normal quantities. A concentration of 20 mg solanine/100g fresh weight is thought to be the maximum safe level for market potatoes, to prevent gastrointestinal and neurologic disorders. Fatalities are rare because sprouted potatoes are peeled. Also, solanine in boiling is leached out into the water (Koelling, 1982), although cooking does not destroy it. Solanine is a cholinesterase inhibitor.

Rhubarb (*Rheum raphaniticum*)

The leaves and edible stalks of rhubarb contains rather high quantities of oxalic acid. This was once thought to be the cause of vomiting and enlargement of the liver that follows ingestion of raw leaves and stems. It is much more likely that the toxicity is due to *anthraquinone glycosides*. These are present in leaves, especially in early summer. About 10 to 20 grams of fresh leaves are highly irritating (Fairbairn, 1976, p. 15). Death has sometimes followed ingestion of rhubarb (Leiner, 1980, p. 453).

Mushroom (*Amanita* spp.)

Mushrooms are unpredictable. Some species are only occasionally poisonous. Some are poisonous only if eaten raw; others if eaten in certain seasons or stages of maturity. Cooking does not destroy all the toxins.

Some people become ill if they drink alcoholic beverages when eating mushrooms. Use of certain drugs may make a harmless mushroom toxic for some people.

The most common poisoning in the United States is from *Amanita verna* and *A. brunnescens*. Poisoning by these and the more rare *A. phalloides* is due to thermostable toxins that destroy cells. Toxins are cyclopeptides which include *phalloin*, *phalloidin*, and *amanitins*. Poisoning by *A. muscaria* is due to *ibotenic acid* and *muscimol* (Kinghorn, 1979, p. 25). There are other mushrooms that contain *psilocybin* and *psilocin* which produce hallucinations. Additional toxicants have been extracted from still other species.

Yams (*Dioscorea* spp.)

Yams are among compounds suspected of causing teratogenesis in man and other animals (Lewis and Elvin-Lewis, 1977, p. 95). Certain yams (*D. hirsuta*) contain an alkaloid, *dioscorine*, which produces paralysis of the central nervous system (Glasby, 1975, p. 490).

Sweet Potato (*Ipomoea batatas*)

Sweet potatoes produce metabolites called *phytoalexins* in response to stress (Salunkhe and Wu, 1977). An abundant metabolite is *ipomeamarone*. This is a

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hepatotoxin in mice and a suspected toxin in man. It is not eliminated by baking or boiling and it is best to avoid potatoes with blemishes or discolorations.

C. Food Colors; Spices

Nearly 65 synthetic colors are known to be in use around the world. Khanna and his co-workers (1980) review these substances and they deplore the fact that some appear to be highly toxic and nevertheless are insufficiently regulated. In the United States there are about eight color additives and the FDA certifies them.

Monosodium Glutamate

This is the responsible agent for the digestive upset known as the "Chinese restaurant syndrome." It is especially dangerous in baby foods because of reported damage to the brain in newborn animals (Grollman and Grollman, 1970).

Hot Peppers (*Capsicum* spp.)

Red chili peppers (*C. annuum*) can irritate the entire gastrointestinal tract. The symptoms are more severe with the tobacco pepper (*C. frutescens*) which is considered to be a hepatocarcinogen in rats.

Nutmeg (*Myristica fragrans*)

Nutmeg is the ripe seed of *M. fragrans*. It is a powerful hallucinogen. This is due to a combination of volatile oils, *myristicin*, *elemicin* and the weak carcinogen *safrole*. Ingestion of large quantities of nutmeg causes stupor and death (Merck Index, 1960, p. 700).

Sage (*Salvia officinalis*)

Sage represents the dried leaves of the plant. Large doses may cause nausea, vomiting and constipation (Merck Index, 1960, p. 918).

D. Milk

Bovine milk is the main source of *orotic acid* in the human diet. Levels in cow's milk increase from 21 to 84 ppm from days 2 to 45 of lactation. Because only 10 ppm may affect liver metabolism and cause liver necrosis in rats, there is need to evaluate its possible toxicity in milk powders used in humans (Okonkwo and Kinsella, 1969). Robinson (1980) does not think that *orotic acid* poses a serious problem in man, but believes that research is needed on this point.

FOODS CONTAMINATED BY TOXICANTS

Milk

There is considerable concern about the level of pesticide residues in both bovine and human milk. Dillon et al. (1981) present a survey of this problem. Lee and Lorenz (1979) note other hazards of milk, i.e., in the cardiac-prone hypercholesterolemic person, in the lactose-intolerant person, and in the allergic person. Infants with hereditary disturbance of galactose metabolism develop serious symptoms with ingestion of milk. Such galactosemia demands rigid exclusion of lactose and galactose from the diet. Speckmann et al. (1981) claim that intolerance to lactose in an 8-ounce serving of milk is rare.

Rye (*Secale cereale*)

Ergot is the toxic chemical constituent of the sclerotium (branching filaments; hyphae) of *Claviceps purpurea*, a fungus that parasitizes kernels of rye (and wheat) chiefly. Ergot contains *ergonovine*, an alkaloid causing human ergotism. This is

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highly controlled today, but it killed millions of people in the Middle Ages. There are burning sensations and gangrene in the extremities, and also abortion and convulsions. Epidemics in the Middle Ages were referred to as "St. Anthony's Fire" because gangrenous limbs looked as though they were burned.

Fish and Shellfish

Fish muscle contains histidine which can form histamine by bacterial action if left standing. Canned tuna fish has been a source of histamine poisoning (Rice et al., 1976), and the disorder called scombroid poisoning is caused chiefly by histamine.

Shellfish such as clams eat the dinoflagellates *Gonyaulax catenella* or *G. tamaris* and thereby acquire a nitrogenous compound called *tetrodotoxin* which does not harm the shellfish, but which is often a fatal neurotoxin to man. At certain times of year the dinoflagellates so multiply as to produce a dangerous "red tide" poisonous to clams, crabs, oysters, shrimp and other marine organisms. A neurotoxin is involved called *saxitoxin* which is highly poisonous when man ingests the shellfish and this toxin is not destroyed by cooking. Fish eggs can also contain toxicants (see Liener, 1974).

Meat Grilled on Wooden Skewers

Wooden skewers used to hold "hot dogs" and other meat for grilling can introduce hazards. There are skewers made from the stems of an evergreen, *Nerium oleander*, which contain cardioactive glycosides that have caused deaths (Liener, 1974). The poisons are *oleandroside* and *nerioside*.

Beer Treated with Cobalt

At one time cobalt was added to beer to stabilize the foaming qualities. It took many years before cobalt was incriminated as the cause of severe heart attacks in heavy beer drinkers and this additive was discontinued (Alexander, 1969).

FOODS ACTING AS TOXICANTS BECAUSE OF INHERITED DEFECTS IN THE INDIVIDUAL

For a person having severe pancreatic diabetes, where from birth insulin function may be deranged, a single ordinary meal containing cane sugar (sucrose) may set off a train of metabolic events leading to disastrous results.

Fructose occurs in many fruits and is the predominant sugar in honey. Some people are born without an enzyme, *aldolase B*, and for them fruits (and also cane sugar) have to be completely excluded to prevent gastrointestinal pain, vomiting and hypoglycemia (Cox et al., 1982). The disorder is acquired from a double dose of a recessive gene.

Persons who by gene defect lack the enzyme *lactase* fail to break down milk sugar (lactose) and they may not tolerate milk.

Fatty foods, especially french fried potatoes and onions, are anathema to most people who suffer by inheritance or otherwise from disorders of the biliary tract. This is expected since many nutrients affect the quality and amount of the bile, the movements of the gallbladder, and the tone of the sphincter of Oddi.

These so-called inborn errors of metabolism also include protein disturbances. In phenylketonuria, there is a deficiency of *phenylalanine hydroxylase* and phenylalanine intake must be sharply reduced by feeding artificial protein substitutes. In "maple syrup urine disease," the infant must ingest an artificial formula free of

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valine, leucine and isoleucine. These are only examples of a class of amino acid toxicities.

FOODS AS VITAMIN ANTAGONISTS

A. Biotin Antagonist — Raw Eggs

Raw eggs contain a glycoprotein called *avidin*, present in the egg white up to 0.05% of the total protein content. One molecule of avidin binds four molecules of biotin (vitamin "H"). This deprives the person ingesting raw eggs of an essential growth factor (Comments, 1982-83).

B. Thiamin Antagonists — Seafoods, Vegetables, Fruits

Fish such as carp, herring, swordfish and lake trout contain thiamin antagonists (Goodhart and Shils, 1973, p. 415). There is also anti-thiamin activity in blackberries, black currants, red beets, Brussels sprouts, red cabbage and spinach. There are antagonists for niacin in corn and millet, for pantothenic acid in yeast and for riboflavin in the akee plum.

C. Multivitamin/Mineral Preparations

Multivitamin/mineral preparations contain analogues for B_{12} which are claimed to be harmful to human vitamin B_{12} metabolism (Herbert et al., 1982). It seems advisable to omit B_{12} from vitamin/mineral preparations.

Vitamin Poisoning — Hypervitaminosis

Only those vitamins are considered herein whose known or probable toxicities have strong documentation. Gilman (1980, p. 1553) lists recommended daily vitamin allowances for the reference man, woman and child. Quantitative values for toxic thresholds are controversial. Poisoning does not occur with ordinary daily consumption.

The recommended adult allowance for vitamin A is 5,000 I.U. Poisoning occurs after ingesting up to 100,000 units (30 mg) daily for several months. Food faddists may ingest toxic levels. Infants need about 1,500 I.U. and doses of 18,500 daily for 1 to 3 months are reported to be toxic (Goodhart and Shils, 1973, p. 153).

For vitamin D, the daily requirement for infants and adults is 400 I.U. There is a danger in giving supplements such as 4,000 I.U. in fortified foods to infants (Goodhart and Shils, 1973, p. 160). Ingestion of 50,000 units daily to children and adults should be avoided for any long periods of time.

For other vitamins, toxicities are either unknown or they occur at such repetitively high values that they may be considered relatively safe. The reports are conflicting.

GOITROGENIC FOODS

Soybean (*Glycine max*)

Goiters have occurred in infants fed soy milk or soy flour (Hydovitz, 1960; van Wyk et al., 1969). There are also many goitrogenic foods in the genus *Allium*, but it is only excess consumption that may be associated with toxicity.

Soybeans are toxic in other regards. One possible toxic factor, which is common to other legumes, is a *trypsin inhibitor*. Trypsin is essential in the small bowel for protein digestion. The inhibitor, present in the raw bean, is inactivated by cooking.

Soybeans are also hemagglutinins (phytoagglutinins or lectins) and this may be partly responsible for the gastrointestinal disturbances if raw soybeans are eaten.

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A valid explanation is still needed to explain the growth-inhibiting action in raw soybeans (Goodhart and Shils, 1973, p. 418).

TOXICITY OF FOOD ADDITIVES

Preservatives and Sweeteners are Emphasized Below.

Preservatives

These substances extend the shelf life of foods. Shtenberg and Ignat'ev (1970) urge that sulfites and benzoates should be restricted, substituting sorbic acid. Sorbic acid and potassium sorbate are considered safe by the FDA.

Sodium nicotinate, a fresh meat color extender, has caused food poisoning, expressed particularly in edema of the extremities. This additive is not legalized in some states.

Sodium nitrite is used to prevent growth of *Clostridium botulinum* and to preserve the color of meat and fish in salting and pickling. Nitrites are potentially poisonous in several ways, one involving methemoglobin formation. Another is in formation of N-nitrosamines, formed either in processing foods or in the digestive tract by reaction with amino compounds. By similar mechanisms, nitrites react with phenolic compounds, forming aromatic nitroso substances, some of these nitrophenols being suspected of carcinogenicity (Challis, 1973).

Sweeteners

There are some toxic synthetic sweeteners. Cyclamates were banned in 1969 because they produced bladder cancer in rats. Saccharin is now a regulated substance with suspected carcinogenicity. Crosby (1976) reviews several sweetening agents.

Cane sugar as a natural sweetener is in a sense an additive and its local dental effects are of interest. Meals high in cane sugar favor colonization of cariogenic organisms on teeth. Restriction of cane sugar reduces bacteria such as *Streptococcus mutans* in dental plaques (Newbrun, 1982). Bibby and Mundorff (1975) blame acid candies, beverages and fruits for the highest dissolution of enamel.

POISONING BY EXCESS MINERAL INGESTION

A. Sodium Chloride (Table Salt)

In the United States, the daily sodium intake per person is three or more grams. An estimated safe and adequate daily intake should range from 1.1 to 3.3 grams (48 to 143 mEq.) (National Research Council, 1980). A moderate restriction is one gram. The hypertensive influence of table salt is firmly documented and the FDA proposes labelling the sodium content of processed foods.

Salt poisoning has occurred in hospitalized infants where salt was mistaken for cane sugar. Renal damage and convulsions followed. In the neonate, the repeated intake of skim milk has produced salt intoxication.

B. Iron

The daily iron requirement is 10 to 20 mg in adult males and 8 to 15 in females (Gilman et al., 1980). Finch (1982) states that a normal person can control the accumulation of body iron despite ingestion of 5 to 10 times the normal amount. Other writers note that excesses can be dangerous because excretion hardly increases, so that iron deposits in vital organs.

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American wines supply several mg daily. The Bantus of South Africa brew a beer in iron kettles and the beer contains more than 40 mg of iron per liter. A Bantu male often consumes 100 mg of iron per day in beer (Emery, 1982). A disease called secondary hemochromatosis, often fatal, occurs.

The Food and Nutrition Board of the National Academy of Sciences recommends a daily allowance of 10 mg daily for children in the first six months to supplement milk. This may be too high. Also, it is inadvisable for an adult to take vitamin/mineral preparations containing iron if iron is being ingested sufficiently from other sources.

C. Calcium

The minimal adult requirement is 800 mg per day (Gilman, 1980, p. 1553). The predominant source is dairy products. Healthy serum contains 10 mg/dl. Levels exceeding 14 are dangerous and 18 mg/dl may cause death. Ingestion of daily quantities of calcium salts is ordinarily unlikely to cause toxicity. The so-called "milk-alkali syndrome" can be exacerbated, however, by ingestion of large quantities of milk.

D. Fluoride

Man obtains fluoride from ingesting plants and water. Incidental sources include food additives, e.g., baking powder. Combinations of fluoridated water, toothpastes and certain foods could conceivably produce a problem especially in children. This is a developmental problem in teeth where 1.7 ppm of fluoride in water produces mottled enamel before the teeth have erupted (Gilman, 1980, p. 1547). At 4 to 6 ppm, mottling approaches 100%. Severe mottling is associated with disorders in bone mineralization. Fluoridated drinking water is restricted to 1 ppm optimally.

E. Selenium

The estimated safe daily intake of selenium is 0.05 to 0.02 mg (Gilman, 1980, p. 1555). Where the soil and vegetation have a high selenium content, livestock develop "alkali disease" or "blind staggers." The human population in such areas is claimed, controversially, to develop gastrointestinal symptoms and liver disease from ingestion of meat, eggs, milk and vegetables (Goodhart and Shils, 1973, p. 393; Salunkhe and Wu, 1977).

FOODS INDUCING MINERAL DEFICIENCIES

There is a large literature on *phytates* as toxins. These commonly occur in food plants and they interfere with the intestinal absorption of essential minerals by forming insoluble complexes with them. This produces the hazard of mineral deficiency in parts of the world that rely on cereal proteins (Reddy et al., 1982).

FOODS AFFECTING CARDIOVASCULAR FUNCTION

The literature is voluminous concerning the development of atherosclerosis as influenced by dietary excess of cholesterol and low density lipids, but these will not be considered further here.

A. The Effects of Coffee

The cardiac effects of coffee are highly controversial. Lewis and Elvin-Lewis (1977, p. 181) cite data to the effect that the risk of developing myocardial infarction

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tion is about twice as great for heavy coffee drinkers than for non-drinkers. This does not imply that caffeine is the cause since tea does not produce the same effect.

B. Orange Oil

Orange oil is expressed from the fresh peel of the ripe fruit of the orange. *Citral* is a constituent of this oil and is thus present in marmalade, fruit juices flavored with the oil, and orange drinks made by compressing the whole fruit. There is a question whether high consumption of such foods might damage blood vessels, as seen in experimental animals (Liener, 1980, p. 442). Citral is found to greater extent in oil of lemon, which is a flavoring agent. Goodhart and Shils (1973, p. 414) discuss the controversial aspects of citral toxicity.

C. Licorice (*Glycyrrhiza glabra*)

Hypertension associated with salt and water retention leading to edema follows licorice ingestion to excesses over time (Koster and David, 1968). Koster's patient ingested 100 grams per day. The noxious agent is a steroid-like compound called *glycyrrhetic acid*.

D. Cheese, Drugs, Wine and Beer

Monoamine oxidase inhibitors are in widespread use to elevate the mood. *Tyramine*, present in certain brands of cheese, wine and beer, can provoke a hypertensive crisis in patients treated with monoamine oxidase inhibitors. It takes only 6 mg of tyramine to provoke hypertension in such situations (Sen, 1969), and 25 mg (which occur in many foods or alcoholic beverages) is a very dangerous level (Hutchison, 1968).

The ripening of cheese breaks down casein, one product being tryptophan. Bacteria involved convert tryptophan to tyramine. A cheese and wine party could be disastrous if high tyramine substances such as cheddar cheese and Chianti wine were freely consumed. There are other high tyramine cheeses as well as wines and beers (Sen, 1969; Bassuk and Schoonover, 1977, p. 41; Maga, 1978). Tyramine releases pressor catecholamines (Editorial, 1965).

It is interesting that some varieties of ripe cheddar and Cheshire cheese, plain chocolate, and to a small extent some wines, precipitate migraine attacks and this is due to *2-phenylethylamine* (Chaytor et al., 1975).

FOODS DISTURBING NEUROLOGIC MECHANISMS

Certain foods are cholinesterase inhibitors and thus disturb nerve impulse transmission. Such foods include potatoes (*solanine* effect), roots of tomatoes, beets, apples and eggplants (Salunkhe and Wu, 1977). Bananas in excess affect transmission of impulses because they contain serotonin and catecholamines.

Some foods produce hallucinations. Examples are nutmeg seeds and mace, which contain *myristicin*.

The methylated xanthines in tea, coffee, cocoa and cola drinks overstimulate sensitive individuals. The drugs include *caffeine*, *theophylline* and *theobromine*.

The neurologic effects of coffee are very controversial. A 5-ounce cup of coffee contains up to 90 mg of caffeine which can produce nervous excitement and cardiac stimulation, but only in a sensitive individual. Ingestion of one gram (11 cups) could seriously affect such an individual. The potential toxicity of caffeine is offset by the fact that when ingested in ordinary amounts it does not accumulate excessively in the body.

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OTHER ASPECTS OF FOOD POISONING

Our discussions hardly scratch the surface. There is a large literature on food and drug interactions, making physicians take caution when prescribing antibiotics, anticoagulants, tranquilizers, and certain laboratory tests. The literature on foods as allergens is extensive. Problems in irradiation of food are still greatly unsolved. Food contamination by microorganisms is a continual and severe threat. The poisoning of animals by food toxicants and the transmission in the food chain to man is under perpetual surveillance.

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In all other instances, very little is known about the sequence of events taking place between exposure to the pesticide and appearance of tumors in animals. The chronic toxicity of DDE, the major metabolite of DDT in human beings, has not been thoroughly studied. It seems that in comparison to human beings, in mice and rats DDT is converted more readily to DDD than to DDE. No tumor production was obtained in mice with either pp'-DDD or op'-DDD under conditions comparable to those which led to the appearance of hepatomas following pp'-DDT. Knowledge on intracellular binding of DDT is extremely scarce.

The estrogenicity of DDT, particularly that of the op'-isomer, is established and is probably partly responsible for the effects of DDT on wild life. Environmental estrogens are considered to have a role in the total carcinogenic load. It is not known whether in the experiments with DDT leading to the appearance of tumors also a hormonal imbalance was induced. In addition, op'-DDD has long been known to produce adrenal changes in dogs and man; an effect on cortisol metabolism by op'-DDT and technical DDT has also recently been reported.

Other possible effects of DDT on laboratory animals which under completely different conditions have been postulated to be somehow related to carcinogenesis are immunodepression and interference with vitamin A metabolism.

Effects Possibly Related to Carcinogenesis Exerted by Pesticides Which Have Not Produced Tumors in Long-term Experiments

Mechanisms of carcinogenesis for man and animals are largely unknown so that, in general, it is not possible to assume that chemicals having particular properties represent a carcinogenic hazard. For instance, biological alkylating agents or mutagens deserve priority in a program of evaluation of carcinogenicity, but it cannot be stated that these properties *per se* indicate carcinogenicity. A different condition is goitrogenicity, since this effect can be fit into one theory on the mechanisms of carcinogenesis. Several dithiocarbamates used as fungicides (for instance, maneb and ziram) have produced thyroid enlargement with nodular hyperplasia in rats and occasionally in dogs, similar to those produced by thiouracil. This effect occurred at high doses but the quantitative aspects of the phenomenon are not sufficiently known.

Finally, enzyme induction and enzyme inhibition can be elicited by pesticides. They are not likely to be directly correlated with carcinogenesis. There is some experimental evidence that these compounds can interfere with the metabolism and carcinogenicity of some known carcinogens. Evaluation *a priori* of risk or benefit is extremely difficult.

Food Contaminants and Carcinogenesis

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THE DIET OF MODERN MAN and his animals is composed of a wide variety of foods gathered from all parts of the world. These foods have been grown, harvested, and processed, sometimes by rather primitive means. The hazards of transportation and storage, coupled with the factor of time have an impact on the quality and wholesomeness of the final product.

One needs only to walk through a supermarket to be aware of the complexities which make up man's food today. On the shelves and in the freezer cases, we have a vast array of foods containing a mixture of seasonings and additives, and processed in a multitude of ways. Many of these are combinations of foods which have been processed, packaged, stored, and later sold, perhaps to be stored again and finally heated and consumed—with other processed foods, equally as complex.

The evolution that has taken place in our food preparation assumes that the producer and processor have taken the necessary steps to assure the safety of all the ingredients which comprise the food and in the final product itself. This responsibility requires the combined efforts, talents, and knowledge of many specialists trained to recognize the potential hazards which may exist or develop in food and its components.

Unexpected incidents involving food contaminants have occurred even with our advanced technology and close attention to food safety. A case in point, fortunately with fish and not man, was the 1960-1961 epizootic of liver cancer in rainbow trout. This incidence of liver cancer was correlated with a change in diet from one composed largely of raw meat and fish to a computerized, highly nutritious diet of dry ingredients processed into conveniently sized pellets. One of the ingredients, cottonseed meal, contained the food contaminant aflatoxin, and rainbow trout proved to have a liver that was highly sensitive to this mold metabolite.⁸⁰

Food contaminants to be discussed are those for which there is some evidence of carcinogenic activity. These may be broadly classified in the following groups: 1. Contaminants introduced during production or processing, e.g., benzo(a)pyrene. 2. Contaminants formed by the interaction of substances in food, e.g., nitrosamines. 3. Contaminants resulting from the action of biological processes in or on foods, e.g., mycotoxins.

Food Contaminants Introduced during Production or Processing

A number of polycyclic aromatic hydrocarbons have been demonstrated to cause cancer in experimental animals. It may be that benzo(a)-pyrene is the most universally distributed carcinogen in our environment. Exhaust gases from the burning of fossil fuels and the combustion engine contribute the major share of benzo(a)pyrene which subsequently finds its way to man, his animals, and their food. A recent report by Shabad⁷⁶ of studies conducted in the U.S.S.R. show the distribution, circulation, and fate of hydrocarbons, particularly benzo(a)-pyrene, in the modern environment.

For cons, since man has used fire to cook his food, benzo(a)pyrene has been present as an unrecognized food contaminant. Smoking of meat and fish, one of the oldest methods of food preservation, may also contribute benzo(a)pyrene and other polycyclic hydrocarbons. The popularity of the backyard barbecue and charcoal-broiled meats featured in American restaurants disregard the recent findings that these procedures may contribute measurable amounts of carcinogens to our food. Table 6-5 presents a list of smoked and barbecued foods with the levels of benzo(a)pyrene found. An extensive review of polycyclic aromatic hydrocarbons in foods was recently prepared by Howard and Fazio.³⁶ Shabad⁷⁶ reported that people who consume large quantities of smoked food or work in meat smoking factories show a particularly high incidence of digestive cancers. In 1961, Dungal⁷⁸ suggested that a correlation existed between the consumption of smoked mutton and trout and the incidence of gastric cancer in Iceland. Dungal's observation, probably more than any other, served to stimulate a series of investigations on the possible relationship of polycyclic aromatic compounds in food and gastrointestinal cancer.^{77, 88}

In Japan, where the incidence of gastric cancer is uniquely high, workers have suggested that the common practice of charcoal broiling fish may produce carcinogenic compounds.⁶³ Lijinsky and Shubik^{62, 63} found benzo(a)pyrene in barbecued spareribs and charcoal-broiled steaks. In a later report, Lijinsky and Ross⁷¹ investigated the effect of cooking methods on the production of benzo(a)pyrene and polynuclear hydrocarbons in meat. They related the production of hydrocarbons with: (1) the pyrolysis of fat, (2) the fat content and exposure of the food to the flames, and (3) the closeness to the heat source. Benzo(a)-pyrene was not produced when the heat source was above the food or if the melted fat was kept from dripping into the heat source.

Howard et al.³⁷ analyzed a number of smoked food products for benzo(a)pyrene and found levels varying from 0.8 to 3.2 $\mu\text{g}/\text{kg}$. Recently, investigators in the U. S. Department of Agriculture and U. S. Food and Drug Administration⁶² conducted an extensive survey to ascertain the types and amounts of polycyclic aromatic hydrocarbons present in 60 smoked products and miscellaneous foods. They selected samples

TABLE 6-5.—BENZO(A)PYRENE IN SMOKED FOOD

TYPE OF FOOD	BENZO(A)PYRENE ($\mu\text{G}/\text{KG}$)	REFERENCE
Mutton	1.3	5
Trout	2.1	5
Cod	0.5	5
Redfish	0.8	5
Salmon	1	53
Haddock	0.3	53
Sausage	1.9-10.5	30
Fish	1.7- 7.5	30
Mutton (home prep.)	20	77
Mutton (commercial)	1	77
Fish, dried herring	1.0	37
Fish, sturgeon	0.8	37
Ham	3.2	37
Horse mackerel	0.5	65
Sheep heads (singled)	trace-28	88
Sea birds (singled)	26-99	88
Ham	0.7	62
Cod	4.0	62
Whiting	6.6	62
Barbecued beef	3.3	62
Sausage	0.1	62
Hamburger	2.6	51
Pork chops	7.9	51
Chicken	3.7	51
Sirloin	11.1	51
T-bone steak (gas)	4.4	51
T-bone steak (charcoal)	50.4	51

to represent the wide variety of treatments used in producing smoked foods and found values of benzo(a)pyrene from 0.4 to 7.0 $\mu\text{g}/\text{kg}$ of food. On the outside (skin) of smoked whiting and whelk, they reported levels of 70 and 53.6 $\mu\text{g}/\text{kg}$, respectively. It seems apparent from these studies that benzo(a)pyrene is derived from the incomplete combustion of wood products in the case of smoked foods, and from the pyrolysis of animal fat in the barbecued samples.

In addition to smoked products, other foods have been shown to contain polynuclear hydrocarbons. Benzo(a)pyrene¹⁵ was found in heavily roasted coffee and it was reported to accumulate in oysters taken from polluted waters.¹¹ Jung and Morand³⁰ reported the occurrence of benzo(a)pyrene as well as other polycyclic hydrocarbons in vegetable oils. This was later confirmed by Howard et al.³⁶ who described an assay method and reported benzo(a)pyrene levels from 0.4 to 1.4 $\mu\text{g}/\text{kg}$. The origin of these compounds in vegetable oils remains to be determined, but it appears that the environment may be the source. Benzo(a)pyrene has been found in soil at levels up to 21,150 $\mu\text{g}/\text{kg}$, presumably derived from the fallout of combustion products, largely from engine exhaust.⁷⁰

We have, at this time, no direct evidence that the ingestion of benzo-

(a)pyrene leads to gastrointestinal cancer in man. Falk et al.^{25, 26} discussed the significance of polycyclic hydrocarbons in the environment as carcinogens for man and stated that evidence supports the conclusion that these compounds are carcinogenic to man just as they are to experimental animals. With regard to pulmonary and gastrointestinal cancer, physiologic defenses may function to prevent the induction of cancer.²⁶ It is conceivable that other factors have a role and Peirce²⁷ was able to initiate tumors in the forestomach of mice with a single dose (50 µg) of benzo(a)pyrene if followed after an interval of three weeks by 0.05 ml of lime oil given once weekly for 40 weeks. Higginson²⁸ in a review of ecological factors in gastrointestinal cancer in man suggests that additional studies be undertaken to exclude a relationship between cooked or heated fats and gastric carcinoma. Dungenl and Sigurdsson¹⁹ suggested that additional dietary factors may also have a role in the incidence of gastric cancer which was observed in Iceland and the possible relationship of vitamin C was mentioned. Ender and Celz²² added another parameter to the occurrence of gastric cancer in Iceland when they reported the occurrence of relatively high levels of nitrosamines (15 µg/kg) in smoked haddock.

Sugai et al.²⁹ described studies in which heated fats that had been thermally oxidized and polymerized promoted the formation of ear duct tumors in rats fed 2-acetylaminofluorene. One might assume that Icelandic smoked fish products, which had been stored for some time, would also contain autoxidized lipids. Whether autoxidized and polymerized lipids and the variety of chemically active compounds, e.g., hydroperoxides, carbonyls, epoxides which they contain, would promote carcinogenicity by benzo(a)pyrene and nitrosamines, all of which are present in smoked foods, would merit further research.

In modern food processing methods where a smoked taste is desirable, steps have been taken to eliminate the carcinogenic hazard. Smoke salt and liquid smokes without benzo(a)pyrene are being used to impart a desirable smoky flavor to foods.

Food Contaminants Formed by Interaction of Substances in Food

The death of a number of domestic animals in Norway which had consumed fish meal drew attention to another class of carcinogens, the N-nitrosamines, present as contaminants in the food supply. However, in this case the toxic agent was formed during processing. Mann provided one of the reactants, sodium nitrite, used as a preservative to prevent spoilage of the fish.

In 1951, Barnes and Magee⁴ reported that dimethylnitrosamine was hepatotoxic to a number of laboratory animals. They found that rats on a diet of 50 ppm dimethylnitrosamine developed malignant liver tumors within a year.³⁰ Their findings prompted a great deal of research

on the carcinogenicity of N-nitroso compounds, and several excellent reviews have been published.^{16, 30, 31} In addition, N-nitrosamines were shown to cause cancer in a wide variety of warm- and cold-blooded animals³² and in virtually every organ in the rat.³³ Magee also demonstrated³⁴ that a single dose induced cancer in the rat. Subsequent studies showed N-nitrosamines capable of causing acute cellular damage and acting as powerful mutagens and teratogens.³⁵ From this accumulation of scientific data on animals, we must presume that man is also sensitive to the action of N-nitrosamines. Certainly the studies of Laqueur³⁶ and his associates on the carcinogenic properties of cycad nuts support this conclusion.

The possibility that carcinogenic N-nitrosamines could be formed on be present in man's food received comparatively little attention prior to the Norway incident. The precursors, nitrites and secondary amines, are known to be present in food and indeed nitrites and nitrites are added as preservatives. The reaction to form N-nitrosamines takes place under mild conditions of temperature and pH. Concerned about the presence of nitrites in food, Druckrey and his colleagues³⁷ reported that sodium nitrite and diethylamine fed simultaneously to rats failed to produce tumors. They concluded that nitrites probably were safe in foods but cautioned against excessive use.

The investigations in Norway by Koppang,⁴⁰⁻⁴⁴ Sakshang⁷⁰ and Ender^{21, 29, 34} and their associates conclusively demonstrated that nitrosamines formed in fish meal during processing were responsible for toxic liver necrosis leading to the death of cattle and sheep. Earlier observations made by Böhler⁷ on a liver disease of mink in Norway were shown to be the result of feeding nitrite-treated fish meal.²³ In 1962-1963, Oregon State University workers witnessed the loss of 22 mink which was diagnosed as a hepatotoxicosis associated with feeding toxic herring meal imported from Norway.⁸³ These widespread findings that N-nitrosamines are present in food in concentrations sufficient to cause the death of domestic animals has caused considerable speculations about their hazard as potential carcinogens for man.^{22, 60, 66}

N-Nitrosamines have also been shown to be present in smoked fish and meat products. Ender and Celz²² reported 40 µg/kg of N-nitrosamine in one sample of kippers and 15 µg/kg in a sample of smoked haddock from Iceland. They suggested the possibility that N-nitrosamines be considered along with benzo(a)pyrene as potential carcinogens in the occurrence of gastric cancer in Iceland.

The occurrence of N-nitrosamines in wheat flour has been the subject of debate by several investigators.^{32, 63, 67} and remains to be resolved. It was also detected in some types of cheese and in milk.³² In Europe, nitrate is often added to cheese milk to prevent butyric acid fermentation and minimize production of gas from lactose. In a report presented before the 53rd Annual Meeting of the International Dairy Federation held in 1968,⁶⁸ the question of N-nitrosamines in cheese and the prob-

lems of analysis were discussed. Levels of N-nitrosamines ranging from 10 to 100 $\mu\text{g}/\text{kg}$ were cited but remain to be confirmed.

The possibility of the formation of N-nitrosamines in roasted foods was suggested by Devik.¹¹ He heated 0.01 mol of amino acid and 0.005 mol of D-glucose absorbed onto 20 g potato starch for several hours and reported a yield of N-nitrosamines from 0 to 1,600 μg . The N-nitrosamines were detected by a polarographic procedure. Workers at Oregon State University⁷² were unable to confirm these findings and a report of their work is in preparation. It is apparent from these reports that methods for the detection of amines and N-nitrosamines are lacking in sensitivity and specificity.

The increasing occurrence of nitrates in our environment, particularly in our water and food supply as a direct result of agricultural practices has been the cause of growing concern. In a review of the subject, Fassett²⁷ recounted a number of cases of poisoning which were attributed to toxic levels of nitrates and nitrites in food and water. Later Phillips⁶⁸ discussed the nitrate problem and its public health implications. He reviewed data citing nitrate levels of 1,074 to 1,668 ppm for spinach and 634 to 2,165 ppm for beets in prepared infant foods.

The preservation of fish and meat with salt has its origin in Antiquity. Later nitrate was added to meat to retard the growth of anaerobic bacteria and to produce a desirable red or pink color. Nitrite has been permitted in meat as a food additive since 1925. In 1939-1940 Tarr and Sunderland^{65, 66} recommended nitrites as a preservative for wet fish and suggested levels of 200 ppm. Its use was permitted in Canada until 1956 when it was replaced by tetracycline antibiotics. The United States restricted the entry of Canadian fish which had been treated with nitrite, but permitted 10 ppm sodium nitrite in smoke-cured tuna fish products processed in the United States. At the present time, up to 200 ppm is permitted in smoke-cured sablefish, salmon, and shad. These three species may also contain up to 500 ppm of sodium nitrate in the finished product. The use of sodium nitrite of not less than 100 ppm and not more than 200 ppm has been proposed for smoked chub. The purpose of nitrite addition to smoked fish is to give an extra measure of protection against the formation of botulinum toxin.

It is difficult to reconcile the use of nitrite in fish products in view of the happenings in Norway. Ender and Ceh²² stated in 1967, "The practice of dipping fish fillets in diluted NaNO_2 -solutions in order to avoid spoilage—should be deemed highly risky."

Since it is known that fresh water species contain a lesser amount of trimethylamine oxide and amines than marine species,²⁰ this may provide some justification for the use of nitrites on fresh water varieties. Recently, Japanese workers demonstrated that a number of species of fish contain enzymes capable of converting the tertiary amine, trimethylamine to dimethylamine and formaldehyde.⁶¹

Other secondary amines are present in fish such as the amino acids pro-

line, hydroxyproline, histidine, tryptophan, and arginine, but little is known about the carcinogenic properties of their N-nitroso derivatives. Hypoxanthine, a secondary amine formed from nucleotide degradation in fish during storage may accumulate at high levels⁸² and a nitrosamine may result from the reaction with nitrite. Boyland et al.⁸ reported nitrosopiperidine to be an active carcinogen and nitrosoproline to be practically inactive, though no experimental data were presented for nitrosoproline. High temperatures, such as those encountered in the cooking of fish, as well as the addition of acid condiments would favor the reaction. However, Ender et al.²² reported the formation of nitrosamine from dimethylamine and nitrite will occur even at temperatures of 0°C and at a pH of 6.5.

The recognition that N-nitrosamines may be formed from a variety of compounds, many of which could be present in meat and other foods, adds a new dimension to the use of nitrite as a food additive. As far as I am aware, no N-nitrosamines have been reported to occur in meat products as the result of nitrite addition. Much of the nitrite binds with hemoglobin and myoglobin; however, some remains as nitrite and would be free to react with secondary amines.

In 1963, Druckrey et al.¹⁷ recognized the possibility that the acidic condition of the stomach could provide the necessary criteria for reaction between nitrites and secondary amines. In a series of experiments with rats, they were unable to induce cancer or cause any teratogenic effects after feeding sodium nitrite and diethylamine for an entire life span and over two generations. It was suggested by them that the pH of the rat stomach which is close to 1 and the basicity of diethylamine could be an explanation for the lack of biological activity. Sander et al.⁷¹ in 1968 detected N-nitrosamine when they incubated nitrite and various secondary amines with human gastric juice which had a pH of 1.3. Sen et al.⁷⁵ in an improved method for the determination of N-nitrosamines in foods confirmed the findings of Sander. Later they⁷⁴ demonstrated that diethylnitrosamine was formed when sodium nitrite and diethylamine were incubated with gastric juice from rats, rabbits, cats, dogs, and man. Gastric juices from man and rabbit with a more acid pH were reported to give a higher yield of N-nitrosamine. They were also able to detect N-nitrosamines formed in vivo after feeding the reactants to cats and rabbits. It was suggested that other species which have gastric juice with a pH similar to that of man be used instead of rats as experimental animals for these studies. Sensitive procedures for the detection, characterization and quantitation of N-nitrosamines and their precursors are needed, particularly in food systems. A combination of gas chromatography and mass spectrometry would appear to offer a promising approach to the analytical problem.

In conclusion, it seems from the accumulated evidence of the past decade that the existence of N-nitrosamines in our food remain a strong possibility. The mode of formation in food and in vivo is less clear and

will require the research efforts of scientists versed in many disciplines. Only then can the benefits to be derived from nitrite addition to food be weighed against the possible hazard.

Contaminants Resulting from Action of Biological Processes in Foods

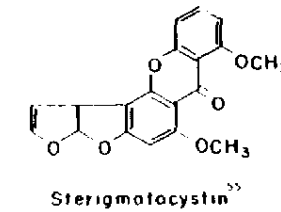
Only during the past decade when it was established that products derived from molds can induce cancer and may be present as contaminants in our foods have they received the concerted attention of investigators concerned with public health. The often quoted turkey incident which occurred in England in 1960 and the simultaneous epizootic of liver cancer in rainbow trout dramatically emphasized our lack of knowledge about molds, their metabolites and particularly their public health significance. One needs only to examine the literature prior to and after the above episodes to appreciate the tremendous scientific effort that was being directed toward solving this problem.

Forgacs and Carl²⁸ reviewed the problem of mycotoxicoses and emphasized the potential hazard of molds and their products. During this period, a continuing series of symposia were held, and reviews and books were published on the subject of molds, mold toxins, and their implications.^{12, 66, 90-92}

In 1969, an entire book devoted to the subject of aflatoxin, a name unknown 10 years ago, was edited by Goldblatt.²⁹ It would be pointless for this writer to attempt to review that which has been done so ably and effectively in this book.

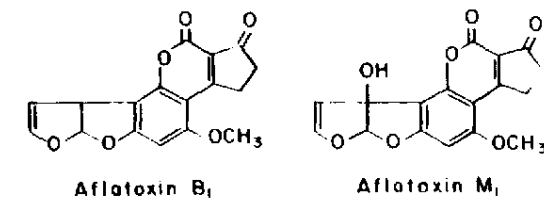
There are a number of mold metabolites which have been demonstrated to be carcinogenic to animals. This discussion will briefly examine sterigmatocystin and aflatoxin M₁ as examples of mycotoxins which may be present as food contaminants and may have the potential to induce cancer.

Sterigmatocystin, a metabolite of *Aspergillus versicolor*⁹ and *Aspergillus nidulans*³⁴ which bears a close structural relationship to aflatoxin B₁, has been found in peanuts and cereals. It has been reported to be less carcinogenic to rats than aflatoxin B₁¹⁵ and to have an LD₅₀ about six times higher than aflatoxin.⁶⁹ The oral LD₅₀ for mice for sterigmatocystin⁵⁵ was determined and found to be of a low order of toxicity and in excess of 800 mg/kg. With tests on ducklings, using bile ductal hyperplasia as the criterion, aflatoxin B₁ was 125 times more toxic than sterigmatocystin.⁶⁹ Purchase and van der Watt⁶⁹ fed sterigmatocystin in a standard diet at levels from 10 to 150 ppm. Two male rats fed 10 ppm for six months and 15 ppm for the next six months developed hepatocellular carcinomas within a year. They suggest that sterigmatocystin is about one-tenth as carcinogenic as aflatoxin B₁ when given orally. However, in their opinion, sterigmatocystin may be just as important a carcinogen to human health as aflatoxin since it is elaborated by several molds and in greater abundance than aflatoxin B₁.



The structure of sterigmatocystin is shown above and it can be noted that it contains the 7,8-dihydrofuro(2,3-b)furan structure which is also present in aflatoxin B₁. Our studies with rainbow trout, which compare aflatoxin B₁ with aflatoxin B₂, indicate that unsaturation at the 2,3 position is important for carcinogenicity.⁴ Preliminary studies with rainbow trout, which I will report elsewhere, show sterigmatocystin to be carcinogenic even at levels of 20 ppb if fed with cyclopropenoid fatty acids. These cyclic fatty acids which occur naturally in certain vegetable oils, including cottonseed, have been demonstrated to increase the incidence and growth of aflatoxin B₁ induced liver cancer in rainbow trout.

Aflatoxin M₁ or 3-hydroxy-6-methoxydifuromcoumarone was reported by Allcroft and Carnaghan² to be present in the milk of cows which consumed groundnut meal containing aflatoxin B₁. Subsequent studies showed the milk to be toxic when tested on ducklings. The chemical structure was reported by Holzapfel et al.³⁶ This toxic factor, M₁, was also purified and isolated from sheep urine.^{3, 64}



Holzapfel et al.³⁶ reported that the oral LD₅₀ dose for day-old Pekin ducklings for aflatoxin B₁ and M₁ was 12 and 16.6 µg, respectively. Studies by de Jongh et al.¹³ established that aflatoxin M₁ was also present in toxic groundnut meal and that the lactating rat converted aflatoxin B₁ to M₁. Butler and Clifford¹⁰ demonstrated that aflatoxin B₁ was partially converted to the milk toxin M₁ in the rat liver. In studies with rainbow trout, we found that trout liver microsomes will convert aflatoxin B₁ to M₁.⁴⁸

In a review of aflatoxicosis in farm animals, Allcroft¹ stated there was a linear relationship between the amount of aflatoxin B₁ ingested and the concentration of aflatoxin M₁ found in the milk. She concluded



FIG. 6-1.—Rainbow trout female (Mt. Shasta strain) fed the semipurified diet plus 16 ppb aflatoxin M_1 for 12 months. Note the greatly enlarged liver with many relatively small hepatomas (arrow). Posterior to the liver is the ovary filled with nearly mature eggs. Length of fish 11 inches, age 27 months.

that the dietary level of aflatoxin B_1 in the cow should not exceed 50 ppb (food intake—13.7 kg) to ensure that only negligible amounts of aflatoxin M_1 would be excreted in the milk. She further states, "Despite the fact that only a very small proportion of ingested aflatoxin appears to be excreted in the milk of cows and sheep, it would seem undesirable to have even traces of this compound present in milk for human consumption and particularly for consumption by children."

Though the acute toxicity of aflatoxin B_1 and M_1 are similar, the lack of sufficient aflatoxin in M_1 has prevented experiments which would determine its carcinogenicity to the usual laboratory animals. Recently milligram quantities of crystalline aflatoxin M_1 were isolated from cultures of *Aspergillus flavus* by investigators at the Western Regional Lab-

TABLE 6-5.—HEPATOMA INCIDENCE IN RAINBOW TROUT FED DIETS CONTAINING AFLATOXIN M_1 .

Diet Description	12 Mo. Incidence
Control test diet*	1/40
+ 4 ppb aflatoxin M_1	6/46
116 ppb aflatoxin M_1	30/43
132 ppb aflatoxin M_1	28/46
164 ppb aflatoxin M_1	30/50
+ 4 ppb aflatoxin B_1	22/46

* J. Nutr. 92:94, 1967.



FIG. 6-2.—Two-year old rainbow trout (Mt. Shasta strain). The upper fish weighed 750 gms., the liver, consisting almost entirely of one large hepatoma, weighed 114 gms. This fish had been fed the semipurified diet plus 1 ppb aflatoxin B_1 and 100 ppb cyclopropenoid fatty acids as *Stearcidia fortida* oil for 12 months. The lower fish had a liver of normal size and color but the three hepatomas it contained were 2.1 mm. diameter. This fish had been fed the same diet with 4 ppb aflatoxin B_1 , without cyclopropene, for 12 months.

oratory of the U.S.D.A. which permitted a comparative study with Oregon State University using the extremely sensitive rainbow trout. These studies reported in 1970⁷⁰ showed aflatoxin M_1 to be highly carcinogenic to rainbow trout (Figure 6-1, Table 6-6).

From these results, it would appear that M_1 is about one-third as carcinogenic to rainbow trout as B_1 . However, when cyclopropenoid fatty acids were fed, as triglycerides, with low levels (4 ppb) of aflatoxin M_1 the incidence increased to 67/98, indicating that cyclopropenoid fatty acids also promote aflatoxin M_1 carcinogenesis in trout as they do aflatoxin B_1 (Figure 6-2).^{49, 70}

Allcroft and Carnaghan⁷ found that pasteurization, roller-drying, spray drying, or freeze drying of milk did not reduce the toxicity of aflatoxin M_1 it contained. Because of the chemical structure of aflatoxin M_1 , one might assume that it is less stable than aflatoxin B_1 . Since both compounds are known to be sensitive to light, perhaps ultraviolet radiation of milk, a process formerly used to increase the vitamin D value, would reduce the aflatoxin M_1 level.

Conclusion

As man's food grows in complexity and sophistication, the opportunities for contamination by adventitious substances are greatly multiplied. This situation places a great burden of responsibility for the maintenance of a wholesome food supply directly in the hands of the producer and the processor. Recent scientific findings have demonstrated that cancer-producing substances in our environment may also be present in our food. Despite the lack of evidence, one must presume that since these compounds can induce cancer in animals they are also carcinogenic to man. This development will require that the food industry join forces with other scientific disciplines to acquire greater information and knowledge about the potential toxicants or contaminants which may occur naturally or develop in food. Only then can appropriate and positive steps be taken to assure the safety and wholesomeness of our food supply.

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Part B

EPIDEMIOLOGY AND DEMOGRAPHY

Acknowledgments

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Briefing

Carcinogens in Scotch

The Scottish whiskey exporters denounced it as an American conspiracy to ruin their trade but, in fact, there was nothing unexpected in the report issued by the National Science Foundation on 7 August saying that a carcinogen known as *N*-nitrosodimethylamine (NDMA) has been discovered in six out of seven brands of Scotch tested this year. NDMA was found in 18 brands of beer as well, but not in any of the wines, sherries, liqueurs, brandies, gins, vodkas, or rums that came under review.

The research, done by David Fine and E. Ulku Goff of the New England Institute for Life Sciences in Waltham,

occurs when nitrosopyrrolidine concentrations reach 10 parts per billion (ppb) after frying. A decade ago, before meat producers were asked to take remedial action, it was not uncommon to find concentrations as high as 100 ppb. The compound in bacon, researchers say, is considered less carcinogenic than NDMA, the one found in beer and Scotch. NDMA has been shown to cause cancer in nearly every laboratory animal tested, and in one experiment, adding 10 ppb of NDMA to the water of tumor-prone mice tripled the incidence of lung tumors.

The important work on beer was done by B. Spiegelhalter at the German national institute for chemical and cancer research in Heidelberg. In the summer of 1978 Spiegelhalter

tamination are under study; the Germans have decided that the point at which NDMA is introduced is in the process of drying or "kilning" the barley malt. The use of hot air drawn directly from a fire is thought to create NDMA in the malt, and indirect heating is thought to inhibit it. Malt for Scotch is prepared in the same way, suggesting that the one Scotch without NDMA in the seven tested—White Label—may use an indirectly heated malt. (The others were Chivas Regal, Black and White, J & B, Ballantine's Sandy Scot, and Cutty Sark.) It is not known which compound in the barley reacts to produce NDMA, although people have suggested it may be a fungicide, pesticide, or anticorrosive agent.

The American work has confirmed the German research and given more weight to the theory that the malting process is the key problem. It also suggests the health risk is lower for Scotch drinkers than for beer drinkers. The concentrations of NDMA are lower in Scotch to begin with, and the volume consumed is lower. The concentrations of NDMA found in the 18 foreign and domestic beers tested at the New England research lab ranged from 0.4 to 7 ppb.

The Distilled Spirits Council, a trade association in the United States, stresses that nitrosamines have not been proved a threat to human health, and a spokesman, Sam Chilcote, claimed recently that it is "practically impossible" to eliminate these compounds from the human diet. The distillers have not decided whether to take any action. However, shortly after the news about nitrosamines in European beer came out last year, the U.S. Brewers Association set up a research committee chaired by William Hardwick of Anheuser-Busch Inc. and asked him to come up with some suggested solutions. A spokesman, refusing to give details, said the brewers have spent "a lot of money" and commissioned research at three labs. Lacking definitive proof that this is a public health hazard, the companies are reluctant to order a complete overhaul of the barley malting process for, as one company official said, that would cost hundreds of millions of dollars and bankrupt half the maltsters in the country.

Worried drinkers always have the



Cartoon by Oliphant. Copyright © 1979 The Washington Star

Massachusetts, did little more than fill a gap in earlier work, most of it done by German researchers, showing that many varieties of beer contain NDMA. Beer and Scotch producers use a similar process of barley malting, and the research on Scotch merely strengthens what was suspected already—that the problem is in the malt.

NDMA is one of a family of carcinogens called nitrosamines, all of which occur widely in the environment. The best known until recently was *N*-nitrosopyrrolidine, a suspected carcinogen in fried bacon. The Department of Agriculture has put into effect a food monitoring program to keep track of nitrosamines, and the "action level" at which bacon is taken off the market

and his colleagues published a report that 70 percent of the 158 European beers they had analyzed contained NDMA in amounts ranging from around 1 to 68 ppb. In general, dark beer contained more than light beer, and the highest concentrations were found in "rauchbier," made from smoked malt. This was deemed a significant health risk for Germans, whose diet is one-quarter beer. Spiegelhalter calculated that, based on average figures, a young German beer drinker might consume as much as 1.2 micrograms of NDMA a day. Other nitrosamines were found, but none so potent or abundant as this one.

Several possible sources of con-

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option of avoiding beer and Scotch. But they should be advised to stay clear of carrot juice and beet juice as well, for they also contain relatively high concentrations of nitrosamines.

Congress Relents, Spares OES Bureau

After a harrowing summer, the State Department's Bureau of Oceans and International Environmental and Scientific Affairs (OES) is expected to glide safely home to port this fall when Congress returns from its August recess and takes up the appropriations bill for the State Department. The OES, which handles negotiations for fishing agreements and scientific and technological exchanges, earlier this year ran afoul of Representative William Alexander (D-Ark.). He slashed both the budget and the staff of the OES in half when they came before a subcommittee on which he sits (*Science*, 8 June 1979).

The funds were restored after a 1½-hour debate on the floor of the House on 12 July in which Alexander reported thirdhand that Henry Kissinger had once told another official that OES "is where the Department of State places its incompetents." He continued: "I would rather have one good horse than a whole team of lame nags that sit grazing at the trough of public expense."

When the harangue was over, several congressmen chided Alexander for failing to hold any hearings on his charges. Representative Clement Zablocki (D-Wis.) spoke of the "unfortunate record" and said the budget slash was adopted "without any consideration and chiefly at the urging of one individual, our good friend, the gentleman from Arkansas." The House then voted to restore the budget; a conference report cleared the House on 2 August; and all that is lacking is the Senate's approval.

An OES official, Leslie Brown, said, "A lot of us were surprised not only by the depth but by the breadth of support" that came through for OES at the last moment. Rep. Alexander had boasted that he would win the battle if it ended up on the House floor. He plainly underestimated the opposition.

Eliot Marshall

(Continued from page 767)

would be doubly concerned about an asthmatic with a common cold."

Greatest concern centers on pilots—and understandably so. The picture of a flight crew incapacitated by ozone is not a very pretty one. Yet most pilots refuse to talk about any problems they may have encountered. This too is understandable. Mere mention of "chest pains" might be equated with "heart trouble," which could mean the loss of a pilot's medical certification to fly.

The most vocal complainers are flight attendants. If they seem to have more problems with ozone than passengers and pilots, Tierney notes, it is probably because they work harder. A person at rest breathes about 5 liters of air per minute. A person pushing a heavy cart up and down an aisle breathes 15 to 20 liters per minute—and thus takes in many times more ozone.

Though flight attendants raise a fuss, the companies they work for are often noncommittal. Trans World Airlines, which operates 11 regular 747's, says it is waiting to see if the FAA regulations go into effect. It will then add ozone-removal equipment.

Pan American says that its 29 regular 747's will get catalytic converters to break down ozone sometime in 1980, and that the ozone problem has already been solved on its very long-range, high-altitude planes. Charcoal filters (each weighing 800 pounds) were installed on all ten of Pan American's 747SP's in March 1978. Since then, there have been only two passenger complaints. The 645 complaints by flight crews in 1978, says a Pan American spokesperson in New York, have to do with politics involving union wage settlements. "As far as we're concerned, there are many more passengers than crew," says James Arey. "Based on the complaint figures, we have done the job. There are no more problems."

Flight attendants, however, say that a wage contract has long been reached—and that complaints are still being sent to New York. "In fact," says Carmen Azopardi of the Independent Union of Flight Attendants, "Pan Am just settled out of court with two flight attendants for ozone-related injuries. One had developed bronchitis, the other asthma." She also notes that passengers have never sent in many complaints, for few understand the problem.

A spokesperson for Boeing says that Pan American is the only airline putting in filters and catalytic converters. The other airlines can get them as optional

equipment, he says, but so far none have. He also says that the FAA regulations will probably never come out, since the problem is so rare. "I've traveled around the world in a 747 and never had a single problem," says Leonard Weiss. "This ozone thing has been vastly overplayed by the news media."

To give credence to their side of the story in the face of industry skepticism, flight attendants presented a 1330-person survey at the House hearing in July. Performed by Dwayne Reed, an epidemiologist with the California State Department of Health Services, the survey looked at flight attendants from Pan American, which flies high-altitude international flights; from Trans World Airlines, which flies both international and lower-altitude flights; and from Pacific Southwest Airlines (PSA), which flies only low-altitude flights within California. The survey tabulated symptoms for five consecutive flight days. With fatigue, backache, nausea, and vomiting, there was little difference between airlines. Chest pain, however, was experienced by 19 percent of the Pan American flight attendants, 13 percent of those on TWA, and 5 percent of those on PSA.

What worries many is lack of data about long-term effects. Ozone, for instance, is a mutagen and is known to cause biochemical changes in the bloodstream of exposed persons. But it is not known if high rates of miscarriages and children with birth defects among flight attendants are a result of ozone exposure.

Even if the FAA does make a ruling on ozone, ALPA fears that the airlines will not take the time and money to put on equipment that really works. As part of the FAA regulation, therefore, ALPA wants a requirement for on-board systems to monitor ozone levels—thus checking the effectiveness of ozone removal equipment.

A few months ago the FAA said the ozone ruling, if it is made, would come in September. Now it has pushed that back to October. Asked how long the airlines would have to comply with a ruling, Ray Ramakis of the FAA's safety regulations division said: "The notice of proposed rule-making said 6 months. It could go to a year. I don't know. There is no set formula." If the airlines have their way, it will take a while. In January the Air Transport Association, which represents the nation's airline companies, told the FAA how long it thought the conversion to ozone-removal equipment would take. The estimate came to somewhere between 4 and 6 years.

—WILLIAM J. BROAD

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Draft

ASSESSMENT OF TECHNOLOGIES FOR DETERMINING CANCER RISKS FROM THE ENVIRONMENT

JUNE 1981

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the number of retail outlets. (Recently, several States have raised drinking ages.)

- Around 1960, total per capita sales of absolute ethanol began to rise significantly registering a 30-percent gain between 1961 and 1971. Since 1971, there has been virtually no change in per capita sales. The effect of the overall increase may not yet have fully manifested itself in cancer rates.
- There is particular concern over increased alcohol consumption in youths. This is heightened by the observation that early drinking behavior predicts drinking habits in later life.
- The proportion of high school students who reported ever having been drunk increased dramatically from 19 percent before 1966 to 45 percent between 1966 and 1975. The proportion reporting being intoxicated at least once a month rose from

10 percent before 1966 to 19 percent between 1966 and 1975.

The importance of alcohol as a health hazard is not limited to its association with a relatively small percentage of cancer. Estimates of the annual number of deaths related to alcohol range from 37,000 to 205,000. Cirrhosis of the liver, the seventh leading cause of death in the United States, was responsible for 30,066 deaths in 1978. As a contributory cause of homicides, suicides, and accidents, alcohol's toll is even greater. The Institute of Medicine (178) recently completed a study of alcoholism and related problems and indicated several opportunities for research. These included further research on alcohol metabolism, development of appropriate animal models, and further efforts through epidemiologic studies to explore the link between alcohol and its adverse health consequences.

DIET

Introduction

Studying the relationship of diet and health is a continued source of frustration and excitement. Food affects all body functions and comes into direct contact with the digestive system and indirect contact with all other organs. Cancer rates for digestive sites vary considerably around the world and have prompted studies of diet's role in cancer causation.

As discussed here, diet encompasses those items ingested as food, including substances added to food, those produced during normal cooking, storage, and digestion, but excluding drinking water and alcohol (discussed in this chapter under *Air and Water Pollution* and *Alcohol*, respectively).

The amounts and balance of the major components of diet are generally believed to be responsible for the lion's share of diet-related cancers. Deficiencies or excesses of microelements, and the presence of additives or contaminants, are probably less important. The various means by which diet may influence the

development of cancer are listed in table 16. Diet also plays a role in the treatment of cancer illustrating the pervasive role of diet with respect to all aspects of the disease.

Unfortunately, dietary studies are plagued with methodological problems and conflicting evidence exists for almost every specific question that has been investigated. The overall association of cancer with diet exists but there is no reliable indication of exactly what dietary changes would be of major importance in reducing cancer incidence and mortality.

The strongest positive associations identified through correlations of dietary patterns and cancer rates are those between total fat intake, particularly animal fat consumption and cancers of the breast and endometrium; and between total protein intake and cancer of the colon. The most dramatic change observed in a diet-related cancer site has been the reduction in incidence and mortality from stomach cancer. In the United States, the 1950 age-adjusted mortality rate from stomach cancer was 24.4/100,000 for males and 13.1/100,000 for females

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Table 16.—Some Currently Attractive Hypothetical or Actual Ways
In Which Diet May Affect the Incidence of Cancer

Possible ways or means	Example ^a
Ingestion of powerful, direct-acting carcinogens or their precursors	Carcinogens in natural foodstuffs (plant products) Carcinogens produced in cooking Carcinogens produced in stored food by microorganisms (bacterial and fungal)
Affecting the formation of carcinogens in the body	Providing substrates for the formation of carcinogens in the body (e.g., nitrites, nitrates, secondary amines) Altering intake or excretion of cholesterol and bile acids (and hence the production of carcinogenic metabolites in the bowel) Altering the bacterial flora of the bowel (and hence the capacity to form carcinogenic metabolites)
Affecting transport, activation, or deactivation of carcinogens	Altering concentration in, or duration of contact with, feces Altering transport of carcinogens to stem cells Induction or inhibition of enzymes (which affect carcinogen metabolism or catabolism) Deactivation, or prevention of formation, of short-lived intracellular species (e.g., by use of selenium, vitamin E, or otherwise trapping free radicals; by use of β -carotene or otherwise quenching singlet oxygen; by use of other antioxidants)
Affecting "promotion" of cells (that are already initiated) ^b	Vitamin A deficiency (clinical or subclinical) Retinol [Binding Protein] (hormonal and other factors determine blood RBP, though vitamin A intake may not affect it much) Otherwise affecting stemcell differentiation (carotenoids: determinants of lipid "profile"?) Age at menarche Adipose-tissue-derived estrogens Other effects
Overnutrition	

^aThere may be considerable overlap between many of the entries in this table.
^bOr, more generally, affecting the probability that a partially transformed stem cell will become fully transformed and will proliferate successfully into cancer.
SOURCE: Doll and Peto (93).

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while in 1977 the rate was 8.8/100,000 for males and 4.0/100,000 for females. This decrease has occurred in many other countries, including those with high initial rates, such as Japan and Iceland, and those with lower initial rates, such as Canada and New Zealand. The factors believed to have contributed to these decreases include: reduction in use of salt and pickling, lower consumption of smoked foods, and increased use of refrigeration, increased consumption of milk, green vegetables, fruit, and antioxidants (237).

In general, as a result of increased intake of calories, proteins, and certain other nutrients, Americans have been growing taller and reaching sexual maturation earlier. A great improvement in the Nation's health has resulted from this change, but increased risk for certain cancer sites may accompany the improvement. For ex-

ample, earlier sexual maturation in women is associated with higher risk of breast cancer later in life, though as yet, no increases in breast cancer has been attributed directly to improved nutrition (see *Sexual Development, Reproductive Patterns, and Sexual Practices* for a more complete discussion of this topic).

Many estimates of the importance of diet to cancer have been put forth. Doll and Peto (93) estimated that altering dietary practices may reduce cancer by as much as 35 percent (stomach and large bowel cancer by 90 percent; endometrium, gallbladder, pancreas, and breast by 50 percent; lung, larynx, bladder, cervix, mouth, pharynx, and esophagus by 20 percent; others by 10 percent). The great uncertainty of this estimate is indicated by the range of 10 to 70 percent which they attach to their estimate. Wynder and Gori (368) estimate, by calculating

the percent difference between U.S. mortality rates and the lowest rates reported worldwide and by considering specific case-control studies, that an even larger proportion of cancer, approximately 40 percent in males and 60 percent in females, could be attributable to diet.

In testimony before the Subcommittee on Nutrition of the Senate Committee on Agriculture, Nutrition, and Forestry, Dr. Arthur Upton (353), then-Director of the National Cancer Institute (NCI), succinctly discussed the extent of involvement of diet and cancer when he stated:

Despite the impression that cancers are linked with dietary patterns and the inability to pinpoint specific dietary carcinogens, scientists generally agree that factors in diet and nutrition—including drinking water contaminants—appear to be related to a large number of human cancers, perhaps approaching 50 percent.

Dietary Intake

Fat/Meat Intake

Examination of cancer rates in different countries show positive correlations between colon cancer and consumption of meat and animal protein and between cancer of the breast and endometrium with total fat consumption (17). These cancers are common in the United States, Canada, and Western Europe and rarer throughout the developing world.

The most widely held theory is that fat in the diet has a promotional effect on the development of cancer. One suggested mechanism is that fats affect hormone levels. Several cancers, including breast, ovarian, and endometrial are, in turn, influenced by hormones. This association of dietary fat with higher cancer rates is, however, not found uniformly. Breast and colorectal cancers are not uncommon among vegetarians, and the observed incidence in Seventh Day Adventists who are largely vegetarian, is matched by the same incidence in Mormons who eat meat (293). This finding and the fact that meat intake among Mormons is not markedly lower than among the general population is often cited as a rebuttal to the meat/fat cancer causation hypothesis. These interpretations

should be viewed cautiously for the studies may not have been adequate to reflect any promotional effect in these low risk groups who are less exposed to many other types of carcinogenic stimuli, such as, cigarettes and alcohol, than is the general population. Additional studies in this area are needed.

More and more, studies have focused on specific types of fat. Evidence suggests that diets with a high ratio of polyunsaturated fats (mainly of vegetable origin) to saturated fats (mainly of animal origin) may increase the risk of cancer. Paradoxically, this type of diet is recommended to lower the risk of heart disease. Results from epidemiological studies (98,290) and animal trials (53,305) have been inconsistent and confusing, and have shown different effects on different cancer sites. In rats, polyunsaturated fats need be only a small proportion of a total high fat intake to promote breast tumor incidence. If the animal model is applicable to humans, virtually all high-fat diets will exert a promotional effect (52).

Serum cholesterol has been investigated in many studies as a risk factor for various cancers. Serum levels are directly affected by intake of cholesterol and fat. A diet with a low saturated fat to polyunsaturated fat ratio decreases the amount of ingested cholesterol that will appear in the serum. The discovery that the stools of colorectal cancer patients contain an abnormally high proportion of acid steroids, derived from bile salts, and cholesterol, supports the hypothesis that certain types of fat play a role in the production of colorectal cancer.

A recent epidemiologic study found an association between high-density lipoproteins and cancer risk (199). On the other hand, in a prospective epidemiologic study of heart disease in Framingham, Mass., serum cholesterol levels were inversely associated with the incidence of colon cancer and other sites in men. Men with the lowest serum cholesterol levels experienced a colon cancer rate which was three times higher than men with the higher cholesterol levels (361). A similar negative association was reported in data from the Paris Prospective Study of Coronary Heart Disease (45).

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The hypothesis that dietary cholesterol plays a direct role in the production of colon cancer is supported by some animal studies. For instance, the addition of cholesterol to the diet of rats on a cholesterol-free liquid diet, had a promotional effect and cholesterol enhanced the carcinogenic effect of a known carcinogen (1,2 dimethylhydrazine).

Fiber Intake

Burkitt (40) observed that several intestinal diseases that are common in developed countries are rare in rural Africa where unprocessed food is consumed, and the stools tend to be soft, bulky, and frequent. It is suggested that dietary fiber may reduce colorectal cancer by decreasing the time stools remain in the bowel, by increasing bulk (thereby decreasing the concentration of carcinogens in stool), or by perhaps altering the distribution of bacteria, some of which may produce or destroy carcinogenic metabolites.

The effects of fiber on cancer incidence have attracted much interest but remain problematical. As in the case of fats, dietary fiber is a term which covers a multitude of different substances, each of which may have different influences on carcinogenesis. The methods by which types of fiber are chemically characterized are still in a primitive state, and analyses of the composition of dietary fiber in many foods is lacking. Despite the drawbacks, some associations have emerged. A close inverse correlation has been found between the pentose fiber content of the diet and mortality from colon cancer in part of the United Kingdom (93). No correlation was shown with any other fiber types, or with dietary fiber as a whole.

Elements and Vitamins

Several metals and vitamins are linked with cancer development. They can either act as carcinogens themselves or they may biologically compete with other dietary constituents to enhance or suppress a cancerous response.

An iron-deficient syndrome was shown to be associated with a high risk of developing cancer of the pharyngeal and esophageal mucosa

in northern Scandinavia. The incidence of gastric cancer is found to be 4 to 5 times higher in countries where iron deficiency is prevalent than in the United States (356).

Selenium, an effective antioxidant, is often found at higher levels in plants, milk, or human blood in sections of the United States with low cancer rates. Selenium deficiency in rats consistently increases the carcinogenic effect of known chemical carcinogens, particularly in animals fed on high polyunsaturated fat diets (190). Supplementation with selenium above dietary requirements decreases tumor yield in animals on both low- and high-polyunsaturated fat diets (188).

Stocks and Davies (24) found that a high zinc-copper ratio in soils was associated with elevated rates of human stomach cancer while Strain et al. (24) reported an association of elevated cancer rates with low zinc-copper ratios. Marginal zinc deficiency is associated with increased esophageal cancer in animals (122) and esophageal cancer patients had lower levels of zinc in their blood, hair, and tumor tissue than controls in a study of Chinese men (211). Zinc deficiency may interact synergistically with alcohol to enhance esophageal carcinogenesis (135).

The overall relationship between cancer and vitamins is not well understood. Vitamin C has been shown to reduce carcinogen formation in experimental animals (359), and this activity may be important in reducing cancer occurrence. Vitamin A (retinol) has been more exhaustively studied than any other vitamin (for review see 292) and it has been suggested that vitamin A, or, more particularly, its vegetable precursor, beta-carotene, may decrease the susceptibility of a variety of epithelial tissues to the development of cancer. Retinol (or its analogs retinoic acid and various retinoids) has been repeatedly demonstrated to diminish the risk of experimentally induced cancer in laboratory animals (93). These results are particularly intriguing because the protective effect is observed even when these substances are fed long after the animal is treated with an initiating carcinogen, and the vitamin and its analogs appear to be effective at a wide variety of sites.

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People with a history of consuming above average amounts of provitamin A (beta-carotene) have a slightly lower incidence of several different types of cancer than people who give a history of consuming less. Beta-carotene is found in carrots, green leafy vegetables and in red palm oil which is used for cooking in many tropical countries. Further epidemiologic studies are now in progress in areas where red palm oil is habitually used. The role of beta-carotene in cancer prevention is still uncertain and this particular hypothesis has been mentioned to illustrate the potential importance of diet.

Local deficiency of folate (a B-complex vitamin) has been demonstrated in the abnormal cervical tissue of some women taking oral contraceptives. The observed abnormalities are often precursors of cervical cancer. Supplementation with folic acid in these women can reverse the abnormality and appears to prevent progression to carcinoma (41). Deficiencies of lipotropes (choline, methionine, and folic acid) increased the susceptibility of animals to a variety of environment carcinogens in several studies (311).

Dietary Balance

Studies in laboratory animals have shown that altering gross aspects of diet can have substantial impact on the risk of an animal developing cancer. Jose (195) reviewed several reports which demonstrate that restricting calorie intake without modifying the proportion of the individual constituents reduces the incidence of spontaneous tumors and of a variety of cancers produced by exposure to known carcinogens. Not only did calorie restriction result in decreased incidence of tumors, but it also delayed the time of appearance of tumors, and when tumors appeared, they grew and metastasized more slowly. The life spans of animals on restricted diets were often increased up to 50 percent compared to normally fed controls. However, decreased calorie intake, when accompanied by inadequate protein intake makes laboratory animals more susceptible to many environmental carcinogens (47).

A suspected association between obesity and the risk of cancer was strengthened in an epi-

demologic study conducted by ACS which followed 750,000 people from 1959 to 1972 (207). Overall cancer mortality was found elevated for those individuals who were more than 40 percent heavier than average. Mortality from cancers of the colon and rectum were increased among men while mortality from cancers of the gallbladder and biliary passages, breast, cervix, endometrium, and ovary were increased among women. The meaning to be attached to the results is not entirely clear since weight is associated with a variety of social and behavioral characteristics that affect the risk of cancer in other ways, including smoking habits and socioeconomic status. Another concern is that diagnosing cancers may be more difficult in obese individuals, and cancers may generally be more advanced when they are detected.

In addition to the quantity and composition of food, the timing of intake has also been shown to be important. For example, Roe and Tucker (cited in 93) randomized mice with a high spontaneous incidence of mammary tumors, between continuous feeding, in which the mice were fed 6 g of food each day, which they consumed in frequent small amounts, and intermittent feeding, in which food was limited to 5 g per day which was eaten at once. No clear difference in longevity was observed, but nonfatal spontaneous mammary tumors arose in 64 percent of the continuously fed mice and in only 8 percent of those fed intermittently.

Immune function in both humans and animals can be severely compromised by deficiencies of certain dietary nutrients—in particular, protein, methionine, choline, folate, vitamin B₁₂, vitamin A, zinc, and pyridoxine (147). Deficiencies in certain of these nutrients, with a concomitant depression of immune system function have been demonstrated in animals and are suspected of increasing the susceptibility of developing certain cancers (356).

Naturally Occurring Carcinogens and Precursors

Along with the major components, thousands of chemical substances occur in small quantities in foods. Most naturally occurring carcinogens

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plants and animals.

Nitrates and Nitrites

Nitrate and nitrite salts naturally occur in
vegetables, fish, and meat, are added to food for
their preservative properties (see *Additives* p.
82), and are present in pesticide and drug
residues in food (127). They react with other
chemicals in the body to produce N-nitro-
samines and N-nitrosamides. N-nitrosamines
are among the most powerful chemical car-
cinogens in laboratory animals, producing tu-
mors at a variety of sites including liver, kidney,
lungs, esophagus, bladder, pancreas, trachea,
nasal cavities, and peripheral nervous system
(221). Experimentally, vitamin C has been
shown to inhibit the formation of nitrosamines
(359).

Epidemiological evidence linking nitrites and
nitrates to cancer is fragmentary. Evidence for a
relationship between gastric cancer and the ni-
trite content of the diet is not wholly consistent.
It is notable that vegetables, which are usually
the main source of dietary nitrates, appear to
protect against the development of the disease,
possibly because of their vitamin C content (93).
On present knowledge, the possible contribu-
tion of dietary nitrates and secondary amines to
the production of cancer is uncertain.

Other Substances

Cycasin in the cycad nut, safrole in sassafras,
pyrrolizidine alkaloids in some plants, and ex-
tracts of coltsfoot and bracken fern are a few
naturally occurring compounds which exhibit
carcinogenic properties in animals. Only
bracken fern has been demonstrated to cause
cancer in man. Bracken fern is commonly eaten
in Japan, and Japanese who eat it daily have
three times the risk of developing cancer of the
esophagus as Japanese who do not eat it at all
(93). It has been postulated that the high bowel
cancer rates in Scotland may be due to the inges-
tion of cattle fed on bracken fern or the leaching
of the carcinogenic components into water or
food (24). Range-fed cattle and sheep pass

pyrrolizidine alkaloids along to humans in their
meat and milk (215).

Certain plant-derived preparations are asso-
ciated with cancer. Extracts of some plants used
to make herbal infusions, for use as home reme-
dies, tonics or beverages, are carcinogenic in
animals, and their tannin-containing fractions
are particularly active. Some population groups
who use these products have high rates of
esophageal cancer, suggesting an association
(297). Perhaps of greater concern, because of its
widespread use, coffee has been associated with
human bladder (24) and pancreatic cancer (220),
but whether the associations are causal is not
yet known. Studies in animal cell cultures have
shown caffeine, a constituent of both coffee and
tea, to potentiate the effect of carcinogenic
substances (96).

Mutagenic substances have also been iden-
tified in cruciferous plants (cabbage, broccoli,
etc.), from cereal grains, and some grazing
range plants in the Southwestern United States
(MacGregor, 1980). Other not-yet-identified
carcinogens and mutagens may occur naturally
in food, but on present evidence, naturally oc-
curring carcinogens are not regarded as an im-
portant cause of cancer in the United States (93).

Carcinogens and Precursors Produced by Cooking

Another possible source of carcinogens is
their production in cooking. Humans are the on-
ly animals that cook their food, and it has been
known for many years that carcinogenic chemi-
cals such as benzo(a)pyrene (B(a)P) and other
polycyclic hydrocarbons are produced when
meat or fish is broiled or smoked or when food
is fried in fat which has been used repeatedly.
Sugimura et al. (336) demonstrated that broiling
also produces powerful mutagens that cannot be
accounted for by the production of B(a)P alone.
Few people eat more broiled foods than Ameri-
cans and while the declining stomach cancer
rate provides some assurance that cancer at that
site is not related to broiled food, the possibility
remains that colorectal cancer, which has not
decreased materially, might be related. Recent

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Contaminants

Natural Contaminants

A less obvious source of carcinogenic activity and one that was overlooked altogether until the early 1960's, is the production of carcinogens by microorganisms in food. Aflatoxin, a product of the fungus *Aspergillus flavus*, is the most powerful liver carcinogen known for some animal species. In addition, human liver cells contain the enzymes necessary to produce the metabolite products that appear to be responsible for its activity. There is evidence for believing that aflatoxin is a major factor in the production of liver cancer in certain tropical coun-

tries where contaminated food. A wide variety of products ranging from pesticides to insecticide residues, to substances used in the production of packaging, such as polychlorinated biphenyls, can pollute food. Organic compounds in food pose the greatest potential for environmental food contamination based on the amount, frequency, and toxicity of organics manufactured in the United States.

Polycyclic aromatic hydrocarbons (PAH) are found widely in all types of foods, sometimes at the same level where they can be present in charcoal-broiled meats and smoked ham, i.e., up to 15 ug B(a)P/kg wet weight. Some shellfish and finfish from polluted waters have been reported to contain up to 1000 ug B(a)P/kg. The importance of ingested PAH in cancer induction is uncertain. Epidemiologically, the relationship has been established that when cancer is produced experimentally in laboratory animals, it either affects the same organ system as found in man or occurs by a mechanism that does not appear relevant (93).

Some polycyclic aromatic hydrocarbons that were found as contaminants of DDT, aldrin, dieldrin) and other pesticides (hepatomas) in mice, and were found to be carcinogenic in laboratory animals. These compounds accumulate in the liver and increase in liver tumors appeared after their introduction. The latent period is not known, but the possibility exists that effects may be seen in years from now. Pesticide residues in the form of secondary amines may constitute a hazard to the formation of nitrosamines in food. It has been proved to be a cause of cancer in laboratory animals.

Many of the pesticides fall into this category of carcinogenic contaminants. The extent to which they have contributed to the production of cancer is difficult to evaluate, but it is likely that they have contributed significantly.

Additives

Chemicals are used to preserve food and give it color, flavor, and consistency. Food dyes were among the first chemicals investigated because of their structural similarities to accepted carcinogens (94). Consumption of

the aflatoxin in the diet is estimated to be about 100 ug/day. This is a very small amount, but it is a constant exposure. The aflatoxin is a potent liver carcinogen, and it is a major factor in the production of liver cancer in certain tropical countries where it is found in food.

The aflatoxin is a potent liver carcinogen, and it is a major factor in the production of liver cancer in certain tropical countries where it is found in food. The aflatoxin is a potent liver carcinogen, and it is a major factor in the production of liver cancer in certain tropical countries where it is found in food.

In the United States, primary cancer of the liver is a rare disease accounting for less than 1 percent of cancer deaths (2,796 deaths in 1978). The amount of aflatoxin in the American diet is small and is only one among several other possible causes of liver cancer. In the American context, the chief importance of the discovery of the aflatoxin is that it is a potent liver carcinogen, and it is a major factor in the production of liver cancer in certain tropical countries where it is found in food. The aflatoxin is a potent liver carcinogen, and it is a major factor in the production of liver cancer in certain tropical countries where it is found in food.

Environmental Contaminants

In late 1979, OTA (Office of Technology Assessment) published a report on the state-of-the-art in the field of environmental contaminants. The report is a summary of the current state of knowledge on the health effects of environmental contaminants.

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chemicals by consumers who are unaware of their presence is partially responsible for the food safety laws. The laws require that newly introduced direct food additives be carefully screened in the laboratory before they may be used.

Some definitely carcinogenic chemicals, which have now been withdrawn from foods, were used for a time before their carcinogenicity in animals was discovered. These include butter yellow, thiourea, and a food preservative used in Japan. The number of cancers, if any, which these additives produced is unknown. Doll and Peto (93) estimated an attribution of less than 1 percent of total cancer mortality to food additives.

Of the many food additives presently used in the United States, three require special consideration: the artificial sweeteners cyclamates and saccharin, butylated hydroxytoluene (BHT), and nitrites and nitrates.

Cyclamates were shown to produce bladder cancer in animals (24) and were removed from commerce in 1969. OTA (282) reviewed data that show saccharin caused cancer of the bladder in rats in two circumstances: 1) in straightforward feeding studies when given over two generations, and 2) when given following the administration of a powerful carcinogen. OTA also reported positive and negative results from a number of short-term tests. Therefore, some evidence supports the conclusion that saccharin causes cancer in defined conditions. The human evidence that has been collected over the last few years fails to distinguish clearly between saccharin and cyclamates, but it is more relevant to the former as the use of saccharin began earlier (in 1902) and has continued longer. An increase in incidence or mortality from bladder cancer could not be attributed to the introduction of saccharin (16).

One epidemiologic study (175) showed that ingestion of artificial sweeteners by males increased their relative risk of bladder cancer to 1.6. (The incidence of bladder cancer among men who did not consume saccharin was taken as relative risk equal to 1.0; saccharin users had a risk 60-percent higher). The results reported in the study were consistent with those from the

laboratory. In both cases cancer occurred in the bladder in males.

Subsequent epidemiologic studies have failed to confirm the relative risk of 1.6. The large case-control study of bladder cancer in the United States conducted by NCI revealed relative risks of 0.99 for males and 1.01 for females among users of artificial sweeteners in any form (174). However, Wilson (362) points out that one projection from animal studies suggests that only about 500 bladder cancer cases annually are to be expected from saccharin consumption in the United States (see also 282). The relative risk represented by 500 cases could not have been detected in the case-control study.

Doll and Peto (93) reviewed five case-control studies which examined saccharin consumption and bladder cancer. With the exception of Howe et al. (175), the relative risks in all the experiments lie very close to 1.0; some slightly higher; some lower. They conclude that the "human evidence could hardly be more null," at least for cancer of the bladder, which was the anatomic site affected by saccharin in rats (93).

BHT has been used extensively as an antioxidant for many years. It is not carcinogenic by itself, but has been reported to enhance the production of lung tumors by urethan in mice (93). Conversely, its antioxidant effect is found to inhibit the formation of active carcinogens in the laboratory (93), and similar effects might be expected to occur in vivo. It has been postulated that its use—and perhaps that of the more widely used butylated hydroxyanisole—have contributed to the decline in mortality from stomach cancer (24).

Nitrites have been used to preserve meat since the last century (also see above). According to Shubik (328), nitrites added to food constitutes only 10 percent of the total nitrite reaching the stomach in vegetables and saliva. However, if the formation of nitrosamines and nitrosamides in the intestinal tract proves to be of practical importance, dietary nitrite may play a role in cancer formation. The National Research Council's Panel on Nitrates (cited in 93) was unable to reach any conclusions about their quantitative effect, but advised that reasonable measures be

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taken to minimize human exposure to N-nitroso compounds, including the restriction of the amounts of nitrate and nitrite added to meat products.

There is great uncertainty regarding the contribution of the compounds discussed above to the formation of cancer. The possibility also exists that other additives might have detrimental effects.

Diet Summary

Dietary components discussed above, and many others, are currently the subjects of intensive research, from which some results should be known within the next few years. The outcomes may show diet to be a factor in determining cancer occurrence at many sites, particularly the stomach, large bowel, endometrium, gallbladder and in tropical countries, the liver. Diet may also be shown to affect the incidence

of cancers of the breast and pancreas, and, through the antipromoting effects of retinoids, to reduce incidence of epithelial cancers in many other tissues. If these or other hypotheses are proven, practicable means of dietary modifications may eventually be identified to reduce cancer rates.

Cairns (43) draws attention to the probable difficulty of changing cancer incidence even if a direct link is shown between a diet constituent and cancer incidence:

Cancer of the lung is due to a pleasant and highly addictive habit, cancer of the large intestine and breast are most common in affluent countries and so are presumably associated with some desirable habit, such as a diet high in animal fats, that the rich nations can afford and the others cannot These are signs, therefore, that the campaign to prevent cancer may come into some conflict with people's immediate desires.

OCCUPATIONAL EXPOSURES

The last several years have seen a heated discussion concerning the contribution of occupational exposures to cancer in the United States. Since the formation of the Occupational Safety and Health Administration (OSHA) in 1970, 20 regulations have been promulgated and two more proposed relating to suspect carcinogenic factors in the workplace. Labor unions and public interest groups have criticized OSHA for moving slowly, while industry and their trade associations claim unnecessary irrational regulation and undue expense. In an effort to implement a comprehensive and rational policy for the regulation of carcinogens in the workplace, OSHA held 2 months of hearings in 1978 which attracted the participation of labor unions, environmental groups and spawned a major new trade association, the American Industrial Health Council (AIHC). Subsequently, OSHA promulgated "a general policy for the identification and regulation of physical and chemical substances that pose a potential occupational carcinogenic risk to humans" (278,279).

The first recognized industrial cancer was identified by Percival Pott, a British surgeon, who observed that scrotal cancer occurred more frequently in men who had been employed as chimney sweeps as boys. This led to the identification of soot as the first chemical and occupational carcinogen. In the ensuing years, many other groups of workers have been found to suffer from occupationally induced cancer. For the purpose of this assessment, occupational exposure is defined as exposure to a substance or physical agent through any route during the course of employment.

The workplace setting has proved to be the single most productive source of information in the discovery of carcinogenic substances. This is because of the defined populations involved and higher exposures which can be more easily monitored and identified. Table 17 lists carcinogens and processes found in the workplace which are associated with increased cancer risk. Occupations known to produce an elevated risk of cancer, though the specific agents responsible

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TRENDS

The Reaganomics of Toxic Chemicals

The Toxic Substances Control Act (TSCA) broke new ground when it became law six years ago, directing the Environmental Protection Agency to balance risks with benefits in managing hazardous chemicals. Though this risk-benefit analysis makes TSCA consistent with the Reagan administration's philosophy of regulation, the government's campaign against waste and overregulation is nevertheless bringing changes to the EPA's stewardship of TSCA.

As a result, the mixed reviews that TSCA earned from the chemical industry during its first five years are giving way to growing enthusiasm for the EPA's initiatives.

One TSCA effort that everyone applauds is the basic inventory—the list of 55,000 chemicals now being manufactured in the U.S. that EPA has assembled since 1977. "No similar document exists in any other country," E. Hamilton Hurst, vice-president for environmental health and safety of Nalco Chemical Co., told the American Chemical Society's 1982 spring meeting. The list is "a valuable tool" for both EPA and the industry, and many foreign nations are busy assembling similar data.

There is also industrial enthusiasm for EPA's changing philosophy concerning the TSCA requirement that chemical companies give "premanufacturing notice" (PMN) 90 days before they put new chemicals into production. EPA is to use that 90-day period to determine if the chemicals have risks greater than their potential benefits.

Douglas G. Bannerman, acting director of EPA's Office of Industry Assistance, told the ACS that over 1,000 PMNs have come to EPA since 1979, when the requirement took effect. The result, he said, "is the only complete and accurate record of the development and commercialization of new chemicals ever compiled . . . a wealth of information."

But C.W. Umland of Exxon Chemical Americas said the PMN requirement has caused "substantial disruption of new chemical development and introduction," and J.R. Yost of the Muskegon Chemical Co. called the PMN requirement "a unique burden for small companies," where most U.S. innovation is supposed to take place. Dr. Bannerman's analysis shows that just over 70 percent of the PMNs have come from large companies (with over \$500 million in annual sales). And he admits that by adding costs and delays, TSCA may have caused "a significant reduction in new-product innovation among smaller specialty chemical firms."

Of more than 1,000 PMNs received, EPA has kept only 9 chemicals off the market. But Dr. Bannerman pointed out that TSCA's greater achievement has been to keep other new chemicals off the market by encouraging companies to take "a more responsible look" on their own.

This low rejection rate, coupled with the Reagan administration's initiatives for regulatory relief, have encouraged EPA to issue some exemptions: PMNs will no longer be required for three classes of new chemicals: high-molecular-weight polymers,

chemicals that are intermediates to other products and used only within one manufacturing plant, and some very low-production specialty items. And Dr. Bannerman brought the ACS a special message from Washington: "Don't let the PMN requirements limit your creative spirit in the development and commercialization of new chemicals."

But J. Clarence Davies of the Conservation Foundation is not entirely comfortable with these initiatives. The long latency period of chronic effects from chemicals means that the pre-Reagan PMN system simply hasn't been proven; even if a dangerous chemical has slipped past EPA's sieve, we wouldn't know it yet.

EPA's other job under TSCA is to study the 55,000 existing chemicals already in production for possible hazards—clearly a prodigious effort that is beyond any reasonable resources EPA might have had even during the Carter administration. That enterprise has now been largely abandoned as just too ambitious.

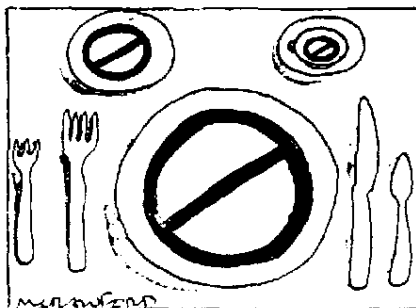
In doing that, Dr. Davies told the ACS, the EPA "surrendered what should be one of the main benefits of TSCA—the opportunity to comprehensively review the universe of commercial chemicals." But he lamented that this retrenchment might be necessary. "Under Reaganomics, asking EPA to look for new chemical problems is akin to complaining about the lack of good French restaurants in San Salvador. If survival is in question, some desirable goals must be sacrificed."—J.M. □

URL 03970

Regulating Food: Cancer on Your Plate?

U.S. food safety laws mandate "zero risk," in contrast to risk-benefit comparisons required by the Toxic Substances Control Act (see above). The Delaney Clause says that no food product can be sold in the United States if it contains a measurable quantity of any known carcinogen.

What is a "measurable quantity"? That depends on when you ask, and of whom, for methods of chemical analysis are constantly becoming better. Analysts are able to identify ever smaller quantities of pollutants in a food, down to parts per billion or even trillion.



It was a somewhat different world when Representative James J. Delaney first heard in 1950 the judgment of Dr. W.C. Heuper, chief of the Environmental Cancer Section of the National Institutes of Health: "I do not believe that one can establish a safe dose of carcinogens. . . . It would be a wise precautionary measure not to add any chemicals to our food supply that produce cancer either in man or in experimental animals." Thus, the Delaney Clause was born.

All this explains why major revisions in the Food Additive Amendments of 1958 to

the Food, Drug, and Cosmetic Act are now before Congress—and why members of the American Chemical Society devoted so much of their 1982 spring meeting to the issue.

The issue arises in part because of the increasing use of plastics in food packaging—and the fact that minute quantities of the polymers in the containers migrate to their contents. It's now possible to detect such migration, and so the offending containers are outlawed under present law. But Howard R. Roberts, former acting director of the Bureau of Foods in the Food and Drug Administration (FDA) who is now with the National Soft Drink Association, told the ACS that many of these constituents "occur at levels so minute as to be toxicologically insignificant."

He joined Michael R. Taylor, formerly a lawyer for the FDA, in appealing for a change. "The public health benefit derived from closely regulating such minute amounts of substances is frequently outweighed by the tremendous resources being expended—by government and industry—in chasing the elusive zero," said Mr. Taylor.

That search for the "elusive zero" can be expensive. William Horwitz, acting director of FDA's Science Policy Staff, described a new method for determining the dioxins in fish down to a level of about 50 parts per trillion—at a cost of about \$1,000 per determination.

And then there's the problem of accura-

cy. Dr. Horwitz cited analyses of identical materials by different laboratories that varied by 55 percent at the 40-parts-per-billion level.

The problem of aflatoxins—the carcinogenic molds sometimes found on corn and peanuts—provides another example of the difficulties, expressed by one participant: "If you can see a carcinogen, it will hurt you; but if you can't see it, it won't."

When analytical chemists could detect aflatoxins at the level of 20 parts per billion in consumer products such as peanut butter, the FDA lowered the limit accordingly. But by now it's possible to detect 1 part per billion of aflatoxin in peanut butter, and enforcing that limit would in some years foreclose marketing as food nearly half of the nation's peanut butter, said Joseph V. Rodricks, vice-president of Cleiment Associates, Inc., who was associated with FDA for 13 years.

Accordingly, FDA has decided with some uneasiness to base its regulatory decision not only on analytical detectability but "on the technical capability of manufacturers to control the aflatoxin content of their products," said Dr. Roberts. The question of risk is not dealt with explicitly.

Two bills now in Congress would change all this. Senator Orrin Hatch would require food manufacturers to tell the FDA in advance what chemicals, and how much of them, would be in new food products, and they would be asked to provide appropriate toxicological data. Ninety days later the

manufacturer would be free to market the product unless FDA had objected.

A different approach, sponsored by Representative Albert Gore, would permit food impurities in "de minimus amounts." Representative Gore doesn't define *de minimus*, but Mr. Taylor says the implication is clear: "*de minimus* refers to an amount of a substance that, taking into account what is known about [its] toxicity . . . poses risks that are so small or so improbable that they can reliably be said to be *de minimus*, or simply not worthy of government concern."

But the question of risk, absent in today's "technical-feasibility" criteria, opens the issue to personal rather than legal or scientific decisions. Judith Rae Brunton of the Washington law firm of Hyman and Phelps, specialists in food, drug, and cosmetic law, told the ACS that "there is no single acceptable risk level for all foods among all consumers." If the "technical-feasibility" criteria are abandoned, "one of the choices among the acceptable levels of risk—no risk—has been eliminated." In that case, she warned, the public will turn to the scientific community "to identify and quantify the risks that must be accepted." And so far, "Science hasn't been able to answer those questions."

Ms. Brunton concluded that the present controversy is merely "the opening argument in what promises to be a long debate about fundamental social change."—J.M. □

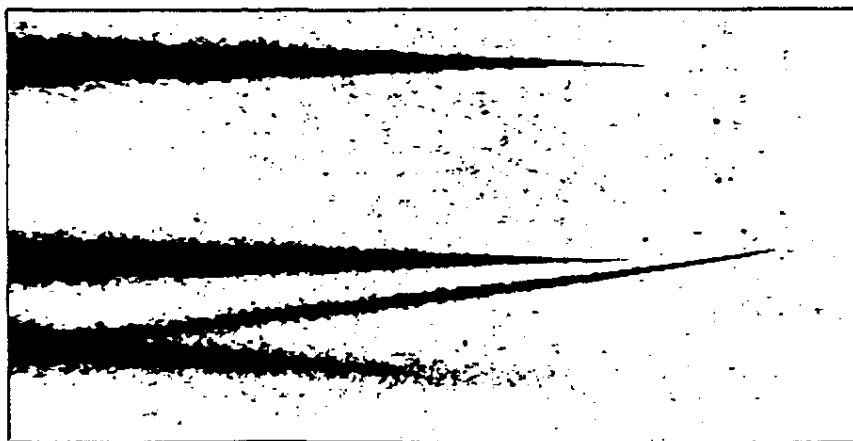
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World's Fastest Uranium

There was jubilation when final confirmation came at 6:00 P.M. Tuesday, May 11. A giant atom smasher at the University of California's Lawrence Berkeley Laboratory—the Bevalac—had become the world's first machine to accelerate uranium particles to nearly the speed of light.

Long a goal of nuclear scientists, this feat provides a vast new capability for exploring the fundamental properties of matter under the extreme conditions of heat and pressure created when nuclei of heavy elements collide. "These collisions," says Herman Grunder, director of LBL's accelerator division, "will momentarily create concentrations of neutrons and protons far bigger, hotter, and denser than any seen previously."

The achievement results from \$6 million



Tracks left by some of the first uranium nuclei ever to be accelerated to nearly the speed of light. The dark streaks are the last one-half millimeter of three tracks as they came to rest in a special

photographic emulsion. The bottom track shows a nucleus splitting into two lighter nuclei. The work was done at the Bevalac located at the University of California's Lawrence Berkeley Laboratory.

Both species face a common enemy at high frequency, (ii) the mechanism by which they compete displays density-dependent effectiveness, and (iii) the two species harm each other less than either is harmed by the common enemy. That this suggestion is sufficiently general to accommodate cooperation between species is supported by observations of plant defense guilds (9), aggregations of tube-building polychaetes (10), associations of hydroids and bryozoans (11), and my own observation that *B. simplex*, a treelike congener of *B. turrita*, settles preferentially into dense stands of young *B. turrita* colonies (12).

I have shown here that a density dependence in interference competition can lead to group living and that the formation of groups in a sessile organism necessitates intraspecific cooperation. Yet cooperation and competition are generally viewed as being virtually opposite extremes of organism interactions. My results suggest the reverse. In certain cases, interspecific competition may provide the very selective pressures that lead to the evolution of cooperation.

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3. Fertile colonies were collected from Eel Pond, Woods Hole, Mass., and induced to release their larvae by light shock. A minimum of 100 larvae were released into clean Nalgene containers. Each container was covered with a piece of plastic mesh (Vexar) divided into six equalized (100 cm²) regions. In each region colonies of *B. turrita* were attached in different densities in a modified Latin Square by looping the stalks through the holes in the mesh. After a period of 6 to 8 hours, the mesh was removed and the number of larvae settled in each region was counted.
4. Colonies that had previously settled on Vexar mesh were attached to a new (10 by 10 cm) Vexar substratum with cable ties and monofilament line. Attachment by monofilament failed in some instances, resulting in the loss of some colonies. Only one loss, however, could not be unambiguously attributed to a failure in the attachment method. All weight gains are expressed as averages of those colonies that remained attached.
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6. Clearly the evolution of group living will be dependent upon the frequency at which interference competition occurs.
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Allee was aware of such risks although he did not incorporate them in his definition of cooperation; for example, see "the costs of under-crowding" [W. C. Allee, *The Social Life of Animals* (Norton, New York, 1939), p. 293, figure 2]. The gregariousness of *B. turrita* is a cooperative act under either definition.
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13. I thank R. Grosberg, G. E. Hutchinson, J. B. C. Jackson, B. Keller, N. Knowlton, J. Moore, C. Wahle, and J. Wulff for technical assistance or comments (or both). Supported by a Steps Toward Independence fellowship from the Marine Biological Laboratory, Woods Hole, Mass.

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Milk of Dairy Cows Frequently Contains a Leukemogenic Virus

Abstract. Milk or viable milk cells collected from 24 dairy cattle naturally infected with bovine leukemia virus were inoculated into lambs, which were subsequently examined for the development of infection. With this bioassay, infectious virus was demonstrated in the milk of 17 of the cows. Bovine leukemia virus is leukemogenic in at least two mammalian species, is widespread in commercial dairy herds, and can infect a wide range of hosts in vivo and cells, including human cells, in vitro.

Bovine leukemia virus (BLV) is a horizontally transmitted, probably insect-borne oncornavirus that differs from the leukemia viruses of other species in several important ways (1, 2). In vivo, BLV is usually present in the lymphocytes in a covert, nonproductive state; the infected lymphocytes do not show viral RNA, viral antigens, or viral particles unless they are grown in vitro for a few hours (2-4). BLV is universally regarded as the

causative agent of the adult enzootic form of bovine leukemia (lymphosarcoma), the most common fatal malignancy of dairy cattle (1). Under experimental conditions BLV infects sheep (5, 6), goats (7), and apparently chimpanzees (8). BLV-infected sheep frequently develop leukemia (5, 6). There is evidence that BLV can cross species barriers under natural conditions and infect both sheep and capybaras (9). In vitro, BLV infects cells of various origins, including human and simian cells (10, 11).

More than 20 percent of the dairy cows and approximately 60 percent of the herds surveyed in the United States were infected with BLV (1). Bovine leukemia virus infection is also prevalent among cattle of most other countries studied. Thus the question of whether dairy cows naturally infected with BLV release infectious virus into milk is an important public health consideration.

Several in vitro infectivity assays for BLV have been developed, but they are not suitable for detecting the virus in milk, mainly because of the composition and frequent bacterial content of this secretion. Sheep provide an alternative means for determining the presence of BLV in milk because they are highly susceptible to BLV infection and seem to resist the bacteria present in various bovine secretions and excretions (12). In a preliminary experiment, BLV was demonstrated in one sheep injected with the milk of one naturally infected cow (12).

We used the sheep bioassay to determine the frequency with which infectious BLV is released into the milk of cows naturally infected with BLV. In our first experiment, BLV-free lambs

Table 1. Detection of BLV in lambs inoculated with whole milk from naturally infected cows. Each milk sample was injected intraperitoneally into one or, in most cases, two lambs less than 7 days old. The lambs were subsequently examined for the presence of BLV antibodies by means of the immunodiffusion test with BLV glycoprotein antigen (23) and, in most cases, for the presence of infectious BLV by means of the syncytia induction assay (14). Symbols: +, one or both sheep positive for BLV 12 or more months after injection; -, sheep negative for BLV.

Milk donor	Day of lactation on which milk was collected		
	10	30	50
G-245	+	-	
BF-157	+	-	
BF-138	+	-	
G-142	-	+	
G-266	-		
BF-269	-		
BF-291		+	
G-255		-	
G-189		-	
G-43		-	+
G-257		-	-
G-256			+
G-263			-
G-24			+
G-265			-
G-198			-

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were injected with fresh milk (100 ml) collected from 16 BLV-infected cows belonging to a dairy herd (13) maintained at the University of Pennsylvania (New Bolton Center) and to a commercial dairy herd. The milk from six cows was assayed on two different lactation days, but, owing to the short lambing season, recipient sheep of appropriate age were not available for use in assaying milk from any one cow more frequently. Seven lambs injected with milk samples from a BLV-free cow and seven lambs that were not inoculated served as controls. All lambs were obtained from the same BLV-free flock and were raised in the same facilities. Transmission of BLV from sheep to sheep has not been observed (5, 6).

As shown in Table 1, the injection of milk from 8 of the 16 infected cows induced BLV antibodies in one or both recipient lambs. These antibodies were usually detected 8 months after inoculation and persisted at least until the sheep were 12 to 16 months old. In no instance was the milk from a cow positive for BLV on more than one of the lactation days. In several instances only one of a pair of sheep inoculated with a milk sample developed BLV antibodies. It appears, therefore, that the quantity of infectious BLV in 100 ml of milk is close to the sheep bioassay's limit of sensitivity. If this is the case, it cannot be determined whether the failure to detect BLV in milk of an infected cow at successive lactation dates is due to intermittent release of the virus or to a constant release at low levels.

The persistence of the antibody response of the recipient sheep indicated that the response was due to infection rather than to immunization by virion antigens in the milk inoculum. Furthermore, infectious virus was detected in the blood lymphocytes of six of nine antibody-positive sheep examined with the syncytia induction assay for BLV (14). None of the control sheep showed BLV antibodies or infectious BLV during the 16 months of observation.

In cattle, BLV seems to infect only the lymphocytes, and BLV-infected lymphocytes do not usually synthesize BLV particles *in vivo* (2-4). We therefore assumed that the infections in milk-injected sheep are due mainly to the presence in milk of BLV-infected lymphocytes rather than to free extracellular virus. Thus, in an attempt to increase the sensitivity of the sheep bioassay, we conducted a second experiment in which lambs were injected with viable cells isolated from milk rather than with whole milk. Of the nine infected dairy cows used as

donors, only one (BF-138) had been used in the first experiment. As shown in Table 2, milk cells collected from the cows at one or more of three sampling times induced BLV infection or BLV antibodies in the recipient lambs. Most of the sheep developed antibodies before their lymphocytes became positive for BLV. However, by 8 to 10 months after inoculation there was a close correlation between the presence of antibodies and the presence of BLV. There were no significant differences among lactation days in the frequency with which BLV-infected cells were present in the cows' milk. Nine sheep injected with lymphocytes from BLV-free cows and eight uninjected sheep housed with the infected sheep remained negative for BLV for at least 16 months.

In most instances, BLV was detected in both sheep inoculated with milk cells

from a BLV-positive cow (Table 2). In contrast, only half of the infected whole-milk samples in the first experiment induced BLV infection in both recipient lambs. Moreover, whereas most of the sheep inoculated with milk cells became infected within 3 months, BLV was usually detected only after 8 months in the sheep inoculated with whole milk. Thus the sensitivity of the sheep bioassay was indeed greatly increased when milk cells were used as the inoculum. It is likely that in the first experiment the percentage of cows identified as having infectious BLV in their milk would have been greater had milk cells rather than whole milk been assayed.

The fact that infectious BLV or BLV-infected cells are present in the milk of most naturally infected cows does not necessarily mean that calves become infected with BLV by the oral route. Some

Table 2. Detection of BLV in lambs inoculated with milk cells from naturally infected cows. The cells were isolated by centrifugation, washed twice, and injected subcutaneously (10^6 viable cells per lamb); N.D., not determined.

Milk donor	Day of lactation	Recipient sheep	3 to 4 months after inoculation		8 to 10 months after inoculation	
			BLV antibodies*	BLV†	BLV antibodies	BLV
G-4182	4	14	+	+	+	+
G-149	1 to 2	23	+	-	N.D.	N.D.
		24	+	+	+	+
	4 to 6	3	+	-	+	+
		60	+	-	+	-
G-312	10 to 13	54	+	-	+	+
		62	+	-	+	+
	1 to 2	64	+	-	+	+
		18	+	-	+	+
G-314	4 to 6	55	+	-	+	+
		59	+	-	+	+
	10 to 13	65	+	+	+	+
G-281	1 to 2	52	-	-	+	+
		67	-	-	-	-
BF-138	4 to 6	57	-	-	-	-
		66	-	-	-	-
	1 to 2	53	+	-	+	+
BF-283	4 to 6	63	+	-	+	+
		8	+	+	+	+
	10 to 13	15	+	+	+	+
		19	+	-	+	+
BF-306	1 to 2	7	+	+	+	+
		9	-	+	+	+
	4 to 6	11	+	+	-	+
		16	+	+	+	-
BF-316	10 to 13	12	+	+	+	+
		22	+	-	+	+
	1 to 2	51	+	-	+	-
BF-316	4 to 6	58	+	-	+	+
		4	+	N.D.	N.D.	N.D.
	1 to 2	2	+	+	N.D.	N.D.
BF-316	10 to 13	1	+	+	N.D.	N.D.
		10	+	+	+	+
BF-316	10 to 13	6	+	+	+	+

*As determined by the immunodiffusion test with BLV glycoprotein antigen. †As determined by the competitive radioimmunoassay for BLV p25 (24). Peripheral blood lymphocytes were cultured for 48 hours with phytohemagglutinin (3), washed by centrifugation, resuspended in buffer (0.02M tris, 0.1M NaCl, 0.001M EDTA (pH 7.5), 0.5 percent Nonidet P40, 0.2 percent sodium deoxycholate, and 2-phenylmethylsulfonyl fluoride) to a final concentration of 3×10^6 cells per milliliter, and frozen and thawed three times. After incubation at 37°C for 15 minutes, the extract was clarified by centrifugation and tested with the competitive radioimmunoassay. An extract was considered to be positive for BLV when 40 μ l (equivalent to 10^6 lymphocytes) displaced more than 20 percent of the labeled antigen.

researchers in Europe have concluded that bovine leukemia, and therefore BLV, is transmitted via milk (15, 16). However, owing to the experimental design and methodology used, the data on which this conclusion was based do not indicate whether the calves became infected prenatally, by milk ingestion, or by contact. Studies conducted under appropriate experimental conditions have shown that milk-borne transmission of BLV, as compared with contact transmission, is rare if it occurs at all (17, 18). The resistance of calves to milk-borne infection with BLV is probably due mainly to the maternal virus-neutralizing antibodies that all calves nursed on BLV-positive dams acquire through colostrum (17-19).

The present study and the data on the prevalence of BLV infection in dairy herds indicate that humans are often orally exposed to BLV. Although the infectivity of BLV is apparently destroyed by pasteurization (20), people in many countries consume unpasteurized milk. Moreover, it is not known whether pasteurization destroys the biological activity of the proviral BLV DNA in the infected cells of milk. There is no evidence that BLV can infect humans, but neither do the data exclude this possibility. Although attempts to demonstrate BLV antibodies, particles, and antigens in humans have been negative (1), the findings are not conclusive because of the limited sensitivity of the assays used and because some BLV-infected cells do not synthesize virus particles or viral antigens (2-4, 10, 11). Molecular hybridization studies have failed to demonstrate BLV-related sequences in human tumors (21), but only a few tumors were examined and the probe used was only partially representative of the viral genome. While earlier epidemiological surveys showed no association between human and bovine leukemia (1), the most recent survey, involving a large number of cases, showed a statistically significant increase in human acute lymphoid leukemia in areas with a high incidence of bovine leukemia and BLV infection (22). Clearly, the question of whether BLV poses a public health hazard deserves thorough investigation with the most sensitive virological and immunological techniques available, particularly highly representative molecular probes.

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Emergence of Posttetanic Potentiation as a Distinct Phase in the Differentiation of an Identified Synapse in *Aplysia*

Abstract. The developmental time course of posttetanic potentiation was studied at an identified chemical synapse. In stage 11 juveniles (3 weeks after metamorphosis), the synaptic connections made by cholinergic neuron L_{10} onto postsynaptic neurons L_2 to L_6 were present but showed no posttetanic potentiation. In stage 13 adults (12 weeks after metamorphosis), the same tetanus resulted in an increase of 300 percent in the synaptic potential. A similar pattern was observed at two other identified synapses in the abdominal ganglion. Thus, the initial steps in synapse formation do not include the expression of this plastic capability. Rather, at least 10 weeks is required between the onset of synaptic function and the final expression of mature synaptic properties.

Many chemical synapses in the adult animal can be altered for long periods as a result of previous stimulation. It is important in the study of neural differentiation to determine whether the capability for plastic change is part of the machinery that is present in the initial establishment of a functioning synapse, or whether these capabilities represent separate regulatory processes that emerge independently later in development.

Although the initial development and maintenance of synapses have been studied extensively (1-4), little is known about how neurons acquire plastic capabilities. Knowledge about the development of synaptic plasticity may help to elucidate the mechanisms of normal behavioral maturation, as well as the mechanisms that contribute to disorders in perception and motor coordination that occur when animals are reared in abnormal perceptual or motor environments (5). We examined posttetanic potentiation (PTP), a common form of plasticity, at an identified chemical synapse in the abdominal ganglion of the marine mollusk *Aplysia californica*. We found that

PTP develops as a late step in the maturation of the synapse and is independent of transmission. The gradual emergence of PTP at an identifiable synapse provides an opportunity for studying the detailed mechanisms underlying this form of synaptic plasticity.

We used laboratory-reared animals at various stages of development, from stage 11 juveniles weighing 3 mg at 3 weeks after metamorphosis to reproductively mature, stage 13 adults weighing 100 g or more at 12 weeks after metamorphosis (6). We examined a specific, identified inhibitory synapse between presynaptic neuron L_{10} and one of its five identified follower cells (L_2 to L_6) located in the rostral quadrant of the left abdominal ganglion (7). Even in the youngest animals studied, both the pre- and postsynaptic neurons were clearly identifiable and large enough (15 μ m in diameter in animals weighing 3 mg) to permit successful intracellular recordings. To obtain reliable postsynaptic potentials, we hyperpolarized the follower cells to at least 50 mV below the reversal potential of the inhibitory postsynaptic

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DIET, NUTRITION, AND CANCER: INTERIM DIETARY GUIDELINES

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ABSTRACT—The Committee on Diet, Nutrition, and Cancer of the National Academy of Sciences recently evaluated the role of diet in carcinogenesis. Both epidemiological and laboratory evidence suggests that a high intake of total fat increases susceptibility to cancer of different sites, particularly the breast and colon. In epidemiological studies frequent consumption of certain fruits and vegetables and in laboratory experiments some components of fruits and vegetables, especially cruciferous vegetables, appear to decrease the incidence of cancers at various sites. In contrast, frequent consumption of salt-cured, salt-pickled, or smoked foods, possibly because they may contain nitrosamines or polycyclic aromatic hydrocarbons, appears to increase the risk of esophageal or stomach cancer. Excessive alcohol consumption among smokers appears to be associated with an elevated risk of cancers of the oral cavity, esophagus, larynx, and respiratory tract. Interim dietary guidelines to reduce the risk of cancer were proposed in accordance with these conclusions. No definitive conclusions were reached for other dietary factors, including total calories, cholesterol, fiber, and selenium, nor could the quantitative contribution of diet to overall cancer risk be estimated.—JNCI 1983; 70:1151-1170.

INTRODUCTION

On June 16, 1982, a committee of scientists¹ appointed by the National Research Council-National Academy of Sciences (the Academy) and operating within the Assembly of Life Sciences completed an analysis of the literature concerning the role of diet in the etiology and prevention of cancer.² This article summarizes the basis for the major conclusions and recommendations of the committee's report "Diet, Nutrition, and Cancer" (1).

The search for the causes of cancer has been an important branch of cancer research. Considerable effort has been devoted to studying the influence of both environmental and genetic factors on the incidence of cancer. In the course of this research, it became clear that most cancers have external causes and in principle, therefore, should be preventable. For example, blacks and Japanese residing in the United States develop the spectrum of cancers that is typical for the United States but different from that in Africa or Japan.

Epidemiologists have demonstrated a correlation between diets consumed in modern affluent societies and the incidence of cancers in such organs as the breast and colon. However, to establish causal relationships and to determine which, if any, of the dietary components is responsible have proved to be difficult. Similar difficulties are encountered in laboratory experiments. Like humans, most animals have a significant incidence of cancer in old age, and the rates of these cancers often tend to be affected by changes in diet. However, the influence of diet on spontaneous and experimentally induced cancers is not easily investigated, partly because the underlying mechanisms and molecular biology of the cancers still are not fully understood. Indeed, the effects of diet were often regarded as a nuisance, i.e., yet another variable standing between the investigators and their measurement of carcinogenicity. As a consequence, researchers only recently have returned to the study of diet as a factor in carcinogenesis.

Despite these difficulties, considerable research effort has been devoted in the last 3-4 decades to understanding whether and how dietary factors affect carcinogenesis. In June 1980, the National Cancer Institute (NCI) commissioned the Academy to evaluate the implications of this research. Lack of consensus among scientists about the interpretation of the research data, and as a consequence heightened confusion and concern among the public and the Congress about the implications of this knowledge for public health, served as a major impetus for the Academy's study.

¹ The Committee on Diet, Nutrition, and Cancer is chaired by Clifford Grobstein. Vice-chairman is John Cairns. Members of the committee are Robert Berliner, Selwyn Broitman, T. Colin Campbell, Joan D. Gussow, Laurence N. Kolonel, David Kritchevsky, Walter Mertz, Anthony B. Miller, Michael J. Prival, Thomas Slaga, and Lee Wattenberg. Takashi Sugimura serves as an adviser to the committee.

² This work was conducted pursuant to Public Health Service contract N01CP-05603 from the Division of Cancer Cause and Prevention, National Cancer Institute.

The Academy established a multidisciplinary 14-member panel, the Committee on Diet, Nutrition, and Cancer, to address NCI's request to 1) "review . . . the state of knowledge and information pertinent to diet/nutrition and the incidence of cancer," 2) "develop a series of recommendations related to dietary components (nutrients and toxic contaminants) and nutritional factors which can be communicated to the public," and 3) "based on the above state-of-the-art appraisals and the identification of gap areas, develop a series of research recommendations related to dietary components and nutritional factors and the incidence of cancer."

The committee's 500-page report summarizes scientific evidence concerning nutritive and nonnutritive dietary factors in carcinogenesis and offers to the public several interim dietary guidelines to reduce the risk of cancer.

THE SCOPE OF WORK AND THE APPROACH TO EVALUATING THE LITERATURE

Scientifically valid data on diet and nutrition in relation to cancer are provided by three major sources: epidemiological studies on human populations, experimental studies on animals, and *in vitro* tests for genetic toxicity. However, there are a number of limitations to the interpretation of data from each type of study. The strengths and weaknesses of epidemiological studies, of dietary methodology used in epidemiological studies, and of laboratory experiments to study the relationship between dietary factors and carcinogenesis are discussed in the report (1).

Epidemiological studies of the association between diet and cancer have examined many common forms of cancer, although most research has been directed to cancers of the gastrointestinal tract and the breast and other tissues susceptible to hormonal influence and less research has been directed to cancers of the respiratory tract and urinary bladder. The committee examined the evidence for each of these sites.

In the laboratory, investigators have attempted to shed light on the mechanisms by which diet may influence carcinogenesis. In this pursuit, they have examined the ability of individual nutrients, food extracts, or nonnutritive components of food to enhance or inhibit carcinogenesis and mutagenesis, thereby providing epidemiologists with testable hypotheses regarding specific components of the diet. Because the data from both epidemiological and laboratory studies are generally grouped according to dietary constituents or cancer sites, the committee decided to organize its report in a similar fashion, focusing on all major nutritive and nonnutritive constituents of the diet and on each major site.

Although the committee considered the evidence from all types of epidemiological studies, it had the most confidence in data derived from case-control studies and from the few cohort studies that have been reported. Instead of relying on aggregate correlation data, these studies are based on the collection and analysis of data on individuals, and the investigators attempt to control for confounding variables. Thus these studies provide more definitive evidence indicative of meaningful associations than do data derived from

correlation and descriptive studies. Greater emphasis was given to the results of case-control or cohort studies that were designed to examine a specific hypothesis. Because most epidemiological studies suffered from methodological weaknesses, the consistency of results among studies of different types and in different settings assumed particular importance in the evaluation of the epidemiological evidence. In evaluating laboratory evidence, the committee placed more confidence in data derived from studies on more than one animal species or test system, in results that have been reproduced in different laboratories, and in the few data that indicated a dose response.

Overall, the strength and preponderance of data and the degree of concordance between the epidemiological and laboratory evidence determined the certainty of the conclusions in the report. Concurrence between epidemiological and laboratory evidence was considered a prerequisite to proposing dietary guidelines to the public.

MAJOR CONCLUSIONS AND INTERIM DIETARY GUIDELINES

Overall, the committee concluded that "the differences in the rates at which various cancers occur in different human populations are often correlated with differences in diet. The likelihood that some of these correlations reflect causality is strengthened by laboratory evidence that similar dietary patterns and components of food also affect the incidence of certain cancers in animals."

This general statement was prefaced with the warning that it is not yet possible to be absolutely certain about some of the associations between diet and cancer because scientists have yet to attain a complete understanding of how diet influences carcinogenesis. The committee likened the current state of knowledge on this subject to the knowledge on cigarettes and lung cancer approximately 20 years ago. However, the evidence was judged to be sufficiently convincing to propose interim dietary guidelines regarding total fat, fruits, vegetables, and whole grain products; salt-cured, salt-pickled, and smoked foods; and alcohol. In addition, some research and regulatory recommendations were proposed concerning food additives, contaminants, and mutagens. A discussion of the major conclusions follows the six interim guidelines presented below.

Interim Guidelines

1) ". . . the committee recommends that the consumption of both saturated and unsaturated fats be reduced in the average U.S. diet. An appropriate and practical target is to reduce the intake of fat from its present level (approximately 40%) to 30% of total calories in the diet. The scientific data do not provide a strong basis for establishing fat intake at precisely 30% of total calories. Indeed, the data could be used to justify an even greater reduction. However, in the judgment of the committee, the suggested reduction (i.e., one-quarter of the fat intake) is a moderate and practical target, and is likely to be beneficial."

2) "The committee emphasizes the importance of including fruits, vegetables, and whole grain cereal products in the

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daily diet." However, the committee cautioned against the use of high and potentially toxic doses of nutrient supplements.

3) "The committee recommends that the consumption of food preserved by salt-curing (including salt-pickling) or smoking be minimized."

4) "The committee recommends that efforts continue to be made to minimize contamination of foods with carcinogens from any source. Where such contaminants are unavoidable, permissible levels should continue to be established and the food supply monitored to assure that such levels are not exceeded. Furthermore, intentional additives (direct and indirect) should continue to be evaluated for carcinogenic activity before they are approved for use in the food supply."

5) "The committee recommends that further efforts be made to identify mutagens in food and to expedite testing for their carcinogenicity. Where feasible and prudent, mutagens should be removed or their concentration minimized when this can be accomplished without jeopardizing the nutritive value of foods or introducing other potentially hazardous substances into the diet."

6) "The committee recommends that if alcoholic beverages are consumed, it be done in moderation."

These interim guidelines were presented with the proviso that "it is not now possible and may never be possible to specify a diet that would protect everyone against all forms of cancer." However, the guidelines are likely to reduce the risk of cancer, and they concur with good nutritional practices and with dietary recommendations issued by other organizations concerned with public health, e.g., the American Heart Association (2), the NCI (3), and the U.S. Department of Agriculture and the U.S. Department of Health, Education, and Welfare (4).

Lipids (Fats and Cholesterol): Basis for Conclusions

Evidence supporting an association between fat intake and cancer is derived both from epidemiological studies and laboratory experiments.

Epidemiological evidence: fats.—Epidemiological studies have repeatedly shown an association between dietary fat and the occurrence of cancer at several sites, but most of the evidence pertains to cancers of the breast, colon, and prostate gland.

Breast cancer is known to be affected by hormonal status, but diet has also been suggested as a contributory factor (5). Several international correlation studies have shown strong direct associations between per-capita fat intake and breast cancer incidence or mortality rates (6–10). In general, the correlations in these studies were higher for total fat than for other dietary factors, such as total protein, animal protein, and specific fat components. Intracountry data sets in the continental United States, England, and Hawaii have generally supported these findings (11–13). For example, on the basis of dietary interview data from a population survey in Hawaii, Kolonel et al. (13) correlated ethnic sex-specific consumption of fat with corresponding patterns of breast cancer incidence and found significant associations for total fat, animal fat, and both saturated and unsaturated fats.

The findings of three case-control studies and one cohort study, in which husbands of wives with and without breast

cancer were interviewed, further support a role for dietary fat in the risk for breast cancer (14–17).

An increased risk of *large bowel cancer* has also been associated with higher fat intake in both correlation and case-control studies. Correlations of international incidence and mortality rates for large bowel cancer in relation to dietary variables suggest that colon cancer, and to a lesser extent rectal cancer, are strongly associated with total dietary fat (6, 10). In contrast to the strong correlations observed in international studies, there is a striking lack of a good correlation within countries. For example, Bingham et al. (18) calculated average intakes of nutrients by populations in different regions of Great Britain and found no significant association between high fat intake and mortality from colon and rectal cancers. Lyon and Sorenson (19) also reported little difference in fat intake between the population of Utah (with a low risk for colon cancer) and that of the United States as a whole. One possible explanation for this discrepancy is that there may be insufficient variability in food intake patterns within a country to demonstrate a strong association with the risk of colon or rectal cancer. In contrast, the variation in fat intake among countries is much greater, and so the overall association is much stronger.

Several case-control studies have shown a direct association between frequent consumption of high-fat foods, total fat intake, especially saturated fat, and colon cancer (14, 20–22). Jain et al. (22), for example, reported a gradient in response between the risk of both colon and rectal cancers and the level of dietary fat, especially saturated fat.

Several investigators also found a direct association between meat consumption (meat is an important source of fat, especially saturated fat) and colon cancer (23–25). However, these results were not reproduced in other studies (26–28).

Some of the conflicting data on fat and colon cancer may be explained by the possible confounding effects of dietary fiber, which has been suggested as exerting a protective effect. The incidence of colon cancer in Denmark and New York is higher than in Finland, although the per-capita fat intake of these three populations is similar. In contrast, the intake of fiber-containing foods among Finns appears to be higher than in the other 2 groups (29, 30). In an ongoing cohort study in Minnesota, Bjelke (26) has found a reduced risk of colorectal cancer in subjects with a high index of vegetable consumption. (Vegetables are good sources of fiber and micronutrients.) However, no such effect has yet been observed in a parallel cohort in Norway (26). Some investigators have suggested that the protective effect of dietary fiber may be due to its effect on adsorption, dilution, or metabolism of carcinogens or promoters or to decreased fecal transit times and thus decreased contact between carcinogens and the colon mucosae (30, 31). However, contrary to many reports, differences in fecal transit times due to differences in fiber intake have not been consistently reported (32, 33).

The data on *prostate cancer* are limited. However, several correlation studies indicate an association between high fat intake and increased prostate cancer incidence and mortality rates (6, 13, 34–36). The results of two case-control studies also support these findings (37, 38). International

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incidence and mortality data also indicate that there are significant correlations between prostate cancer and cancers of other sites associated with a high-fat diet, including cancers of the breast, colon, and corpus uteri (34, 39, 40). However, there are important exceptions; e.g., the Mormons in Utah have a high incidence of prostate cancer but a low incidence of breast cancer (41).

Other cancer sites that have been associated with dietary fat include testis, corpus uteri, ovary, and pancreas (6, 13, 36, 42, 43).

The epidemiological data relating fat intake to different cancers are not entirely consistent, and the data for breast and prostate cancer are more consistent than for colon cancer. In the case of breast cancer, the magnitude of the association appears greater in the correlation data than in the case-control studies; e.g., the results of the most thorough case-control study yet reported by Miller et al. (15) were only weakly positive, perhaps partly reflecting the fact that recent dietary intake was assessed, whereas dietary patterns much earlier in life may have had a greater influence on breast cancer risk (44). Studies of changing breast cancer incidence among Japanese migrants to Hawaii and their descendants support this hypothesis (45). The data for colon cancer also are not altogether consistent. Some studies of large bowel cancer may have failed to demonstrate an association with dietary fat, either because they focused on relatively homogeneous populations or because they were not specifically designed to examine the association of fat intake with cancer. Indeed, the studies that were designed specifically to test this hypothesis tended to show the most striking direct associations, especially when the possible confounding effects of dietary fiber were taken into consideration (20, 22, 29, 30).

Another difficulty is distinguishing clearly between the effect of protein and fat in epidemiological studies. Several of the same international correlation studies and case-control studies that have shown an association between high dietary fat intake and cancer at several sites have also suggested possible associations between high intake of total protein or animal protein and increased risk of cancers at a number of different sites, including the breast (6, 8-10, 12, 13, 15), large bowel (6, 22, 46), pancreas (6, 42), prostate gland (13, 36), and endometrium (6, 13). However, the data for protein, especially from analytical studies, are much more limited than for fats. In addition, the more consistent and often stronger association of these cancers with fat intake makes it likely that dietary fat is the more active component. Nevertheless, because of the very high correlation between fat and protein intake in Western diets, the evidence does not completely preclude an independent effect of protein.

Epidemiological evidence: cholesterol.—A relationship between dietary or serum cholesterol and cancer, especially colon cancer, has been reported by many investigators. Most of these studies were designed to examine the association between serum cholesterol and cardiovascular disease.

Several prospective cohort studies and some intervention trials indicate an inverse association between serum cholesterol and cancer, usually mortality from colon cancer in males but not in females (47-55). However, in general, in

the intervention trials, low cholesterol levels per se do not appear to be associated with increased mortality from cancer (54, 55). Elimination of early cases of cancer in some of the cohort studies suggests that the lower serum cholesterol levels may be the result of metabolic change accompanying tumor growth rather than the cause of the disease (52, 56, 57). The only case-control study (58) designed specifically to examine the relationship of serum cholesterol to cancer reported a significant inverse association between serum cholesterol levels among controls and male cases with advanced tumors only; the study, therefore, supports the hypothesis that lower serum levels may be due to metabolic changes associated with tumor growth. However, in other studies, the inverse association, especially with colon cancer, persisted despite adjustment for this possibility (48-50). Five cohort studies and three intervention trials demonstrated no association between serum cholesterol and cancer (59-62). Dietary and serum cholesterol levels are frequently not well correlated in individuals, and dietary cholesterol has usually been associated directly with colon cancer (22, 63).

Experimental evidence: fats.—The first demonstration that fat could influence tumorigenesis was reported in 1930 by Watson and Mellanby (64). Since then, numerous experiments have been conducted in which different levels and components of fat were used to study their effects in various tissues and animal species.

In studies of *mammary carcinogenesis*, the influence of lipid nutriture on tumorigenesis appears to follow a consistent pattern, regardless of whether the tumors are chemically induced, occur spontaneously, or result from tumor cell implantation.

Tannenbaum (65) demonstrated that high dietary fat enhanced the development of either spontaneous or chemically induced mammary tumors in mice. Tannenbaum and Silverstone (66) noted that tumor incidence was greater in obese mice than in normal mice and that caloric restriction inhibited mammary tumorigenesis in normal mice. Feeding mice isocaloric high- and low-fat diets provided evidence that fat rather than calories per se was responsible for enhancing tumorigenesis (65). However, Lavik and Baumann (67) found that calories appear to have a greater effect than fat on 3-methylcholanthrene-induced skin tumors.

High-fat diets appear to enhance breast carcinogenesis induced by several carcinogens, e.g., 7,12-dimethylbenz[*a*]anthracene (68-71), *N*-nitroso-*N*-methylurea (NMU) (72), and X-rays (73). Increasing the level of dietary fat up to 20% by weight of the diet appears to increase the incidence or multiplicity of chemically induced breast tumors, depending on the carcinogen used (74-76). Breast tumorigenesis appears to be enhanced when high-fat diets are fed after, but not before, tumor initiation (75). This is consistent with the concept that dietary fat exerts a promoting effect on tumorigenesis rather than an effect at the initiation stage.

The levels of fat and of the carcinogen appear to be important determinants of the outcome. In some experiments that used low doses of the carcinogen, high-fat diets decreased the latent period and increased tumor incidence. At high doses of the carcinogen, high-fat diets increased tumor incidence and tumor multiplicity but appeared to

have no effect on latency (75, 76).

The type of fat also has been shown to be an important factor in breast tumorigenesis. Mammary tumor incidence appears to be uniformly high with all types of fat at 20% of the diet, but diets containing 20% polyunsaturated fat enhance tumorigenesis more effectively than do diets containing 20% saturated fat, provided that the fat serves as an adequate source of essential fatty acids (68). Supplementation of a diet high in saturated fat (17%) with 3% polyunsaturated fat provides the same tumor-enhancing effects as a 20% polyunsaturated fat diet (77, 78). Thus the possible promoting effects of high-lipid diets on breast carcinogenesis appear to depend on the total quantity of fat in the diet (with the maximum effect achieved at 20%) and sufficient polyunsaturated fat to serve as an adequate source of essential fatty acids.

Lipid requirements for maximum rate of growth of transplantable breast adenocarcinomas are essentially the same as those described above (79, 80). Limited data also indicate that polyunsaturated fats enhance the growth of both normal and neoplastic mammary epithelial cells from rats (81, 82).

In studies of *intestinal cancer*, increasing the quantity of dietary fat (generally from 5 to 20% by weight) increases the incidence of bowel tumors induced by a variety of carcinogens: 1,2-dimethylhydrazine (DMH) (83-86), methylazoxymethanol (MAM) acetate, 2',3-dimethyl-4-amino-biphenyl (DMAB), or NMU (86, 87). A promoting effect of dietary fat was suggested by studies in which high-fat diets increased the frequency of small and large bowel tumors when fed to rats after administration of azoxymethane (AOM) but not before or during administration of the carcinogen (88). However, in studies in which the effects of high and low levels of dietary fat on bowel tumorigenesis were compared, the animals rarely were "pair fed" or fed isocaloric diets. Consequently, it is possible that the tumor-enhancing effect of high-fat diets on the bowel is related to increased caloric intake. An intake of 5% polyunsaturated fat appears to have a greater tumor-enhancing effect than does the equivalent level of saturated fat. At a 20% fat level, no clear difference was apparent between the effects of polyunsaturated fat and saturated fat (83, 84).

Some studies suggest that bowel tumorigenesis is associated with increased concentration of fecal bile acids that accompanies high levels of fat intake. Through the efforts of many investigators, the concept has evolved that fecal bile acids and metabolites of cholesterol may function as cocarcinogens, carcinogens, or promoters in tumorigenesis of the large bowel (84, 89-93). To date, however, no active carcinogen derived from bile acids has been isolated from human or animal feces, and, therefore, the effect of bile acids on bowel tumorigenesis is not fully resolved.

Increasing dietary lipids appears to increase the incidence of carcinogen-induced *hepatomas* in rats (74, 94). Differing effects of the quality and quantity of dietary fat depend on the stage of hepatoma development at the time of exposure (74). Hepatic tumorigenesis, unlike breast carcinogenesis, is enhanced by a deficiency of lipotropic factors in rats fed high-fat diets (95). Limited data indicate that the yield of *pancreatic adenocarcinomas* is increased more effectively by

polyunsaturated fat than by saturated fat (96, 97).

Experimental evidence: cholesterol.—Some investigators have suggested that dietary cholesterol is cocarcinogenic in the bowel (98), but other studies have not confirmed these findings (85, 99).

Lipids (fats and cholesterol): conclusion.—The committee concluded that of all the dietary components it considered, the combined epidemiological and experimental evidence is most suggestive for a causal relationship between high-fat intake and the occurrence of cancer. Both epidemiological studies and experiments in animals provide strong evidence that increasing the intake of total fat increases the incidence of cancer at certain sites, particularly the breast and colon and to a lesser extent the prostate gland, and, conversely, that the risk is lower with lower intakes of fat. Laboratory data suggest that when fat intake is low, polyunsaturated fats are more effective than saturated fats in enhancing tumorigenesis, whereas the data on humans do not permit a clear distinction to be made between the effects of different components of fat. In general, however, the evidence from epidemiological and laboratory studies is consistent and, therefore, strengthens confidence in conclusions about the importance of fat in carcinogenesis. Although most of the data suggest that dietary fat has promoting activity, there is not enough evidence to warrant the complete exclusion of an effect on initiation.

Data concerning the association between serum cholesterol levels and colon cancer are inconsistent. An inverse association between serum cholesterol levels and colon cancer in males, but not in females, has been noted in several studies, but not in others, and alternative explanations of these findings have been suggested. Furthermore, the experimental data on cholesterol and cancer risk also are too limited for any inferences to be drawn.

Fruits and Vegetables

Fruits, vegetables, and whole grain products are important sources of many nutrients, especially vitamins and their precursors (such as β -carotene) and minerals. They are also a good source of dietary fiber and nonnutritive inhibitors of carcinogenesis. The recommendation emphasizing their daily consumption is based on the following epidemiological and laboratory evidence.

Epidemiological evidence: cruciferous vegetables.—Several case-control and a few cohort studies point specifically to an inverse association between the consumption of vegetables in general, raw vegetables (e.g., lettuce and celery), or cruciferous vegetables in particular (e.g., cabbage, cauliflower, brussels sprouts, and broccoli) and cancers of the alimentary tract [e.g., cancer of the stomach (26, 36, 100-102) and large bowel (27)].

Epidemiological evidence: vitamin A and carotene.—A growing accumulation of epidemiological evidence from several case-control and a few cohort studies indicates an inverse relationship between vegetable consumption or estimated "vitamin A" intake and cancers of the lung (103-106), larynx (107), bladder (108), esophagus (109-112), stomach (36, 100, 101), colorectum (26), and prostate gland (38). With few exceptions, the estimates of vitamin A intake were

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derived from the frequency of ingestion of groups of foods (e.g., green and yellow vegetables) known to be rich in precursors of vitamin A (notably, β -carotene) and a few foods such as whole milk and liver containing preformed retinol (vitamin A). Thus to a large extent, these studies have measured indirect indices of vitamin A intake.

Two case-control studies found an inverse relationship between serum vitamin A levels and the risk of bronchial carcinoma (113, 114), and, recently, three cohort studies in the United States and England have also reported a similar relationship for serum vitamin A and subsequent overall cancer risk (49, 52, 115).

Epidemiological evidence: vitamin C.—As in the studies for vitamin A, several case-control studies and a few correlation studies suggest an inverse association between fresh-fruit consumption or estimated vitamin C intake and cancers of different sites, especially cancers of the stomach (13, 26, 116–118), esophagus (110–112, 119, 120), and larynx (107), and uterine cervical dysplasia (121). In contrast, for colon cancer Jain et al. (22) found no association with vitamin C consumption in a case-control study.

The observations for gastric cancer are consistent with the hypothesis that vitamin C offers protection by blocking the reaction of secondary and tertiary amines with nitrite to form nitrosamines (122). As described later in this paper, several of the same studies have indicated a direct association between consumption of cured or smoked foods and stomach cancer (26, 116, 117). These studies mostly provide indirect evidence of a protective role for vitamin C against gastric cancer, inasmuch as they are based on the consumption of fresh fruits and vegetables, known to contain high concentrations of vitamin C, rather than on actual measurements of vitamin C intake.

Epidemiological evidence: fiber.—Some evidence concerning the protective role of fiber-containing foods in colon cancer was also discussed earlier in this paper. Some correlation data support the hypothesis that dietary fiber affords protection against cancer of the large bowel (29, 123). However, other correlation studies have not supported this hypothesis (7, 19, 63). Bingham and colleagues (18) found no significant correlation between total fiber intake and mortality from colon cancer, although they observed that the mean intake of the pentosan fraction of total dietary fiber was inversely correlated with mortality from colon cancer. Case-control studies also have yielded mixed results; some studies indicated a protective effect of fiber on colon carcinogenesis (20, 25, 124), whereas others found no inverse association (22) or reported a direct association (21).

Laboratory evidence.—In laboratory experiments, a number of nutrients (especially vitamin A and vitamin C) and other constituents of fruits and vegetables (e.g., fiber components, indoles, and isothiocyanates) have been tested for their effect on chemically induced carcinogenesis in vivo.

Laboratory evidence: nonnutritive components of fruits and vegetables.—Besides the presence of vitamins in fruits and vegetables, which may offer protection, there is some evidence that nonnutritive components of some plant products, especially cruciferous vegetables such as cabbage, broccoli, brussels sprouts, and cauliflower, afford protection against chemically induced neoplasia in vivo (125, 126). These compounds

include aromatic isothiocyanates (127), flavones (128), indoles (129), protease inhibitors (130), β -sitosterol (131, 132), and naturally occurring phenols (133). The possible modes of action of these nonnutritive substances have been reviewed by Wattenberg (125, 126, 134).

Laboratory evidence: vitamin A, retinoids, and β -carotene.—Studies on animals indicate that a deficiency of vitamin A can result in an increased susceptibility to carcinogen-induced neoplasia (135–139), whereas higher levels of vitamin A or its synthetic analogues, the retinoids, appear to have a protective effect against the induction of cancer by chemical carcinogens (135, 140–145) or to lead to regression of skin papillomas (146). In contrast, some investigators observed an increase in hormone-induced or chemically induced neoplasia by an enhanced intake of the vitamin (138, 147, 148).

In vitro experiments in organ cultures support the concept that a deficiency of vitamin A enhances neoplastic response to chemical carcinogens (149) and that its addition inhibits carcinogenesis (150). Recently, carotenoids (e.g., β -carotene) have been shown to protect against the development of UV light-induced skin tumors in hairless mice (151), whereas an opposite effect of β -carotene was observed earlier by Shamberger (141) for chemically induced carcinogenesis.

Laboratory evidence: vitamin C.—Ascorbic acid in vitro and in vivo can prevent the reaction of nitrites with amino compounds, thus inhibiting the formation of carcinogenic N-nitroso compounds and indirectly inhibiting tumor formation (152–156). In contrast, the effect of vitamin C on in vivo carcinogenesis from already formed carcinogens has been inhibitory in some studies (157–159) but not in others (160–162). In cell cultures, ascorbic acid appears to prevent chemically induced transformation of cells or to induce reversion of transformed cells (163).

Laboratory evidence: fiber.—Studies on the role of dietary fiber in chemically induced carcinogenesis have produced mixed results; e.g., bran administered by different routes appears to protect against DMH-induced colon cancer in animals (164–166). Cellulose also was found to protect rats against DMH-induced tumors (167, 168) but not against tumors induced by AOM or NMU (169, 170). Pectin did not inhibit DMH-induced colon tumors (168). Recently, Glauert et al. (171) reported that dietary agar enhanced DMH-induced colon cancer in mice.

Although some data suggest that some types of fiber (e.g., bran and cellulose) can inhibit the action of certain chemical carcinogens in vivo, the data from different studies are difficult to compare, primarily because of lack of uniform experimental protocols.

Fruit and vegetables: summary and conclusions.—Numerous epidemiological studies indicate an inverse association between the consumption of foods containing carotene, vitamin A, or vitamin C or of vegetables in the cruciferous family and the risk of cancer of the urinary bladder, large bowel, lung, stomach, or esophagus. The epidemiological evidence was derived from several case-control studies as well as cohort studies in different parts of the world, including the United States, Hawaii, Japan, Singapore, Norway, and Iran. In laboratory experiments, vitamin A, its synthetic analogues (the retinoids), indoles, and other nonnutritive chemicals in cruciferous vegetables tend to suppress the induction of

chemically induced tumors. The evidence for an inhibitory effect of β -carotene is limited, and that for fiber is inconclusive. There is evidence that vitamin C can inhibit the formation of some carcinogens *in vitro* and *in vivo*. Because investigators have not yet clearly identified which components of vegetables or fruits are responsible for the protective effect observed in epidemiological studies and because some nutrients like vitamin A can be toxic in high doses, the committee recommended against the use of high-dose supplements of individual nutrients.

Salt-Cured, Salt-Pickled, and Smoked Foods

The recommendation to minimize the consumption of cured, pickled, and smoked foods is based on evidence from some parts of the world (especially China, Japan, Hawaii, Hungary, Norway, and Iceland) that populations frequently consuming salt-cured (including salt-pickled) or smoked foods have a greater incidence of cancer, especially esophageal or stomach cancer. Laboratory investigations support the epidemiological observations by demonstrating carcinogenicity and/or mutagenicity of polycyclic aromatic hydrocarbons (PAH) or N-nitroso compounds that are produced by some methods of smoking or curing foods. These compounds cause mutations in bacteria and cancer in animals and are suspected of being carcinogenic in humans.

Epidemiological evidence: pickled, smoked, and cured foods.—Correlation studies in China on increased susceptibility to esophageal cancer have implicated trace mineral deficiencies (especially molybdenum), diets low in animal products, high intakes of pickles, pickled vegetables, and moldy foods containing N-nitroso compounds (possibly produced by fungal contaminants), and hot foods (172, 173). However, studies in Iran have shown no differences in nitrosamine levels in foods in regions of high and low risk for esophageal cancer (112).

In contrast to the limited evidence for esophageal cancer, a number of studies in different parts of the world have associated frequent consumption of cured, pickled, or smoked foods with stomach cancer, e.g., salted fish in Norway (26), home-smoked meat products containing high levels of benzo[a]pyrene (BP) in the Vend population in Hungary (174, 175), smoked trout and smoked mutton containing high levels of PAH in northwestern Iceland (176), and occupational exposure to curing smoke and frequent consumption of smoked fish in coastal regions of Latvia (177).

Studies of migrants to Canada and the United States have supported this evidence. Choi et al. (178) reported that mortality from gastric cancer among Icelandic immigrants who consumed large amounts of smoked and pickled foods in Manitoba was twice as high as that among people born in Manitoba. Kriebel and Jowett (179) attributed the disproportionately high rates of stomach cancer in Minnesota, Wisconsin, and the Upper Peninsula of Michigan to high rates among migrants from northern Europe and suggested that native-born Americans have shared the increased risk of the migrants, possibly by adopting the "high-risk" diet of the foreign-born residents.

In a case-control study, Haenszel and colleagues (101)

studied Japanese living in Hawaii. They reported an elevated stomach cancer risk for both Issei (migrants) and Nisei (offspring) who frequently used pickled vegetables and dried and or salted fish. These findings were not confirmed in a similar study in Japan. The authors interpreted this difference in findings to difficulty in demonstrating associations with dietary factors in nonmigrant populations and the frequent failure of case-control studies to establish the significance of small differences in food habits among cases and controls (102). However, other case-control studies have reported more frequent consumption of bacon in the Netherlands (116), fried foods, especially bacon drippings, in the United States (117), and frequent use of salted pickled foods in Japan (180) among patients with gastric cancer, as compared to consumption by controls.

Epidemiological evidence: salt.—Except for the few instances in which salt, nitrate, nitrite, N-nitroso compounds, or PAH have been labeled as the potential cancer-causing agents, in general, the specific causative agents in cured, pickled, or smoked foods have not been identified. The evidence relating salt intake *per se* to gastric cancer is tenuous. Joossens and Geboers (181) suggested that both gastric cancer and cerebrovascular disease are related to salt intake. However, salt is generally used in combination with nitrate or nitrite to preserve food (182). Furthermore, Okamura and Matsu-hisa (183) found no correlation between the salt content of salted fish and the death rate for gastric cancer in Japan.

Epidemiological evidence: nitrate, nitrite, and N-nitroso compounds.—Because many N-nitroso compounds are strong carcinogens under a variety of conditions and in many species (184, 185), much concern has been expressed during the past 10–15 years about their role in the etiology of human cancer. The most important sources of nitrosamines in the American diet currently are cured meats, especially bacon (186). Humans can be exposed to N-nitroso compounds indirectly from the use of nitrate and nitrite as preservatives in meats and other cured products. Nitrate can be reduced to nitrite, which can interact with dietary substrates such as amines or amides to produce N-nitroso compounds. Vegetables contribute most of the nitrate ingested, but they also contain inhibitors of nitrosation such as ascorbic acid. More than one-third of the average daily intake of nitrite is contributed by the ingestion of cured meats, approximately one-third by baked goods and cereals, and less than one-fifth by vegetables (186).

Findings from epidemiological studies conducted in Colombia, Chile, Japan, Iran, China, England, and the United States (Hawaii) have been consistent with the hypothesis that exposure of humans to high levels of nitrate or nitrite in the diet or drinking water is associated with increased incidence of cancers of the stomach and the esophagus. [See review by the Academy (186); for examples, see (101, 116, 117, 187–189).] The associations with cancer in these studies were based either on correlations of high-risk population groups with corresponding exposures to food and water supplies or on comparisons of the frequency of consumption of foods containing these substances (plus secondary amines) by gastric cancer patients and controls rather than on direct measures of exposure. The Academy's Committee on Nitrite and Alternative Curing Agents in Food (186) recently con-

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cluded that these studies do not provide conclusive evidence for a causal relationship between nitrate and nitrite and human cancer. However, because nitrate can be converted to nitrite and subsequently to N-nitroso compounds that are suspected human carcinogens, the committee recommended that exposure to these agents be reduced (186).

A number of investigators have suggested a possible mechanism for the role of N-nitroso compounds in human gastric cancer (182, 190-192): bacterial colonization of the stomach, especially in cases of achlorhydria, consequent reduction of dietary nitrate to nitrite, and endogenous synthesis of N-nitroso compounds. Weisburger et al. (182) postulated and Ohshima and Bartsch (193) recently demonstrated that reducing agents such as ascorbic acid may interfere with the endogenous production of N-nitroso compounds that occurs when dietary nitrite reacts with amines or amides. Such a mechanism could explain the protective effect of green or yellow vegetables and of other vitamin C-containing foods mentioned earlier in this paper.

Experimental evidence: nitrate, nitrite, and N-nitroso compounds.—The few experiments conducted in animals have provided no evidence that nitrate is carcinogenic (194-196) or mutagenic or that nitrite is directly carcinogenic (186, 197-201). However, nitrite can interact with dietary amines or amides or with endogenous metabolites to produce N-nitroso compounds that induce cancer. Nitrite is mutagenic, at least in microbial systems. A majority of the approximately 300 different N-nitroso compounds tested, with the use of any of several routes of administration, have been shown to induce cancer in various tissues of one or more species of laboratory animals (186).

Experimental evidence: PAH.—Small amounts of PAH are present as contaminants in a variety of grilled, roasted, or conventionally smoked foods. Vegetables can become contaminated by PAH from air, soil, or water; fish and shellfish can assimilate such chemicals from their marine environments (202). However, unless vegetables or seafood are obtained from highly contaminated environments, the major sources of PAH appear to be the smoking, cooking, or other methods of processing foods (202, 203).

Of the more than 100 PAH found in the environment, approximately 20 are carcinogenic in laboratory animals. Of the five PAH found to be carcinogenic when administered orally, three—BP, dibenz[*a,h*]anthracene (DBA), and benz[*a*]anthracene (BA)—may occur in the diet. The carcinogenicity of BP in various animal species, including mice, rats, and hamsters, has been well established (204-206). BP is mutagenic in microbial and mammalian cell systems (207, 208). DBA is carcinogenic in mice (209) and mutagenic in bacteria (210). BA also is carcinogenic in mice (211) and mutagenic in a variety of cell systems (208, 210).

Pickled, smoked, and cured foods: summary and conclusions.—Evidence is limited that the consumption of pickled foods, possibly containing N-nitroso compounds, is associated with esophageal cancer in China. The evidence for gastric cancer is more extensive. A number of correlation studies in different parts of the world, a few studies of migrants to Canada and the United States, and some case-control studies suggest that a high risk for gastric cancer is associated with frequent consumption of smoked foods (which may lead to increased

exposure to PAH), with frequent ingestion of salt-pickled foods or cured foods containing N-nitroso compounds or nitrate and nitrite (which may result in subsequent in vivo production of nitrosamines), and possibly with ingestion of other carcinogens produced by food processing, including cooking. PAH and N-nitroso compounds cause mutations in various systems, are carcinogenic in animals, and are suspected of causing cancer in humans.

Food Additives and Contaminants

Lack of adequate data on a large number of substances precluded a comprehensive assessment of the risk to humans from exposure to additives and contaminants in the diet. The report (1) contains an evaluation of the carcinogenicity of selected chemicals in both categories.

Naturally occurring carcinogens.—In addition to nutrients, a variety of nonnutritive substances (e.g., hydrazines) are natural constituents of foods. Furthermore, metabolites of molds (e.g., mycotoxins such as the potent carcinogen aflatoxin) and of bacteria (e.g., carcinogenic nitrosamines) may contaminate foods. Many of these are occasional contaminants, whereas others are normal components of relatively common foods.

Aflatoxin, a mycotoxin that occurs in grains and other food commodities, is carcinogenic in several species of animals, including mice, hamsters, rats, trout, and ducks, and there is evidence of a dose response (1, 212, 213). In addition, aflatoxin has been shown to be mutagenic in bacterial and mammalian species (214, 215). Several other mycotoxins that are found in food are carcinogenic and/or mutagenic in laboratory tests (1, 216). However, with the exception of aflatoxin, which has been implicated in liver cancer in humans in some parts of the world (1, 217, 218), there is no reliable epidemiological evidence relating other mycotoxins to neoplasia in humans.

The experimental and epidemiological evidence concerning nitrate, nitrite, and N-nitroso compounds was discussed earlier in this paper.

Several hydrazine derivatives of two mushrooms—*Agaricus bisporus* and *Gyromitra esculenta*, both of which are consumed in various parts of the world—appear to be carcinogenic in mice and, under certain conditions, in hamsters, and they are mutagenic in bacteria (219-221). However, the significance of these findings for risk to humans cannot be determined inasmuch as there are no epidemiological data.

Several pyrrolizidine alkaloids (e.g., symphytine and senkirkine) are carcinogenic in animals and/or mutagenic in several test systems (216, 222). Tumors develop in rats fed plants such as coltsfoot and comfrey, which contain these alkaloids (1, 216). Cycad nuts, which are eaten in some parts of the world, contain cycasin (methylazoxymethanol- β -D-glucoside), a compound known to be carcinogenic in animals (216). Cycasin is also mutagenic in the Ames test after addition of β -D-glucosidase (223). However, no direct evidence has been presented for the carcinogenicity of pyrrolizidine alkaloids and cycasin in humans (1, 216).

Consumption of bracken fern, which occurs widely in nature, has been associated with a higher risk of esophageal

cancer in Japan (224). However, in a case-control study no association was observed between consumption of fiddlehead greens (related to bracken fern) and bladder cancer (225).

Coffee drinking has been associated with an elevated risk for bladder cancer in several case-control studies (225-227), but, with some possible exceptions (228), there is no evidence of a dose response and, therefore, the evidence is inconclusive. Coffee drinking also has been associated with pancreatic cancer (229, 230), but its association with other cancer sites is inconsistent (231-234). Coffee, whether brewed, instant, or decaffeinated, has been found to be mutagenic (235-237), but not carcinogenic in animal bioassays (238, 239).

Other plant constituents, such as methylxanthines, thiourea, tannins, coumarin, parasorbic acid, safrole, and estragole, are carcinogenic in laboratory animals and mutagenic in bacterial or mammalian cell systems (1, 216). However, the significance of these findings for human health is not known because there are no epidemiological data.

In conclusion, many of the naturally occurring substances discussed above have been found to be carcinogenic in laboratory animals and mutagenic in bacterial and other systems, thereby posing a potential risk to humans. However, no pertinent epidemiological studies have been made except for those on coffee and aflatoxins and those on nitrate, nitrite, and N-nitroso compounds. The compounds thus far shown to be carcinogenic in animals have been reported to occur in small amounts in the average U.S. diet; however, there is no evidence that any of these substances individually makes a major contribution to the total risk of cancer in the United States. This lack of sufficient data should not be interpreted as an indication that these or other compounds subsequently found to be carcinogenic do not present a hazard. Further investigations are necessary.

Food additives.—Nearly 3,000 substances are intentionally used to process foods in the United States. Another estimated 12,000 chemicals, such as vinyl chloride and acrylonitrile, which are used in food production and food processing, are classified as indirect additives. Some additives, such as sugar, are consumed in large amounts by the general population. However, the annual per-capita exposure to most of these substances constitutes a minute portion of the diet (240). Although the Food Safety Provisions and, in many cases, the Delaney Clause of the Federal Food, Drug, and Cosmetic Act (241) prohibit the addition of known carcinogens to foods, only a small proportion of substances added to foods has been tested for carcinogenicity according to protocols that are considered acceptable by current standards. Moreover, except for the studies on nonnutritive sweeteners (242), very few epidemiological studies have been conducted to examine the effect of food additives on cancer incidence (1).

Of the few direct food additives that have been tested and found to be carcinogenic in animals, all except saccharin have been banned from use in the food supply. Minute residues of a few indirect additives that are known either to produce cancer in animals [e.g., vinyl chloride and acrylonitrile (243, 244)] or to be carcinogenic in humans [e.g., vinyl chloride (245)] occasionally are detected in foods [(246, 247); Breder CV: Personal communication]. There is

no evidence to suggest that the increasing use of food additives has contributed significantly to the overall risk of cancer for humans. However, the lack of a detectable effect may be due to the relatively recent use of these substances, to their lack of carcinogenicity, or to the inability of epidemiological techniques to detect the effects of additives against the background of common cancers from other causes. Therefore, no definitive conclusion can be reached until more data become available (1).

Environmental contaminants.—Very low levels of a large and chemically diverse group of substances—environmental contaminants—may be present in a variety of foods. The dietary levels of some of these substances are monitored through the Total Diet Studies conducted by the Food and Drug Administration (248). Many of these contaminants have been extensively tested for carcinogenicity and mutagenicity.

The results of standard chronic toxicity tests indicate that a number of environmental contaminants (e.g., some organochlorine pesticides and polychlorinated biphenyls) cause cancer in laboratory animals. There is no epidemiological evidence to suggest that these compounds individually make a major contribution to the risk of cancer in humans. However, the possibility that they act synergistically and may thereby create a greater carcinogenic risk cannot be excluded (1).

Mutagens in Food

It is generally agreed, but not without some controversy, that there is a high degree of correlation between the mutagenicity of compounds in the *Salmonella*-microsome assay and their carcinogenicity in laboratory animals (210, 249-252). Recent studies show that this correlation is dependent on the class of chemical being investigated, and one must exercise judgment in evaluating the significance of negative or positive tests. A chemical that is mutagenic to bacteria or other organisms is generally suspected of being carcinogenic, although carcinogenicity must be confirmed in long-term tests in whole animals.

Considerable attention recently has been directed toward the presence of mutagenic activity in foods. Many categories of mutagenic compounds have been identified in the diet, including food additives, plant constituents such as flavonoids, and contaminants such as mycotoxins, nitrosamines, residues of pesticides, and PAH. In addition, cooking and other processing of food lead to the production of numerous mutagens, many of which have not yet been identified. Most of the studies to identify mutagens in food have used the Ames *Salmonella* assay.

Naturally occurring mutagens in foods.—Among the most widespread of the known naturally occurring mutagens that are normal constituents of many foods are the mutagenic flavonoids (253). Among the flavonol aglycones that have been shown to be mutagenic are quercetin, kaempferol, and galangin (253-256). The mutagenic activity of many foods or drinks (e.g., green tea, Japanese pickles, and dillweed) has been attributed to the flavonoids contained in them (257-259), and the mutagenicity of onions and raisins has been shown to be due to quercetin (260). Quercetin was found to be carcinogenic in one study (261) but noncarcinogenic in three other studies (262-264).

The mutagenicity of coffee has been mentioned earlier. Although caffeine has been reported to be mutagenic in bacteria (265), it probably is not responsible for the mutagenicity of coffee because decaffeinated coffee is reported to be as mutagenic as regular coffee under test conditions (237). Grape juice and several varieties of tea also have been shown to be mutagenic (237, 266). Most of the alcoholic beverages from China (267) and whiskies from Japan and North America (268) and some French brandies have been shown to be mutagenic (269).

The mutagenicity of some mycotoxins and some plant constituents was discussed earlier in this paper.

Mutagens resulting from cooking of foods.—Different methods of cooking and processing foods appear to affect their mutagenic activity. Charcoal-broiled and grilled beef contains PAH, many of which are mutagenic and/or carcinogenic (270). The smoking of foods and roasting of coffee are major sources of PAH activity (202).

Much of the mutagenicity of well-cooked foods has been shown to be due to pyrolysis of amino acids in proteins (271). Pyrolysates of most amino acids tested yielded some mutagenic activity, the pyrolysate of tryptophan being the most mutagenic (272, 273).

Several of the mutagenic pyrolysates of amino acids or proteins have been tested for carcinogenicity in vivo. For example, pyrolysates of tryptophan—3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1) and 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2)—pyrolysates of glutamic acid—2-amino-6-methyldipyrro[1,2-*a*:3',2'-*d*]imidazole (Glu-P-1) and 2-aminodipyrro[1,2-*a*:3',2'-*d*]imidazole (Glu-P-2)—and pyrolysates of soybeans—2-amino-9*H*-pyrido[2,3-*b*]indole (AaC) and 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole (MeAaC)—which are all produced during pyrolysis of proteins and amino acids, were found to be carcinogenic (274, 275). Thus it appears that the identification of several of the mutagenic compounds found in pyrolysates of proteins and amino acids was an accurate prediction of carcinogenicity. However, the presence of a carcinogenic chemical in a pyrolyzed amino acid or protein mixture does not necessarily imply that the carcinogen also will be present in normally cooked, uncharred food.

Modifiers of mutagenic activity.—A number of substances in food have been reported to either enhance or diminish the mutagenic activity of other substances. Most of these effects have been observed in vitro, but their relevance to effects on intact mammals is unknown.

Norharman and harman present in some foods (e.g., broiled beef and sardines) appear to act as comutagens (276–280) in in vitro test systems. However, juices prepared from some common vegetables, fruits, and spices (including cabbage, broccoli, green pepper, mint leaf, ginger, apple, and pineapple) reduced the mutagenic activity of tryptophan pyrolysates (281, 282). Antimutagenic activity also has been found in extracts of wheat sprouts, leaf lettuce, parsley, brussels sprouts, mustard greens, spinach, cabbage, broccoli, and other vegetables (282, 283).

A number of antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), were antimutagenic in the presence of a series of mutagens that require metabolic activation in vitro but not in the presence

of direct-acting mutagens (284, 285). However, the mutagenicity of direct-acting mutagens, such as β -propiolactone and *N*-methyl-*N*-nitrosoguanidine, also was reported to be inhibited by antioxidants such as ascorbate (286, 287). These findings probably reflect an underlying common mechanism concerning mutagenicity inhibition by antioxidants.

Mutagens in food: summary and conclusions.—Many foods contain mutagenic flavonoids, such as quercetin, kaempferol, and their glycosides, and some substances found in foods can enhance or inhibit the mutagenic activity of other compounds. Mutagens in charred meat and fish are produced during the pyrolysis of proteins that occurs when foods are cooked at very high temperatures. Mutagens can also be produced during normal cooking of meat at lower temperatures. Smoking of foods as well as charcoal broiling results in the deposition of mutagenic and carcinogenic polynuclear organic compounds such as BP on the surface of the food.

Most mutagens detected in foods have not been adequately tested for their carcinogenic activity. Thus the committee concluded that it is not yet possible to assess whether such mutagens are likely to contribute significantly to the incidence of cancer in the United States (1).

Alcohol

In certain populations throughout the world there appears to be an association between esophageal cancer and the consumption of certain locally prepared alcoholic beverages, e.g., in Japan (288), in some African populations (289, 290), in France (291, 292), and in China (293, 294). Gastric cancer (295) has been associated with red-wine consumption in France.

An association between beer drinking and colorectal cancers appears to be statistically significant in certain countries. Excessive beer consumption has been correlated with colorectal or rectal cancer in the mainland United States (296, 297), with colorectal cancer in 20 countries (298), and with bowel cancer in males in Great Britain (299). Two cohort studies [(300); Bjelke E: Unpublished Ph.D. thesis, 1973] reported a direct relationship between colorectal cancer and the frequency of beer or liquor consumption in Dublin and Norway. However, case-control studies of bowel cancer in Finland (301), in the United States (117), and in Norway [(302); Bjelke E: Unpublished Ph.D. thesis] showed no significant relationship with beer drinking. Similarly, no associations with bowel cancer were observed for beer drinking in a correlation analysis by Hinds et al. (303) or in a cohort study by Jensen (304).

An association between alcohol abuse and cancer at various sites (e.g., esophagus, tongue, pharynx, hypopharynx, larynx, and lung) has been recognized for some time (305–309). In the United States, case-control studies have established that excessive consumption of alcoholic beverages increases the risk of incurring cancer of the oral cavity (excluding the lip, glottis, and supraglottic region), larynx, and esophagus (310–321). Excessive alcohol consumption has also been linked with the development of hepatomas (322, 323), although hepatomas generally are found in

individuals with cirrhosis. In addition to alcohol, hepatitis B virus and aflatoxin have been associated with liver cancer. Because all three of these agents produce hepatic injury that may lead to cirrhosis, they may contribute to the development of hepatomas.

Synergism between alcohol and smoking.—Alcohol consumers (especially abusers) are, more often than not, smokers. Flammant et al. (324), assessing both variables, stressed the interaction between alcohol and smoking in cancers of the oral cavity and the esophagus. Studies completed since then have confirmed these findings of an interactive role between tobacco and alcohol in tumorigenesis of the oral cavity, larynx, lung, and esophagus (311, 314, 317, 319, 325–329). Avoidance of tobacco and alcohol by males could effect a marked reduction of these cancers (317, 328). Excessive alcohol consumption may also augment other disease processes such as impaired nutritional status, which may be associated with the development of cancer at these sites (326).

Alcohol: summary and conclusions.—In some countries, including the United States, beer drinking has been correlated with an increased risk of colorectal cancer, especially rectal cancer. Some evidence exists that excessive alcohol consumption may contribute to the formation of hepatomas by a mechanism involving hepatic injury and cirrhosis. Alcohol appears to act synergistically with smoking in increasing the risk for cancers of the mouth, larynx, esophagus, and respiratory tract.

Other Dietary Components

The extensive analysis of the literature, only part of which is cited in this paper, encompassed all of the major nutrients and nonnutritive components of diet. However, because of insufficient or inconsistent data or because of lack of concurrence between the epidemiological and laboratory evidence, no definitive conclusions could be drawn about the role in carcinogenesis of many dietary components: total caloric intake, protein, dietary fiber, carbohydrates, cholesterol, most minerals (including selenium), vitamin E, and the B-vitamins. Discussion of the literature on these subjects can be found in (1).

Contribution of Diet to Overall Risk of Cancer

The NCI had requested that the Academy attempt to determine the percentage of cancers that are diet related. Higginson and Muir (330) had suggested that in some regions of England and Wales possibly 30% of cancers occurring in men and 60% in women could be attributed to life-style (including diet). Wynder and Gori (331) were more specific, concluding that in the United States more than 40% of cancers in men and almost 60% in women could be related to dietary factors. Using data on mortality but employing a similar approach to that of Wynder and Gori, Doll and Peto (332) provided a wide range (i.e., 10–70%; average, 35%) for the proportion of deaths from cancer that could be reduced by practical dietary means. They concluded that dietary modification would have the greatest effect on the incidence of cancers of the stomach and large

bowel and would affect, to a lesser extent, the incidence of cancers of the breast, endometrium, and lung.

The Committee on Diet, Nutrition, and Cancer concluded that cancers of most major sites are influenced by dietary patterns but that the data are not sufficient to quantitate the contribution of diet to the overall cancer risk or to determine the percent reduction in risk that might be achieved by dietary modifications (1).

COMMENTS

One of the major objectives in examining the literature on diet, nutrition, and cancer was to determine whether any guidance could be provided to the public. The committee held the strong conviction that dietary recommendations are justified only if the evidence is sufficiently convincing that guidelines to reduce the risk of cancer have a high likelihood of benefit and are without discernible risk. The committee was also aware of the potential impact of its report on scientists, the public, and the food industry. However, having been persuaded that the evidence was sufficiently convincing to warrant certain conclusions, the committee believed that not to offer certain interim guidelines would be a dereliction of its responsibility. Furthermore, the guidelines are consistent with good nutrition and with other recommendations from public health authorities.

The committee called for the assistance of the NCI, other federal agencies concerned with public health, and the food industry in disseminating and implementing the interim guidelines. Although in the judgment of the committee these guidelines are likely to reduce the risk of certain diet-related cancers, the committee concluded:

"Since the current data base is incomplete, future epidemiological and experimental research is likely to provide new insights into the relationship between diet and cancer. Therefore, the committee suggests that the National Cancer Institute establish mechanisms to review these dietary guidelines at least every 5 years."

The final report, to be published by the Academy in June 1983, will be concerned with future research on diet, nutrition, and cancer.

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Dietary Carcinogens

In this issue of *Science*, Bruce Ames reviews the increasing body of evidence that large numbers of potent carcinogens arise from natural processes. Mutagens are present in substantial quantities in fruits and vegetables. Carcinogens are formed in cooking as a result of reactions involving proteins or fats. Dietary practices may be an important determinant of current cancer risks.

Ames describes the role of plant materials as follows: Plants in nature synthesize toxic chemicals in large amounts, apparently as a primary defense against the hordes of bacterial, fungal, and insect and other animal predators. Plants in the human diet are no exception. The variety of toxic chemicals is so great that organic chemists have been characterizing them for over 100 years, and new plant chemicals are still being discovered. Recent widespread use of short-term tests for detecting mutagens and the increased testing of plant substances for carcinogenicity in animals have contributed to the identification of many natural mutagens, teratogens, and carcinogens in the human diet.

Safrrole and related compounds are present in many edible plants. Safrrole is a carcinogen in rodents and some of its metabolites are mutagens. Oil of sassafras, once used to flavor some root beer, is about 75 percent safrrole. Black pepper contains about 10 percent by weight of a closely related compound, piperine. Extracts of black pepper at a dose equivalent to 4 milligrams of dried pepper per day cause tumors in mice at many sites. Many hydrazines are carcinogens and mutagens, and large amounts of them are found in edible mushrooms. One carcinogenic hydrazine is present in the false morel at a concentration of 50 milligrams per 100 grams. It causes lung tumors in mice at a level of 20 micrograms per mouse per day.

Carcinogens and mutagens are present in mold-contaminated foods such as corn, nuts, peanut butter, bread, cheese, and fruit. Some of these contaminants, such as aflatoxin, are among the most potent known carcinogens and mutagens. Nitrosamines and nitroso compounds are suspect as causative agents of stomach and esophageal cancer in humans. In the digestive system these nitrogen compounds are formed from nitrate and nitrite. Beets, celery, lettuce, spinach, radishes, and rhubarb all contain about 200 milligrams of nitrate per 100-gram portion.

Rancid fats are possible causative agents of colon and breast cancer in humans. These forms account for a substantial fraction of all the cancer deaths in the United States. Unsaturated fats are easily oxidized on standing and in cooking to form mutagens, promoters, and carcinogens. Among the numerous products of such oxidations are fatty acid hydroperoxides and cholesterol epoxide. Thus the colon and digestive tract are exposed to many fat-derived carcinogens. Human breast fluid can contain high levels of cholesterol epoxide.

Burnt and browned materials formed by heating proteins during cooking are highly mutagenic. Chemicals isolated from such products have been found to be carcinogenic when fed to rodents. In addition, the browning reaction products from caramelization of sugars or the reaction of amino acids and sugars during cooking contain a large variety of DNA-damaging agents.

The view that dietary practices might be a causative factor in cancer is not new. Epidemiologists have noted marked differences in cancer rates between population groups. Effects from changes in diet following migration have also been observed. Results of current studies are beginning to delineate more sharply specific causative agents. When more definitive information is available, it should be possible for prudent people to choose fruits and vegetables that present minimal hazards. In the meantime, there is persuasive evidence that charred meats and rancid fats should not be part of the diet.—PHILIP H. ABELSON

Dietary Carcinogens and Anticarcinogens

Oxygen radicals and degenerative diseases

Bruce N. Ames

Comparison of data from different countries reveals wide differences in the rates of many types of cancer. This leads to hope that each major type of cancer may be largely avoidable, as is the case for cancers due to tobacco, which constitute 30 percent of the cancer deaths in the United States and the United Kingdom (1). Despite numerous suggestions to the contrary, there is no convincing evidence of any generalized increase in U.S. (or U.K.) cancer rates other than what could plausibly be ascribed to the delayed effects of previous increases in tobacco usage (1-3). Thus, whether or not any recent changes in life-style or pollution in industrialized countries will substantially affect future cancer risks, some important determinants of current risks remain to be discovered among long-established aspects of our way of life. Epidemiologic studies have indicated that dietary practices are the most promising area to explore (1, 4). These studies suggest that a general increase in consumption of fiber-rich cereals, vegetables, and fruits and decrease in consumption of fat-rich products and excessive alcohol would be prudent (1, 4). There is still a lack of definitive evidence about the dietary components that are critical for humans and about their mechanisms of action. Laboratory studies of natural foodstuffs and cooked food are beginning to uncover an extraordinary variety of mutagens and possible carcinogens and anticarcinogens. In this article I discuss dietary mutagens and carcinogens and anticarcinogens that seem of importance and speculate on relevant biochemical mechanisms, particularly the role of oxygen radicals and their inhibitors in the fat-cancer relationship, promotion, anticarcinogenesis, and aging.

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Natural Mutagens and Carcinogens in Food

Plant material. Plants in nature synthesize toxic chemicals in large amounts, apparently as a primary defense against the hordes of bacterial, fungal, and insect and other animal predators (5-10). Plants in the human diet are no exception. The variety of these toxic chemi-

Summary. The human diet contains a great variety of natural mutagens and carcinogens, as well as many natural antimutagens and anticarcinogens. Many of these mutagens and carcinogens may act through the generation of oxygen radicals. Oxygen radicals may also play a major role as endogenous initiators of degenerative processes, such as DNA damage and mutation (and promotion), that may be related to cancer, heart disease, and aging. Dietary intake of natural antioxidants could be an important aspect of the body's defense mechanism against these agents. Many antioxidants are being identified as anticarcinogens. Characterizing and optimizing such defense systems may be an important part of a strategy of minimizing cancer and other age-related diseases.

cals is so great that organic chemists have been characterizing them for over 100 years, and new plant chemicals are still being discovered (12, 24, 25). However, toxicological studies have been completed for only a very small percentage of them. Recent widespread use of short-term tests for detecting mutagens (41, 42) and the increased number of animal cancer tests on plant substances (6) have contributed to the identification of many natural mutagens, teratogens, and carcinogens in the human diet (5-40). Sixteen examples are discussed below.

1) *Safrole*, *estragole*, *methyleugenol*, and related compounds are present in many edible plants (5). *Safrole*, *estragole*, and *methyleugenol* are carcinogens in rodents, and several of their metabolites are mutagens (5). Oil of *sassafras*, which had been used in "natural" *sassa-parilla* root beer, is about 75 percent *safrole*. Black pepper contains small

amounts of *safrole* and large amounts (close to 10 percent by weight) of the closely related compound *piperine* (26). Extracts of black pepper cause tumors in mice at a variety of sites at a dose of extract equivalent to 4 mg of dried pepper per day (about 160 mg/kg per day) for 3 months; an estimate of the average human intake of black pepper is over 140 mg per day (about 2 mg/kg per day) for life (26).

2) Most *hydrazines* that have been tested are carcinogens and mutagens, and large amounts of carcinogenic *hydrazines* are present in edible *mushrooms*. The widely eaten false morel (*Gyromitra esculenta*) contains 11 *hydrazines*, three of which are known *carcinogens* (28). One of these, *N-methyl-N-formylhydrazine*, is present at a concentration of 50 mg per 100 g and causes lung tumors in mice at the extremely low dietary level of 20 µg per mouse per day (28). The most common commercial mushroom, *Agaricus bisporus*, contains about 300 mg of *agaritine*, the δ-glutamyl derivative of the mutagen 4-hydroxy-

methylphenylhydrazine, per 100 g of mushrooms, as well as smaller amounts of the closely related carcinogen *N-acetyl-4-hydroxymethylphenylhydrazine* (28). Some *agaritine* is metabolized by the mushroom to a diazonium derivative which is a very potent carcinogen (a single dose of 400 ng/g gave 30 percent of mice stomach tumors) and which is also present in the mushroom in smaller amounts (28). Many *hydrazine* carcinogens may act by producing oxygen radicals (43).

3) Linear *furocoumarins* such as *psoralen derivatives* are potent light-activated carcinogens and mutagens and are widespread in plants of the Umbelliferae family, such as *celery*, *parsnips*, *figs*, and *parsley* (for instance, 4 mg per 100 g of parsnip) (17, 19, 44). The level in *celery* (about 100 µg per 100 g) can increase about 100-fold if the *celery* is stressed or diseased (19). *Celery* pickers and handlers commonly develop skin

rashes on their arms when exposed to diseased celery (19). Oil of bergamot, a citrus oil, is very rich in a psoralen and was used in the leading suntan lotion in France (17). Psoralens, when activated by sunlight, damage DNA and induce tanning more rapidly than the ultraviolet component of sunlight, which is also a carcinogen (17). Psoralens, (plus light) are also effective in producing oxygen radicals (18).

4) The potato glycoalkaloids solanine and chaconine are strong cholinesterase inhibitors and possible teratogens and are present at about 15 mg per 200 g of potato (12, 13). When potatoes are diseased, bruised, or exposed to light, these and other (24) glycoalkaloids reach levels that can be lethal to humans (12). Plants typically respond to damage by making more (and often different) toxic chemicals as a defense against insects and fungi (19, 24, 25). The different cultivars of potatoes vary in the concentration of these toxic glycoalkaloids (the concentration is a major determinant of insect and disease resistance); one cultivar bred for insect resistance had to be withdrawn from use because of its toxicity to humans (> 40 mg of glycoalkaloids in a 200-g potato is considered to be a toxic level) (12).

5) Quercetin and several similar flavonoids are mutagens in a number of short-term test systems. Flavonoids are extremely widespread (daily levels close to 1 g) in the human diet (8, 16, 20, 21). There is evidence for the carcinogenicity of quercetin in two strains of rats (8), although it was negative in other experiments (21).

6) Quinones and their phenol precursors (9, 14, 16, 23, 43) are widespread in the human diet. Quinones are quite toxic as they can act as electrophiles or accept a single electron to yield the semiquinone radical, which can either react directly with DNA (14, 46) or participate in a redox cycle of superoxide radical generation by transferring the electron to O_2 (47). The superoxide radical and its metabolic product H_2O_2 can, in turn, lead to the oxidation of fat in cellular membranes by a lipid peroxidation chain reaction, thus generating mutagens and carcinogens, as discussed below. A number of quinones and dietary phenols have been shown to be mutagens (7, 9, 16, 23, 44). Mutagenic anthraquinone derivatives are found in plants such as rhubarb and in mold toxins (7, 16, 48). Many dietary phenols can spontaneously autoxidize to quinones, generating hydrogen peroxide at the same time [examples are catechol derivatives such as the caf-

feic acid component of chlorogenic acid (9), which is present at about 250 mg per cup of coffee]. The amounts of these phenols in human urine (and in the diet) are appreciable (45). Catechol, for example, is excreted in urine at about 10 mg per day and appears to be mainly derived from metabolism of plant substances (45). Catechol is a potent promoter of carcinogenesis (45), an inducer of DNA damage, a likely active metabolite of the carcinogen benzene (46), and a toxic agent in cigarette smoke (45). Catecholamine induction of cardiomyopathy is thought to occur through generation of oxygen radicals (49).

7) Theobromine, a relative of caffeine, has been shown to be genotoxic in a variety of tests, to potentiate (as does caffeine) DNA damage by various carcinogens in human cells, and to cause testicular atrophy and spermatogenic cell abnormalities in rats (27). Cocoa powder is about 2 percent theobromine, and therefore humans may consume hundreds of milligrams of theobromine a day from chocolate. Theobromine is also present in tea.

8) Pyrolizidine alkaloids are carcinogenic, mutagenic, and teratogenic and are present in thousands of plant species (often at > 1 percent by weight), some of which are ingested by humans, particularly in herbs and herbal teas and occasionally in honey (7, 29). Pyrolizidine alkaloid poisonings in humans (as well as in other mammals) cause lung and liver lesions and are commonly misdiagnosed (29).

9) The broad (fava) bean (*Vicia faba*), a common food of the Mediterranean region, contains the toxins *vicine* and *convicine* at a level of about 2 percent of the dry weight (30). Pythagoras forbade his followers to eat the beans, presumably because he was one of the millions of Mediterranean people with a deficiency of glucose-6-phosphate dehydrogenase. This deficiency results in a low glutathione concentration in blood cells, which causes increased resistance to the malarial parasite, probably accounting for the widespread occurrence of the mutant gene in malarial regions. However, the low glutathione concentration also results in a marked sensitivity to agents that cause oxidative damage, such as the fava bean toxins and a variety of drugs and viruses. Sensitive individuals who ingest fava beans develop a severe hemolytic anemia caused by the enzymatic hydrolysis of *vicine* to its aglycone, *divicine*, which forms a quinone that generates oxygen radicals (30).

10) Allyl isothiocyanate, a major flavor

ingredient in oil of mustard and horse-radish, is one of the main toxins of the mustard seed and has been shown to cause chromosome aberrations in hamster cells at low concentration (50) and to be a carcinogen in rats (31).

11) Gossypol is a major toxin in cottonseed and accounts for about 1 percent of its dry weight (32). Gossypol causes pathological changes in rat and human testes, abnormal sperm, and male sterility (32, 33). Genetic damage has been observed in embryos sired by gossypol-treated male rats: dominant lethal mutations in embryos were measured after males were taken off gossypol treatment and allowed to mate (33). Gossypol appears to be a carcinogen as well: it has been reported to be a potent initiator and also a promoter of carcinogenesis in skin painting studies with mice (34). Crude, unrefined cottonseed oil contains considerable amounts of gossypol (100 to 750 mg per 100 ml). Thus human consumption may be appreciable in countries, such as Egypt, where fairly crude cottonseed oil is commonly used in cooking. Gossypol is being tested as a male contraceptive in over 10,000 people in China (at an oral dose of about 10 mg per person per day), as it is inexpensive and causes sterility during use (33). Gossypol's mode of action as a spermicide may be through the production of oxygen radicals (35).

Plant breeders have developed "glandless cotton," a new strain with low levels of gossypol, but seeds from this strain are much more susceptible to attack by the fungus *Aspergillus flavus*, which produces the potent carcinogen aflatoxin (36).

12) Sterculic acid and malvalic acid are widespread in the human diet. They are toxic cyclopropenoid fatty acids present in cottonseed oil and other oils from seeds of plants in the family Malvaceae (for instance, cotton, kapok, okra, and durian) (51). Another possible source of human exposure is consumption of fish, poultry, eggs, and milk from animals fed on cottonseed (51). Cyclopropenoid fatty acids are carcinogens in trout, markedly potentiate the carcinogenicity of aflatoxin in trout, cause atherosclerosis in rabbits, are mitogenic in rats, and have a variety of toxic effects in farm animals (51). The toxicity of these fatty acids could be due to their ease of oxidation to form peroxides and radicals (51).

13) Leguminous plants such as lupine contain very potent teratogens (22). When cows and goats forage on these plants, their offspring may have severe teratogenic abnormalities; an example is

the characteristic "crooked calf" abnormality due to the ingestion of *anagyrine* from lupine (22). In addition, significant amounts of these teratogens are transferred to the animals' milk, so that drinking the milk during pregnancy is a serious teratogenic hazard (22). In one rural California family, a baby boy, a litter of puppies, and goat kids all had "crooked" bone birth-defect abnormalities. The pregnant mother and the dog had both been drinking milk obtained from the family goats, which had been foraging on lupine (the main forage in winter) (22). It was at first mistakenly thought that the birth defects were caused by spraying of 2,4-D.

14) *Sesquiterpene lactones* are widespread in many plants (37), although because they are bitter they are not eaten in large amounts. Some have been shown to be mutagenic (37). They are a major toxin in the white sap of *Lactuca virosa* (poison lettuce), which has been used as a folk remedy. Plant breeders are now transferring genes from this species to commercial lettuce to increase insect resistance (38).

15) The *phorbol esters* present in the Euphorbiaceae, some of which are used as folk remedies or herb teas, are potent promoters of carcinogenesis and may have been a cause of nasopharyngeal cancer in China and esophageal cancer in Curaçao (39).

16) Alfalfa sprouts contain canavanine, a highly toxic arginine analog that is incorporated into protein in place of arginine. Canavanine, which occurs in alfalfa sprouts at about 1.5 percent of their dry weight (40), appears to be the active agent in causing the severe lupus erythematosus-like syndrome seen when monkeys are fed alfalfa sprouts (40). Lupus in man is characterized by a defect in the immune system which is associated with autoimmunity, antinuclear antibodies, chromosome breaks, and various types of pathology (40). The chromosome breaks appear to be due to oxygen radicals as they are prevented by superoxide dismutase (52). The canavanine-alfalfa sprout pathology could be due in part to the production of oxygen radicals during phagocytization of antibody complexes with canavanine-containing protein.

The 16 examples above, plus coffee (discussed below), illustrate that the human dietary intake of "nature's pesticides" is likely to be several grams per day—probably at least 10,000 times higher than the dietary intake of man-made pesticides (53).

Levels of plant toxins that confer insect and fungal resistance are being increased or decreased by plant breeders

(38). There are health costs for the use of these natural pesticides, just as there are for man-made pesticides (41, 54), and these must be balanced against the costs of producing food. However, little information is available about the toxicology of most of the natural plant toxins in our diet, despite the large doses we are exposed to. Many, if not most, of these plant toxins may be "new" to humans in the sense that the human diet has changed drastically with historic times. By comparison, our knowledge of the toxicological effects of new man-made pesticides is extensive, and general exposure is exceedingly low (53).

Plants also contain a variety of anticarcinogens (55), which are discussed below.

Alcohol. Alcohol has long been associated with cancer of the mouth, esophagus, pharynx, larynx, and, to a lesser extent, liver (1, 56), and it appears to be an important human teratogen, causing a variety of physical and mental defects in babies of mothers who drink (57). Alcohol drinking causes abnormalities in mice (57a) and is a synergist for chromosome damage in humans (58). Alcohol metabolism generates acetaldehyde, which is a mutagen and teratogen (59), a cocarcinogen, and possibly a carcinogen (60), and also radicals that produce lipid hydroperoxides (61) and other mutagens and carcinogens (62; see below). In some epidemiologic studies on alcohol (56), it has been suggested that dietary green vegetables are a modifying factor in the reduction of cancer risk.

Mold carcinogens. A variety of mold carcinogens and mutagens are present in mold-contaminated food such as corn, grain, nuts, peanut butter, bread, cheese, fruit, and apple juice (15, 63). Some of these, such as sterigmatocystin and aflatoxin, are among the most potent carcinogens and mutagens known (15, 63). Dietary glutathione has been reported to counteract aflatoxin carcinogenicity.

Nitrite, nitrate, and nitrosamines. A number of human cancers, such as stomach and esophageal cancer, may be related to nitrosamines and other nitroso compounds formed from nitrate and nitrite in the diet (64, 65). Beets, celery, lettuce, spinach, radishes, and rhubarb all contain about 200 mg of nitrate per 100-g portion (65). Anticarcinogens in the diet may be important in this context as well (66).

Fat and cancer: possible oxidative mechanisms. Epidemiologic studies of cancer in humans suggest, but do not prove, that high fat intake is associated with colon and breast cancer (1, 4, 67). A

number of animal studies have shown that high dietary fat is a promoter and a presumptive carcinogen (4, 67, 68). Colon and breast cancer and lung cancer (which is almost entirely due to cigarette smoking) account for about half of all U.S. cancer deaths. In addition to the cyclopropanoid fatty acids already discussed, two other plausible mechanisms involving oxidative processes could account for the relation (69) between high fat and both cancer and heart disease.

1) *Rancid fat.* Fat accounts for over 40 percent of the calories in the U.S. diet (67), and the amount of ingested oxidized fat may be appreciable (70, 71). Unsaturated fatty acids and cholesterol in fat are easily oxidized, particularly during cooking (70, 71). The lipid peroxidation chain reaction (rancidity) yields a variety (71–73) of mutagens, promoters, and carcinogens such as fatty acid hydroperoxides (62), cholesterol hydroperoxide (74), endoperoxides, cholesterol and fatty acid epoxides (74–77), enals and other aldehydes (44, 59, 78), and alkoxy and hydroperoxy radicals (44, 72). Thus the colon and digestive tract are exposed to a variety of fat-derived carcinogens. Human breast fluid can contain enormous levels (up to 780 μM) (75) of cholesterol epoxide (an oxidation product of cholesterol), which could originate from either ingested oxidized fat or oxidative processes in body lipids. Rodent feeding studies with oxidized fat (79) have not yielded definitive results.

2) *Peroxisomes* oxidize an appreciable percentage of dietary fatty acids, and removal of each two-carbon unit generates one molecule of hydrogen peroxide (a mutagen, promoter, and carcinogen) (80, 81). Some hydrogen peroxide escapes the catalase in the peroxisome (80, 82, 83), thus contributing to the supply of oxygen radicals, which also come from other metabolic sources (72, 83–85). Hydroperoxides generate oxygen radicals in the presence of iron-containing compounds in the cell (72). Oxygen radicals, in turn, can damage DNA and can start the rancidity chain reaction which leads to the production of the mutagens and carcinogens listed above (72). Drugs such as clofibrate, which cause lowering of serum lipids and proliferation of peroxisomes in rodents, result in age pigment (lipofuscin) accumulation (a sign of lipid peroxidation in tissues) and liver tumors in animals (80). Some fatty acids, such as $\text{C}_{22:1}$ and certain *trans* fatty acids, appear to cause peroxisomal proliferation because they are poorly oxidized in mitochondria and are preferentially oxidized in the peroxisomes, although they may be selective for heart or

liver (86). There has been controversy about the role of *trans* fatty acids in cancer and heart disease, and recent evidence suggests that *trans* fatty acids might not be a risk factor for atherosclerosis in experimental animals (87). Americans consume about 12 g of *trans* fatty acids a day (87) and a similar amount of unnatural *cis* isomers [which need further study (88)], mainly from hydrogenated vegetable fats. Dietary C_{22:1} fatty acids are also obtained from rapeseed oil and fish oils (86). Thus oxidation of certain fatty acids might generate grams of hydrogen peroxide per day within the peroxisome (86). Another source of fat toxicity could be perturbations in the mitochondrial or peroxisomal membranes caused by abnormal fatty acids, yielding an increased flux of superoxide and hydrogen peroxide. Mitochondrial structure is altered when rats are fed some abnormal fatty acids from partially hydrogenated fish oil (89). Dietary C_{22:1} fatty acids and clofibrate also induce ornithine decarboxylase (86), a common attribute of promoters.

A recent National Academy of Sciences committee report suggests that a reduction of fat consumption in the American diet would be prudent (4), although other scientists argue that, until we know more about the mechanism of the fat-cancer relation and about which types of fat are dangerous, it is premature to recommend dietary changes (90).

Cooked Food as a Source of Ingested Burnt and Brownd Material

Work of Sugimura and others has indicated that the burnt and browned material from heating protein during cooking is highly mutagenic (21, 91). Several chemicals isolated on the basis of their mutagenicity from heated protein or pyrolyzed amino acids were found to be carcinogenic when fed to rodents (21). In addition, the browning reaction products from the caramelization of sugars or the reaction of amino acids and sugars during cooking (for instance, the brown material on bread crusts and toasted bread) contain a large variety of DNA-damaging agents and presumptive carcinogens (23, 38, 92). The amount of burnt and browned material in the human diet may be several grams per day. By comparison about 500 mg of burnt material is inhaled each day by a smoker using two packs of cigarettes (at 20 mg of tar per cigarette) a day. Smokers have more easily detectable levels of mutagens in their urine than nonsmokers (93), but so do people who have consumed a meal of

fried pork or bacon (94). In the evaluation of risk from burnt material it may be useful (in addition to carrying out epidemiologic studies) to compare the activity of cigarette tar to that of the burnt material from cooked food (or polluted air) in short-term tests and animal carcinogenicity tests involving relevant routes of exposure. Route of exposure and composition of the burnt material are critical variables. The risk from inhaled cigarette smoke can be one reference standard: an average life shortening of about 8 years for a two-pack-a-day smoker. The amount of burnt material inhaled from severely polluted city air, on the other hand, is relatively small: it would be necessary to breathe smoggy Los Angeles air (111 µg/m³ total particulates; 31 µg/m³ soluble organic matter) for 1 to 2 weeks to equal the soluble organic matter of the particulates or the mutagenicity from one cigarette (20 mg of tar) (95). Epidemiologic studies have not shown significant risks from city air pollution alone (1, 96). Air in the houses of smokers is considerably more polluted than city air outside (97).

Coffee, which contains a considerable amount of burnt material, including the mutagenic pyrolysis product methylglyoxal, is mutagenic (21, 98). However, one cup of coffee also contains about 250 mg of the natural mutagen chlorogenic acid (9) [which is also an antinitrosating agent (66)], highly toxic atracylosides (10), the glutathione transferase inducers kahweol palmitate and cafestol palmitate (11), and about 100 mg of caffeine [which inhibits a DNA-repair system and can increase tumor yield (99) and cause birth defects at high levels in several experimental species (100)]. There is preliminary, but not conclusive, epidemiologic evidence that heavy coffee drinking is associated with cancer of the ovary, bladder, pancreas, and large bowel (101).

Cooking also accelerates the rancidity reaction of cooking oils and fat in meat (70, 71), thus increasing consumption of mutagens and carcinogens.

Anticarcinogens

We have many defense mechanisms to protect ourselves against mutagens and carcinogens, including continuous shedding of the surface layer of our skin, stomach, cornea, intestines, and colon (102). Understanding these mechanisms should be a major goal of cancer, heart, and aging research. Among the most important defenses may be those against oxygen radicals and lipid peroxidation if, as discussed here, these agents are major

contributors to DNA damage (103). Major sources of endogenous oxygen radicals are hydrogen peroxide (83) and superoxide (72, 104) generated as side products of metabolism, and the oxygen radical burst from phagocytosis after viral or bacterial infection or the inflammatory reaction (105). A variety of environmental agents could also contribute to the oxygen radical load, as discussed here and in recent reviews (72, 106). Many enzymes protect cells from oxidative damage; examples are superoxide dismutase (104), glutathione peroxidase (107), DT-diaphorase (108), and the glutathione transferases (109). In addition, a variety of small molecules in our diet are required for antioxidative mechanisms and appear to be anticarcinogens; some of these are discussed below.

1) *Vitamin E* (tocopherol) is the major radical trap in lipid membranes (72) and has been used clinically in a variety of oxidation-related diseases (110). Vitamin E ameliorates both the cardiac damage and carcinogenicity of the quinones adriamycin and daunomycin, which are mutagenic, carcinogenic, cause cardiac damage, and appear to be toxic because of free radical generation (111). Protective effects of tocopherols against radiation-induced DNA damage and mutation and dimethylhydrazine-induced carcinogenesis have also been observed (112). Vitamin E markedly increases the endurance of rats during heavy exercise, which causes extensive oxygen radical damage to tissues (113).

2) *β-Carotene* is another antioxidant in the diet that could be important in protecting body fat and lipid membranes against oxidation. Carotenoids are free-radical traps and remarkably efficient quenchers of singlet oxygen (114). Singlet oxygen is a very reactive form of oxygen which is mutagenic and particularly effective at causing lipid peroxidation (114). It can be generated by pigment-mediated transfer of the energy of light to oxygen, or by lipid peroxidation, although the latter is somewhat controversial. *β-Carotene* and similar polyenes are present in carrots and in all food that contains chlorophyll, and they appear to be the plants' main defense against singlet oxygen generated as a by-product from the interaction of light and chlorophyll (115). Carotenoids have been shown to be anticarcinogens in rats and mice (116). Carotenoids (in green and yellow vegetables) may be anticarcinogens in humans (1, 56, 117). Their protective effects in smokers might be related to the high level of oxidants in both cigarette smoke and tar (45, 118). Carotenoids have been used medically in

the treatment for some genetic diseases, such as porphyrias, where a marked photosensitivity is presumably due to singlet oxygen formation (119).

3) *Selenium* is another important dietary anticarcinogen. Dietary selenium (usually selenite) significantly inhibits the induction of skin, liver, colon, and mammary tumors in experimental animals by a number of different carcinogens, as well as the induction of mammary tumors by viruses (120). It also inhibits transformation of mouse mammary cells (121). Low selenium concentrations may be a risk factor in human cancer (122). A particular type of heart disease in young people in the Keshan area of China has been traced to a selenium deficiency, and low selenium has been associated with cardiovascular death in Finland (123). Selenium is in the active site of glutathione peroxidase, an enzyme essential for destroying lipid hydroperoxides and endogenous hydrogen peroxide and thus helping to prevent oxygen radical-induced lipid peroxidation (107), although not all of the effects of selenium may be accounted for by this enzyme (120). Several heavy-metal toxins, such as Cd^{2+} (a known carcinogen) and Hg^{2+} , lower glutathione peroxidase activity by interacting with selenium (107). Selenite (and vitamin E) has been shown to counter the oxidative toxicity of mercuric salts (124).

4) *Glutathione* is present in food and is one of the major antioxidants and anti-mutagens in the soluble fraction of cells. The glutathione transferases (some of which have peroxidase activity) are major defenses against oxidative and alkylating carcinogens (109). The concentration of glutathione may be influenced by dietary sulfur amino acids (125, 126). *N*-Acetylcysteine, a source of cysteine, raises glutathione concentrations and reduces the oxidative cardiotoxicity of adriamycin and the skin reaction to radiation (127). Glutathione concentrations are raised even more efficiently by L-2-oxothiazolidine-4-carboxylate, which is an effective antagonist of acetaminophen-caused liver damage (126). Acetaminophen is thought to be toxic through radical and quinone oxidizing metabolites (128). Dietary glutathione may be an effective anticarcinogen against aflatoxin (129).

5) Dietary *ascorbic acid* is also important as an antioxidant. It was shown to be anticarcinogenic in rodents treated with ultraviolet radiation, benzo[a]pyrene, and nitrite (forming nitroso carcinogens) (64, 65, 130), and it may be inversely associated with human uterine cervical dysplasia (although this is not

proof of a cause-effect relationship) (131). It was recently hypothesized that ascorbic acid may have been supplemented and perhaps partially replaced in humans by uric acid during primate evolution (132).

6) *Uric acid* is a strong antioxidant present in high concentrations in the blood of humans (132). The concentration of uric acid in the blood can be increased by dietary purines; however, too much causes gout. Uric acid is also present in high concentrations in human saliva (132) and may play a role in defense there as well, in conjunction with lactoperoxidase. A low uric acid level in blood may possibly be a risk factor in cigarette-caused lung cancer in humans (133).

7) Edible plants and a variety of substances in them, such as phenols, have been reported to inhibit (cabbage) or to enhance (beets) carcinogenesis (11, 55, 134) or mutagenesis (23, 66, 92, 135) in experimental animals. Some of these substances appear to inhibit by inducing cytochrome P-450 and other metabolic enzymes [(134); see also (11)], although on balance it is not completely clear whether it is generally helpful or harmful for humans to ingest these inducing substances.

The hypothesis that as much as 80 percent of cancer could be due to environmental factors was based on geographic differences in cancer rates and studies of migrants (136). These differences in cancer rates were thought to be mainly due to life-style factors, such as smoking and dietary carcinogens and promoters (136), but they also may be due in good part [see also (1)] to less than optimum amounts of anticarcinogens and protective factors in the diet.

The optimum levels of dietary antioxidants, which may vary among individuals, remain to be determined; however, at least for selenium (120), it is important to emphasize the possibility of deleterious side effects at high doses.

Oxygen Radicals and Degenerative Diseases Associated with Aging

Aging. A plausible theory of aging holds that the major cause is damage to DNA (102, 137) and other macromolecules and that a major source of this damage is oxygen radicals and lipid peroxidation (43, 84, 103, 138-141). Cancer and other degenerative diseases, such as heart disease (102), are likely to be due in good part to this same fundamental destructive process. Age pigment (lipofuscin) accumulates aging in all mammalian

species and has been associated with lipid peroxidation (73, 84, 138, 139). The fluorescent products in age pigment are thought to be formed by malondialdehyde (a mutagen and carcinogen and a major end product of rancidity) cross-linking protein and lipids (138). Metabolic rate is directly correlated with the rate of lipofuscin formation (and inversely correlated with longevity) (139).

Cancer increases with about the fourth power of age, both in short-lived species such as rats and mice (about 30 percent of rodents have cancer by the end of their 2- to 3-year life-span) and in long-lived species such as humans (about 30 percent of people have cancer by the end of their 85-year life-span) (142). Thus, the marked increase in life-span that has occurred in 60 million years of primate evolution has been accompanied by a marked decrease in age-specific cancer rates; that is, in contrast to rodents, 30 percent of humans do not have cancer by the age of 3 (142). One important factor in longevity appears to be basal metabolic rate (139, 141), which is much lower in man than in rodents and could markedly affect the level of endogenous oxygen radicals.

Animals have many antioxidant defenses against oxygen radicals. Increased levels of these antioxidants, as well as new antioxidants, may also be a factor in the evolution of man from short-lived prosimians (143). It has been suggested that an increase in superoxide dismutase is correlated (after the basal metabolic rate is taken into account) with increased longevity during primate evolution, although this has been disputed (141). Ames *et al.* proposed (132) that as uric acid was an antioxidant and was present in much higher concentrations in the blood of humans than in other mammals, it may have been one of the innovations enabling the marked increase in life span and consequent marked decrease in age-specific cancer rates which occurred during primate evolution. The ability to synthesize ascorbic acid may have been lost at about the same time in primate evolution as uric acid levels began to increase (144).

Cancer and promotion. Both DNA-damaging agents (initiating mutagens) (21, 41, 42) and promoters (145) appear to play an important role in carcinogenesis (21, 146). It has been postulated that certain promoters of carcinogenesis act by generation of oxygen radicals and resultant lipid peroxidation (73, 146-149). Lipid peroxidation cross-links proteins (43, 150) and affects all aspects of cell organization (72), including membrane and surface structure, and the mi-

totic apparatus. A common property of promoters may be their ability to produce oxygen radicals. Some examples are fat and hydrogen peroxide (which may be among the most important promoters) (67, 68, 81), TCDD (151), lead and cadmium (152), phorbol esters (147, 149, 153), wounding of tissues (154), asbestos (155), peroxides (156), catechol (45) (see quinones above), mezcrcin and teleocidin B (147), phenobarbital (157), and radiation (72, 158). Inflammatory reactions involve the production of oxygen radicals by phagocytes (105), and this could be the basis of promotion for asbestos (155) or wounding (154). Some of the antioxidant anticarcinogens (discussed above) are also antipromoters (73, 121, 146, 159, 160), and phorbol ester-induced chromosome damage (149) or promotion of transformation (159) is suppressed by superoxide dismutase, as would be expected if promoters were working through oxidative mechanisms. Many "complete" carcinogens cause the production of oxygen radicals (73, 161); examples are nitroso compounds, hydrazines, quinones, polycyclic hydrocarbons (through quinones), cadmium and lead salts, nitro compounds, and radiation. A good part of the toxic effects of ionizing radiation damage to DNA and cells is thought to be due to generation of oxygen radicals (103, 162), although only a tiny part of the oxygen radical load in humans is likely to be from this source.

Recent studies give some clues as to how promoters might act. Promoters disrupt the mitotic apparatus, causing hemizygosity and expression of recessive genes (163). Phorbol esters generate oxygen radicals, which cause chromosome breaks (164) and increase gene copy number (165). Promoters also cause formation of the peroxide hormones of the prostaglandin and leukotriene family by oxidation of arachidonic acid and other C₂₀ polyenoic fatty acids, and inhibitors of this process appear to be antipromoters (160). These hormones are intimately involved in cell division, differentiation, and tumor growth (166) and could have arisen in evolution as signal molecules warning the cell of oxidative damage. Effects on the cell membrane have also been suggested as the important factor in promotion, causing inhibition of intercellular communication (167) or protein kinase activation (167a).

Heart disease. It has been postulated that atherosclerotic lesions, which are derived from single cells, are similar to benign tumors and are of somatic mutational origin (102, 168). Fat appears to be one major risk factor for heart disease as

well as for colon and breast cancer (69). In agreement with this, a strong correlation has been observed between the frequency of atherosclerotic lesions and adenomatous polyps of the colon (69). Thus, the same oxidative processes involving fat may contribute to both diseases. Oxidized forms of cholesterol have been implicated in heart disease (169), and atherosclerotic-like lesions have been produced by injecting rabbits with lipid hydroperoxide or oxidized cholesterol (169). The anticarcinogens discussed above could be anti-heart disease agents as well. As pointed out in the preceding section, vitamin E ameliorates both the cardiac damage and carcinogenicity of the free-radical-generating quinones adriamycin and daunomycin; N-acetylcysteine reduces the cardiotoxicity of adriamycin; and selenium is an antirisk factor for one type of heart disease.

Other diseases. The brain uses 20 percent of the oxygen consumed by man and contains an appreciable amount of unsaturated fat. Lipid peroxidation (with consequent age pigment) is known to occur readily in the brain (72), and possible consequences could be senile dementia or other brain abnormalities (84). Several inherited progressive diseases of the central nervous system, such as Batten's disease, are associated with lipofuscin accumulation and may be due to a lipid peroxidation caused by a high concentration of unbound iron (170). Mental retardation is one consequence of an inherited defective DNA repair system (XP complementation group D) for depurinated sites in DNA (171).

Senile cataracts have been associated with light-induced oxidative damage (172). The retina and an associated layer of cells, the pigment epithelium, are extremely sensitive to degeneration in vitamin E and selenium deficiency (173). The pigment epithelium accumulates massive amounts of lipofuscin in aging and dietary antioxidant deficiency (173). The eye is well known to be particularly rich in antioxidants.

The testes are quite prone to lipid peroxidation and to the accumulation of age pigment. A number of agents, such as gossypol, which cause genetic birth defects (dominant lethals) may be active by this mechanism. The various agents known to cause cancer by oxidative mechanisms are prospective mutagenic agents for the germ line. Thus, vitamin E, which was discovered 60 years ago as a fertility factor (72), and other antioxidants such as selenium (174), may help both to engender and to protect the next generation.

Risks

There are large numbers of mutagens and carcinogens in every meal, all perfectly natural and traditional [see also (21, 23)]. Nature is not benign. It should be emphasized that no human diet can be entirely free of mutagens and carcinogens and that the foods mentioned are only representative examples. To identify a substance, whether natural or man-made, as a mutagen or a carcinogen, is just a first step. Beyond this, it is necessary to consider the risks for alternative courses of action and to quantitate the approximate magnitude of the risk, although the quantification of risk poses a major challenge. Carcinogens differ in their potency in rodents by more than a millionfold (175), and the levels of particular carcinogens to which humans are exposed can vary more than a billionfold. Extrapolation of risk from rodents to humans is difficult for many reasons, including the longevity difference, antioxidant factors, and the probable multi-causal nature of most human cancer.

Tobacco smoking is, without doubt, a major and well-understood risk, causing about 30 percent of cancer deaths and 25 percent of fatal heart attacks (as well as other degenerative diseases) in the United States (1). These percentages may increase even more in the near future as the health effects of the large increase in women smokers become apparent (1). Diet, which provides both carcinogens and anticarcinogens, is extremely likely to be another major risk factor. Excessive alcohol consumption is another risk, although it does not seem to be of the same general importance as smoking and diet. Certain other high-dose exposures might also turn out to be important for particular groups of people—for instance, certain drugs, where consumption can reach hundreds of milligrams per day; particular cosmetics; and certain occupational exposures (2), where workers inhale dusts or solvents at high concentration. We must also be prudent about environmental pollution (41, 54). Despite all of these risks, it should be emphasized that the overall trend in life expectancy in the United States is continuing steadily upward (176).

The understanding of cancer and degenerative disease mechanisms is being aided by the rapid progress of science and technology, and this should help to dispel confusion about how important health risks can be identified among the vast number of minor risks. We have many methods of attacking the problem of environmental carcinogens (and anticarcinogens), including human epidemi-

ology (1), short-term tests (41, 42, 177), and animal cancer tests (175). Powerful new methods are being developed [for instance, see (58, 177)] for measuring DNA damage or other pertinent factors with great sensitivity in individuals. These methods, which are often noninvasive as they can be done on blood or urine (even after storage), can be combined with epidemiology to determine whether particular factors are predictive of disease. Thus, more powerful tools will be available for optimizing antioxidants and other dietary anti-risk factors, for identifying human genetic variants at high risk, and for identifying significant health risks.

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RESEARCH ARTICLE

Imaging Dopamine Receptors in the Human Brain by Positron Tomography

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One of the most intriguing problems in biomedical research today is that of relating manifestations of neuropsychiatric diseases to chemical processes in different parts of the brain. The neurotrans-

mitter dopamine appears to be associated with abnormalities related to disorders such as Parkinson's disease and schizophrenia. The highest density of dopamine neurons occurs in the nigrostriatal dopamine pathway which degenerates in Parkinson's disease (1). Neuroleptic drugs elicit extrapyramidal parkinsonian side effects by blocking dopamine receptors in the corpus striatum and also exert antischizophrenic action by blocking dopamine receptors, perhaps in limbic areas (2). Numbers of dopamine receptors are increased by chronic neuroleptic treatment (3) and are also increased in some schizophrenics, perhaps

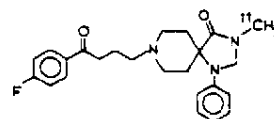
as a result of neuroleptic therapy (4). The development of positron emission tomography (PET) and appropriate radioactive tracers labeled with positron-emitting radionuclides has now made it

Abstract. *Neurotransmitter receptors may be involved in a number of neuropsychiatric disease states. The ligand 3-N-¹¹C]methylspiperone, which preferentially binds to dopamine receptors in vivo, was used to image the receptors by positron emission tomography scanning in baboons and in humans. This technique holds promise for noninvasive clinical studies of dopamine receptors in humans.*

possible to relate regional biochemistry within the human brain to measurements of behavior in normal subjects and to elucidate abnormalities in patients with Alzheimer's disease (5), Huntington's disease (6), depression (7), and multiple infarct dementia (8). The technique consists of intravenous injection of a substance such as ¹⁸F-labeled deoxyglu-

cose, [¹¹C]carboxyhemoglobin, ionic rubidium-82, ⁶⁸Ga-labeled EDTA, and other radiopharmaceuticals, and subsequent imaging of the distribution of the radioactive label in the brain by means of the tomographic method, based on detection of the annihilation radiation produced during positron emission (9).

The butyrophenone neuroleptic drug spiperone has been useful in binding studies for measuring dopamine receptors both in vitro (10) and in vivo (11). We now report initial results obtained with 3-N-¹¹C]methylspiperone (¹¹C-NMSP), a spiperone derivative, in PET



scanning studies to visualize the distribution of dopamine receptors in the brains of baboons and a human being. All studies were performed with a NeuroECAT scanner (Ortec, Inc., Oak Ridge, Tennessee), which has a spatial resolution of approximately 8 mm (full width at half maximum) in the plane of the slice. The distance between slices is 3 cm.

The newly developed tracer ¹¹C-NMSP was synthesized by N-alkylation of spiperone with [¹¹C]methyl iodide; the iodide was produced from ¹¹CO₂, which in turn had been produced with an in-hospital cyclotron (model RNP-16, Scanditronix Cyclotron, Sweden). Carbon-11 is a positron-emitting isotope with a physical half-life of 20 minutes. The entire synthesis was accomplished with material ready for injection within 55 minutes after the end of the cyclotron

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Natural carcinogenic products

Not all cancer-causing substances are man-made. Nature does a fair share of producing compounds that are potential toxins when ingested; fortunately, many of these toxins are weak carcinogens.



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There are many statements in the popular press that cancer results from exposure to pollutants emanating from the increase in technological developments. However, certain historical documents indicate that lung cancer, for example, often associated with air pollution or excess smoking, was also known in Germany during the Middle Ages.

Lung cancer, which occurred among

the cobalt miners of the Schneeberg in Saxony, was described by Paracelsus in 1531, and by Agricola and others who called it "Bergkrankheiten" (mountain disease). At that time the disease was mistaken for tuberculosis of the lung. This ailment, which also occurred in the miners working in the Joachimsthal of eastern Germany, was caused by exposure to uranium, radium, and their daughter products present in the ores mined in these locations.

It is currently evident that modern miners on the Colorado Plateau encounter the same risks as the German miners, especially when exposure to

uranium and its daughters is combined with smoking. Thus, an occupational disease related to a "natural" product was first noted almost 450 years ago.

Culprits: natural organics

Other naturally occurring inorganic compounds are associated with an increase in cancer rate. Examples are arsenic and several other metals present in drinking water in certain areas of the world. However, naturally occurring organic compounds are much more prevalent among the lists of natural carcinogenic products.

A research group showed in 1942 that crude ergot was tumorigenic in

animals fed this compound for six months or more. Since the tumors generally regressed when the ergot was discontinued, this was indicative of a relatively benign effect. The constituents of ergot responsible for this action have not been investigated.

In the early 1950's, it was reported that subcutaneous injections of tannic acid or tannins induced an appreciable number of liver tumors in both rats and mice. The data were somewhat contradictory and have not been fully confirmed, especially in view of the somewhat artificial method of administration used. For this reason, the carcinogenicity of tannins and tannic acid for a route pertinent to human exposure is questionable.

During the 1960s, it was first reported that safrole caused liver tumors in animals fed the substance at high dose levels. Safrole, which occurs in many natural oils such as anise, camphor or sassafras oil, was previously allowed as a synthetic flavoring agent in beverages and foods.

As a result of the tumor-inducing properties of safrole, reexamination of the GRAS (Generally Recognized as Safe) list, which includes safrole, was deemed necessary by the FDA. Safrole is partially metabolized by hydroxylation to 1'-hydroxysafrole, which is further metabolized to 1-acetoxysafrole, which is considered the likely active carcinogenic intermediate.

In an attempt to identify the agent responsible for the high incidence of amyotrophic lateral sclerosis in Guam, the National Institute of Neurological Diseases and Blindness tested various plant products used on that island, including the nuts of the cycad plant. These nuts were used as an emergency starch when typhoons had destroyed other food sources. Many different varieties of the cycad plant (*Cycas circinalis*), which is a member of an ancient plant family, grow in tropical or subtropical areas.

The nuts, about the size of a lemon, and the outer husk are the parts used in Guam. The husk is chewed by people working in the fields; the nut is used as a starch after it is soaked in water to remove the toxin. The toxic material is cycasin, the glucoside of methylazoxymethanol, which is the active moiety.

Cycasin causes liver, kidney and intestinal tumors in rats and other laboratory animals. It also has neurological effects since cattle grazing on cycads, often the only green forage in times of drought, may show paralysis.

In Kenya, the kernels of the plant *Encephalartos*, also a member of the



Cycasin. The nuts and husks (above) of the cycad (below) plant, which contain the toxin cycasin, are used as food by the people of Guam; husks are chewed, and the nuts are soaked and used as a starch



Peanuts. Moldy peanuts contain the deadly toxin aflatoxin

cycad family, are consumed both by humans and by cattle. In this very large kernel, the toxic compound is macrozamin, which is also carcinogenic in rats, causing kidney, liver and lung tumors similar to the effect of cycasin.

Edible but toxic

In many areas of the world poisoning of domestic animals by plants of the *Senecio*, *Heliotrope* and *Crotalaria* species has been reported. *Senecio* species are quite widespread, and some forms, such as "golden ragwort" or *Senecio aureus*, are found in North America. These aster-like plants are quite toxic to the liver because detoxification of ammonia from dietary nitrogen is blocked. Thus grazing animals which eat these plants often die from ammonia intoxication.

In many African countries the *Senecio* plants, especially *S. jacobaea*, are used as herbal medicines despite their toxicity. Most of these plants contain one or the other of the pyrrolizidine alkaloids such as retrorsine, isatidine and lasiocarpine. Honey made from such plants may be contaminated by these alkaloids, some of which are also liver carcinogens in rats.

In Japan, plants known as coltsfoot are used as herbal remedies or as vegetables. Several of these are also carcinogenic to the livers of rats. Apparently, one compound responsible is an alkaloid, petastenine, which has a structure similar to that of the pyrrolizidine alkaloids isolated from the *Senecio* plants. Also, there is a recent report that a plant called comfrey, which is used as a poultice or as a tea, has a carcinogenic effect in animals.

One may question the use of herbal remedies or sassafras tea in modern society. Nevertheless, until several years ago one of the materials used to flavor vermouth, a constituent of popular drinks such as martinis or Manhattans, was a root of an Indian (Jammu) variety of calamus.

The American variety of this plant is *Acorus calamus* or sweet flag. This plant was used by the American Indians and others as a flavoring agent or folk medicine. In 1967 it was found that β -asarone, about 85% of the flavoring material in Jammu calamus, caused intestinal tumors in rats. Thus the use of calamus as a flavoring material for American vermouth was no longer allowed by the FDA. Because of certain structural similarities between safrole and β -asarone, one might assume analogous pathways for activation of β -asarone and safrole.

Another compound with similar features is estragole, a component of oil or estragen or oil of tarragon, obtained from the tarragon plant, *Artemisia dracunculus*. Recent studies have shown that estragole has a definite but weak liver carcinogenic effect in young male mice. Since tarragon is often used as a flavoring agent in gourmet types of vinegar, naturally occurring carcinogens may be consumed by those living sophisticated lifestyles.

Another gourmet-type food is a wild mushroom, the false morel (*Gyromitra esculenta*). This mushroom contains N-methyl-N-formylhydrazine, which causes several types of tumors when given to mice in their drinking water for their lifespan.

Active ingredient unknown

In the previously mentioned plants, the carcinogenic components have generally been identified. However, there are other carcinogenic plants in which the active component has not been characterized. One of these is the bracken fern, a common inhabitant of open woods and waste places. The young shoots, called the fiddleheads or croziers, are eaten as a springtime delicacy in many countries.

Cattle that often graze on bracken fern are found to have bladder or stomach cancer; this is especially so in Turkey, Scotland and Wales. To some extent the effect of bracken fern in experimental animals can be inhibited by simultaneous administration of the enzyme-inducer phenothiazine.

Various compounds have been proposed as the active component in bracken fern. However, numerous efforts to fractionate and characterize the active material(s) have not been productive.

Of all carcinogenic plants, the one with the greatest exposure is probably the tobacco plant. There continues to be much controversy over the excess risk of lung and bladder cancer from smoking cigarettes. Since tobacco smoke contains dimethylnitrosamine, benzo(a)pyrene and numerous other known carcinogens, the probability of a risk from continued long-term exposure would seem high.

The gas phase contains nitrosamines, hydrazine, vinyl chloride, and several other toxic materials. The particulate phase contains numerous polycyclic aromatic hydrocarbons of varying carcinogenic potency. In addition, some of these substances, at least in animal experiments, have an organ-specific effect, especially for the esophagus, lung, pancreas and bladder.

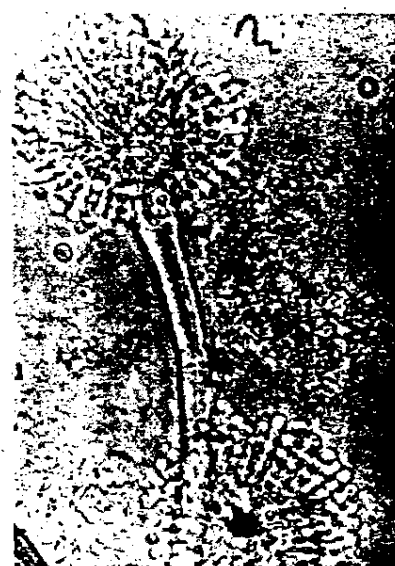
Further studies indicate that at least four nitrosamines are present in unburned tobacco itself. One of these, N-nitrosodiethanolamine, was found previously to induce liver cancer in rats.

Endogenously formed carcinogens may also represent a hazard. There has been much publicity and controversy lately over N-nitroso compounds formed in the digestive system from nitrite and secondary or tertiary amines.

Another case of interest is the formation of ethyl carbamate or urethane in foods or beverages processed by fermentation. Ethyl carbamate is a weak carcinogen but does cause lung tumors in mice, or liver tumors in mice or newborn rats, although adult rats are resistant to its action. Foods and beverages such as beer, wine, bread, olives and yogurt all contain measurable levels of the compound, which is probably formed by reaction of endogenous carbamyl phosphate with ethanol to yield ethyl carbamate.

Microbes make carcinogens

Even microorganisms can synthesize carcinogens. Some of these toxins, when ingested, affect more people than any other unintentionally ingested natural carcinogen.



Fungus. *Aspergillus flavus* on moldy grains produces aflatoxin, which is a potent liver carcinogen

In 1960, an outbreak in England of a fatal liver disease in turkey poult was traced to peanut meal in their feed which had been infested with the fungus *Aspergillus flavus*. This fungus is capable of producing some very toxic compounds such as aflatoxin B₁ and

B₂, lesser quantities of aflatoxin G₁ and G₂, and several other compounds which have not been thoroughly investigated.

Aflatoxin B₁ is one of the most potent liver carcinogens in rats or rainbow trout. In certain strains of rats fed 0.1 ppm of aflatoxin B₁ for 50–80 weeks there was a 50% incidence of liver cancer; even 0.015 ppm was effective after 68–80 weeks. Rainbow trout were affected by levels as low as 0.1 ppb. On the other hand, adult mice are quite resistant to aflatoxin.

The fungus can contaminate many types of grains or oil seeds such as rice, corn, cottonseed meal, and copra. Cows fed meal containing aflatoxin B₁ or B₂ excrete aflatoxin M₁ or M₂ in the milk. These compounds represent detoxification products; they are much less toxic to rats, and do not induce tumors in rats under conditions where aflatoxin B₁ does.

In warm, humid areas of the world the aflatoxin contamination of food crops can often be correlated with liver cancer incidence in the population. Data supporting this supposition have been obtained from Swaziland, Kenya and Thailand.

Another compound, similar to aflatoxin, is sterigmatocystin, produced by *Aspergillus versicolor* or *Aspergillus nidulans*. Although this compound is carcinogenic in rats and mice, it is considered for use as a chemotherapeutic agent in the treatment of neoplastic (tumor-related) diseases.

Certain other microorganisms, however, are capable of synthesizing quite unique structures. Elaiomycin, produced by *Streptomyces hepaticus* has an azoxy structure similar to that of cycasin, and has weak carcinogenic activity in rats. Griseofulvin from *Penicillium griseofulvum* is useful in the treatment of fungal skin infections. This compound has induced liver tumors and other liver-specific effects such as cirrhosis and protoporphyrin in mice. Streptozotocin, from *Streptomyces achromogenes* possesses an N-nitrosourea structure, a most unusual type of compound. While this compound induces kidney and lung tumors in rats, it is a useful drug in treatment of neoplastic disease. Still other mycotoxins which may exhibit some carcinogenicity are luteoskyrin (from *P. islandicum*) and Actinomycin D (from *S. chrysomallus* or *S. antibioticus*).

Precautions to take

What measures can be taken to protect against some of these naturally occurring carcinogens? As an example, aflatoxin can be considered.

Naturally occurring carcinogens

Toxin	Source	Species affected	Organ developing tumors
Actinomycin D	<i>Streptomyces chrysomallus</i> or <i>S. antibioticus</i>	Mouse Rat	Local reaction
Aflatoxin B ₁	<i>Aspergillus flavus</i> from moldy grains	Man?	Liver
Aflatoxin B ₂		Monkey	Liver
Aflatoxin G ₁		Mouse (infant)	Liver
Aflatoxin G ₂		Rainbow trout Rat	Liver Liver
Asarone	Calamus root	Rat	Intestine
Cycasin	Cycads	Guinea pig Mouse Rat	Intestine Kidney Liver
Elaiomycin	<i>Streptomyces hepaticus</i>	Rat	Various sites
Ergot (crude)	<i>Claviceps purpurea</i> fungus on rye	Rat	Fibrous tissue
Estragole	Oil of tarragon	Mouse	Liver
Ethyl carbamate	Fermented products	Mouse	Lung
Griseofulvin	<i>Penicillium griseofulvum</i>	Mouse	Liver
Luteoskyrin	<i>Penicillium islandicum</i>	Mouse Rat	Liver Liver
Macrozamin	Encephalartos and zamia plants	Rat	Kidney Liver Lung
N-Methyl-N-formylhydrazine	False morel	Mouse	Liver Lung
Nitrosamines	Tobacco	Rat	Liver
Petastenine	Coltsfoot plant	Rat	Liver
Pyrrolizidine alkaloids	Senecio plants	Rat	Liver
Safrole	Natural plant oils	Mouse Rat	Liver Liver
Sterigmatocystin	<i>Aspergillus versicolor</i>	Mouse Rat	Liver Liver
Streptozotocin	<i>Streptomyces achromogenes</i>	Rat	Kidney Lung
Unknown	Bracken fern	Cattle Mouse Rat	Bladder Intestine Lung

Growth of the fungus on seed crops can be inhibited by proper harvesting, by preventing insect damage, by avoiding damage to the kernels and by ensuring proper drying and storing.

Many simple compounds such as *p*-aminobenzoic acid; potassium sulfite; potassium fluoride; and acetic, citric and lactic acids inhibit the growth of the fungus or production of the toxin. Furthermore, an extensive search by the Southern Regional Research Laboratory has shown that aflatoxin B₁ can be inactivated by various treatments.

A promising one involves treatment with ammonia (at two or three atmospheres of pressure) to cleave the lactone ring of aflatoxin with the loss of

one carbon. Further degradation can occur with complete loss of two rings in the aflatoxin molecule. Compounds of this type are not carcinogenic when tested under conditions where aflatoxin B₁ is active. Furthermore, the ammonia treatment does not seem to affect the protein level of treated peanut meal, for example. Thus the process offers a means to salvage large lots of contaminated oil seeds for animal feed.

Although there are many naturally occurring carcinogens in the environment—except for tobacco and aflatoxin—data relating these substances to cancer in people are lacking. However, the normal mammalian detoxification systems can usually handle

small quantities of many toxins fairly well, especially if the nutritional quality of the diet is adequate. Furthermore, repair enzymes are available to handle isolated damage.

Except for aflatoxin and cycasin, many of the naturally occurring carcinogens are relatively weak. Also, many of our foodstuffs contain substances that can inhibit the action of these carcinogens. Vegetables such as brussels sprouts, alfalfa, and cabbage contain certain indole derivatives that protect against carcinogens.

Thus, a moderate, nutritionally adequate diet affords protection against some toxins. In addition, the avoidance of obviously deleterious materials such as tobacco or moldy foods is advantageous in protecting one against naturally occurring carcinogens.

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Dietary Carcinogens and Anticarcinogens

Oxygen radicals and degenerative diseases

Bruce N. Ames

Comparison of data from different countries reveals wide differences in the rates of many types of cancer. This leads to hope that each major type of cancer may be largely avoidable, as is the case for cancers due to tobacco, which constitute 30 percent of the cancer deaths in the United States and the United Kingdom (1). Despite numerous suggestions to the contrary, there is no convincing evidence of any generalized increase in U.S. (or U.K.) cancer rates other than what could plausibly be ascribed to the delayed effects of previous increases in tobacco usage (1-3). Thus, whether or not any recent changes in life-style or pollution in industrialized countries will substantially affect future cancer risks, some important determinants of current risks remain to be discovered among long-established aspects of our way of life. Epidemiologic studies have indicated that dietary practices are the most promising area to explore (1, 4). These studies suggest that a general increase in consumption of fiber-rich cereals, vegetables, and fruits and decrease in consumption of fat-rich products and excessive alcohol would be prudent (1, 4). There is still a lack of definitive evidence about the dietary components that are critical for humans and about their mechanisms of action. Laboratory studies of natural foodstuffs and cooked food are beginning to uncover an extraordinary variety of mutagens and possible carcinogens and anticarcinogens. In this article I discuss dietary mutagens and carcinogens and anticarcinogens that seem of importance and speculate on relevant biochemical mechanisms, particularly the role of oxygen radicals and their inhibitors in the fat-cancer relationship, promotion, anticarcinogenesis, and aging.

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Natural Mutagens and Carcinogens in Food

Plant material. Plants in nature synthesize toxic chemicals in large amounts, apparently as a primary defense against the hordes of bacterial, fungal, and insect and other animal predators (5-40). Plants in the human diet are no exception. The variety of these toxic chemi-

Summary. The human diet contains a great variety of natural mutagens and carcinogens, as well as many natural antimutagens and anticarcinogens. Many of these mutagens and carcinogens may act through the generation of oxygen radicals. Oxygen radicals may also play a major role as endogenous initiators of degenerative processes, such as DNA damage and mutation (and promotion), that may be related to cancer, heart disease, and aging. Dietary intake of natural antioxidants could be an important aspect of the body's defense mechanism against these agents. Many antioxidants are being identified as anticarcinogens. Characterizing and optimizing such defense systems may be an important part of a strategy of minimizing cancer and other age-related diseases.

icals is so great that organic chemists have been characterizing them for over 100 years, and new plant chemicals are still being discovered (12, 24, 25). However, toxicological studies have been completed for only a very small percentage of them. Recent widespread use of short-term tests for detecting mutagens (41, 42) and the increased number of animal cancer tests on plant substances (6) have contributed to the identification of many natural mutagens, teratogens, and carcinogens in the human diet (5-40). Sixteen examples are discussed below.

1) *Safrole, estragole, methyleugenol*, and related compounds are present in many edible plants (5). Safrole, estragole, and methyleugenol are carcinogens in rodents, and several of their metabolites are mutagens (5). Oil of sassafras, which had been used in "natural" sarsaparilla root beer, is about 75 percent safrole. Black pepper contains small

amounts of safrole and large amounts (close to 10 percent by weight) of the closely related compound *piperine* (26). Extracts of black pepper cause tumors in mice at a variety of sites at a dose of extract equivalent to 4 mg of dried pepper per day (about 160 mg/kg per day) for 3 months; an estimate of the average human intake of black pepper is over 140 mg per day (about 2 mg/kg per day) for life (26).

2) Most *hydrazines* that have been tested are carcinogens and mutagens, and large amounts of carcinogenic hydrazines are present in edible mushrooms. The widely eaten false morel (*Gyromitra esculenta*) contains 11 hydrazines, three of which are known carcinogens (28). One of these, *N*-methyl-*N*-formylhydrazine, is present at a concentration of 50 mg per 100 g and causes lung tumors in mice at the extremely low dietary level of 20 µg per mouse per day (28). The most common commercial mushroom, *Agaricus bisporus*, contains about 300 mg of *agaritine*, the δ-glutamyl derivative of the mutagen 4-hydroxy-

methylphenylhydrazine, per 100 g of mushrooms, as well as smaller amounts of the closely related carcinogen *N*-acetyl-4-hydroxymethylphenylhydrazine (28). Some agaritine is metabolized by the mushroom to a diazonium derivative which is a very potent carcinogen (a single dose of 400 ng/g gave 30 percent of mice stomach tumors) and which is also present in the mushroom in smaller amounts (28). Many hydrazine carcinogens may act by producing oxygen radicals (43).

3) Linear *furocoumarins* such as *psoralen derivatives* are potent light-activated carcinogens and mutagens and are widespread in plants of the Umbelliferae family, such as celery, parsnips, figs, and parsley (for instance, 4 mg per 100 g of parsnip) (17, 19, 44). The level in celery (about 100 µg per 100 g) can increase about 100-fold if the celery is stressed or diseased (19). Celery pickers and handlers commonly develop skin

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rashes on their arms when exposed to diseased celery (19). Oil of bergamot, a citrus oil, is very rich in a psoralen and was used in the leading suntan lotion in France (17). Psoralens, when activated by sunlight, damage DNA and induce tanning more rapidly than the ultraviolet component of sunlight, which is also a carcinogen (17). Psoralens (plus light) are also effective in producing oxygen radicals (18).

4) The potato glycoalkaloids *solanine* and *chaconine* are strong cholinesterase inhibitors and possible teratogens and are present at about 15 mg per 200 g of potato (12, 13). When potatoes are diseased, bruised, or exposed to light, these and other (24) glycoalkaloids reach levels that can be lethal to humans (12). Plants typically respond to damage by making more (and often different) toxic chemicals as a defense against insects and fungi (19, 24, 25). The different cultivars of potatoes vary in the concentration of these toxic glycoalkaloids (the concentration is a major determinant of insect and disease resistance); one cultivar bred for insect resistance had to be withdrawn from use because of its toxicity to humans (> 40 mg of glycoalkaloids in a 200-g potato is considered to be a toxic level) (12).

5) *Quercetin* and several similar flavonoids are mutagens in a number of short-term test systems. Flavonoids are extremely widespread (daily levels close to 1 g) in the human diet (8, 16, 20, 21). There is evidence for the carcinogenicity of quercetin in two strains of rats (8), although it was negative in other experiments (21).

6) *Quinones* and their phenol precursors (9, 14, 16, 23, 45) are widespread in the human diet. Quinones are quite toxic as they can act as electrophiles or accept a single electron to yield the semiquinone radical, which can either react directly with DNA (14, 46) or participate in a redox cycle of superoxide radical generation by transferring the electron to O_2 (47). The superoxide radical and its metabolic product H_2O_2 can, in turn, lead to the oxidation of fat in cellular membranes by a lipid peroxidation chain reaction, thus generating mutagens and carcinogens, as discussed below. A number of quinones and dietary phenols have been shown to be mutagens (7, 9, 16, 23, 44). Mutagenic anthraquinone derivatives are found in plants such as rhubarb and in mold toxins (7, 16, 48). Many dietary phenols can spontaneously autoxidize to quinones, generating hydrogen peroxide at the same time [examples are catechol derivatives such as the caf-

feic acid component of chlorogenic acid (9), which is present at about 250 mg per cup of coffee]. The amounts of these phenols in human urine (and in the diet) are appreciable (45). Catechol, for example, is excreted in urine at about 10 mg per day and appears to be mainly derived from metabolism of plant substances (45). Catechol is a potent promoter of carcinogenesis (45), an inducer of DNA damage, a likely active metabolite of the carcinogen benzene (46), and a toxic agent in cigarette smoke (45). Catecholamine induction of cardiomyopathy is thought to occur through generation of oxygen radicals (49).

7) *Theobromine*, a relative of caffeine, has been shown to be genotoxic in a variety of tests, to potentiate (as does caffeine) DNA damage by various carcinogens in human cells, and to cause testicular atrophy and spermatogenic cell abnormalities in rats (27). Cocoa powder is about 2 percent theobromine, and therefore humans may consume hundreds of milligrams of theobromine a day from chocolate. Theobromine is also present in tea.

8) *Pyrolizidine* alkaloids are carcinogenic, mutagenic, and teratogenic and are present in thousands of plant species (often at > 1 percent by weight), some of which are ingested by humans, particularly in herbs and herbal teas and occasionally in honey (7, 29). Pyrolizidine alkaloid poisonings in humans (as well as in other mammals) cause lung and liver lesions and are commonly misdiagnosed (29).

9) The broad (fava) bean (*Vicia faba*), a common food of the Mediterranean region, contains the toxins *vicine* and *convicine* at a level of about 2 percent of the dry weight (30). Pythagoras forbade his followers to eat the beans, presumably because he was one of the millions of Mediterranean people with a deficiency of glucose-6-phosphate dehydrogenase. This deficiency results in a low glutathione concentration in blood cells, which causes increased resistance to the malarial parasite, probably accounting for the widespread occurrence of the mutant gene in malarial regions. However, the low glutathione concentration also results in a marked sensitivity to agents that cause oxidative damage, such as the fava bean toxins and a variety of drugs and viruses. Sensitive individuals who ingest fava beans develop a severe hemolytic anemia caused by the enzymatic hydrolysis of *vicine* to its aglycone, *divicine*, which forms a quinone that generates oxygen radicals (30).

10) *Allyl isothiocyanate*, a major flavor

ingredient in oil of mustard and horseradish, is one of the main toxins of the mustard seed and has been shown to cause chromosome aberrations in hamster cells at low concentration (50) and to be a carcinogen in rats (31).

11) *Gossypol* is a major toxin in cottonseed and accounts for about 1 percent of its dry weight (32). Gossypol causes pathological changes in rat and human testes, abnormal sperm, and male sterility (32, 33). Genetic damage has been observed in embryos sired by gossypol-treated male rats: dominant lethal mutations in embryos were measured after males were taken off gossypol treatment and allowed to mate (33). Gossypol appears to be a carcinogen as well: it has been reported to be a potent initiator and also a promoter of carcinogenesis in skin painting studies with mice (34). Crude, unrefined cottonseed oil contains considerable amounts of gossypol (100 to 750 mg per 100 ml). Thus human consumption may be appreciable in countries, such as Egypt, where fairly crude cottonseed oil is commonly used in cooking. Gossypol is being tested as a male contraceptive in over 10,000 people in China (at an oral dose of about 10 mg per person per day), as it is inexpensive and causes sterility during use (33). Gossypol's mode of action as a spermicide may be through the production of oxygen radicals (35).

Plant breeders have developed "glandless cotton," a new strain with low levels of gossypol, but seeds from this strain are much more susceptible to attack by the fungus *Aspergillus flavus*, which produces the potent carcinogen aflatoxin (36).

12) *Sterculic acid* and *malvalic acid* are widespread in the human diet. They are toxic cyclopropanoid fatty acids present in cottonseed oil and other oils from seeds of plants in the family Malvaceae (for instance, cotton, kapok, okra, and durian) (51). Another possible source of human exposure is consumption of fish, poultry, eggs, and milk from animals fed on cottonseed (51). Cyclopropanoid fatty acids are carcinogens in trout, markedly potentiate the carcinogenicity of aflatoxin in trout, cause atherosclerosis in rabbits, are mitogenic in rats, and have a variety of toxic effects in farm animals (51). The toxicity of these fatty acids could be due to their ease of oxidation to form peroxides and radicals (51).

13) Leguminous plants such as lupine contain very potent teratogens (22). When cows and goats forage on these plants, their offspring may have severe teratogenic abnormalities; an example is

the characteristic "crooked calf" abnormality due to the ingestion of *anagyrine* from lupine (22). In addition, significant amounts of these teratogens are transferred to the animals' milk, so that drinking the milk during pregnancy is a serious teratogenic hazard (22). In one rural California family, a baby boy, a litter of puppies, and goat kids all had "crooked" bone birth-defect abnormalities. The pregnant mother and the dog had both been drinking milk obtained from the family goats, which had been foraging on lupine (the main forage in winter) (22). It was at first mistakenly thought that the birth defects were caused by spraying of 2,4-D.

14) *Sesquiterpene lactones* are widespread in many plants (37), although because they are bitter they are not eaten in large amounts. Some have been shown to be mutagenic (37). They are a major toxin in the white sap of *Lactuca virosa* (poison lettuce), which has been used as a folk remedy. Plant breeders are now transferring genes from this species to commercial lettuce to increase insect resistance (38).

15) The *phorbol esters* present in the Euphorbiaceae, some of which are used as folk remedies or herb teas, are potent promoters of carcinogenesis and may have been a cause of nasopharyngeal cancer in China and esophageal cancer in Curaçao (39).

16) Alfalfa sprouts contain *canavanine*, a highly toxic arginine analog that is incorporated into protein in place of arginine. Canavanine, which occurs in alfalfa sprouts at about 1.5 percent of their dry weight (40), appears to be the active agent in causing the severe lupus erythematosus-like syndrome seen when monkeys are fed alfalfa sprouts (40). Lupus in man is characterized by a defect in the immune system which is associated with autoimmunity, antinuclear antibodies, chromosome breaks, and various types of pathology (40). The chromosome breaks appear to be due to oxygen radicals as they are prevented by superoxide dismutase (52). The canavanine-alfalfa sprout pathology could be due in part to the production of oxygen radicals during phagocytization of antibody complexes with canavanine-containing protein.

The 16 examples above, plus coffee (discussed below), illustrate that the human dietary intake of "nature's pesticides" is likely to be several grams per day—probably at least 10,000 times higher than the dietary intake of man-made pesticides (53).

Levels of plant toxins that confer insect and fungal resistance are being increased or decreased by plant breeders

(38). There are health costs for the use of these natural pesticides, just as there are for man-made pesticides (41, 54), and these must be balanced against the costs of producing food. However, little information is available about the toxicology of most of the natural plant toxins in our diet, despite the large doses we are exposed to. Many, if not most, of these plant toxins may be "new" to humans in the sense that the human diet has changed drastically with historic times. By comparison, our knowledge of the toxicological effects of new man-made pesticides is extensive, and general exposure is exceedingly low (53).

Plants also contain a variety of anticarcinogens (55), which are discussed below.

Alcohol. Alcohol has long been associated with cancer of the mouth, esophagus, pharynx, larynx, and, to a lesser extent, liver (1, 56), and it appears to be an important human teratogen, causing a variety of physical and mental defects in babies of mothers who drink (57). Alcohol drinking causes abnormalities in mice (57a) and is a synergist for chromosome damage in humans (58). Alcohol metabolism generates acetaldehyde, which is a mutagen and teratogen (59), a cocarcinogen, and possibly a carcinogen (60), and also radicals that produce lipid hydroperoxides (61) and other mutagens and carcinogens (62; see below). In some epidemiologic studies on alcohol (56), it has been suggested that dietary green vegetables are a modifying factor in the reduction of cancer risk.

Mold carcinogens. A variety of mold carcinogens and mutagens are present in mold-contaminated food such as corn, grain, nuts, peanut butter, bread, cheese, fruit, and apple juice (15, 63). Some of these, such as sterigmatocystin and aflatoxin, are among the most potent carcinogens and mutagens known (15, 63). Dietary glutathione has been reported to counteract aflatoxin carcinogenicity.

Nitrite, nitrate, and nitrosamines. A number of human cancers, such as stomach and esophageal cancer, may be related to nitrosamines and other nitroso compounds formed from nitrate and nitrite in the diet (64, 65). Beets, celery, lettuce, spinach, radishes, and rhubarb all contain about 200 mg of nitrate per 100-g portion (65). Anticarcinogens in the diet may be important in this context as well (66).

Fat and cancer: possible oxidative mechanisms. Epidemiologic studies of cancer in humans suggest, but do not prove, that high fat intake is associated with colon and breast cancer (1, 4, 67). A

number of animal studies have shown that high dietary fat is a promoter and a presumptive carcinogen (4, 67, 68). Colon and breast cancer and lung cancer (which is almost entirely due to cigarette smoking) account for about half of all U.S. cancer deaths. In addition to the cyclopropanoid fatty acids already discussed, two other plausible mechanisms involving oxidative processes could account for the relation (69) between high fat and both cancer and heart disease.

1) *Rancid fat.* Fat accounts for over 40 percent of the calories in the U.S. diet (67), and the amount of ingested oxidized fat may be appreciable (70, 71). Unsaturated fatty acids and cholesterol in fat are easily oxidized, particularly during cooking (70, 71). The lipid peroxidation chain reaction (rancidity) yields a variety (71–73) of mutagens, promoters, and carcinogens such as fatty acid hydroperoxides (62), cholesterol hydroperoxide (74), endoperoxides, cholesterol and fatty acid epoxides (74–77), enals and other aldehydes (44, 59, 78), and alkoxy and hydroperoxy radicals (44, 72). Thus the colon and digestive tract are exposed to a variety of fat-derived carcinogens. Human breast fluid can contain enormous levels (up to 780 μM) (75) of cholesterol epoxide (an oxidation product of cholesterol), which could originate from either ingested oxidized fat or oxidative processes in body lipids. Rodent feeding studies with oxidized fat (79) have not yielded definitive results.

2) *Peroxisomes* oxidize an appreciable percentage of dietary fatty acids, and removal of each two-carbon unit generates one molecule of hydrogen peroxide (a mutagen, promoter, and carcinogen) (80, 81). Some hydrogen peroxide escapes the catalase in the peroxisome (80, 82, 83), thus contributing to the supply of oxygen radicals, which also come from other metabolic sources (72, 83–85). Hydroperoxides generate oxygen radicals in the presence of iron-containing compounds in the cell (72). Oxygen radicals, in turn, can damage DNA and can start the rancidity chain reaction which leads to the production of the mutagens and carcinogens listed above (72). Drugs such as clofibrate, which cause lowering of serum lipids and proliferation of peroxisomes in rodents, result in age pigment (lipofuscin) accumulation (a sign of lipid peroxidation in tissues) and liver tumors in animals (80). Some fatty acids, such as C_{22:1} and certain *trans* fatty acids, appear to cause peroxisomal proliferation because they are poorly oxidized in mitochondria and are preferentially oxidized in the peroxisomes, although they may be selective for heart or

liver (86). There has been controversy about the role of *trans* fatty acids in cancer and heart disease, and recent evidence suggests that *trans* fatty acids might not be a risk factor for atherosclerosis in experimental animals (87). Americans consume about 12 g of *trans* fatty acids a day (87) and a similar amount of unnatural *cis* isomers [which need further study (88)], mainly from hydrogenated vegetable fats. Dietary C_{22:1} fatty acids are also obtained from rapeseed oil and fish oils (86). Thus oxidation of certain fatty acids might generate grams of hydrogen peroxide per day within the peroxisome (86). Another source of fat toxicity could be perturbations in the mitochondrial or peroxisomal membranes caused by abnormal fatty acids, yielding an increased flux of superoxide and hydrogen peroxide. Mitochondrial structure is altered when rats are fed some abnormal fatty acids from partially hydrogenated fish oil (89). Dietary C_{22:1} fatty acids and clofibrate also induce ornithine decarboxylase (86), a common attribute of promoters.

A recent National Academy of Sciences committee report suggests that a reduction of fat consumption in the American diet would be prudent (4), although other scientists argue that, until we know more about the mechanism of the fat-cancer relation and about which types of fat are dangerous, it is premature to recommend dietary changes (90).

Cooked Food as a Source of

Ingested Burnt and Browned Material

Work of Sugimura and others has indicated that the burnt and browned material from heating protein during cooking is highly mutagenic (21, 91). Several chemicals isolated on the basis of their mutagenicity from heated protein or pyrolyzed amino acids were found to be carcinogenic when fed to rodents (21). In addition, the browning reaction products from the caramelization of sugars or the reaction of amino acids and sugars during cooking (for instance, the brown material on bread crusts and toasted bread) contain a large variety of DNA-damaging agents and presumptive carcinogens (23, 38, 92). The amount of burnt and browned material in the human diet may be several grams per day. By comparison about 500 mg of burnt material is inhaled each day by a smoker using two packs of cigarettes (at 20 mg of tar per cigarette) a day. Smokers have more easily detectable levels of mutagens in their urine than nonsmokers (93), but so do people who have consumed a meal of

fried pork or bacon (94). In the evaluation of risk from burnt material it may be useful (in addition to carrying out epidemiologic studies) to compare the activity of cigarette tar to that of the burnt material from cooked food (or polluted air) in short-term tests and animal carcinogenicity tests involving relevant routes of exposure. Route of exposure and composition of the burnt material are critical variables. The risk from inhaled cigarette smoke can be one reference standard: an average life shortening of about 8 years for a two-pack-a-day smoker. The amount of burnt material inhaled from severely polluted city air, on the other hand, is relatively small: it would be necessary to breathe smoggy Los Angeles air (111 µg/m³ total particulates; 31 µg/m³ soluble organic matter) for 1 to 2 weeks to equal the soluble organic matter of the particulates or the mutagenicity from one cigarette (20 mg of tar) (95). Epidemiologic studies have not shown significant risks from city air pollution alone (1, 96). Air in the houses of smokers is considerably more polluted than city air outside (97).

Coffee, which contains a considerable amount of burnt material, including the mutagenic pyrolysis product methylglyoxal, is mutagenic (21, 98). However, one cup of coffee also contains about 250 mg of the natural mutagen chlorogenic acid (9) [which is also an antinitrosating agent (66)], highly toxic atracylosides (10), the glutathione transferase inducers kahweol palmitate and cafestol palmitate (11), and about 100 mg of caffeine [which inhibits a DNA-repair system and can increase tumor yield (99) and cause birth defects at high levels in several experimental species (100)]. There is preliminary, but not conclusive, epidemiologic evidence that heavy coffee drinking is associated with cancer of the ovary, bladder, pancreas, and large bowel (101).

Cooking also accelerates the rancidity reaction of cooking oils and fat in meat (70, 71), thus increasing consumption of mutagens and carcinogens.

Anticarcinogens

We have many defense mechanisms to protect ourselves against mutagens and carcinogens, including continuous shedding of the surface layer of our skin, stomach, cornea, intestines, and colon (102). Understanding these mechanisms should be a major goal of cancer, heart, and aging research. Among the most important defenses may be those against oxygen radicals and lipid peroxidation if, as discussed here, these agents are major

contributors to DNA damage (103). Major sources of endogenous oxygen radicals are hydrogen peroxide (83) and superoxide (72, 104) generated as side products of metabolism, and the oxygen radical burst from phagocytosis after viral or bacterial infection or the inflammatory reaction (105). A variety of environmental agents could also contribute to the oxygen radical load, as discussed here and in recent reviews (72, 106). Many enzymes protect cells from oxidative damage; examples are superoxide dismutase (104), glutathione peroxidase (107), DT-diaphorase (108), and the glutathione transferases (109). In addition, a variety of small molecules in our diet are required for antioxidative mechanisms and appear to be anticarcinogens: some of these are discussed below.

1) *Vitamin E* (tocopherol) is the major radical trap in lipid membranes (72) and has been used clinically in a variety of oxidation-related diseases (110). Vitamin E ameliorates both the cardiac damage and carcinogenicity of the quinones adriamycin and daunomycin, which are mutagenic, carcinogenic, cause cardiac damage, and appear to be toxic because of free radical generation (111). Protective effects of tocopherols against radiation-induced DNA damage and mutation and dimethylhydrazine-induced carcinogenesis have also been observed (112). Vitamin E markedly increases the endurance of rats during heavy exercise, which causes extensive oxygen radical damage to tissues (113).

2) *β-Carotene* is another antioxidant in the diet that could be important in protecting body fat and lipid membranes against oxidation. Carotenoids are free-radical traps and remarkably efficient quenchers of singlet oxygen (114). Singlet oxygen is a very reactive form of oxygen which is mutagenic and particularly effective at causing lipid peroxidation (114). It can be generated by pigment-mediated transfer of the energy of light to oxygen, or by lipid peroxidation, although the latter is somewhat controversial. *β-Carotene* and similar polyenes are present in carrots and in all food that contains chlorophyll, and they appear to be the plants' main defense against singlet oxygen generated as a by-product from the interaction of light and chlorophyll (115). Carotenoids have been shown to be anticarcinogens in rats and mice (116). Carotenoids (in green and yellow vegetables) may be anticarcinogens in humans (1, 56, 117). Their protective effects in smokers might be related to the high level of oxidants in both cigarette smoke and tar (45, 118). Carotenoids have been used medically in

the treatment for some genetic diseases, such as porphyrias, where a marked photosensitivity is presumably due to singlet oxygen formation (119).

3) *Selenium* is another important dietary anticarcinogen. Dietary selenium (usually selenite) significantly inhibits the induction of skin, liver, colon, and mammary tumors in experimental animals by a number of different carcinogens, as well as the induction of mammary tumors by viruses (120). It also inhibits transformation of mouse mammary cells (121). Low selenium concentrations may be a risk factor in human cancer (122). A particular type of heart disease in young people in the Keshan area of China has been traced to a selenium deficiency, and low selenium has been associated with cardiovascular death in Finland (123). Selenium is in the active site of glutathione peroxidase, an enzyme essential for destroying lipid hydroperoxides and endogenous hydrogen peroxide and thus helping to prevent oxygen radical-induced lipid peroxidation (107), although not all of the effects of selenium may be accounted for by this enzyme (120). Several heavy-metal toxins, such as Cd^{2+} (a known carcinogen) and Hg^{2+} , lower glutathione peroxidase activity by interacting with selenium (107). Selenite (and vitamin E) has been shown to counter the oxidative toxicity of mercuric salts (124).

4) *Glutathione* is present in food and is one of the major antioxidants and antimutagens in the soluble fraction of cells. The glutathione transferases (some of which have peroxidase activity) are major defenses against oxidative and alkylating carcinogens (109). The concentration of glutathione may be influenced by dietary sulfur amino acids (125, 126). *N*-Acetylcysteine, a source of cysteine, raises glutathione concentrations and reduces the oxidative cardiotoxicity of adriamycin and the skin reaction to radiation (127). Glutathione concentrations are raised even more efficiently by L-2-oxothiazolidine-4-carboxylate, which is an effective antagonist of acetaminophen-caused liver damage (126). Acetaminophen is thought to be toxic through radical and quinone oxidizing metabolites (128). Dietary glutathione may be an effective anticarcinogen against aflatoxin (129).

5) Dietary *ascorbic acid* is also important as an antioxidant. It was shown to be anticarcinogenic in rodents treated with ultraviolet radiation, benzo(a)pyrene, and nitrite (forming nitroso carcinogens) (64, 65, 130), and it may be inversely associated with human uterine cervical dysplasia (although this is not

proof of a cause-effect relationship) (131). It was recently hypothesized that ascorbic acid may have been supplemented and perhaps partially replaced in humans by uric acid during primate evolution (132).

6) *Uric acid* is a strong antioxidant present in high concentrations in the blood of humans (132). The concentration of uric acid in the blood can be increased by dietary purines; however, too much causes gout. Uric acid is also present in high concentrations in human saliva (132) and may play a role in defense there as well, in conjunction with lactoperoxidase. A low uric acid level in blood may possibly be a risk factor in cigarette-caused lung cancer in humans (133).

7) Edible plants and a variety of substances in them, such as phenols, have been reported to inhibit (cabbage) or to enhance (beets) carcinogenesis (11, 55, 134) or mutagenesis (23, 66, 92, 135) in experimental animals. Some of these substances appear to inhibit by inducing cytochrome P-450 and other metabolic enzymes [(134); see also (11)], although on balance it is not completely clear whether it is generally helpful or harmful for humans to ingest these inducing substances.

The hypothesis that as much as 80 percent of cancer could be due to environmental factors was based on geographic differences in cancer rates and studies of migrants (136). These differences in cancer rates were thought to be mainly due to life-style factors, such as smoking and dietary carcinogens and promoters (136), but they also may be due in good part [see also (1)] to less than optimum amounts of anticarcinogens and protective factors in the diet.

The optimum levels of dietary antioxidants, which may vary among individuals, remain to be determined; however, at least for selenium (120), it is important to emphasize the possibility of deleterious side effects at high doses.

Oxygen Radicals and Degenerative Diseases Associated with Aging

Aging. A plausible theory of aging holds that the major cause is damage to DNA (102, 137) and other macromolecules and that a major source of this damage is oxygen radicals and lipid peroxidation (43, 84, 103, 138-141). Cancer and other degenerative diseases, such as heart disease (102), are likely to be due in good part to this same fundamental destructive process. Age pigment (lipofuscin) accumulates aging in all mammalian

species and has been associated with lipid peroxidation (73, 84, 138, 139). The fluorescent products in age pigment are thought to be formed by malondialdehyde (a mutagen and carcinogen and a major end product of rancidity) cross-linking protein and lipids (138). Metabolic rate is directly correlated with the rate of lipofuscin formation (and inversely correlated with longevity) (139).

Cancer increases with about the fourth power of age, both in short-lived species such as rats and mice (about 30 percent of rodents have cancer by the end of their 2- to 3-year life-span) and in long-lived species such as humans (about 30 percent of people have cancer by the end of their 85-year life-span) (142). Thus, the marked increase in life-span that has occurred in 60 million years of primate evolution has been accompanied by a marked decrease in age-specific cancer rates; that is, in contrast to rodents, 30 percent of humans do not have cancer by the age of 3 (142). One important factor in longevity appears to be basal metabolic rate (139, 141), which is much lower in man than in rodents and could markedly affect the level of endogenous oxygen radicals.

Animals have many antioxidant defenses against oxygen radicals. Increased levels of these antioxidants, as well as new antioxidants, may also be a factor in the evolution of man from short-lived prosimians (143). It has been suggested that an increase in superoxide dismutase is correlated (after the basal metabolic rate is taken into account) with increased longevity during primate evolution, although this has been disputed (141). Ames *et al.* proposed (132) that as uric acid was an antioxidant and was present in much higher concentrations in the blood of humans than in other mammals, it may have been one of the innovations enabling the marked increase in life span and consequent marked decrease in age-specific cancer rates which occurred during primate evolution. The ability to synthesize ascorbic acid may have been lost at about the same time in primate evolution as uric acid levels began to increase (144).

Cancer and promotion. Both DNA-damaging agents (initiating mutagens) (21, 41, 42) and promoters (145) appear to play an important role in carcinogenesis (21, 146). It has been postulated that certain promoters of carcinogenesis act by generation of oxygen radicals and resultant lipid peroxidation (73, 146-149). Lipid peroxidation cross-links proteins (43, 150) and affects all aspects of cell organization (72), including membrane and surface structure, and the mi-

totic apparatus. A common property of promoters may be their ability to produce oxygen radicals. Some examples are fat and hydrogen peroxide (which may be among the most important promoters) (67, 68, 81), TCDD (151), lead and cadmium (152), phorbol esters (147, 149, 153), wounding of tissues (154), asbestos (155), peroxides (156), catechol (45) (see quinones above), mezerein and teleocidin B (147), phenobarbital (157), and radiation (72, 158). Inflammatory reactions involve the production of oxygen radicals by phagocytes (105), and this could be the basis of promotion for asbestos (155) or wounding (154). Some of the antioxidant anticarcinogens (discussed above) are also antipromoters (73, 121, 146, 159, 160), and phorbol ester-induced chromosome damage (149) or promotion of transformation (159) is suppressed by superoxide dismutase, as would be expected if promoters were working through oxidative mechanisms. Many "complete" carcinogens cause the production of oxygen radicals (73, 161); examples are nitroso compounds, hydrazines, quinones, polycyclic hydrocarbons (through quinones), cadmium and lead salts, nitro compounds, and radiation. A good part of the toxic effects of ionizing radiation damage to DNA and cells is thought to be due to generation of oxygen radicals (103, 162), although only a tiny part of the oxygen radical load in humans is likely to be from this source.

Recent studies give some clues as to how promoters might act. Promoters disrupt the mitotic apparatus, causing hemizygosity and expression of recessive genes (163). Phorbol esters generate oxygen radicals, which cause chromosome breaks (164) and increase gene copy number (165). Promoters also cause formation of the peroxide hormones of the prostaglandin and leukotriene family by oxidation of arachidonic acid and other C₂₀ polyenoic fatty acids, and inhibitors of this process appear to be antipromoters (160). These hormones are intimately involved in cell division, differentiation, and tumor growth (166) and could have arisen in evolution as signal molecules warning the cell of oxidative damage. Effects on the cell membrane have also been suggested as the important factor in promotion, causing inhibition of intercellular communication (167) or protein kinase activation (167a).

Heart disease. It has been postulated that atherosclerotic lesions, which are derived from single cells, are similar to benign tumors and are of somatic mutational origin (102, 168). Fat appears to be one major risk factor for heart disease as

well as for colon and breast cancer (69). In agreement with this, a strong correlation has been observed between the frequency of atherosclerotic lesions and adenomatous polyps of the colon (69). Thus, the same oxidative processes involving fat may contribute to both diseases. Oxidized forms of cholesterol have been implicated in heart disease (169), and atherosclerotic-like lesions have been produced by injecting rabbits with lipid hydroperoxide or oxidized cholesterol (169). The anticarcinogens discussed above could be anti-heart disease agents as well. As pointed out in the preceding section, vitamin E ameliorates both the cardiac damage and carcinogenicity of the free-radical-generating quinones adriamycin and daunomycin; *N*-acetylcysteine reduces the cardiotoxicity of adriamycin; and selenium is an antirisk factor for one type of heart disease.

Other diseases. The brain uses 20 percent of the oxygen consumed by man and contains an appreciable amount of unsaturated fat. Lipid peroxidation (with consequent age pigment) is known to occur readily in the brain (72), and possible consequences could be senile dementia or other brain abnormalities (84). Several inherited progressive diseases of the central nervous system, such as Batten's disease, are associated with lipofuscin accumulation and may be due to a lipid peroxidation caused by a high concentration of unbound iron (170). Mental retardation is one consequence of an inherited defective DNA repair system (XP complementation group D) for depurinated sites in DNA (171).

Senile cataracts have been associated with light-induced oxidative damage (172). The retina and an associated layer of cells, the pigment epithelium, are extremely sensitive to degeneration in vitamin E and selenium deficiency (173). The pigment epithelium accumulates massive amounts of lipofuscin in aging and dietary antioxidant deficiency (173). The eye is well known to be particularly rich in antioxidants.

The testes are quite prone to lipid peroxidation and to the accumulation of age pigment. A number of agents, such as gossypol, which cause genetic birth defects (dominant lethals) may be active by this mechanism. The various agents known to cause cancer by oxidative mechanisms are prospective mutagenic agents for the germ line. Thus, vitamin E, which was discovered 60 years ago as a fertility factor (72), and other antioxidants such as selenium (174), may help both to engender and to protect the next generation.

Risks

There are large numbers of mutagens and carcinogens in every meal, all perfectly natural and traditional [see also (21, 23)]. Nature is not benign. It should be emphasized that no human diet can be entirely free of mutagens and carcinogens and that the foods mentioned are only representative examples. To identify a substance, whether natural or man-made, as a mutagen or a carcinogen, is just a first step. Beyond this, it is necessary to consider the risks for alternative courses of action and to quantitate the approximate magnitude of the risk, although the quantification of risk poses a major challenge. Carcinogens differ in their potency in rodents by more than a millionfold (175), and the levels of particular carcinogens to which humans are exposed can vary more than a billionfold. Extrapolation of risk from rodents to humans is difficult for many reasons, including the longevity difference, antioxidant factors, and the probable multicausal nature of most human cancer.

Tobacco smoking is, without doubt, a major and well-understood risk, causing about 30 percent of cancer deaths and 25 percent of fatal heart attacks (as well as other degenerative diseases) in the United States (1). These percentages may increase even more in the near future as the health effects of the large increase in women smokers become apparent (1). Diet, which provides both carcinogens and anticarcinogens, is extremely likely to be another major risk factor. Excessive alcohol consumption is another risk, although it does not seem to be of the same general importance as smoking and diet. Certain other high-dose exposures might also turn out to be important for particular groups of people—for instance, certain drugs, where consumption can reach hundreds of milligrams per day; particular cosmetics; and certain occupational exposures (2), where workers inhale dusts or solvents at high concentration. We must also be prudent about environmental pollution (41, 54). Despite all of these risks, it should be emphasized that the overall trend in life expectancy in the United States is continuing steadily upward (176).

The understanding of cancer and degenerative disease mechanisms is being aided by the rapid progress of science and technology, and this should help to dispel confusion about how important health risks can be identified among the vast number of minor risks. We have many methods of attacking the problem of environmental carcinogens (and anticarcinogens), including human epidemi-

ology (1), short-term tests (41, 42, 177), and animal cancer tests (175). Powerful new methods are being developed [for instance, see (58, 177)] for measuring DNA damage or other pertinent factors with great sensitivity in individuals. These methods, which are often noninvasive as they can be done on blood or urine (even after storage), can be combined with epidemiology to determine whether particular factors are predictive of disease. Thus, more powerful tools will be available for optimizing antioxidants and other dietary anti-risk factors, for identifying human genetic variants at high risk, and for identifying significant health risks.

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RESEARCH ARTICLE

Imaging Dopamine Receptors in the Human Brain by Positron Tomography

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One of the most intriguing problems in biomedical research today is that of relating manifestations of neuropsychiatric diseases to chemical processes in different parts of the brain. The neurotrans-

mitter dopamine appears to be associated with abnormalities related to disorders such as Parkinson's disease and schizophrenia. The highest density of dopamine neurons occurs in the nigrostriatal dopamine pathway which degenerates in Parkinson's disease (1). Neuroleptic drugs elicit extrapyramidal parkinsonian side effects by blocking dopamine receptors in the corpus striatum and also exert antischizophrenic action by blocking dopamine receptors, perhaps in limbic areas (2). Numbers of dopamine receptors are increased by chronic neuroleptic treatment (3) and are also increased in some schizophrenics, perhaps

as a result of neuroleptic therapy (4). The development of positron emission tomography (PET) and appropriate radioactive tracers labeled with positron-emitting radionuclides has now made it

Abstract. Neurotransmitter receptors may be involved in a number of neuropsychiatric disease states. The ligand 3-N-¹¹C-methylspiperone, which preferentially binds to dopamine receptors *in vivo*, was used to image the receptors by positron emission tomography scanning in baboons and in humans. This technique holds promise for noninvasive clinical studies of dopamine receptors in humans.

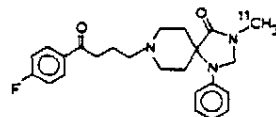
possible to relate regional biochemistry within the human brain to measurements of behavior in normal subjects and to elucidate abnormalities in patients with Alzheimer's disease (5), Huntington's disease (6), depression (7), and multiple infarct dementia (8). The technique consists of intravenous injection of a substance such as ¹⁸F-labeled deoxyglu-

cose, [¹¹C]carboxyhemoglobin, ionic rubidium-82, ⁶⁸Ga-labeled EDTA, and other radiopharmaceuticals, and subsequent imaging of the distribution of the radioactive label in the brain by means of the tomographic method, based on detection of the annihilation radiation produced during positron emission (9).

The butyrophenone neuroleptic drug spiperone has been useful in binding studies for measuring dopamine receptors both *in vitro* (10) and *in vivo* (11). We now report initial results obtained with 3-N-¹¹C-methylspiperone (¹¹C-NMSP), a spiperone derivative, in PET

scanning studies to visualize the distribution of dopamine receptors in the brains of baboons and a human being. All studies were performed with a NeuroECAT scanner (Ortec, Inc., Oak Ridge, Tennessee), which has a spatial resolution of approximately 8 mm (full width at half maximum) in the plane of the slice. The distance between slices is 3 cm.

The newly developed tracer ¹¹C-NMSP was synthesized by N-alkylation of spiperone with [¹¹C]methyl iodide: the iodide was produced from ¹¹CO₂, which in turn had been produced with an in-hospital cyclotron (model RNP-16, Scanditronix Cyclotron, Sweden). Carbon-11 is a positron-emitting isotope with a physical half-life of 20 minutes. The entire synthesis was accomplished with material ready for injection within 55 minutes after the end of the cyclotron



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Dietary Carcinogens

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Dietary Carcinogens

In this issue of *Science*, Bruce Ames reviews the increasing body of evidence that large numbers of potent carcinogens arise from natural processes. Mutagens are present in substantial quantities in fruits and vegetables. Carcinogens are formed in cooking as a result of reactions involving proteins or fats. Dietary practices may be an important determinant of current cancer risks.

Ames describes the role of plant materials as follows: Plants in nature synthesize toxic chemicals in large amounts, apparently as a primary defense against the hordes of bacterial, fungal, and insect and other animal predators. Plants in the human diet are no exception. The variety of toxic chemicals is so great that organic chemists have been characterizing them for over 100 years, and new plant chemicals are still being discovered. Recent widespread use of short-term tests for detecting mutagens and the increased testing of plant substances for carcinogenicity in animals have contributed to the identification of many natural mutagens, teratogens, and carcinogens in the human diet.

Safrole and related compounds are present in many edible plants. Safrole is a carcinogen in rodents and some of its metabolites are mutagens. Oil of sassafras, once used to flavor some root beer, is about 75 percent safrole. Black pepper contains about 10 percent by weight of a closely related compound, piperine. Extracts of black pepper at a dose equivalent to 4 milligrams of dried pepper per day cause tumors in mice at many sites. Many hydrazines are carcinogens and mutagens, and large amounts of them are found in edible mushrooms. One carcinogenic hydrazine is present in the false morel at a concentration of 50 milligrams per 100 grams. It causes lung tumors in mice at a level of 20 micrograms per mouse per day.

Carcinogens and mutagens are present in mold-contaminated foods such as corn, nuts, peanut butter, bread, cheese, and fruit. Some of these contaminants, such as aflatoxin, are among the most potent known carcinogens and mutagens. Nitrosamines and nitroso compounds are suspect as causative agents of stomach and esophageal cancer in humans. In the digestive system these nitrogen compounds are formed from nitrate and nitrite. Beets, celery, lettuce, spinach, radishes, and rhubarb all contain about 200 milligrams of nitrate per 100-gram portion.

Rancid fats are possible causative agents of colon and breast cancer in humans. These forms account for a substantial fraction of all the cancer deaths in the United States. Unsaturated fats are easily oxidized on standing and in cooking to form mutagens, promoters, and carcinogens. Among the numerous products of such oxidations are fatty acid hydroperoxides and cholesterol epoxide. Thus the colon and digestive tract are exposed to many fat-derived carcinogens. Human breast fluid can contain high levels of cholesterol epoxide.

Burnt and browned materials formed by heating proteins during cooking are highly mutagenic. Chemicals isolated from such products have been found to be carcinogenic when fed to rodents. In addition, the browning reaction products from caramelization of sugars or the reaction of amino acids and sugars during cooking contain a large variety of DNA-damaging agents.

The view that dietary practices might be a causative factor in cancer is not new. Epidemiologists have noted marked differences in cancer rates between population groups. Effects from changes in diet following migration have also been observed. Results of current studies are beginning to delineate more sharply specific causative agents. When more definitive information is available, it should be possible for prudent people to choose fruits and vegetables that present minimal hazards. In the meantime, there is persuasive evidence that charred meats and rancid fats should not be part of the diet.—PHILIP H. ABELSON

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Dietary Carcinogens and Anticarcinogens

Bruce N. Ames

URL 04020

Dietary Carcinogens and Anticarcinogens

Oxygen radicals and degenerative diseases

Bruce N. Ames

Comparison of data from different countries reveals wide differences in the rates of many types of cancer. This leads to hope that each major type of cancer may be largely avoidable, as is the case for cancers due to tobacco, which constitute 30 percent of the cancer deaths in the United States and the United Kingdom (1). Despite numerous suggestions to the contrary, there is no convincing evidence of any generalized increase in U.S. (or U.K.) cancer rates other than what could plausibly be ascribed to the delayed effects of previous increases in tobacco usage (1-3). Thus, whether or not any recent changes in life-style or pollution in industrialized countries will substantially affect future cancer risks, some important determinants of current risks remain to be discovered among long-established aspects of our way of life. Epidemiologic studies have indicated that dietary practices are the most promising area to explore (1, 4). These studies suggest that a general increase in consumption of fiber-rich cereals, vegetables, and fruits and decrease in consumption of fat-rich products and excessive alcohol would be prudent (1, 4). There is still a lack of definitive evidence about the dietary components that are critical for humans and about their mechanisms of action. Laboratory studies of natural foodstuffs and cooked food are beginning to uncover an extraordinary variety of mutagens and possible carcinogens and anticarcinogens. In this article I discuss dietary mutagens and carcinogens and anticarcinogens that seem of importance and speculate on relevant biochemical mechanisms, particularly the role of oxygen radicals and their inhibitors in the fat-cancer relationship, promotion, anticarcinogenesis, and aging.

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Natural Mutagens and Carcinogens in Food

Plant material. Plants in nature synthesize toxic chemicals in large amounts, apparently as a primary defense against the hordes of bacterial, fungal, and insect and other animal predators (5-40). Plants in the human diet are no exception. The variety of these toxic chemi-

Summary. The human diet contains a great variety of natural mutagens and carcinogens, as well as many natural antimutagens and anticarcinogens. Many of these mutagens and carcinogens may act through the generation of oxygen radicals. Oxygen radicals may also play a major role as endogenous initiators of degenerative processes, such as DNA damage and mutation (and promotion), that may be related to cancer, heart disease, and aging. Dietary intake of natural antioxidants could be an important aspect of the body's defense mechanism against these agents. Many antioxidants are being identified as anticarcinogens. Characterizing and optimizing such defense systems may be an important part of a strategy of minimizing cancer and other age-related diseases.

cals is so great that organic chemists have been characterizing them for over 100 years, and new plant chemicals are still being discovered (12, 24, 25). However, toxicological studies have been completed for only a very small percentage of them. Recent widespread use of short-term tests for detecting mutagens (41, 42) and the increased number of animal cancer tests on plant substances (6) have contributed to the identification of many natural mutagens, teratogens, and carcinogens in the human diet (5-40). Sixteen examples are discussed below.

1) *Safrole, estragole, methyleugenol*, and related compounds are present in many edible plants (5). Safrole, estragole, and methyleugenol are carcinogens in rodents, and several of their metabolites are mutagens (5). Oil of sassafras, which had been used in "natural" sarsaparilla root beer, is about 75 percent safrole. Black pepper contains small

amounts of safrole and large amounts (close to 10 percent by weight) of the closely related compound *piperine* (26). Extracts of black pepper cause tumors in mice at a variety of sites at a dose of extract equivalent to 4 mg of dried pepper per day (about 160 mg/kg per day) for 3 months; an estimate of the average human intake of black pepper is over 140 mg per day (about 2 mg/kg per day) for life (26).

2) Most *hydrazines* that have been tested are carcinogens and mutagens, and large amounts of carcinogenic hydrazines are present in edible mushrooms. The widely eaten false morel (*Gyromitra esculenta*) contains 11 hydrazines, three of which are known carcinogens (28). One of these, *N-methyl-N-formylhydrazine*, is present at a concentration of 50 mg per 100 g and causes lung tumors in mice at the extremely low dietary level of 20 µg per mouse per day (28). The most common commercial mushroom, *Agaricus bisporus*, contains about 300 mg of *agaritine*, the δ-glutamyl derivative of the mutagen 4-hydroxy-

methylphenylhydrazine, per 100 g of mushrooms, as well as smaller amounts of the closely related carcinogen *N-acetyl-4-hydroxymethylphenylhydrazine* (28). Some agaritine is metabolized by the mushroom to a diazonium derivative which is a very potent carcinogen (a single dose of 400 ng/g gave 30 percent of mice stomach tumors) and which is also present in the mushroom in smaller amounts (28). Many hydrazine carcinogens may act by producing oxygen radicals (43).

3) Linear *furocoumarins* such as *psoralen derivatives* are potent light-activated carcinogens and mutagens and are widespread in plants of the Umbelliferae family, such as celery, parsnips, figs, and parsley (for instance, 4 mg per 100 g of parsnip) (17, 19, 44). The level in celery (about 100 µg per 100 g) can increase about 100-fold if the celery is stressed or diseased (19). Celery pickers and handlers commonly develop skin

rashes on their arms when exposed to diseased celery (19). Oil of bergamot, a citrus oil, is very rich in a psoralen and was used in the leading suntan lotion in France (17). Psoralens, when activated by sunlight, damage DNA and induce tanning more rapidly than the ultraviolet component of sunlight, which is also a carcinogen (17). Psoralens (plus light) are also effective in producing oxygen radicals (18).

4) The potato glycoalkaloids *solanine* and *chaconine* are strong cholinesterase inhibitors and possible teratogens and are present at about 15 mg per 200 g of potato (12, 13). When potatoes are diseased, bruised, or exposed to light, these and other (24) glycoalkaloids reach levels that can be lethal to humans (12). Plants typically respond to damage by making more (and often different) toxic chemicals as a defense against insects and fungi (19, 24, 25). The different cultivars of potatoes vary in the concentration of these toxic glycoalkaloids (the concentration is a major determinant of insect and disease resistance); one cultivar bred for insect resistance had to be withdrawn from use because of its toxicity to humans (> 40 mg of glycoalkaloids in a 200-g potato is considered to be a toxic level) (12).

5) *Quercetin* and several similar flavonoids are mutagens in a number of short-term test systems. Flavonoids are extremely widespread (daily levels close to 1 g) in the human diet (8, 16, 20, 21). There is evidence for the carcinogenicity of quercetin in two strains of rats (8), although it was negative in other experiments (21).

6) *Quinones* and their phenol precursors (9, 14, 16, 23, 45) are widespread in the human diet. Quinones are quite toxic as they can act as electrophiles or accept a single electron to yield the semiquinone radical, which can either react directly with DNA (14, 46) or participate in a redox cycle of superoxide radical generation by transferring the electron to O_2 (47). The superoxide radical and its metabolic product H_2O_2 can, in turn, lead to the oxidation of fat in cellular membranes by a lipid peroxidation chain reaction, thus generating mutagens and carcinogens, as discussed below. A number of quinones and dietary phenols have been shown to be mutagens (7, 9, 16, 23, 44). Mutagenic anthraquinone derivatives are found in plants such as rhubarb and in mold toxins (7, 16, 48). Many dietary phenols can spontaneously autoxidize to quinones, generating hydrogen peroxide at the same time [examples are catechol derivatives such as the caf-

feic acid component of chlorogenic acid (9), which is present at about 250 mg per cup of coffee]. The amounts of these phenols in human urine (and in the diet) are appreciable (45). Catechol, for example, is excreted in urine at about 10 mg per day and appears to be mainly derived from metabolism of plant substances (45). Catechol is a potent promoter of carcinogenesis (45), an inducer of DNA damage, a likely active metabolite of the carcinogen benzene (46), and a toxic agent in cigarette smoke (45). Catecholamine induction of cardiomyopathy is thought to occur through generation of oxygen radicals (49).

7) *Theobromine*, a relative of caffeine, has been shown to be genotoxic in a variety of tests, to potentiate (as does caffeine) DNA damage by various carcinogens in human cells, and to cause testicular atrophy and spermatogenic cell abnormalities in rats (27). Cocoa powder is about 2 percent theobromine, and therefore humans may consume hundreds of milligrams of theobromine a day from chocolate. Theobromine is also present in tea.

8) *Pyrrolizidine* alkaloids are carcinogenic, mutagenic, and teratogenic and are present in thousands of plant species (often at > 1 percent by weight), some of which are ingested by humans, particularly in herbs and herbal teas and occasionally in honey (7, 29). Pyrrolizidine alkaloid poisonings in humans (as well as in other mammals) cause lung and liver lesions and are commonly misdiagnosed (29).

9) The broad (fava) bean (*Vicia faba*), a common food of the Mediterranean region, contains the toxins *vicine* and *convicine* at a level of about 2 percent of the dry weight (30). Pythagoras forbade his followers to eat the beans, presumably because he was one of the millions of Mediterranean people with a deficiency of glucose-6-phosphate dehydrogenase. This deficiency results in a low glutathione concentration in blood cells, which causes increased resistance to the malarial parasite, probably accounting for the widespread occurrence of the mutant gene in malarial regions. However, the low glutathione concentration also results in a marked sensitivity to agents that cause oxidative damage, such as the fava bean toxins and a variety of drugs and viruses. Sensitive individuals who ingest fava beans develop a severe hemolytic anemia caused by the enzymatic hydrolysis of *vicine* to its aglycone, *divicine*, which forms a quinone that generates oxygen radicals (30).

10) *Allyl isothiocyanate*, a major flavor

ingredient in oil of mustard and horseradish, is one of the main toxins of the mustard seed and has been shown to cause chromosome aberrations in hamster cells at low concentration (50) and to be a carcinogen in rats (31).

11) *Gossypol* is a major toxin in cottonseed and accounts for about 1 percent of its dry weight (32). Gossypol causes pathological changes in rat and human testes, abnormal sperm, and male sterility (32, 33). Genetic damage has been observed in embryos sired by gossypol-treated male rats: dominant lethal mutations in embryos were measured after males were taken off gossypol treatment and allowed to mate (33). Gossypol appears to be a carcinogen as well: it has been reported to be a potent initiator and also a promoter of carcinogenesis in skin painting studies with mice (34). Crude, unrefined cottonseed oil contains considerable amounts of gossypol (100 to 750 mg per 100 ml). Thus human consumption may be appreciable in countries, such as Egypt, where fairly crude cottonseed oil is commonly used in cooking. Gossypol is being tested as a male contraceptive in over 10,000 people in China (at an oral dose of about 10 mg per person per day), as it is inexpensive and causes sterility during use (33). Gossypol's mode of action as a spermicide may be through the production of oxygen radicals (35).

Plant breeders have developed "glandless cotton," a new strain with low levels of gossypol, but seeds from this strain are much more susceptible to attack by the fungus *Aspergillus flavus*, which produces the potent carcinogen aflatoxin (36).

12) *Sterculic acid* and *malvalic acid* are widespread in the human diet. They are toxic cyclopropenoid fatty acids present in cottonseed oil and other oils from seeds of plants in the family Malvaceae (for instance, cotton, kapok, okra, and durian) (51). Another possible source of human exposure is consumption of fish, poultry, eggs, and milk from animals fed on cottonseed (51). Cyclopropenoid fatty acids are carcinogens in trout, markedly potentiate the carcinogenicity of aflatoxin in trout, cause atherosclerosis in rabbits, are mitogenic in rats, and have a variety of toxic effects in farm animals (51). The toxicity of these fatty acids could be due to their ease of oxidation to form peroxides and radicals (51).

13) Leguminous plants such as lupine contain very potent teratogens (22). When cows and goats forage on these plants, their offspring may have severe teratogenic abnormalities: an example is

the characteristic "crooked calf" abnormality due to the ingestion of *anagyrine* from lupine (22). In addition, significant amounts of these teratogens are transferred to the animals' milk, so that drinking the milk during pregnancy is a serious teratogenic hazard (22). In one rural California family, a baby boy, a litter of puppies, and goat kids all had "crooked" bone birth-defect abnormalities. The pregnant mother and the dog had both been drinking milk obtained from the family goats, which had been foraging on lupine (the main forage in winter) (22). It was at first mistakenly thought that the birth defects were caused by spraying of 2,4-D.

14) *Sesquiterpene lactones* are widespread in many plants (37), although because they are bitter they are not eaten in large amounts. Some have been shown to be mutagenic (37). They are a major toxin in the white sap of *Lactuca virosa* (poison lettuce), which has been used as a folk remedy. Plant breeders are now transferring genes from this species to commercial lettuce to increase insect resistance (38).

15) The *phorbol esters* present in the Euphorbiaceae, some of which are used as folk remedies or herb teas, are potent promoters of carcinogenesis and may have been a cause of nasopharyngeal cancer in China and esophageal cancer in Curaçao (39).

16) Alfalfa sprouts contain *canavanine*, a highly toxic arginine analog that is incorporated into protein in place of arginine. Canavanine, which occurs in alfalfa sprouts at about 1.5 percent of their dry weight (40), appears to be the active agent in causing the severe lupus erythematosus-like syndrome seen when monkeys are fed alfalfa sprouts (40). Lupus in man is characterized by a defect in the immune system which is associated with autoimmunity, antinuclear antibodies, chromosome breaks, and various types of pathology (40). The chromosome breaks appear to be due to oxygen radicals as they are prevented by superoxide dismutase (52). The canavanine-alfalfa sprout pathology could be due in part to the production of oxygen radicals during phagocytization of antibody complexes with canavanine-containing protein.

The 16 examples above, plus coffee (discussed below), illustrate that the human dietary intake of "nature's pesticides" is likely to be several grams per day—probably at least 10,000 times higher than the dietary intake of man-made pesticides (53).

Levels of plant toxins that confer insect and fungal resistance are being increased or decreased by plant breeders

(38). There are health costs for the use of these natural pesticides, just as there are for man-made pesticides (41, 54), and these must be balanced against the costs of producing food. However, little information is available about the toxicology of most of the natural plant toxins in our diet, despite the large doses we are exposed to. Many, if not most, of these plant toxins may be "new" to humans in the sense that the human diet has changed drastically with historic times. By comparison, our knowledge of the toxicological effects of new man-made pesticides is extensive, and general exposure is exceedingly low (53).

Plants also contain a variety of anticarcinogens (55), which are discussed below.

Alcohol. Alcohol has long been associated with cancer of the mouth, esophagus, pharynx, larynx, and, to a lesser extent, liver (1, 56), and it appears to be an important human teratogen, causing a variety of physical and mental defects in babies of mothers who drink (57). Alcohol drinking causes abnormalities in mice (57a) and is a synergist for chromosome damage in humans (58). Alcohol metabolism generates acetaldehyde, which is a mutagen and teratogen (59), a cocarcinogen, and possibly a carcinogen (60), and also radicals that produce lipid hydroperoxides (61) and other mutagens and carcinogens (62; see below). In some epidemiologic studies on alcohol (56), it has been suggested that dietary green vegetables are a modifying factor in the reduction of cancer risk.

Mold carcinogens. A variety of mold carcinogens and mutagens are present in mold-contaminated food such as corn, grain, nuts, peanut butter, bread, cheese, fruit, and apple juice (15, 63). Some of these, such as sterigmatocystin and aflatoxin, are among the most potent carcinogens and mutagens known (15, 63). Dietary glutathione has been reported to counteract aflatoxin carcinogenicity.

Nitrite, nitrate, and nitrosamines. A number of human cancers, such as stomach and esophageal cancer, may be related to nitrosamines and other nitroso compounds formed from nitrate and nitrite in the diet (64, 65). Beets, celery, lettuce, spinach, radishes, and rhubarb all contain about 200 mg of nitrate per 100-g portion (65). Anticarcinogens in the diet may be important in this context as well (66).

Fat and cancer: possible oxidative mechanisms. Epidemiologic studies of cancer in humans suggest, but do not prove, that high fat intake is associated with colon and breast cancer (1, 4, 67). A

number of animal studies have shown that high dietary fat is a promoter and a presumptive carcinogen (4, 67, 68). Colon and breast cancer and lung cancer (which is almost entirely due to cigarette smoking) account for about half of all U.S. cancer deaths. In addition to the cyclopropanoid fatty acids already discussed, two other plausible mechanisms involving oxidative processes could account for the relation (69) between high fat and both cancer and heart disease.

1) *Rancid fat.* Fat accounts for over 40 percent of the calories in the U.S. diet (67), and the amount of ingested oxidized fat may be appreciable (70, 71). Unsaturated fatty acids and cholesterol in fat are easily oxidized, particularly during cooking (70, 71). The lipid peroxidation chain reaction (rancidity) yields a variety (71–73) of mutagens, promoters, and carcinogens such as fatty acid hydroperoxides (62), cholesterol hydroperoxide (74), endoperoxides, cholesterol and fatty acid epoxides (74–77), enals and other aldehydes (44, 59, 78), and alkoxy and hydroperoxy radicals (44, 72). Thus the colon and digestive tract are exposed to a variety of fat-derived carcinogens. Human breast fluid can contain enormous levels (up to 780 μM) (75) of cholesterol epoxide (an oxidation product of cholesterol), which could originate from either ingested oxidized fat or oxidative processes in body lipids. Rodent feeding studies with oxidized fat (79) have not yielded definitive results.

2) *Peroxisomes* oxidize an appreciable percentage of dietary fatty acids, and removal of each two-carbon unit generates one molecule of hydrogen peroxide (a mutagen, promoter, and carcinogen) (80, 81). Some hydrogen peroxide escapes the catalase in the peroxisome (80, 82, 83), thus contributing to the supply of oxygen radicals, which also come from other metabolic sources (72, 83–85). Hydroperoxides generate oxygen radicals in the presence of iron-containing compounds in the cell (72). Oxygen radicals, in turn, can damage DNA and can start the rancidity chain reaction which leads to the production of the mutagens and carcinogens listed above (72). Drugs such as clofibrate, which cause lowering of serum lipids and proliferation of peroxisomes in rodents, result in age pigment (lipofuscin) accumulation (a sign of lipid peroxidation in tissues) and liver tumors in animals (80). Some fatty acids, such as $\text{C}_{22:1}$ and certain *trans* fatty acids, appear to cause peroxisomal proliferation because they are poorly oxidized in mitochondria and are preferentially oxidized in the peroxisomes, although they may be selective for heart or

liver (86). There has been controversy about the role of *trans* fatty acids in cancer and heart disease, and recent evidence suggests that *trans* fatty acids might not be a risk factor for atherosclerosis in experimental animals (87). Americans consume about 12 g of *trans* fatty acids a day (87) and a similar amount of unnatural *cis* isomers [which need further study (88)], mainly from hydrogenated vegetable fats. Dietary C_{22:1} fatty acids are also obtained from rapeseed oil and fish oils (86). Thus oxidation of certain fatty acids might generate grams of hydrogen peroxide per day within the peroxisome (86). Another source of fat toxicity could be perturbations in the mitochondrial or peroxisomal membranes caused by abnormal fatty acids, yielding an increased flux of superoxide and hydrogen peroxide. Mitochondrial structure is altered when rats are fed some abnormal fatty acids from partially hydrogenated fish oil (89). Dietary C_{22:1} fatty acids and clofibrate also induce ornithine decarboxylase (86), a common attribute of promoters.

A recent National Academy of Sciences committee report suggests that a reduction of fat consumption in the American diet would be prudent (4), although other scientists argue that, until we know more about the mechanism of the fat-cancer relation and about which types of fat are dangerous, it is premature to recommend dietary changes (90).

Cooked Food as a Source of Ingested Burnt and Brownd Material

Work of Sugimura and others has indicated that the burnt and browned material from heating protein during cooking is highly mutagenic (21, 91). Several chemicals isolated on the basis of their mutagenicity from heated protein or pyrolyzed amino acids were found to be carcinogenic when fed to rodents (21). In addition, the browning reaction products from the caramelization of sugars or the reaction of amino acids and sugars during cooking (for instance, the brown material on bread crusts and toasted bread) contain a large variety of DNA-damaging agents and presumptive carcinogens (23, 38, 92). The amount of burnt and browned material in the human diet may be several grams per day. By comparison about 500 mg of burnt material is inhaled each day by a smoker using two packs of cigarettes (at 20 mg of tar per cigarette) a day. Smokers have more easily detectable levels of mutagens in their urine than nonsmokers (93), but so do people who have consumed a meal of

fried pork or bacon (94). In the evaluation of risk from burnt material it may be useful (in addition to carrying out epidemiologic studies) to compare the activity of cigarette tar to that of the burnt material from cooked food (or polluted air) in short-term tests and animal carcinogenicity tests involving relevant routes of exposure. Route of exposure and composition of the burnt material are critical variables. The risk from inhaled cigarette smoke can be one reference standard: an average life shortening of about 8 years for a two-pack-a-day smoker. The amount of burnt material inhaled from severely polluted city air, on the other hand, is relatively small: it would be necessary to breathe smoggy Los Angeles air (111 µg/m³ total particulates; 31 µg/m³ soluble organic matter) for 1 to 2 weeks to equal the soluble organic matter of the particulates or the mutagenicity from one cigarette (20 mg of tar) (95). Epidemiologic studies have not shown significant risks from city air pollution alone (1, 96). Air in the houses of smokers is considerably more polluted than city air outside (97).

Coffee, which contains a considerable amount of burnt material, including the mutagenic pyrolysis product methylglyoxal, is mutagenic (21, 98). However, one cup of coffee also contains about 250 mg of the natural mutagen chlorogenic acid (9) [which is also an antinitrosating agent (66)], highly toxic atracylosides (10), the glutathione transferase inducers kahweol palmitate and cafestol palmitate (11), and about 100 mg of caffeine [which inhibits a DNA-repair system and can increase tumor yield (99) and cause birth defects at high levels in several experimental species (100)]. There is preliminary, but not conclusive, epidemiologic evidence that heavy coffee drinking is associated with cancer of the ovary, bladder, pancreas, and large bowel (101).

Cooking also accelerates the rancidity reaction of cooking oils and fat in meat (70, 71), thus increasing consumption of mutagens and carcinogens.

Anticarcinogens

We have many defense mechanisms to protect ourselves against mutagens and carcinogens, including continuous shedding of the surface layer of our skin, stomach, cornea, intestines, and colon (102). Understanding these mechanisms should be a major goal of cancer, heart, and aging research. Among the most important defenses may be those against oxygen radicals and lipid peroxidation if, as discussed here, these agents are major

contributors to DNA damage (103). Major sources of endogenous oxygen radicals are hydrogen peroxide (83) and superoxide (72, 104) generated as side products of metabolism, and the oxygen radical burst from phagocytosis after viral or bacterial infection or the inflammatory reaction (105). A variety of environmental agents could also contribute to the oxygen radical load, as discussed here and in recent reviews (72, 106). Many enzymes protect cells from oxidative damage; examples are superoxide dismutase (104), glutathione peroxidase (107), DT-diaphorase (108), and the glutathione transferases (109). In addition, a variety of small molecules in our diet are required for antioxidative mechanisms and appear to be anticarcinogens; some of these are discussed below.

1) *Vitamin E* (tocopherol) is the major radical trap in lipid membranes (72) and has been used clinically in a variety of oxidation-related diseases (110). Vitamin E ameliorates both the cardiac damage and carcinogenicity of the quinones adriamycin and daunomycin, which are mutagenic, carcinogenic, cause cardiac damage, and appear to be toxic because of free radical generation (111). Protective effects of tocopherols against radiation-induced DNA damage and mutation and dimethylhydrazine-induced carcinogenesis have also been observed (112). Vitamin E markedly increases the endurance of rats during heavy exercise, which causes extensive oxygen radical damage to tissues (113).

2) *β-Carotene* is another antioxidant in the diet that could be important in protecting body fat and lipid membranes against oxidation. Carotenoids are free-radical traps and remarkably efficient quenchers of singlet oxygen (114). Singlet oxygen is a very reactive form of oxygen which is mutagenic and particularly effective at causing lipid peroxidation (114). It can be generated by pigment-mediated transfer of the energy of light to oxygen, or by lipid peroxidation, although the latter is somewhat controversial. *β-Carotene* and similar polyenes are present in carrots and in all food that contains chlorophyll, and they appear to be the plants' main defense against singlet oxygen generated as a by-product from the interaction of light and chlorophyll (115). Carotenoids have been shown to be anticarcinogens in rats and mice (116). Carotenoids (in green and yellow vegetables) may be anticarcinogens in humans (1, 56, 117). Their protective effects in smokers might be related to the high level of oxidants in both cigarette smoke and tar (45, 118). Carotenoids have been used medically in

the treatment for some genetic diseases, such as porphyrias, where a marked photosensitivity is presumably due to singlet oxygen formation (119).

3) *Selenium* is another important dietary anticarcinogen. Dietary selenium (usually selenite) significantly inhibits the induction of skin, liver, colon, and mammary tumors in experimental animals by a number of different carcinogens, as well as the induction of mammary tumors by viruses (120). It also inhibits transformation of mouse mammary cells (121). Low selenium concentrations may be a risk factor in human cancer (122). A particular type of heart disease in young people in the Keshan area of China has been traced to a selenium deficiency, and low selenium has been associated with cardiovascular death in Finland (123). Selenium is in the active site of glutathione peroxidase, an enzyme essential for destroying lipid hydroperoxides and endogenous hydrogen peroxide and thus helping to prevent oxygen radical-induced lipid peroxidation (107), although not all of the effects of selenium may be accounted for by this enzyme (120). Several heavy-metal toxins, such as Cd^{2+} (a known carcinogen) and Hg^{2+} , lower glutathione peroxidase activity by interacting with selenium (107). Selenite (and vitamin E) has been shown to counter the oxidative toxicity of mercuric salts (124).

4) *Glutathione* is present in food and is one of the major antioxidants and anti-mutagens in the soluble fraction of cells. The glutathione transferases (some of which have peroxidase activity) are major defenses against oxidative and alkylating carcinogens (109). The concentration of glutathione may be influenced by dietary sulfur amino acids (125, 126). *N*-Acetylcysteine, a source of cysteine, raises glutathione concentrations and reduces the oxidative cardiotoxicity of adriamycin and the skin reaction to radiation (127). Glutathione concentrations are raised even more efficiently by L-2-oxothiazolidine-4-carboxylate, which is an effective antagonist of acetaminophen-caused liver damage (126). Acetaminophen is thought to be toxic through radical and quinone oxidizing metabolites (128). Dietary glutathione may be an effective anticarcinogen against aflatoxin (129).

5) Dietary *ascorbic acid* is also important as an antioxidant. It was shown to be anticarcinogenic in rodents treated with ultraviolet radiation, benzo[*a*]pyrene, and nitrite (forming nitroso carcinogens) (64, 65, 130), and it may be inversely associated with human uterine cervical dysplasia (although this is not

proof of a cause-effect relationship) (131). It was recently hypothesized that ascorbic acid may have been supplemented and perhaps partially replaced in humans by uric acid during primate evolution (132).

6) *Uric acid* is a strong antioxidant present in high concentrations in the blood of humans (132). The concentration of uric acid in the blood can be increased by dietary purines; however, too much causes gout. Uric acid is also present in high concentrations in human saliva (132) and may play a role in defense there as well, in conjunction with lactoperoxidase. A low uric acid level in blood may possibly be a risk factor in cigarette-caused lung cancer in humans (133).

7) Edible plants and a variety of substances in them, such as phenols, have been reported to inhibit (cabbage) or to enhance (beets) carcinogenesis (11, 55, 134) or mutagenesis (23, 66, 92, 135) in experimental animals. Some of these substances appear to inhibit by inducing cytochrome P-450 and other metabolic enzymes [(134); see also (11)], although on balance it is not completely clear whether it is generally helpful or harmful for humans to ingest these inducing substances.

The hypothesis that as much as 80 percent of cancer could be due to environmental factors was based on geographic differences in cancer rates and studies of migrants (136). These differences in cancer rates were thought to be mainly due to life-style factors, such as smoking and dietary carcinogens and promoters (136), but they also may be due in good part [see also (1)] to less than optimum amounts of anticarcinogens and protective factors in the diet.

The optimum levels of dietary antioxidants, which may vary among individuals, remain to be determined; however, at least for selenium (120), it is important to emphasize the possibility of deleterious side effects at high doses.

Oxygen Radicals and Degenerative Diseases Associated with Aging

Aging. A plausible theory of aging holds that the major cause is damage to DNA (102, 137) and other macromolecules and that a major source of this damage is oxygen radicals and lipid peroxidation (43, 84, 103, 138-141). Cancer and other degenerative diseases, such as heart disease (102), are likely to be due in good part to this same fundamental destructive process. Age pigment (lipofuscin) accumulates aging in all mammalian

species and has been associated with lipid peroxidation (73, 84, 138, 139). The fluorescent products in age pigment are thought to be formed by malondialdehyde (a mutagen and carcinogen and a major end product of rancidity) cross-linking protein and lipids (138). Metabolic rate is directly correlated with the rate of lipofuscin formation (and inversely correlated with longevity) (139).

Cancer increases with about the fourth power of age, both in short-lived species such as rats and mice (about 30 percent of rodents have cancer by the end of their 2- to 3-year life-span) and in long-lived species such as humans (about 30 percent of people have cancer by the end of their 85-year life-span) (142). Thus, the marked increase in life-span that has occurred in 60 million years of primate evolution has been accompanied by a marked decrease in age-specific cancer rates; that is, in contrast to rodents, 30 percent of humans do not have cancer by the age of 3 (142). One important factor in longevity appears to be basal metabolic rate (139, 141), which is much lower in man than in rodents and could markedly affect the level of endogenous oxygen radicals.

Animals have many antioxidant defenses against oxygen radicals. Increased levels of these antioxidants, as well as new antioxidants, may also be a factor in the evolution of man from short-lived prosimians (143). It has been suggested that an increase in superoxide dismutase is correlated (after the basal metabolic rate is taken into account) with increased longevity during primate evolution, although this has been disputed (141). Ames *et al.* proposed (132) that as uric acid was an antioxidant and was present in much higher concentrations in the blood of humans than in other mammals, it may have been one of the innovations enabling the marked increase in life span and consequent marked decrease in age-specific cancer rates which occurred during primate evolution. The ability to synthesize ascorbic acid may have been lost at about the same time in primate evolution as uric acid levels began to increase (144).

Cancer and promotion. Both DNA-damaging agents (initiating mutagens) (21, 41, 42) and promoters (145) appear to play an important role in carcinogenesis (21, 146). It has been postulated that certain promoters of carcinogenesis act by generation of oxygen radicals and resultant lipid peroxidation (73, 146-149). Lipid peroxidation cross-links proteins (43, 150) and affects all aspects of cell organization (72), including membrane and surface structure, and the mi-

otic apparatus. A common property of promoters may be their ability to produce oxygen radicals. Some examples are fat and hydrogen peroxide (which may be among the most important promoters) (67, 68, 81), TCDD (151), lead and cadmium (152), phorbol esters (147, 149, 153), wounding of tissues (154), asbestos (155), peroxides (156), catechol (45) (see quinones above), mezerein and teleocidin B (147), phenobarbital (157), and radiation (72, 158). Inflammatory reactions involve the production of oxygen radicals by phagocytes (105), and this could be the basis of promotion for asbestos (155) or wounding (154). Some of the antioxidant anticarcinogens (discussed above) are also antipromoters (73, 121, 146, 159, 160), and phorbol ester-induced chromosome damage (149) or promotion of transformation (159) is suppressed by superoxide dismutase, as would be expected if promoters were working through oxidative mechanisms. Many "complete" carcinogens cause the production of oxygen radicals (73, 161); examples are nitroso compounds, hydrazines, quinones, polycyclic hydrocarbons (through quinones), cadmium and lead salts, nitro compounds, and radiation. A good part of the toxic effects of ionizing radiation damage to DNA and cells is thought to be due to generation of oxygen radicals (103, 162), although only a tiny part of the oxygen radical load in humans is likely to be from this source.

Recent studies give some clues as to how promoters might act. Promoters disrupt the mitotic apparatus, causing hemizygosity and expression of recessive genes (163). Phorbol esters generate oxygen radicals, which cause chromosome breaks (164) and increase gene copy number (165). Promoters also cause formation of the peroxide hormones of the prostaglandin and leukotriene family by oxidation of arachidonic acid and other C₂₀ polyenoic fatty acids, and inhibitors of this process appear to be antipromoters (160). These hormones are intimately involved in cell division, differentiation, and tumor growth (166) and could have arisen in evolution as signal molecules warning the cell of oxidative damage. Effects on the cell membrane have also been suggested as the important factor in promotion, causing inhibition of intercellular communication (167) or protein kinase activation (167a).

Heart disease. It has been postulated that atherosclerotic lesions, which are derived from single cells, are similar to benign tumors and are of somatic mutational origin (102, 168). Fat appears to be one major risk factor for heart disease as

well as for colon and breast cancer (69). In agreement with this, a strong correlation has been observed between the frequency of atherosclerotic lesions and adenomatous polyps of the colon (69). Thus, the same oxidative processes involving fat may contribute to both diseases. Oxidized forms of cholesterol have been implicated in heart disease (169), and atherosclerotic-like lesions have been produced by injecting rabbits with lipid hydroperoxide or oxidized cholesterol (169). The anticarcinogens discussed above could be anti-heart disease agents as well. As pointed out in the preceding section, vitamin E ameliorates both the cardiac damage and carcinogenicity of the free-radical-generating quinones adriamycin and daunomycin; *N*-acetylcysteine reduces the cardiotoxicity of adriamycin; and selenium is an antirisk factor for one type of heart disease.

Other diseases. The brain uses 20 percent of the oxygen consumed by man and contains an appreciable amount of unsaturated fat. Lipid peroxidation (with consequent age pigment) is known to occur readily in the brain (72), and possible consequences could be senile dementia or other brain abnormalities (84). Several inherited progressive diseases of the central nervous system, such as Batten's disease, are associated with lipofuscin accumulation and may be due to a lipid peroxidation caused by a high concentration of unbound iron (170). Mental retardation is one consequence of an inherited defective DNA repair system (XP complementation group D) for depurinated sites in DNA (171).

Senile cataracts have been associated with light-induced oxidative damage (172). The retina and an associated layer of cells, the pigment epithelium, are extremely sensitive to degeneration in vitamin E and selenium deficiency (173). The pigment epithelium accumulates massive amounts of lipofuscin in aging and dietary antioxidant deficiency (173). The eye is well known to be particularly rich in antioxidants.

The testes are quite prone to lipid peroxidation and to the accumulation of age pigment. A number of agents, such as gossypol, which cause genetic birth defects (dominant lethals) may be active by this mechanism. The various agents known to cause cancer by oxidative mechanisms are prospective mutagenic agents for the germ line. Thus, vitamin E, which was discovered 60 years ago as a fertility factor (72), and other antioxidants such as selenium (174), may help both to engender and to protect the next generation.

Risks

There are large numbers of mutagens and carcinogens in every meal, all perfectly natural and traditional [see also (21, 23)]. Nature is not benign. It should be emphasized that no human diet can be entirely free of mutagens and carcinogens and that the foods mentioned are only representative examples. To identify a substance, whether natural or man-made, as a mutagen or a carcinogen, is just a first step. Beyond this, it is necessary to consider the risks for alternative courses of action and to quantitate the approximate magnitude of the risk, although the quantification of risk poses a major challenge. Carcinogens differ in their potency in rodents by more than a millionfold (175), and the levels of particular carcinogens to which humans are exposed can vary more than a billionfold. Extrapolation of risk from rodents to humans is difficult for many reasons, including the longevity difference, antioxidant factors, and the probable multicausal nature of most human cancer.

Tobacco smoking is, without doubt, a major and well-understood risk, causing about 30 percent of cancer deaths and 25 percent of fatal heart attacks (as well as other degenerative diseases) in the United States (1). These percentages may increase even more in the near future as the health effects of the large increase in women smokers become apparent (1). Diet, which provides both carcinogens and anticarcinogens, is extremely likely to be another major risk factor. Excessive alcohol consumption is another risk, although it does not seem to be of the same general importance as smoking and diet. Certain other high-dose exposures might also turn out to be important for particular groups of people—for instance, certain drugs, where consumption can reach hundreds of milligrams per day; particular cosmetics; and certain occupational exposures (2), where workers inhale dusts or solvents at high concentration. We must also be prudent about environmental pollution (41, 54). Despite all of these risks, it should be emphasized that the overall trend in life expectancy in the United States is continuing steadily upward (176).

The understanding of cancer and degenerative disease mechanisms is being aided by the rapid progress of science and technology, and this should help to dispel confusion about how important health risks can be identified among the vast number of minor risks. We have many methods of attacking the problem of environmental carcinogens (and anticarcinogens), including human epidemi-

ology (1), short-term tests (41, 42, 177), and animal cancer tests (175). Powerful new methods are being developed [for instance, see (58, 177)] for measuring DNA damage or other pertinent factors with great sensitivity in individuals. These methods, which are often noninvasive as they can be done on blood or urine (even after storage), can be combined with epidemiology to determine whether particular factors are predictive of disease. Thus, more powerful tools will be available for optimizing antioxidants and other dietary anti-risk factors, for identifying human genetic variants at high risk, and for identifying significant health risks.

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CANCER EPIDEMIOLOGY and PREVENTION

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*Chapter 18. Occupation
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CHAPTER EIGHTEEN

Occupation

Pierre Decoufle

INTRODUCTION

Epidemiologic studies of occupational groups provide a unique opportunity to observe the experience of people in continual and intimate contact with a host of potentially hazardous substances. Occupational exposures to physical and chemical agents are usually heavy and extend over a relatively long period of time. These circumstances suggest that if a particular substance has the potential for inducing a carcinogenic effect in humans, the best opportunity of observing it is in an occupational setting. Occupational exposures are often associated with well-defined and circumscribed populations that make natural study groups. For example, one might define a study group from the work force of a particular industry, plant, or labor union if there is information on duration of employment in specific jobs and, possibly, a qualitative or quantitative assessment of exposures to particular agents. In a cohort approach, these individuals are followed forward to determine the status of their health at subsequent intervals and its relationship to prior exposures.

There are at least four reasons for conducting epidemiologic studies of industrial populations. One is the monitoring or surveillance of various groups to detect previously unsuspected hazards by noting unusual disease patterns. This has been done in the steel and rubber industries in the United States (Lloyd and Ciocco, 1969;

McMichael et al, 1974; Monson and Nakano, 1976; Andjelkovich et al, 1976). Epidemiologic studies also assist in the re-evaluation of current thinking on what are "safe" levels for known hazards. As was the case with vinyl chloride (Creech and Johnson, 1974), our thinking may change because new and more serious health effects are found from observations in industry. Epidemiologic studies of workers can provide leads for experimental research. Thus, identification of high-risk occupational groups suggests a set of potentially carcinogenic substances that can be tested individually and jointly in laboratory animals. Finally, there will always be work environments in the real world of industrial processes that will be difficult to re-create under experimental conditions. Human exposure to complex mixtures of different materials produced during industrial processes may defy replication in the laboratory.

HISTORY

The first realization that contact with materials in the work environment could cause malignant disease was Percivall Pott's observation of an unusually high frequency of scrotal cancer among London chimney sweeps in 1775. Although heavy exposure to soot due to the lack of protective clothing and the poor personal hygiene of the young workers were considered causal factors by contemporary observers, ex-

perimental verification of the carcinogenicity of soot derived from bituminous coal did not come until this century (Passey, 1922). A hundred years after Pott's report, it was recognized that skin cancer (including scrotal) could be induced by a variety of coal tar products in different industries (Volkman, 1875; Manouvriez, 1876; Ball, 1885; Butlin, 1892; Lueke, 1907).

Early in the 19th century, Paris described cancers of the scrotum among Cornish smelter workers, presumably due to arsenic liberated in the smelting process (Paris, 1822). However, there are no subsequent reports confirming his observations, and later investigators have doubted the accuracy of this report (Butlin, 1892; Henry, 1946). It was not until this century that inorganic arsenic, in the form of a pesticide, was definitely established as a cause of occupational skin cancer (Hill and Faning, 1948).

Certain forms of mineral oil constitute the third major occupational exposure capable of causing skin cancer. The earliest report was Dr. Joseph Bell's description of scrotal cancer cases among "paraffin pressmen" in the Scottish shale oil industry (Bell, 1876). Early in the 20th century, the risk of scrotal cancer from mineral oil was found among mule spinners in the cotton industry (Southam and Wilson, 1922). Subsequently, cutting oils used in the machine tool industry were associated with an unusual frequency of skin cancer (Cruickshank and Squire, 1950).

In 1879, Harting and Hesse identified pulmonary cancer as an occupational disease among metal miners in central Europe — apparently the first instance of an occupationally induced tumor of this organ system. In the early part of this century, radioactivity was discovered in these mines and was eventually conceded to be the causal agent (Hueper, 1966b). Between 1930 and 1950, there was increased awareness of occupational environments associated with high risks of respiratory cancer. During this period, nickel refining (Annual Report of the Chief Inspector of Factories and Workshops, 1933), coal carbonization processes (Kuroda and Kawahata, 1936), arsenic (Hill and Faning, 1948), chromates (Machle and Gregorius, 1948), and asbestos (Merewether, 1949) were implicated as respiratory cancer hazards.

In the case of asbestos, suspicions were first aroused when an unusually high frequency of lung cancer appeared at autopsy among asbestos workers dying of asbestosis, a form of pulmonary fibrosis induced by the fibrous dust (Merewether, 1949). Although there were early case reports in the German literature, the first

epidemiologic study of the chromate-producing industry was not instigated until the 1940s, when the management of one of the large United States firms became concerned about an apparently high incidence of lung cancer among its employees (Machle and Gregorius, 1948).

An analysis of occupations reported on death certificates between 1921 and 1932 in England and Wales identified several groups of coal carbonization and by-products workers to be at high risk of lung cancer (Kennaway and Kennaway, 1936). These findings were later confirmed by conventional epidemiologic studies.

Tumors of the urinary bladder were recognized as having an industrial origin when Rehn studied workers in the German dyestuffs industry in 1895. The process involved production of synthetic dyes utilizing various chemicals produced from coal tar. For many years, these malignancies were mistakenly referred to as "aniline cancers" after the principal dye intermediate that was used. Only after repeated epidemiologic and experimental studies were two specific carcinogenic aromatic amines, benzidine and β -naphthylamine, discovered.

CURRENT STATUS OF EPIDEMIOLOGIC FINDINGS

To date, most of the known occupational causes of cancer have been identified through epidemiologic observations with subsequent confirmation by laboratory studies, although arsenic and benzene still lack experimental evidence. In the cases of 4-aminobiphenyl, mustard gas, and vinyl chloride, the risks were detected in man after the substances had been shown to induce tumors in laboratory animals, although little attention was given to the experimental studies when first reported.

Table 1 outlines well-established relationships between specific sites of cancer and various chemical and physical agents or industrial processes based on direct observations of various occupational groups. For some industrial processes, discrete carcinogenic agents have not been identified yet, such as iron ore mining and the manufacture of the dyes auramine and magenta. Coal carbonization processes (e.g., coke ovens) liberate a multitude of chemicals (including polycyclic aromatic hydrocarbons, some of which are carcinogenic in experimental animals), but the specific compound or combination of compounds responsible for the excess of lung cancer among exposed workers

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TABLE 1. Cancer Sites for Which Relationships with Occupational Exposures are Well-Established in Human Studies

Site	Agent or Industrial Process	References
Bladder	Benzidine, β -naphthylamine, 4-Aminobiphenyl (xenylamine)	Rehn, 1895; Ferguson et al, 1934; Scott, 1952; Case et al, 1954; Melick et al, 1955; Vigliani and Barsotti, 1961; Lieben, 1963; Goldwater et al, 1965; Mancuso and El-Attar, 1967; Melick et al, 1971; Zaron et al, 1973; Tsuchiya et al, 1975 Case and Pearson, 1954
	Manufacture of certain dyes (e.g., auramine and magenta)	Doll et al, 1965; Doll et al, 1972
	Gas retorts	Case and Hosker, 1954; Davies, 1965
Blood (leukemia)	Rubber and cable making industries	Girard et al, 1971; Aksoy et al, 1974; Vigliani, 1976; Infante et al, 1977
	Benzene	Warren, 1956; Seltzer and Sartwell, 1965; Warren and Lombard, 1966;
	X-radiation	Malenowski et al, 1975
Bone	Radium, Mesothorium	Martland, 1931; Polednak et al, 1978
Larynx	Ethanol (ethyl alcohol) manufacture by strong acid process (diethyl sulfate?)	Lynch et al, 1979
	Isopropyl alcohol manufacture by strong acid process (diisopropyl sulfate?)	Well et al, 1952; Eckardt, 1974
	Mustard gas	Wada et al, 1968
Liver (angiosarcoma)	Arsenic (Inorganic compounds)	Roth, 1958
	Vinyl chloride	Creech and Johnson, 1974; Waxweiler et al, 1976; Spirtas and Kaminski, 1978
Lung, Bronchus	Arsenic (Inorganic compounds)	Hill and Fanning, 1948; Roth, 1958; Galy et al, 1963; Lee and Fraumeni, 1969; Ott et al, 1974; Milham and Strong, 1974; Kuratsune et al, 1974; Tokudome and Kuratsune, 1976; Rencher et al, 1977; Pinto et al, 1978; Axelson et al, 1978; Mabuchi et al, 1980
	Asbestos	Merewether, 1949; Doll, 1955; Mancuso and Coulter, 1963; Selikoff et al, 1964; Jacob and Anspach, 1965; Lieben, 1966; Enterline and Kendrick, 1967; Selikoff et al, 1968; Knox et al, 1968; Newhouse, 1969; Tabershaw et al, 1970; Elmes and Simpson, 1971; Fletcher, 1972; Selikoff et al, 1972; Newhouse et al, 1972; Enterline et al, 1973; Selikoff et al, 1973; Edge, 1976; Martischniig et al, 1977; Peto et al, 1977; Robinson et al, 1979; McDonald et al, 1980; Selikoff et al, 1980
	Bis(chloromethyl) ether	Figueroa et al, 1973; Lemen et al, 1976; DeFonso and Kelton, 1976; Pasternack et al, 1977
	Chromium compounds	Machle and Gregorius, 1948; Baetjer, 1950a; Baetjer, 1950b; Mancuso and Hueper, 1951; Brinton et al, 1952; Bidstrup and Case, 1956; Enterline, 1974; Langard and Norseth, 1975; Royle, 1975; Michel- Briand and Simonin, 1977; Davies, 1978; Hayes et al, 1979; Dalager et al, 1980

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	Coal carbonization processes (coke ovens, gas retorts, producer gas manufacture)	Kuroda and Kawahata, 1936; Kennaway and Kennaway, 1936; Kennaway and Kennaway, 1947; Doll, 1952; Christian, 1962; Doll et al, 1965; Kawai et al, 1967; Lloyd, 1971; Redmond et al, 1972; Doll et al, 1972; Mazumdar et al, 1975
	Coal tar pitch volatiles (roofing materials, aluminum reduction plants)	Hemmond et al, 1976; Gibbs and Horowitz, 1979
	Iron ore (hematite) mining	Faulds and Stewart, 1956; Boyd et al, 1970
	Mustard gas	Wada et al, 1968
	Nickel refining	Doll, 1958; Morgan, 1958; Mastromatteo, 1967; Doll et al, 1970; Pedersen et al, 1973; Doll et al, 1977
	Radiation (radioactive ores)	Harting and Hesse, 1879; Peller, 1939; Lorenz, 1944; DeVilliers and Windish, 1964; Wagoner et al, 1965; Lundin et al, 1969; Lundin et al, 1971
Nasal Cavity, Sinuses	Isopropanol (isopropyl alcohol) manufacture by strong acid process (diisopropyl sulfate?)	Well et al, 1952; Eckardt, 1974
	Mustard gas	Wada et al, 1968
	Nickel refining	Annual Report, 1933; Doll, 1958; Morgan, 1958; Mastromatteo, 1967; Doll et al, 1970; Pedersen et al, 1973; Doll et al, 1977
	Radium, Mesothorium	Hasterlik et al, 1964
	Shoe manufacturing (leather dust?)	Acheson et al, 1972; Acheson, 1976
	Woodworking (wood dust?)	Acheson et al, 1972; Acheson, 1976; Brinton et al, 1977
Peritoneum (mesothelioma)	Asbestos	Selikoff et al, 1965; Newhouse and Thompson, 1965; Selikoff et al, 1972; Selikoff et al, 1973; Greenberg and Lloyd Davies, 1974
Pharynx	Mustard gas	Wada et al, 1968
Pleura (mesothelioma)	Asbestos	Wagner et al, 1960; Selikoff et al, 1965; Newhouse and Thompson, 1965; Selikoff et al, 1972; Selikoff et al, 1973; Greenberg and Lloyd Davies, 1974
Skin (including scrotum) (Epitheliomas)	Arsenic (inorganic compounds)	Paris, 1822; Kennaway, 1925; Henry, 1946; Hill and Fanning, 1948; Roth, 1958; Mabuchi et al, 1960
	Coal tar products (mainly coal tar, creosote, pitch, soot)	Pott, 1775; Volkmann, 1875; Manouvriez, 1876; Ball, 1885; Butlin, 1892; Lueke, 1907; Kennaway, 1925; Henry, 1946; Henry, 1947; Kipling and Waldron, 1976; Doll et al, 1972
	Coal hydrogenation	Sexton, 1960
	Mineral oils (from coal, petroleum, shale)	Volkmann, 1875; Ball, 1876; Scott, 1922; Southam and Wilson, 1922; Kennaway, 1925; Henry, 1946; Henry, 1947; Cruickshank and Squire, 1950; Cruickshank and Gourevitch, 1952; Hendricks et al, 1959; Kipling, 1968; Kipling and Waldron, 1976; Jarvholm et al, 1981
	X-radiation	Court Brown and Doll, 1958; Hunter, 1969; Matanoski et al, 1975

is not known. Similarly, the precise constituents of wood and leather dusts that produce tumors of the nasal cavity and sinuses are unknown, although in the case of woodworking the use of hardwoods in the furniture industry seems to be associated with the greatest risk (Acheson, 1976).

The original report of upper respiratory system cancers among workers engaged in the manufacture of isopropyl alcohol implicated isopropyl oil, a volatile by-product of the process, as the likely carcinogen (Weil et al, 1952). However, a recent report of laryngeal tumors among workers engaged in a similar process suggests that alkyl sulfates may be the common denominator (Lynch et al, 1979).

There is disagreement on exactly which compounds of chromium are carcinogenic for man. Studies in the chromate-producing industry (i.e., alkaline roasting of chromite ore) and in the manufacture of chromate pigments (lead and zinc chromate) provide convincing evidence that at least certain hexavalent chromium compounds are human carcinogens (Enterline, 1974; Langard and Norseth, 1975; Davies, 1978). Experimental work indicates that some chromium compounds show very little carcinogenic activity while others (e.g., calcium chromate) are very active (Sunderman, 1976). Recently, several reports suggest that exposure to chromic acid mist in chromium plating operations may be associated with an unusual frequency of respiratory cancer (Royle, 1975; Michel-Briand and Simonin, 1977).

The situation with nickel and its compounds is much the same as with chromium. Epidemiologic studies have established beyond a doubt that the nickel refining process is associated with very high risks of respiratory cancer. However, workers in this industry who developed cancers of the nasal passages or lung were exposed to mixtures of several different nickel compounds as well as other carcinogenic agents (e.g., arsenic). Animal testing has shown at least one nickel compound (nickel subsulfide) to be a potent carcinogen (Sunderman, 1976). As yet there are no definitive reports of increased respiratory cancer among nickel-exposed workers outside the refining industry.

Mineral oils represent a complex class of materials ranging from crude petroleum through various fractions produced at refineries to products used as coolants and lubricants in various industries. Not all mineral oils exhibit carcinogenicity for the skin either in man or in laboratory animals. Extraction and refining of oil from Scottish shale (Scott, 1922) and old methods of

recovering paraffin from petroleum (Hendricks et al, 1959) were associated with the occurrence of skin cancer among workers exposed to certain fractions of unrefined oils.

Skin cancer has also been seen among machinists who came into intimate and prolonged contact with oils used in cutting and grinding operations (Cruickshank and Squire, 1950; Cruickshank and Gourevitch, 1952; Kipling, 1968). Experimental work has shown that the carcinogenic component of mineral oils can be greatly reduced by the method and degree of refining they undergo (Bingham et al, 1965). In general, the more refined a mineral oil product is, the less is its carcinogenic potential. Thus, Great Britain adopted a highly refined "white" lubricating oil as the lubricant of choice in the cotton industry to control the "epidemic" of skin cancer among mule spinners (Kipling, 1968).

Epidemiologic studies of industrial populations have implicated additional substances as "suspect" carcinogens. A sample of these is listed in Table 2. The quality and quantity of epidemiologic evidence vary from fairly strong in the cases of cadmium with prostatic cancer and coke oven emissions with kidney cancer to suggestive for lead, ethylene oxide, and acrylonitrile. In fact, some of these agents might appear on other lists of "recognized" carcinogens.

In addition to expanding the previous list of agents, Table 2 indicates other cancer sites that may be affected by the materials shown in Table 1. Thus, asbestos is linked with cancers of the esophagus, larynx, stomach, and large bowel in addition to the three sites previously listed, a remarkable finding. However, it should be noted that all seven sites have not necessarily been seen in every asbestos-exposed population studied. Coke oven emissions are associated with increased mortality from kidney cancer (hypernephroma) and possibly prostatic cancer. An unusual pattern of cancers of the brain and respiratory system has been seen in workers exposed to vinyl chloride, now a well-established human liver carcinogen. Mineral oil, in the form of cutting oils, is related to cancers of the lung and digestive organs among workers exposed to mists arising from machining operations. For most of these materials, there are supporting data from studies in experimental animals, although the animal tumors may not be at the same site as observed in humans.

With the proliferation of epidemiologic studies of different occupational groups during the

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TABLE 3. Occupational Groups Associated with High Risks for Cancer, With No Specific Agents Identified

Occupational Group	Site(s)	References
Benzoyl Chloride Manufacture	Lung	Sakabe et al, 1976
Chemists	Brain Lymphatic and Hematopoietic Tissues Pancreas	Olin and Ahlbom, 1980 Li et al, 1969; Olin and Ahlbom, 1980 Li et al, 1969
Coal Miners	Stomach	Rockette, 1977
Coke By-Product Plant Workers	Colon Pancreas	Redmond et al, 1976 Redmond et al, 1976
Foundry Workers	Lung	Turner and Grace, 1938; McLaughlin and Harding, 1956; Koskela et al, 1976; Gibson et al, 1977; Decoufle and Wood, 1979; Egan et al, 1979; Tola et al, 1979
Leather Workers	Bladder Larynx Mouth, Pharynx	Henry et al, 1931; Versluys, 1949; Wynder et al, 1963; Cole et al, 1972; Decoufle, 1979 Decoufle, 1979 Decoufle, 1979
Metal Miners	Lung	Wagoner et al, 1963
Oil Refinery/Petrochemical Workers	Brain Brain, Leukemia, Multiple Myeloma, Stomach Esophagus, Lung, Stomach	Theriault and Goulet, 1979; Alexander et al, 1980 Thomas et al, 1980 Hanis et al, 1979
Painters	Blood (leukemia)	Viadana and Bross, 1972
Printing Workers	Lung Mouth, Pharynx	Moss et al, 1972; Greenberg, 1972 Lloyd et al, 1977
Rubber Industry Work Areas	Bladder Blood (leukemia) Brain Lung Prostate Stomach	Monson and Nakano, 1976; McMichael et al, 1976; Monson and Fine, 1978 Monson and Nakano, 1976; McMichael et al, 1976; Andjelkovich et al, 1977; Monson and Fine, 1978 Monson and Nakano, 1976; Monson and Fine, 1978 Monson and Nakano, 1976; McMichael et al, 1976; Fox and Collier, 1976; Andjelkovich et al, 1977 Monson and Fine, 1978 McMichael et al, 1976; Andjelkovich et al, 1977 Monson and Nakano, 1976; McMichael et al, 1976 Andjelkovich et al, 1977; Monson and Fine, 1978
Textile Workers	Nasal Cavity and Sinuses	Acheson et al, 1972
Woodworkers	Lymphatic Tissue (Hodgkin's disease)	Milham and Hesser, 1967; Petersen and Milham, 1974; Grufferman et al, 1976; Greene et al, 1978

last 10 to 15 years, many industrial populations have been shown to be at high risk for certain forms of cancer. Table 3 lists some of these groups. Although the reports suggest that occupational factors are responsible for the observed excesses, causative agents in the work environment have not been identified yet. Additional epidemiologic studies may be needed to confirm some of these findings, as in the case of painters with leukemia for which there is only one known report (Viadana and Bross, 1972).

An important reason for the inability to pinpoint discrete carcinogenic agents in these studies is the multiplicity of exposures encountered in the subject industries. In addition to materials known to be used in various work settings, many other substances generated as by-products of industrial processes have not been characterized completely. Industrial hygiene investigations of the past and present work environments in high-risk industries can be important, since they may uncover the unsuspected presence of known carcinogens. In addition, experimental studies in which test animals are exposed to the different materials found in high-risk industries may assist in sorting out likely carcinogenic substances.

The foundry industry is a particularly interesting example. Since early reports of an unusual frequency of respiratory cancer among foundry workers and metal grinders (Turner and Grace, 1938; Kennaway and Kennaway, 1947; McLaughlin and Harding, 1956), iron oxide has been considered a possible etiologic agent. However, there are now data to suggest a link with polycyclic aromatic hydrocarbons present in certain casting processes (Tola et al, 1979). Furthermore, exposure to iron oxide in the absence of known carcinogens produced a negative association with lung cancer in a recent study (Axelson and Sjoberg, 1979). Perhaps both these materials play a role in the cancer experience of foundry workers.

CHARACTERISTICS OF OCCUPATIONAL CANCER

Latent Period

Tumors of occupational origin become clinically manifest only after a definite time period (measured in years) has elapsed from first exposure to the carcinogenic agent. Latent period is the term commonly used to characterize this phenomenon, and it can be viewed in several

ways. For tumors of very low normal frequency (e.g., angiosarcoma of the liver, mesothelioma, osteosarcoma) or those of moderate frequency (bladder cancer, leukemia) when the relative risk is very high, virtually every case in an exposed group can be considered occupationally induced. Thus, the individual appearance times of the cases can be analyzed and a minimum or "average" latent period determined, keeping in mind that cases with the longest latency may not have been observed yet. On the other hand, for the more common tumors (e.g., lung cancer) when the relative risk is not very large, the latent period may be viewed as the time interval during which a significant increase in risk first occurs, or the time period of maximum risk, if one exists.

The minimum latent period is usually five years for noncutaneous cancers, with a large proportion of cases appearing between 10 and 30 years after first exposure. Occupational skin cancers exhibit a wide span of latent periods ranging from intervals of less than one year (Sexton, 1960) to well over 50 years (Henry, 1947). An interesting illustration of exposure-specific latent periods is Henry's data on cutaneous epithelioma of occupational origin. He noted that the "average" latent period for mineral oil-induced lesions was significantly longer than for cases produced by pitch, tar, and tar products (Henry, 1947), suggesting that the latter substances are more potent carcinogens.

The latency phenomenon has an important impact on the design of epidemiologic studies of occupational groups exposed to potential or recognized carcinogens. Thus, studies covering a limited number of years since the work force was first exposed to the material (e.g., 10 years) may fail to show an increase in risk simply because enough time has not elapsed for occupationally related tumors to become manifest. This problem may be compounded if the workers are very young, since they will not have reached the peak years of normal cancer incidence during the course of a relatively short follow-up study.

Dose-Response Relationships

Where data are available indicating the amount of exposure to a carcinogenic substance received by workers, whether it be a qualitative or quantitative estimate, an increasing risk with increasing exposure is generally observed. For example, this has been seen for lung cancer in relation to arsenic (Lee and

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Fraumeni, 1969), asbestos (Knox et al, 1968; Newhouse, 1969; Enterline et al, 1973; McDonald et al, 1980), uranium mining (Wagoner et al, 1965; Lundin et al, 1971), and coke oven emissions (Lloyd, 1971; Mazumdar et al, 1975). Thus, reduction in environmental levels of carcinogenic agents should reduce the relative risk of disease. Epidemiologic studies have failed to provide adequate evidence for the existence of "thresholds" for occupationally related cancers, i.e., environmental levels of exposure below which there is no detectable increase in the incidence of cancer related to specific agents. Both biologic and statistical considerations enter the discussion of this point (Schneiderman et al, 1979).

Low levels of exposure to particular agents may not appear to be associated with an unusual incidence of cancer within a given period of time, but with additional follow-up a definite pattern of increased incidence may be noticed. For example, men who worked at the sides of coke ovens, who presumably had much less exposure to the emissions than topside workers, did not at first exhibit a significant increase in lung cancer (Lloyd, 1971), but after a longer follow-up period, they did experience a real increase (Redmond et al, 1972). Thus, it may be that dose and latent period are inversely related, at least for some carcinogenic agents.

Multiple Sites

Industrial materials capable of causing cancer in exposed workers may produce tumors at more than one anatomic site. This appears to be true for asbestos, coal carbonization processes, mustard gas, nickel refining, and radiation. Sometimes this is not immediately evident because epidemiologic studies are not always designed to examine a variety of cancer sites simultaneously. An excess risk for two or more cancers may be due to interactions of the putative agent with nonoccupational factors or with other agents present in the work environment.

For certain agents, evidence of an association with more than one cancer site is either weak or so recent that confirmatory data from other studies are desirable. Thus, arsenic has been linked to tumors of the lymphatic tissues based on a study of pesticide makers (Ott et al, 1974), although such a link has not been reported among smelter workers (Lee and Fraumeni, 1969). Cancers of the upper respiratory tract have been mentioned among chromate work-

ers, but the data are very sparse (Hueper, 1966a). The fact that nonmalignant nasal conditions (e.g., perforation of the nasal septum) do occur frequently among heavily exposed chromate workers (Mancuso, 1951) may have lent credence to this possibility. It should be noted that exposure to aromatic amines is associated with cancers of the upper urinary tract (ureter, kidney), although the number of reported cases is small in relation to bladder tumors (Zavon et al, 1973; Tsuchiya et al, 1975).

Age at Diagnosis or Death

Generally, the age at which occupational tumors appear is a function of the age at first exposure and the attained age distribution of the particular group under study. Latency and amount of exposure are determining factors also.

Table 4 shows data pertaining to age at diagnosis (or death) and latent period for selected occupational cancers. It can be seen that tumors have arisen as early as the fourth decade of life, some even in the twenties. Part of the apparent shift to younger ages in these data (compared to the general population) is due to the method of case ascertainment. Most of these cases are persons who were actively employed in the industry when the cancer was first diagnosed, i.e., the easiest cases to identify. Cases appearing after retirement or among persons who left the industry are generally more difficult to trace.

Occupational cancers are still manifest after age 65 among individuals who have retired from hazardous industries. Thus, increased lung cancer has been seen among retirees from asbestos plants (Enterline et al, 1973), smelters (Pinto et al, 1978), and coke plants (Collins and Redmond, 1976). On the other hand, studies confined to pensioners may not always demonstrate the true magnitude of risk associated with specific work environments because of selective employment factors related to health (Reid and Buck, 1956).

Histologic Types

Several occupational cancers are associated with particular histologic types. The most extreme example is that of vinyl chloride with liver cancer where angiosarcoma (hemangioendotheliosarcoma) is the exclusive cell type (Creech and Johnson, 1974; Spirtas and Ka-

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TABLE 4. Ages at Diagnosis or Death, and Latency Periods for Selected Occupational Cancers

Agent and Cancer Site	Number in Series	Age at Diagnosis or Death		Latency Period (Years)		References
		RANGE	AVERAGE	RANGE	AVERAGE	
Chromates Respiratory System	35	33-72	Mean = 51.7 (at death)	4-47	Mean = 17.2 (as of death)	Machle and Gregorius, 1948
Bis(chloromethyl) ether Lung, Bronchus	38	31-66	Mean = 48.3 (at diagnosis or death)	8-26	Mean = 14.5 (as of diagnosis or death)	Lemen et al, 1976 DeFonso and Kelton, 1976
Vinyl chloride Angiosarcoma of Liver	64	37-71	Median = 49 (at diagnosis)	9-38	Median = 21 (as of diagnosis)	Spirtas and Kaminski, 1978
Aromatic amines Urinary Bladder	81	25-72	Mean = 52.1	2-42	Mean = 22.9	Goldwater et al, 1965
	66	25-66		5-32	Mean = 16.5	Scott, 1952
	100	<30 to 60+	Mean = 44.9	<5 to 30+	Mean = 16.0	Tsuchiya et al, 1975
	13	34-56	Mean = 45.6 (at diagnosis)	9-29	Mean = 16.6 (as of diagnosis)	Zavon et al, 1973

minski, 1978). Myeloid leukemia (mainly acute) is the predominant type found among workers exposed to benzene (Vigliani, 1976; Infante et al, 1977) and among the early radiologists (Warren and Lombard, 1966). Most nasal cancers seen among furniture workers are adenocarcinomas, whereas other cell types are prevalent in the shoe industry (Acheson, 1976).

The following lung carcinogens appear to be associated with an unusual frequency of a particular cell type:

Agent	Cell Type	Reference
Arsenic	Poorly differentiated epidermoid carcinomas	Newman et al, 1976
Bis (chloromethyl) ether	Small cell undifferentiated carcinomas	Figueroa et al, 1973
Mustard gas	Undifferentiated and squamous cell carcinomas	Wada et al, 1968
Radiation	Small cell undifferentiated carcinomas	Saccomanno et al, 1971

Particularly striking in these data is the predominance of small cell, undifferentiated carcinomas attributable to bis(chloromethyl) ether and radiation.

Virtually all of the occupational skin cancers are epitheliomas (carcinomas), with the possible exception of melanoma following exposure to polychlorinated biphenyls (Bahn et al, 1976). Mesotheliomas of the pleura and peritoneum have been consistently observed in populations exposed to asbestos (Wagner et al, 1960; Selikoff et al, 1965; Newhouse and Thompson, 1965). Moreover, all three major commercial varieties of asbestos (amosite, chrysotile, and crocidolite) have been linked to these tumors (Selikoff et al, 1972; Greenberg and Lloyd Davies, 1974).

EXPERIMENTAL CARCINOGENESIS AND EPIDEMIOLOGY

Many industrial materials found to be carcinogenic in laboratory animals have not been confirmed in humans (Tomatis et al, 1978). Table 5 shows a group of such chemicals. Part of the reason for a deficiency in epidemiologic data is related to problems of finding appropriate study groups with adequate information to characterize their exposure. Also, there may be a lack of personal data needed to trace workers over extended time periods to observe long-term health effects. Furthermore, human exposure to a chemical of interest may be confounded by simultaneous exposure to other

TABLE 5. Substances Showing Carcinogenic Activity in Laboratory Animals*

Substance	Industrial Uses
Carbon Tetrachloride	Solvent, degreasing agent, fire extinguishing agent
Chloroform	Solvent, fumigant, insecticide ingredient
Hydrazine and Derivatives	Oxygen scavenger in boiler water systems, drug manufacture (isoniazid), solder fluxes
Perchloroethylene	Dry cleaning, vapor degreasing
Polychlorinated Biphenyls	Dielectrics in capacitors and transformers, heat exchange fluids, hydraulic fluids
Trichloroethylene	Metal degreasing; extraction solvent for oils, fats, waxes; dry cleaning
Urethane	Chemical intermediate for manufacture of pharmaceuticals, pesticides, fungicides, textile finishes

*No definitive human evidence is available, however.

toxic agents. In other instances, an inordinate amount of effort is required to obtain sufficient numbers of exposed workers, or it may be that no information exists to define who has been exposed.

Table 6 outlines important differences between experimental and epidemiologic studies. Three general features of each approach are highlighted: selection of exposed and control groups, exposure to the hazardous material, and determination of health effects. In human studies, the first and third phases are implemented in a less than ideal fashion scientifically, whereas the exposure period is exactly the reality we wish to evaluate. On the other hand, the first and last steps are carried out to the ultimate degree of scientific precision in the laboratory, whereas the exposure phase is a contrived but, hopefully, useful approximation to the human counterpart.

Thus, it seems that for some industrial materials epidemiologic studies will be invaluable for risk assessment, while for others laboratory testing will be the most expeditious means of evaluating carcinogenicity because epidemiologic studies would be impossible or very difficult to carry out.

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TABLE 6. Experimental and Epidemiologic Approaches to the Study of Occupational Cancer

Feature	Animal Testing	Human Studies
Selection of exposure and control groups	Random allocation of homogeneous populations of animals to "exposure" or "no exposure" conditions	Usually, a nonrandom selection of a heterogeneous group of "exposed" individuals and, if you are lucky, an "unexposed" group approximating a true control group might be available
Exposure period	Administration of well-defined levels of single agents through unique routes of entry. Careful control of other factors (e.g., diet)	Individual exposure levels variable and usually unknown. Multiple exposures may complicate the situation. Other factors may be operating (e.g., smoking).
Determination of health effects	Detailed pathologic examination of all body systems	In studies of fatal disease (e.g., cancer) the only health effect information may be death certification diagnosis.

DATA NEEDS

Exposure Data

In planning an epidemiologic study of occupational exposure to a suspected carcinogenic material, many questions should be raised con-

cerning the nature and degree of exposure in the workplace. Figure 1 outlines some of these considerations. Not all of the questions may have answers, and the prospects for initiating the study may depend on whether or not the key ones can be addressed. These include number of exposed workers, level of exposure, and

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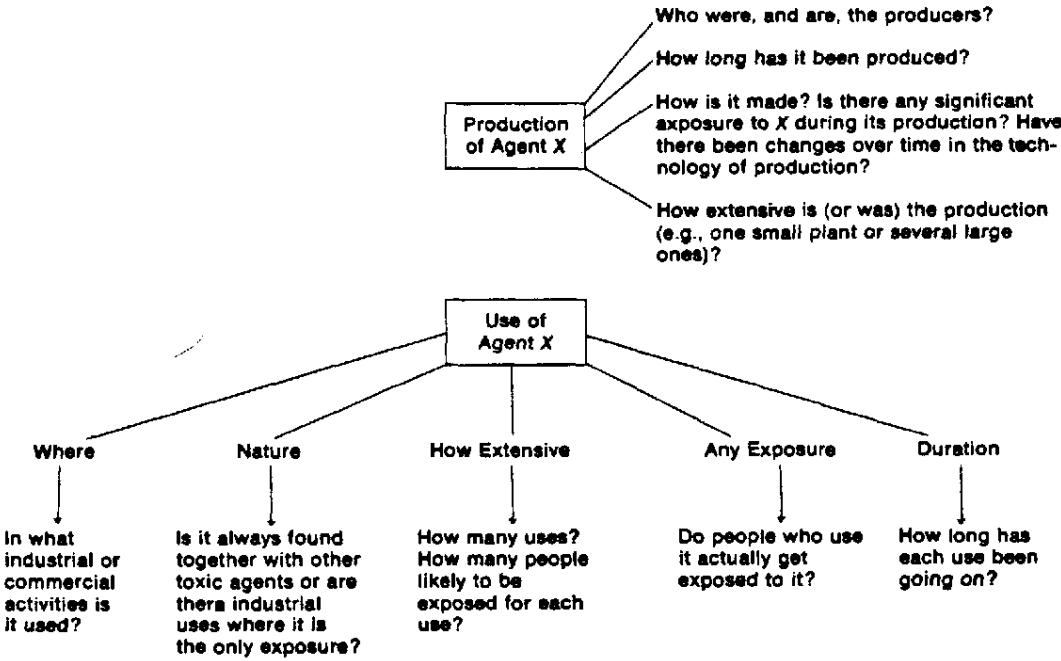


Figure 1. Considerations in planning an epidemiologic study of exposure to agent X.

duration of use. After going through this process, one must make a decision as to whether an exposed working population really does (or did) exist. This involves a judgment as to what constitutes an exposure that is likely to produce a detectable effect, if indeed the material is carcinogenic for man. Input from several disciplines, including industrial hygiene and toxicology, is valuable at this point.

Worker Data

The conduct of epidemiologic studies of cancer in industry requires data that specify who worked where and when, and to what were they exposed. Thus, records must exist that define the population at risk in terms of demographic and occupational factors. Industry is in the best position to provide this information from data contained in administrative files such as personnel and employment records. These records have been used to define groups of workers employed in the past in suspect industries (historical cohort studies). Personal data are needed to trace terminated employees so their subsequent disease experience can be analyzed through ascertainment of morbidity or mortality. Employment information, such as duration of employment in specific jobs and departments, is utilized to characterize an employee's potential exposure to various hazardous agents. In addition, industry often has access to death information (sometimes even death certificates) for individuals who die while still on the employment rolls or as pensioners. These data can be analyzed in their own right using the proportionate mortality approach for preliminary indication of an unusual disease pattern.

Labor unions are another potential source of data that should not be overlooked. In particular, trade unions may represent the only central source of information on their membership, because skilled tradesmen and construction workers tend to be scattered throughout many industries. Several trade unions operate death benefit plans for their members, which provide a mechanism for compiling cause of death information on large numbers of workers in a particular trade. Studies of several such groups have been reported based on these data systems (Decoufle et al, 1977; Lloyd et al, 1977; Egan et al, 1979). Furthermore, membership mailing lists have proved useful in establishing contemporary cohorts for forward studies (Dunn and

Weir, 1968). Other unions maintain valuable information on their members in the form of dues records. These files depict weekly or monthly payments of dues so that approximate dates and duration of employment may be estimated. In addition, they may provide sufficient identifying information to trace persons for current vital status. The existence and availability of these records could be a major determining factor in undertaking a study of an industry whose work force is distributed among thousands of small establishments.

Identification of High-Risk Groups

A number of data sources exist that provide valuable information on relationships between cancer at specific sites and occupation on a broad-scale basis.

Vital Statistics Data

Henry and the Kennaways examined the occupational distribution of persons dying from selected cancers based on death certificate entries for England and Wales during the 1920s and 1930s (Henry et al, 1931; Kennaway and Kennaway, 1936, 1947). Among lung cancer deaths in those two decades, they identified several high-risk occupations exposed to coal tar products, which were subsequently confirmed by formal epidemiologic studies. Analysis of bladder cancer deaths also showed an association with coal tar occupations. Leather tanning and dyeing were two other trades linked to high rates of bladder cancer in these data, and both processes have come under recent scrutiny.

Since those early studies, similar data have been available for analysis every ten years in England and Wales (Registrar General, 1958, 1971, 1978) and represent a valuable resource for screening purposes, keeping in mind that interpretation must be done with caution. In other countries, investigators have used death certificate occupational data in the same way (Versluys, 1949; Morrison, 1957), but only once has it been done on a national scale in the United States (Guralnick, 1963). However, a comprehensive analysis of occupation and cause of death for the State of Washington has been completed (Milham, 1976). Furthermore, surveys limited to a specific cancer site in an area where the mortality rate is unusually high are useful (Gottlieb et al, 1979).

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Cancer Surveys

As part of the Third National Cancer Survey in the United States, occupational histories were obtained on a random sample of incident cases. These data were analyzed in such a way as to draw attention to strong associations between specific cancer sites and broad occupational groups (Williams et al, 1977). Thus, high-risk occupations could be identified for further, in-depth study. Unfortunately, the amount of available data limited the specificity of occupational information that could be examined.

Cancer Registries

Unfortunately, cancer registries in the United States have not systematically gathered occupational information on their cases. Hopefully, in the future, steps will be taken to collect and analyze job histories in at least certain types of cancer registry systems.

Hospital-based Data

Special surveys of bladder cancer in various localities have produced clues to occupational relationships (Wynder et al, 1963; Dunham et al, 1968; Anthony and Thomas, 1970; Cole et al, 1972). In addition, studies of lung cancer cases and prior work history have provided leads for further research (Wynder and Graham, 1951; Breslow et al, 1954; Menck and Henderson, 1976; Blot et al, 1978).

Roswell Park Memorial Institute, a major cancer treatment and research center located in Buffalo, New York, gathered lifetime occupational histories on all incoming patients before a diagnosis was made. These data were the subject of a comprehensive analysis (Houten et al, 1977a) and several specialized reports (Viadana et al, 1976; Houten et al, 1977b; Bross et al, 1978; Decoufle, 1979). Some previously unsuspected relationships were identified (e.g., dairy farmers with bladder cancer; hairdressers with cancers of the ovary and corpus uteri and lymphomas) and remain to be confirmed. Other findings were consistent with previous reports (e.g., leather workers with bladder cancer), demonstrating the value of such a data source.

CONCLUSION

Occupational hazards have implications beyond the work force because many chemical and physical agents are not confined to a plant

but may spread into the general environment. In this way, large populations may be at unusual risk of cancer from such agents as arsenic, asbestos, polycyclic hydrocarbons, radioactive materials, and vinyl chloride. Although the extent of the hazard resulting from environmental pollution or contaminated consumer products is not clear, there is no question that occupational groups are invaluable as sentinels for identifying and evaluating risks to the general population.

The importance of epidemiologic studies within occupational groups can be appreciated in the case of asbestos, in which regulatory agencies have been forced to evaluate the potential public health impact of the ever increasing instances of general population exposure to this material. A long series of epidemiologic studies, dating back to 1955, have documented the carcinogenic effects of exposure to asbestos in the workplace. These data provide the basis for assessing the potential impact of general environmental asbestos exposures in the form of (1) crushed stone used on roads and public recreation areas, (2) insulation sprayed on structural supports for new buildings, (3) decaying insulation materials in already existing buildings, and (4) asbestos-contaminated water, drugs and beverages.

It would be difficult to document, directly, the carcinogenic effects of asbestos attributable to these intermittent or low-level exposures. Instead, inferences are often drawn from occupational studies in choosing a prudent course of action.

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CANCER EPIDEMIOLOGY and PREVENTION

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*Chapter 2. Causation and Causal Inference
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CHAPTER TWO

Causation and Causal Inference

Kenneth J. Rothman

CAUSATION

The Evolution of the Concept of Causation

The understanding of causation has developed in parallel with the growth of science. Aristotle, whose scientific views dominated Western thought for centuries, described the following four types of cause for each object: (1) *material cause*, the matter of the object, (2) *formal cause*, the pattern imposed on the matter, (3) *efficient cause*, the force producing the object, and (4) *final cause*, the purpose of the object. Aristotle's description of causes did little to enhance understanding, at least when judged by today's standards. Coupled with the weight of his authority, Aristotle's views probably repressed the further development of philosophic inquiry about causation for many centuries.

During these centuries, the prevailing doctrine of scientific philosophy was *rationalism*, which regarded reason as the source of all knowledge of the physical world. Rationalism flourished in an era when religion overshadowed science, but as modern science took root in the seventeenth century, *empiricism* replaced rationalism as the accepted philosophy of science. Empiricism substituted observation for reason, or rather combined observation with reason in a new methodology, inductive

logic. For the empiricists, causation was an inferred relation between two events that were repeatedly observed to occur in the same time sequence. To infer causation required observation, prediction, and additional observation to verify the prediction. This profound change in scientific thought characterized the rise of modern science.

Empiricism was not without its critics. Foremost among them was David Hume, who argued that the mere temporal conjunction of two events cannot impart to an observer any knowledge about a causal connection between them. Hume revealed the process of inductive inference to be a faulty process: without direct evidence for a causal connection, which was impossible, induction could lead to incorrect inferences. Inductive logic, therefore, was not really a "logic" at all. With this simple but profound skepticism, Hume shook the new foundation of empiric science.

Ever since Hume, defenders of empiricism have attempted to answer his criticism. The first defender was Kant, whose answer to Hume was imbedded in his concept of the *synthetic a priori*. He distinguished between two types of judgments: *analytic*, which were logical truisms unrelated to empiric judgments, and *synthetic*, which were judgments that extended knowledge. Kant believed that some synthetic judg-

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ments are made without reference to experience. For him, the explanation of the existence of these *synthetic a priori* judgments was the central issue in metaphysics. Causation was one of Kant's *synthetic a priori* judgments: that events had causes was known on the basis of *synthetic a priori* judgment, and induction was merely the process of identifying the actual cause.

Though Hume died before Kant's arguments were published, Reichenbach (1951) supplies us with the hypothetical response that Hume might have given to Kant:

How does it help us to know there is a cause if we wish to know what the cause is? It is true, if we knew there were no cause it would be nonsensical to search for one; but that is not our situation. We do not know whether there is a cause; in this situation we make inductive inferences, based on observation, and conclude, say, that the moon is the cause of the tides. This inductive inference is what I question; and it would remain just as questionable if you could prove the general statement that there is a cause.

Reichenbach himself proposed a different solution to "Hume's problem" (as Kant described it), a solution he contended could not have been proposed before the development of probability theory and quantum mechanics. He argued that Heisenberg's principle of uncertainty, by which individual atomic phenomena are explainable only in probabilistic terms, serves as a proof that a concept of strict causality is not tenable. According to his view, natural laws are, in effect, only idealized statistical laws, and causality is not an ultimate principle, but only a substitute for statistical regularity. He proposed that

... the idea of a strict causality is to be abandoned, and that the laws of probability take over the place once occupied by the law of causality.

Reichenbach concluded that scientific philosophy has led us to the truth that empiric knowledge can never be certain. Hume's objection to the use of inductive logic was the inconsistency of a "logic" that is uncertain. For Hume, such a methodology was invalid; for Reichenbach, it is the best we can achieve, which validates it. Carnap (1962) took Reichenbach's argument further by attempting to formalize inductive logic with a set of probability laws quantifying the degree of confirmation of a hypothesis.

A different answer to "Hume's problem" was proposed by Popper (1968), who rejected the

view that the validity of scientific statements can be quantified by probabilities. He thought such a compromise position about the verifiability of a causal proposition to be only a sidestepping of Hume's criticism of inductive logic. Popper found that the inadmissibility of inductive logic leads to a contradiction with the basic premise of empiric science only if it is assumed that all empiric scientific statements are conclusively verifiable. Popper maintained that scientific statements are conclusively decidable only in the negative — they can be falsified — but no scientific statement can be conclusively verified. Falsification, unlike verification, requires no inductive inference, only deductive logic. Popper's view coincides with the historical view of science; widely accepted theories have been subsequently replaced, after falsification, with new theories. No conclusive verification has ever occurred.

Although Popper resolved Hume's problem by arguing that scientific progress does not involve inductive logic, whereas Reichenbach and Carnap attempted to modify and formalize that logic, all would agree that scientific knowledge is uncertain. For Reichenbach and Carnap the nature of the uncertainty was that of probability; for Popper, it was that of tentativeness. Popper emphasized that the utility or content of a scientific statement rests on the degree of falsifiability of the statement: statements such as "God is one" or "Levitation is possible for those who try hard enough" have no scientific content, because neither is falsifiable. Popper's emphasis on the falsifiability of a theory has implications for current thought in carcinogenesis, as we shall see: the multi-stage theory, which is much discussed, is so general a statement that it cannot be falsified; it therefore has little scientific content. Specific variants of the theory, however, such as Knudson's (1971) two-mutation model, are more easily falsifiable and therefore of greater scientific interest.

Epidemiologic Concepts of Causation

Philosophers of science have improved our understanding of the process of causal inference, but there remains the need, at least in epidemiology, to formulate a general and coherent model of causation to facilitate the conceptualization of epidemiologic problems. A first step is to distinguish conceptually between sufficient causes and components of sufficient causes (Rothman, 1976). A sufficient cause is one that by definition inevitably produces the

effect. Each occurrence of the effect is presumed to result from a sufficient cause. Precisely because a sufficient cause implies inevitability of the effect, sufficient causes are difficult to identify in their entirety. Components of sufficient causes, however, imply by their presence only an increased probability of the effect; the actual outcome depends on the occurrence of the complementary component causes that are needed to complete a sufficient cause. The causes that epidemiologists study as causal risk factors are component causes. Though such causes are not "complete," blocking the action of a component cause will render an otherwise sufficient cause insufficient, and thereby prevent the effect, at least through one mechanism. It is still possible that the effect will occur anyway through another mechanism, i.e., a different sufficient cause, which does not include as a component cause the factor that has been blocked.

Consider lung cancer as an example. Smoking is a causal risk factor, but it is not a sufficient cause, because most smokers (more than 90 per cent) never get lung cancer. Most cases of lung cancer, however, are attributable to a causal mechanism that involves smoking as a component cause. By preventing smoking, or by making a nontoxic cigarette, most cases of lung cancer could be prevented. A few cases of lung cancer would still occur, though, because there are other sufficient causes of lung cancer that do not involve smoking, as evinced by the occurrence of lung cancer among nonsmokers.

Some component causes of a sufficient cause may be passive components, those that contribute appropriate conditions for the other component causes to act. Genetic characteristics that are risk factors fall into this category of component causes. Nearly every complete cause has components that are genetic, and these can be thought of as susceptibility factors. Nongenetic component causes also may contribute to the effect in a similar way by providing the right conditions for other component causes to act. Ingested nitrite, for example, may cause cancer if it is chemically converted to nitrosamine in the stomach. The reaction is dependent on pH, with the highest yield occurring at a pH of 3.4, and lesser yields at a lower or higher pH (Mirvish, 1970). Very little conversion occurs below a pH of 1.5 or above a pH of 5.0. The pH of stomach contents depends on the type and quantity of food consumed; it is usually much lower than 3.4 when the stomach is empty, but may be near 3.4 after a large meal. Sufficient alkalinity of the gastric contents may be thought

of as a passive component cause of cancer, permitting dietary nitrite to be converted to an active carcinogen, which may then induce cancer.

Generally speaking, only active component causes of cancer are considered carcinogens. Passive component causes create the conditions in which active causes produce the transitions between stages; their passive role corresponds to that of a susceptibility factor, but not to what is usually meant by a carcinogen. This distinction between carcinogenic agents and other component causes of cancer is obviously not conceptually rigorous, but rather only a distinction that derives from common usage.

CARCINOGENESIS

The Multi-stage Theory of Carcinogenesis

While some component causes thus establish a set of susceptible conditions, others actively play a role in converting the system from one stage to the next along a causal sequence. The precise number of stages in a causal path cannot be determined meaningfully, because the causal sequence can always be described in finer detail, and the designation of stages is ultimately an arbitrary classification. Even a single abrupt transition event could be dissected into many graduations. Often, however, the transitions in a causal path appear to be reasonably categorized into one or more distinct events. Carcinogenesis has been demonstrated to have at least two and possibly many such transitions; carcinogenic agents are considered *initiators* if they act on an early stage in the sequence, and *promoters* if they act on a later stage. (Some scientists restrict the use of the term carcinogen to include only initiators and not promoters, but few epidemiologists adhere to this restriction.)

A formalization of the implications of sequentially acting component causes as applied to carcinogenesis has been discussed in terms of the *multi-stage theory of carcinogenesis*. Under this theory, the development of a cancer is the *end stage of a sequence* of stochastic transitional cellular events, for which the transition probabilities are usually assumed to be constant over time. While theoreticians have not reached any consensus as to which form or forms of such a theory might actually apply in carcinogenesis, the consequences of various forms of the general theory have been examined in detail and compared with epidemiologic evidence. (For a

review, see Peto, 1977.) Such comparisons of theory and observations are difficult to interpret, because time trends in incidence, inaccuracies in reporting, and multiple etiologies, among other concerns, all work to distort the observations predicted by the theory. The mathematical assumptions and approximations for some forms of the theory have also been misunderstood (Moolgavkar, 1978).

Despite these reservations, many scientists have been attracted to the multi-stage theory because of its generality and the close correspondence between age-incidence curves predicted from the theory and those actually observed (Cook et al, 1969). The utility of the theory is questionable, however, in view of the difficulty with which the theory might be falsified. Indeed, the multi-stage theory in a general form could be used, as I have already indicated, as a general model of causation to apply to all etiologic situations. What makes the theory interesting and gives it scientific content are the restrictions that are incorporated into it for any given situation. For example, Moolgavkar and Venzon (1979) have recently shown that a two-stage model can generate age-incidence curves similar in shape to those generated from more complicated multi-stage models, thus extending the applicability of Knudson's (1971) two-stage model for embryonal tumors to include adult tumors. They argue that biologic interpretation (i.e., initiation and promotion) can be given to no more than two distinct stages, making their two-stage model more plausible than multi-stage alternatives.

Induction Period and Latent Period

Some scientists use the terms *induction period* and *latent period* interchangeably (*incubation period* is also used), but the time interval between exposure to an etiologic agent and appearance of disease symptoms can be meaningfully partitioned in a way that suggests a distinction between these terms. *The Oxford English Dictionary* (1971) defines *latent* as "hidden," whereas *induction* is defined as the "act of causing or producing." Conceptually, then, the *induction period* is the time interval during which a sufficient cause is completed. In reference to a particular component cause, the induction period would begin at the time of action of the component cause and end when all the other component causes that constitute a sufficient cause have completed their action to initiate the disease. At the time of disease

initiation, when the induction period ends, the *latent period* begins. In principle, the latent period could be reduced to near zero with more accurate means to detect presymptomatic disease; the length of the latent period is simply the time it takes for a disease, once initiated (by a sufficient cause), to be detected.

The latent period is conceptually part of the causal process only to the extent that during the latent period disease may "spontaneously" disappear or fail to progress, in which case clinical disease would not occur. Logically, however, such alterations in the progression of disease should be considered part of the causal process of *clinical* disease, thus implying that where the natural history of disease is not that of inevitable progression, the latent period is subsumed within the induction period, which accordingly would end not at the initiation of preclinical disease, but at the start of symptoms. For diseases that, once initiated, progress inevitably to become clinically apparent, however, the latent period would follow the induction period. It is impossible, of course, to determine when the induction period ends and the latent period begins for any individual case, though this can be estimated for large groups of people. The conceptual distinction between induction period and latent period is worth maintaining, because these two time periods define the intervals during which primary prevention (i.e., blocking a sufficient cause) and secondary prevention (i.e., preclinical detection and treatment) would be applicable.

For exposures that are continuous or intermittent, there remains the difficulty in determining when the exposure acts etiologically. Customarily, epidemiologists measure induction periods from the time of first exposure, because the relevant time of action is unknown, whereas the time of first exposure can usually be determined. A better practice, however, is to choose an arbitrary accumulation of exposure, and determine the time at which that level is reached. It is possible to vary the estimates for the sufficient dose of exposure and learn from the epidemiologic analysis which estimate is most appropriate. This procedure can also incorporate variations in exposure dose with time.

It is known that some carcinogens have lengthy induction periods for certain cancers; an example is the occurrence of adenocarcinoma of the vagina 15 to 30 years after exposure of female fetuses to diethylstilbestrol. It appears probable, on the basis of the age distribution of cases, that a sufficient cause is completed dur-

ing puberty or adolescence for women who develop this disease; therefore the time before puberty or adolescence would be the induction period, and the interval following that period until symptoms appear would be the latent period.

Frequently some types of cancer are described as diseases of lengthy induction period, the implication being that the action of any carcinogen causing that cancer precedes the development of the tumor by a long interval. The disease itself, however, should not be classified as having a lengthy induction period; the induction period refers to the interval between the action of a specific component cause and the disease initiation. Because a component cause might act at any "stage" during carcinogenesis, the length of the induction period for a specific component or carcinogen would depend on the point during the causal sequence at which the carcinogen acted. Carcinogens acting during a late stage (promoters) would have a short induction period, even if the complete causal chain involves a long sequence of stages requiring many years or decades to complete. From animal experiments, it appears that estrogens act as late-stage carcinogens; this finding has been confirmed from epidemiologic research on replacement estrogen therapy and endometrial cancer, which indicates that estrogens have relatively brief (3 to 10 year) induction and latent periods before the occurrence of endometrial cancer (Jick et al, 1979). In addition, it has been found that the risk for endometrial cancer increases with the length of time estrogens have been used, but drops quickly if estrogen therapy is discontinued, a pattern that also agrees with the results from animal studies of promoters.

The Implications of Epidemiologic Observations

One prediction from certain multi-stage models is that each of two carcinogens acting at different stages in the causal process will have effects that are, under some conditions, proportional to the effect of the other; i.e., the effects are "multiplicative." Asbestos and smoking seem to interact in exactly this way in causing lung cancer (Saracci, 1977); the implication, given the appropriateness of the multi-stage model, is that asbestos and smoking act at different stages. Smoking and alcohol also show an interaction that may be multiplicative in causing cancer of the mouth and pharynx (Rothman and Keller, 1972).

Some epidemiologic evidence is suggestive of a single cause affecting more than one stage of the carcinogenic process. One example, described by Peto (1977), is smoking and lung cancer. Ex-smokers maintain a constant incidence of lung cancer from the time of cessation, whereas continuing smokers show a steadily increasing incidence. According to Peto, this pattern suggests that smoking affects the penultimate stage of carcinogenesis. Other data, however, suggest that new smokers do not exhibit a change in incidence for many years, indicating an effect on an early stage. Of course, other interpretations might be given to these findings, but the implication that smoking affects both an early and a late stage in the carcinogenic process for lung cancer is both interesting and plausible.

Land and Norman (1978), analyzing the distribution of induction periods for various cancers after exposure to ionizing radiation, concluded that, if a two-stage model is assumed, radiation affects only the first stage for lung and breast cancer (resulting in a typically long induction period), but the second stage for chronic granulocytic leukemia (resulting in a short induction period).

CAUSAL INFERENCE

The Process of Scientific Inference

Having examined the theoretical basis for scientific inference and a conceptual model of causation that can be modified as needed to describe carcinogenesis, it is appropriate to consider in more specific and practical terms how scientific inference proceeds. The process of generalizing beyond a set of observations requires a judgment about what features of the observations may be extrapolated to a target zone (in epidemiologic studies, to a target population). Such judgments require an understanding of which conditions are relevant and which are irrelevant to the generalization. For example, from a study of smoking and lung cancer in men, one might generalize the results to a target population of women. To do so presumes that being male is irrelevant to the carcinogenic action that smoking has on lung tissue, a judgment based on knowledge about the likely mechanism of carcinogenesis and the biologic similarity between male and female lungs. On the other hand, a study of diet and myocardial infarction in men might not be considered generalizable to women, because

physiologic differences between the sexes may play a role in the causal process. The validity of a generalization is ultimately a matter of informed judgment.

Some epidemiologists have taught that generalization from a study group depends on the study group being a representative subgroup of the target population, in the sense of a sample (the confusion on this point is deeply rooted, as indicated by the use of the term sample-size to refer to the number of subjects in a study). Were this the case, generalization would be limited literally to those individuals who might have been included as study subjects. This misconception has influenced the design of many epidemiologic studies. If the notion were valid, there would be no application to humans of any results obtained from animal research. In addition, every population would require its own set of epidemiologic studies, and these studies would have to be repeated for every new generation!

The tendency to use "representative" study groups probably derives from surveys for which the inferential process is purely statistical (descriptive) and not scientific (synthetic). Social scientists often rely on statistical inference because the decision as to what is relevant and irrelevant for generalization is more difficult in the social sciences, and populations are considerably more diverse in sociologic phenomena than in biologic phenomena. In the biologic sciences, however, investigators conduct experiments using animals with characteristics selected to enhance the validity of the experimental work, rather than to represent the target population. Epidemiologic study designs are usually stronger if the subject selection is guided by the need for making a valid comparison, which may call for severe restriction of admissible subjects to a narrow range of characteristics, rather than a futile attempt to make the subjects representative, in a sampling sense, of the potential target populations.

The preceding discussion implies that to achieve the goal of valid generalization, a preliminary requirement is to reach a valid interpretation of the observations at hand. In the domain of nonexperimental research, the central issue in attaining internal validity in a study is the question of confounding bias. Simply expressed, the problem is to separate the effect under study from the effects of extraneous factors associated with the study factor. In experiments, confounding can be prevented by allocating the treatment (using methods such as randomization and blocking) so that there is no

association between the treatment and the extraneous factors. In nonexperimental research, the epidemiologist must rely on judicious subject selection and data analysis to prevent or remove confounding.

Practical Causal Inference

Though science does not admit conclusive inferences that one event causes another, in epidemiology the exigencies of public health problems require action despite imperfect knowledge. Consequently many people have attempted to codify the basis for making practical decisions about cause and effect. Among those who have examined the aspects of an observed association that lead us to believe it is causal is Sir Austin Bradford Hill (1965). He suggested the following criteria: (1) Strength, (2) Consistency, (3) Specificity, (4) Temporality, (5) Biologic gradient, (6) Plausibility, (7) Coherence, (8) Experimental evidence, and (9) Analogy.

Strength. By strength of association, Hill refers to the magnitude of the ratio of incidences. His argument is essentially that strong associations are likely to be causal, because if they were due to confounding or some other bias, the biasing association would have to be even stronger, and would therefore presumably be more evident. Weak associations are more likely to be explained by biases, but the fact that an association is weak does not rule out a causal connection. It has been pointed out that the strength of an association is not a biologically consistent feature, but rather a characteristic that depends on the relative prevalence of other causes (Rothman, 1976).

Consistency. Consistency refers to the repeated observation of an association in different populations under different circumstances. Lack of consistency, however, does not rule out a causal association, because some effects are produced by their causes only under unusual circumstances.

Specificity. The criterion of specificity requires that a cause should lead to a single, but not multiple, effects. This argument has often been advanced, especially by those seeking to exonerate smoking as a cause of lung cancer. Causes of a given effect, however, cannot be expected to be without other effects on any logical grounds (Sartwell, 1960). In fact, everyday experience teaches us repeatedly that single events may have many effects.

Temporality. Temporality refers to the necessity that the cause precede the effect in time.

This is an inviolable condition, which stems from the very concept of a cause.

Biologic Gradient. Biologic gradient refers to the presence of a dose-response curve. If the response is taken as an epidemiologic measure of effect, measured as a function of comparative disease incidence, then this condition will ordinarily be met. Some causal associations, however, show no apparent trend of effect with dose; an example is the association between diethylstilbestrol and adenocarcinoma of the vagina. A possible explanation is that the doses of diethylstilbestrol administered were all sufficiently great to produce the maximum effect, with actual development of disease depending on other component causes. Associations that do show a dose-response trend are not necessarily causal; confounding can result in such a trend between a noncausal risk factor and disease.

Plausibility. Plausibility refers to biologic plausibility, an important criterion, but one that may be difficult to judge. Sartwell (1960), emphasizing this point, cited the remarks of Cheever in 1861 (this statement was also noted by Hill):

It could be no more ridiculous for the stranger who passed the night in the steerage of an emigrant ship to ascribe the typhus, which he there contracted, to the vermin with which bodies of the sick might be infested. An adequate cause, one reasonable in itself, must correct the coincidences of simple experience.

Coherence. Taken from the Surgeon General's report on smoking and health (USDHEW, 1964), the term coherence implies that a cause and effect interpretation for an association does not conflict with what is known of the natural history and biology of the disease. The examples Hill gives for coherence, such as the histopathologic effect of smoking on bronchial epithelium (in reference to the association between smoking and lung cancer) or the difference in lung cancer incidence by sex, could reasonably be considered as examples of plausibility as well as coherence; the distinction appears to be fine. Hill emphasizes that the absence of coherent information, as distinguished, apparently, from the presence of conflicting information, should not be taken as evidence against an association being considered as causal.

Experimental Evidence. Such evidence is often telling but seldom available for human populations.

Analogy. The criterion of analogy seems to be handicapped by the inventive imagination of

scientists who can find analogies everywhere. Nevertheless, the simple analogies that Hill offers — if one drug can cause birth defects, perhaps another one can also — could conceivably enhance the credibility that an association is causal.

As is evident, these nine criteria offered by Hill to judge whether an association is causal are saddled with reservations and exceptions; some may be wrong (*specificity*) or occasionally irrelevant (*experimental evidence* and perhaps *analogy*). Hill admits that

None of my nine viewpoints can bring indisputable evidence for or against the cause-and-effect hypothesis and none can be required as a *sine qua non*.

In describing the inadequacy of these criteria, Hill goes too far. The fourth criterion, *temporality* of an association, is a *sine qua non*: if the "cause" does not precede the effect, that indeed is indisputable evidence that the association is not causal. Other than this one condition, which is part of the concept of causation, there are no reliable criteria for determining whether an association is causal. Nevertheless, Hill's list is a helpful guide to practical causal inference, if the criterion of *specificity*, which is clearly wrong, is deleted. No rule exists, however, to weigh the extent to which the evidence conforms to these criteria, nor is there any formal method by which one criterion can be weighed relative to the others. Ultimately, causal inference is an informal, rather than a rigorous, judgment.

The failure of some to recognize the theoretical impossibility of "proving" the causal nature of an association has led to fruitless debates pitting skeptics who await such proof against scientists who are persuaded to make a practical inference on the basis of existing evidence. The responsibility that scientists have for making causal judgments was Hill's (1965) final emphasis in his discussion of causation:

All scientific work is incomplete — whether it be observational or experimental. All scientific work is liable to be upset or modified by advancing knowledge. That does not confer upon us a freedom to ignore the knowledge we already have, or to postpone the action that it appears to demand at a given time.

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Introduction

CANCER AND THE ENVIRONMENT

An Academic Review of the Environmental Determinants of Cancer Relevant to Prevention

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*This Symposium was held in cooperation with the
AMERICAN CANCER SOCIETY, INC.*

Symposium Committee:

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**INTRODUCTION: DETERMINANTS OF CANCER RELEVANT TO
PREVENTION, IN THE WAR ON CANCER**

Joseph A. Cimino

President, New York Medical College

Harry B. Demopoulos

New York University Medical Center

We are gathered here today because we don't know all the questions, let alone the answers when it comes to environmental determinants of cancer relevant to prevention. By the end of this three-day international symposium, I hope we will have outlined one possible course and direction in this war on cancer.

1. We should be able to identify the cancer culprits that are amenable to successful intervention.
2. We must pay more attention to the factor of multiplicity and synergistic causes.
3. We must be more concerned with nutrition's role in cancer prevention.
4. We must once and for all put into their proper place the role that urban environments and occupational exposures play in the total cancer picture.
5. We must devote more energies and monies to research on life styles and know how that offers us one of our greatest prevention opportunities.
6. We must correlate intervention with the most effective cost-benefit relationships and know how to maximize the number of lives that can be saved.

Given a few minutes, most of you could easily describe numerous major cancer causing agents. As a group, I am sure that we could come up with the major culprits. I suggest that the day is here for us to say we've wasted too much time and money going after too many suspected culprits and our war on cancer is beginning to look a lot like another Viet Nam. Too much money. Too much manpower. Too much government intervention. Too much shooting in the dark. Too much waste. Too much time being devoted to snipers.

The observation from where I sit is that we have wasted a decade by concentrating on viruses and hoping to develop a miracle cure with a miracle vaccine, while letting the big cancer killers get through our front lines. Granted, significant victories have been achieved in the medical arena: improved surgical techniques, better utilization of radiation, chemotherapy, the develop-

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ment of immunotherapy, and most important an aggressive and somewhat successful promotion by the American Cancer Society of the benefits of early detection.

To be objective, with Proposition 13, national health care debates and soaring medical costs, we know that cancer research money will no longer flow as freely as before. Like Viet Nam it has been a long war and we have yet to come close to achieving victory, but we can't pull out of this one. The military objective of this conference should be, and must be, to marshal our forces and attack the major culprits, give them a priority rating and go after the big ones.

In fighting the war, let us recognize the fact that it is counterproductive to use your major resources hunting down snipers. Let us spend our cancer war dollars where they will produce some significant casualties among the cancer culprits.

A personal experience that vividly illustrates some of the problems we all face in waging this war on cancer concerns the control of air pollution in New York City. It is pertinent because it focuses on the people and forces that seem to get involved: environmentalists; city, state and federal regulatory agencies; private industry; physicians; scientists; the general public and the news media.

During my tenure as Commissioner of Health of New York City, the big debate was raging over air pollution as a major cause of disease in urban America and a possible contributing factor to lung cancer. It escalated to a point where many Americans were convinced that by eliminating this "sniper," a major health hazard would disappear.

The marker chosen for control of air pollution was sulfur dioxide and although no major voice was blaming sulfur dioxide as a cancer causing agent directly, it was blamed for about everything else. At one point the citizens of New York probably believed that the City would be perfect if only SO_2 could be reduced. Hundreds of millions of dollars have been spent in New York City to burn low sulfur fuel. And I suggest to you that to this day I have yet to be convinced by any evidence that sulfur dioxide at levels reached in our urban setting is a major health hazard. Granted, on a clear day you can see the Twin Towers of the new World Trade Center but we went after SO_2 for the wrong reason. In my view the amount of money spent on this one battle may have contributed to New York City's financial plight and represents a colossal cost-benefit error.

Because of the mandatory use of low sulfur fuel, Con Edison had to convert, and the bill was paid by homeowners, renters, industry and taxpayers.

Con Ed wasn't the only victim. City facilities, of course, had to be converted as well as apartment house incinerators, which naturally raised rents.

Whether it be the campaign for cleaner air or the fight to ban saccharin, we must stop wasting money going after suspected snipers and concentrate on the major determinants of cancer. The regulation of sulfur dioxide was one of the most expensive health bullets ever fired.

The scenario on sulfur dioxide is typical of many of the same controversies over cancer causing agents and the disruptive debates now going on.

First, a regulatory agency or environmental group raises a public issue about a suspected health hazard. This is followed by some quickly gathered

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scientific studies — and I use that terminology loosely — which are quoted as Gospel. Next, an activist group clamors for immediate change. The news media highlights and sensationalizes, and then we have the crisis period. Conflicting reports. Confusing counter charges. Shoddy scientific data. And finally mandated change is forced upon us, or a product is withdrawn.

I have not mentioned an important step that unfortunately is a typical flaw in our war on cancer. No one ever stops to make any real attempt to quantify the costs or effects of such a ban. It is time we apply a tourniquet to this kind of thinking and stem the flow of wasted effort and money.

If the proper effort was expended in determining the cost benefit relationship and if the best scientific data available were utilized, the conversion to low sulfur fuel in New York City would have never come about in the crisis oriented manner in which it did.

There must be objective evaluation of the risks and benefits involved and a defense against outbursts of emotionalism.

More emphasis must be placed on rational decision-making concerning the prevention of cancer.

There is little doubt that Washington continues to give the war on cancer a stamp of highest priority. However, what is most disturbing is the accusatory climate this priority has taken. Much controversy swirls around maximum tolerated levels or the pinpointing of thresholds. Things get so confusing that the EPA, OSHA, NIOSH, FDA and CPSC aren't too sure of what the other is doing. In any war, be it Viet Nam or cancer, the allies should have some idea of what each other is doing.

It may be that most of the foods we eat, the chemicals we use, the materials we manufacture will all be illegal by current or proposed Government regulations. And perhaps that's not such a bad idea, for then everyone in Washington will have to return to reality, change the ground rules and step back and take a good long look at costs and effects.

My remarks are not anti-Washington or anti-Government. Quite the contrary. We in science and education must be more aggressive in communicating with federal agencies. All too often we seem to sit back and react, usually negatively, to Government findings and regulations instead of being involved before such findings are made public policy.

I maintain that in the past many of us went along with Government to achieve our own personal and professional goals without asking a word about cost-benefit relationships. We too are to blame for some of the past sins as we strived to keep research monies flowing into our laboratories and institutions. It is time for us professionals to make the commitment that the war on cancer warrants.

The causes of cancer must be put into perspective. Normal life would not be possible if we attempted to eliminate all agents that had the potential for harm, such as Vitamins A and D, essential elements like copper and iron, sodium chloride, etc. The thought that the potentially harmful chemicals that occur naturally in food and in the air we breathe (oxygen can be toxic) are somewhat safe, while comparatively low levels of food additives or common air pollutants are dangerous, is naive.

Let us not perpetuate the tactic of going after the snipers, but rather concentrate efforts on finding out how to intervene in the major lethal cancers. Let's move viruses to a side burner and concentrate on prevention. As a physician and educator I am deeply concerned that the funds for the war on cancer continue to short-change those projects that deal with cancer prevention. We should agree that there is no absolute safety in human life and deal with the unreasonable risks to human health. Let's make a commitment that we can prevent cancer via better directed research. Let's refrain from the irresponsible misuse of cancer statistics, and agree that we have wasted millions and millions of dollars and man years in past efforts and concentrate on those areas, such as life style and diet, that can help prevent cancer now. Let's make a more objective evaluation of the risks and benefits involved and relate our efforts to the cost/benefit concept. Let's start working with, rather than against, governmental agencies. Finally, to repeat my major point, let's give prevention the major status it so richly deserves. It is not necessary to know the exact cause and pathogenesis of a disease in order to prevent its occurrence or progression. By intervening at some point in the development of a disease, at the host, vector, or agent level, we may alter its course. Examples in the history of public health are numerous: quarantines; sanitary water supplies; protection against food spoilage; the first vaccines, as against smallpox; pest and vermin control; swamp drainage; incest taboos; vitamin supplements; hypertension control.

As a result of the efforts of numerous basic scientists, clinicians and epidemiologists, we have begun to understand the major causes of cancer.

That most human cancers are caused predominantly by environmental factors is hardly disputed in present times. For the purposes of prevention, which in the long run is our most effective weapon in the War on Cancer, it is of central importance to describe what is meant by the term, environmental, since it is this term which suggests that most human cancers might be prevented or markedly delayed. An appropriate description perhaps is that given by Dr. John Higginson who helped to popularize the concept of "Environmental Cancer" starting in the 1950's:

"...when I used the term environment in those days, I was considering the total environment, cultural as well as chemical. By cultural, I meant mode of life...I've checked it in every dictionary and every dictionary gives the same: Environment is what surrounds people and impinges on them. The air you breathe, the culture you live in, the agricultural habits of your community, the social-cultural habits, the social pressures, the physical chemicals with which you come in contact, the diet, and so on. A lot of confusion has arisen in later days because most people have not gone back to the early literature, but have used the word environment purely to mean chemicals" (Science, 1979).

Aside from describing what academic scientists mean when referring to the term, "Environmental Cancer", it is relevant for the purposes of launching some prevention efforts now, to identify specific predominant causes of human cancers, if at all possible. Many situations may be associated with an increased

snipers, but rather major lethal cancers. In prevention. As a funds for the war on with cancer preven- human life and deal ommitment that we refrain from the ir- ave wasted millions ncentrate on those er now. Let's make lved and relate our rather than against, et's give prevention ow the exact cause nce or progression. isease, at the host, he history of public protection against nd vermin control; ntension control, its, clinicians and uses of cancer. by environmental es of prevention, ron Cancer, it is of nvironmental, since it be prevented or :given by Dr. John nvironmental Cancer'

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cancer risk, but a comprehensive listing of all of these is probably not compatible with prevention because the length of such a compendium of contributing circumstances would be too long. The concept of predominant causes or factors, on the other hand, is useful for the purpose of initiating reasonable prevention efforts at the present time. The definition of a predominant cause is that in the absence of such a factor, the disease would not occur, or would be markedly less frequent. An example is smoking high-tar cigarettes and lung cancer, in both sexes, and all races. The analogy of tuberculosis and its predominant cause is also helpful in delineating the concept. In this disease, the predominant factor is the tubercle bacillus and the size of the infectious inoculum. Associated factors are crowding, malnutrition, lower socio-economic status, predisposition by diabetes mellitus or steroid therapy, inclement weather, and so on. The control of tuberculosis is most effective when aim is taken at the tubercle bacillus and the size of the inoculum; the associated societal factors must be alleviated for their own humanitarian reasons.

Any academic contributions that are made for the purposes of cancer prevention among the public must be clear cut so as not to confound individuals into apathy and inaction. Prevention messages, carried out on a massive scale, must be relatively free of confusion and therefore, the concept of specific, predominant causes has value since the potential list may be shorter than a compendium of all of the possible contributing circumstances.

The term, predominant causes, has been conceptually advanced, despite the fact that cancer is a multifactorial set of diseases. It is necessary, in order to have a high likelihood of developing cancer, to have multiple factors and circumstances operating conjointly; however, avoiding, or greatly decreasing the risk of developing cancer may only require the control of one or two predominant factors out of the whole constellation of contributing causes and circumstances.

Some of the predominant causes do fit together, such as excess use of high-tar cigarettes and heavy alcohol consumption; in such cases it is reasonable to refer to them as one set of predominant factors, especially since the two are very often used at the same time. Similarly, dietary excesses appear to constitute a constellation of sub-factors that are in co-use, and therefore comprise a second set of predominant factors that could be termed, disordered nutrition. Described in this manner, such sets of predominant factors would generally not have substantial cross-over in terms of the major types of cancers caused, and can therefore be considered, to a large extent, as separate from one another. For example, smoking high-tar cigarettes with concomitant heavy alcohol consumption constitutes one predominant cause responsible for approximately 35% of all cancer deaths in the United States (Hammond and Seidman, 1980), the cancers thought to be induced include nearly 90% of epithelial primaries in the lungs, mouth, larynx, esophagus, and liver, as well as approximately 40% of urinary bladder cancers. Disordered nutrition, which is comprised of several factors including a low-fibre diet with a high-fat intake, excess calories, plus obesity, is another predominant cause which is thought by some to account for nearly 45% of all cancer deaths in the U.S.A. (Hammond and Seidman, 1980; Upton, 1979; Nutrition and Cancer, 1977); these cancers

include between 80-90% of the epithelial primaries in the colon, rectum, breast, ovaries, and endometrium. These two categories: (1) high-tar cigarettes/alcoholism and (2) disordered nutrition may have some validity as separate, predominant causes since the types of cancers found are different. While most individuals who smoke high-tar cigarettes and drink heavily have poor nutrition, especially nutritional deficiencies, their dietary patterns are not generally associated with excess calories and obesity. Most heavy smokers/drinkers have poor appetites and tend to be thin. Many of these individuals undergo major weight gains and uncontrolled appetites upon cessation of smoking and will use this to reinforce the smoking habit. The two types of lifestyles: (1) smoking and drinking to excess, and (2) eating to excess with a predominant high-fat, low-fibre diet, seem to be somewhat segregated and are associated with different types of cancers. This is undoubtedly an over-simplification, especially since heavy smokers/drinkers tend to be malnourished, and therefore also have "disordered nutrition"; however, this is of a different type from the disordered nutrition that is linked to the causation of cancers of colon, rectum, breast, ovaries, and endometrium.

The area of nutritional deficiencies is probably applicable to both types of predominant causes discussed so far, i.e., (1) excess smoking/drinking and (2) low-fibre diet with excess calories/fat ingestion/obesity. Deficiencies of vitamin A, other retinoids, antioxidants, selenium etc. can occur as a result of poor appetites, as occurs in heavy smokers/drinkers, or as a result of consuming excess quantities of only a few types of foods. Obese individuals can and often do have nutritional deficiencies, especially of antioxidants. At a symposium sponsored by the American Health Foundation, in 1979, a group of medical scientists concurred that the dramatic decline in stomach cancers, from the major lethal cancer to its present comparative rareness, is due largely to the greater availability of low-cost, fresh produce, made available by an efficient infrastructure of rapid trucking and refrigerated freight carriers; the net result being the greater consumption of more varied, fresh produce with, presumably, enhanced intake of different trace nutrients and antioxidants (Preventive Medicine, Vol. 9, 1980).

The value of attempting to segregate the predominant causes, and the types of related cancers, centers on the effectiveness of prevention-education and, to some extent, targeted early detection programs. Public health messages must be clear and specific. If predominant causes can be identified, in general, with specific types of major, lethal cancers, then public education for prevention and targeted early detection will be easier. The overall purpose in trying to simplify the predominant causes of cancer is to initiate prevention education now. As further research is performed to verify the major causes of cancer, new and more appropriate public health education can be developed.

Several symposia (Nutrition and Cancer, 1977; Preventive Medicine, Vol. 9, 1980) and individuals like J. Higginson (Science, 1979) and A. Upton (Upton, 1979) have developed relatively short lists of specific predominant causes, quite independently of one another. The concordance among these observers is good; Table 1 reproduces these with a relative percent contribu-

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ENVIRONMENTAL DETERMINANTS OF CANCER

TABLE 1. Predominant Sets of Factors in Developing Cancer **

Estimated Relative Percentage	Approximate Number of Such Cancer Deaths*
• 35% of cancer deaths are predominantly due to smoking high-tar cigarettes and consuming excess amounts of distilled liquor (this includes 90% of the cancers of the lungs, mouth, larynx, esophagus and liver, plus 40% of cancers of the urinary bladder)	350 cancer deaths per day
• 45% are predominantly caused by disordered nutrition, with the following subcategories: a) excess calories b) excess fat ingestion, including saturated and unsaturated fats, as well as cholesterol c) obesity, of a magnitude of 40 lbs. or more overweight, for the average individual d) nutritional deficiencies, especially dietary fiber and retinoids (the latter includes vitamin A deficiency; however, only the recommended daily allowance is needed; excesses are extremely dangerous) (the cancers that relate to disordered nutrition are most of the cancers of the colon, rectum, stomach, breast, and many of the cancers of the ovaries and endometrium)	450 cancer deaths per day
• 5% or less of the present deaths are due predominantly to occupational exposures that occurred in the past when the dangers were not known (the cancers that are generally included represent a small proportion of the malignancies of the urinary bladder, lung, nasopharynx, stomach, hemolymphatic system, liver, bones and skin)	50 cancer deaths per day
• 3% are predominantly due to ionizing radiation; about 2/3 of these are due to "background", or naturally occurring radiation, and 1/3 to medical radiation	30 cancer deaths per day
• 2% are due predominantly to pre-existing, benign medical disorders such as chronic fibrocystic disease of the breast, ulcerative colitis, regional enteritis, and chronic atrophic gastritis	20 cancer deaths per day
• 1% are due predominantly to the administration of prescribed pharmaceutical agents such as cancer chemotherapeutics, estrogens for the menopause, and possibly some anti-hypertensive drugs	10 cancer deaths per day

*Approximately 1,000 individuals in the United States die every day from cancer, i.e., about 365,000/year.
**(Editor's Interview, 1979; Hammond and Seidman, 1980; Upton, 1979; Wynder, 1980).

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tion, in a highly tentative manner. These percentages represent working figures and are only estimates to be probed and tested through further research.

Since these sets of predominant causes in Table 1 are largely segregated, it is valid to add them separately to develop an idea of approximately how much of the cancer burden might be preventable, or put off to a later time in life. It is fair to say that the preponderant causes of most of the lethal cancers in the U.S.A. are known, and present varying degrees of difficulty in terms of public health education. A comparatively small relative percent of cancers have unknown predominant causes, about 10%.

While public health education in cancer prevention requires clarity and simplification, academic standards require full and more complex considerations. Therefore, tabular estimates of the relative percent contribution by various predominant causes have limited academic value and must be modulated by some scientific input in order to avoid mistaken simplifications. For example, while low-fibre diets are thought to be a major factor in the development of colon/rectal cancer, the simplistic corollary that indiscriminate high-fibre intakes would offer prevention requires an answer to the question of "...what type of fiber?". It is entirely possible to experimentally enhance mucosal contact with carcinogens adsorbed on certain types of fibres. Rather than focusing on one aspect of disordered nutrition, it would be perhaps more relevant to realize that low-fibre diets are generally also high fat and lack sufficient fresh produce. The fibre content relates to enteric transit time, and possible also to the adsorptive capacity of the fibre. Transit time may be important since it may affect the duration of exposure of the mucosal epithelium to enteric carcinogens and "promoters"; however, copious fluid intake, high carbohydrate diets and physical activity can also decrease enteric transit time. Therefore, it is possible to see how lifestyle and diet interface and why the term disordered nutrition is a constellation of factors that includes obesity, with its presumed relative inactivity, together with excess calories, high fat intake and low fibre.

Against this type of background, a number of academicians decided that the complexities involved in the development of the major lethal cancers had to be presented to the relevant segments of society, and to the public at large through the media. The Cancer Symposium was under the co-sponsorship of the New York Academy of Sciences and the Cancer Symposium Committee, and was held in cooperation with the American Cancer Society. The Speakers and Chairpersons were selected by the Cancer Symposium Committee and reviewed by the New York Academy of Sciences and the American Cancer Society. These Speakers and Chairpersons reported on work that was supported by peer-reviewed grants/contracts from the N.I.H., N.S.F., W.H.O., the American Cancer Society, the National Research Council, and granting sources in several countries.

The Cancer Symposium was organized into sequential sessions that attempted to answer the following questions:

- How do chemical/physical agents in the personal and general environment cause normal cells to become cancer cells?
- Are there clues in existing human data that can direct the basic

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researchers to the causes of cancer? For example, are there groups of people who follow particular lifestyles that either predispose, or protect them? Are there areas of our country where cancer rates are particularly high, and if so are there consistent factors when scientific controls are studied?

- What are the causes of the lethal cancers, as shown by human epidemiology and animal experiments?
- What are the contributions of high-tar versus low-tar cigarettes, excessive alcohol consumption, general pollution, disordered nutrition, nitrites/nitrates, saccharin/cyclamate, administration of pharmaceutical substances?
- Are there ways to scientifically extrapolate data, since there is a real need to protect workers, communities and consumers right now? Are there systems available now that can be logically used to help determine risk?
- If exposures have occurred, is it possible to intercede during the well-known lag phase (between exposures and development of cancer) with chemicals that might act as "antidotes"? For example, can anything be done to prevent the progression of asbestos-related diseases among the individuals who were exposed 15-30 years ago, at a time when the dangers of heavy exposures were not clear?

The presentations did provide significant portions of the answers to the above questions and additionally offered data on the following points which are discussed and summarized following the Conference Conclusions; these include the questions:

- Is there a cancer epidemic involving most anatomic sites, or is it just lung cancer that is epidemic?
- Is there a reasonable biologic basis for practical thresholds to many of the predominant causes of cancer?
- Is synergism a significant factor, since most humans have multiple exposures? Is there a way of approaching this question on a practical basis?
- Is it likely that industrial and general pollution of air and water will lead to major increases in cancer rates (per 100,000) in the future, in view of increases in industrial production and in population?
- What are the percent relative contributions of widely publicized factors such as general community air pollution, general water pollution and saccharin/cyclamates?

ACKNOWLEDGEMENTS

It is imperative to understand that the advances in the War on Cancer have come about by the concerted efforts of basic scientists, clinicians, biostatisticians, epidemiologists, lay lobbyists, legislators, and administrators in many parts of the world. It is hoped that their efforts will continue, particularly

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RETHINKING THE ENVIRONMENTAL CAUSATION OF HUMAN CANCER

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(Received 19 December 1980)

Summary—Many of the hypotheses regarding the aetiology of cancer in man were suggested by the early 1950s and much recent effort has been directed to defining causes and mechanisms in more detail through more sophisticated epidemiological and laboratory approaches. Although there are many factors involved in its aetiology cancer may be prevented by controlling a single predominant factor. Epidemiology has contributed considerably to our understanding not only of the aetiological factors but also of the possible mechanisms involved, especially those associated with life-style. For cancers believed to be related to the latter, notably those of the gastro-intestinal tract and endocrine-dependent organs, there is evidence for the involvement of cultural, behavioural and dietary factors rather than direct exposures to carcinogens alone. Future efforts should be directed towards a better understanding of factors modulating carcinogenesis since it seems improbable at present that the initiating factors for many cancers can be identified.

Introduction

It is a great pleasure to contribute to this issue dedicated to Dr Leon Golberg. I have known Leon Golberg for many years, and admire not only his scientific work but also his erudite and objective studies on the complex interactions between toxicology and human health and welfare.

Present concepts of the aetiology of human cancer have developed gradually, reflecting new ideas and hypotheses arising from both epidemiological and laboratory studies. Thus, the isolation of pure carcinogenic polycyclic aromatic hydrocarbons was essentially dependent on the original observations of Percivall Pott. The later demonstration of other occupational hazards provided the foundations of modern chemical carcinogenesis. The recognition of oncogenic animal viruses and the development of pure strains of mice as our understanding of inherited susceptibility has increased has had considerable impact on aetiological theories. Willis (1948), while drawing attention to the possible environmental background of many human cancers, largely emphasized industrial chemical carcinogens. It is only comparatively recently that the role of environmental factors in human cancer in a wider sense has been accepted. Moreover it appears that progress in environmental carcinogenesis has been as much dependent on the confirmation or exclusion of hypotheses through more sophisticated investigations as on the promulgation of completely new theories.

In 1950, a group of distinguished experimentalists and laboratory workers meeting in Oxford (Clemmesen, 1950) concluded that geographical variations in cancer incidence predominantly reflected environmental influences. In that term, however, they included all exogenous factors which impinge on man, i.e. the dietary, social and cultural environments as well as discrete chemical carcinogens. Stimulated by the Oxford report, George Oettlé and myself decided to collect accurate statistics in Southern Africa to pro-

vide a baseline of cancer incidence in a newly urbanized population in an attempt to evaluate the relative aetiological importance of lifestyle, diet and industrialization. The results of this survey (Higginson & Oettlé, 1960) in combination with the migrant studies of Kennaway (1944), Haenszel (1961) and Haenszel & Kurihara (1968), and the reports of others (Boyland, 1969; Doll, 1967), strongly suggested that most human cancers were related to environmental factors and not to racial or hereditary factors (Higginson, 1960). Further, the survey suggested that "way of life" was of major significance for many cancers, especially those of the gastro-intestinal tract and endocrine-dependent organs. These estimates were later extended using a wider range of populations (Higginson, 1969).

Carcinogenesis in man

Definition of cause

The massive increase in the production and use of synthetic chemicals in industry, agriculture and medicine since 1940 caused renewed interest in the potential carcinogenic effects of chemical pollutants in the human environment (Carson, 1962). Since the concept of a discrete carcinogenic chemical, physical agent or virus as a direct cause of cancer was widely comprehended, the possibilities for prevention through simple legislative action were readily accepted. In contrast, there was a tendency to neglect the inherent complexities of chemical carcinogenesis, although such complexities and the many associated modulating factors involved had already been demonstrated by many workers (Berenblum, 1978; Clemmesen, 1950; Miller, E. C., 1978). Moreover, for many cancers in humans, the data were inconsistent with an aetiology based only on simple chemical exposures (Higginson & Oettlé, 1960). In addition, an increasing number of parameters related to life-style were identified and associated with increases or decreases in

cancer incidence. Such parameters, now called "carcinogenic risk factors", included absence of fibre in the diet, age at first marriage, etc., and could not readily be defined as carcinogens in the classical sense. Both laboratory and epidemiological studies, however, suggested that the role of such factors including their possible relationship to individual susceptibility should be explicable in more objective biological terms through a better understanding of the multi-stage and molecular basis of carcinogenesis.

The multifactorial nature of carcinogenesis tends theoretically to complicate the definition of cause, since, in addition to the role of complex modulating factors, combinations of carcinogens may show synergistic (Hammond & Selikoff, 1973) or inhibitory effects (Miller, Miller, Brown & MacDonald, 1958). In practice, however, a single factor may be so predominant as to be regarded as the practical cause in the public health sense, in that in its absence a significant proportion of related cancers would not arise. This in no way excludes a role for modulating factors (Cole & Merletti, 1980) or necessarily implies understanding of the basic mechanisms involved. Thus, although the action of cigarette smoking may be multiplied many times by asbestos exposure, the former is the practical cause of 85% of lung cancers in males whether or not exposed to asbestos (Hammond & Seidman, 1980; Hammond & Selikoff, 1973). In contrast, asbestos would be the predominant cause of mesothelioma in exposed shipyard workers. Although oestrogens are believed to be promoters, they can be regarded as the practical cause of a significant proportion of endometrial cancers in parts of the United States (Gusberg, 1980). Thus, in considering aetiology, it is important not to confuse multifactorial mechanisms, i.e. relative role of initiator versus promoter in cigarettes, individual susceptibility, etc., with practical causes especially since the latter may have more immediate health relevance. Routine epidemiology and long-term experimental testing essentially demonstrate the overall impact of numerous events, and usually the effect of individual modulating factors cannot be estimated.

Role of epidemiology in evaluating aetiological hypotheses

While defined aetiological hypotheses in human cancer have been largely developed through case-history epidemiological studies, it is less widely appreciated that epidemiology may also contribute to the analysis of possible mechanisms for those environmental cancers of uncertain aetiology, through deductions made from geographical and temporal variations in incidence and associated migrant studies (Clemmesen, 1950; Doll, 1967; Higginson, 1969; Higginson & Muir, 1979; Higginson & Oettlé, 1960; Wynder & Gori, 1977). In this context the study of low-risk populations is particularly important in allowing the evaluation of those environmental factors especially related to lifestyle (Enstrom, 1980; Higginson & Oettlé, 1960; Lyon, Gardner & West, 1980; Phillips, Kuzma & Lotz, 1980). Epidemiology is of course complemented by laboratory studies which may not only suggest new aetiological hypotheses for testing in man but also the nature of the mechanisms involved.

It should be emphasized that no epidemiology nor laboratory study, whether relating to discrete carcinogens or lifestyle factors, can now be completely conclusive since all results (positive or negative) are dependent on statistical probabilities (IARC Working Group, 1979a; Vesell, 1980).

Hypotheses on the causation of human cancer

Prior to 1950 the cause of very few human cancers had been firmly established, but considerable data are now available. Most cancers can be described as tumours of well-defined environmental origin, or those for which the environmental background can only be deduced. For a smaller group no satisfactory causal hypotheses are available. Estimates of the proportion of cancers in each aetiological group in different communities are reported elsewhere (Higginson, & Muir, 1979).

Cancers caused by defined exogenous factors

The majority of tumours in this group are epithelial cancers of the skin, respiratory and upper digestive systems, liver and bladder. In addition, the causes of a small number of cancers of the endocrine-dependent organs, haemopoietic system, bone and soft tissues have also been identified.

Personal habits are by far the most important stimuli established, especially cigarette smoking. Studies in all countries emphasize the overwhelming role of this habit which causes between 25-35% of all cancers in males in North America, Europe and Japan (Hammond & Seidman, 1980). The proportion in females is small but increasing rapidly. Cigarette smoking is not only carcinogenic *per se* but also enhances multiplicatively the effects of such other factors as asbestos and alcohol (Hammond & Selikoff, 1973; Tuyns, 1978). In most countries this habit is predominantly responsible for the increases in cancer incidence reported since 1950. Excess alcohol consumption, sunbathing, and in Asia betel-quid chewing are other carcinogenic habits. A much smaller part of the cancer burden even in industrialized countries is related to occupational (probably less than 5%) and iatrogenic and radiation exposures. It is estimated that approximately 39 of the 537 chemicals and related industrial processes evaluated in the IARC Monographs Series are probable or definite human carcinogens, of which the most important of industrial origin is asbestos, especially in association with cigarette smoking. However, the list is not definitive and further iatrogenic and occupational hazards will probably be detected in the future.

Although less well-documented, there is considerable evidence in Africa and Asia that primary liver cancer arises in hepatitis B virus carriers who are exposed to aflatoxin (Larouze, Blumberg, London, Lustbader, Sankalé & Payet, 1977; Linsell & Peers, 1977).

Cancers of probable environmental origin

This group comprises tumours of the gastro-intestinal tract, e.g. stomach and large intestine, and of the endocrine-dependent organs, e.g. breast, body and cervix of the uterus, and ovary, etc. Although the definitive stimuli have not been identified, the most

rational interpretation of the available epidemiological data would indicate a direct or indirect association with environmental factors (Higginson & Muir, 1976). While it is probable that further strong exogenous agents will eventually be identified for other tumours, e.g. oesophagus in parts of China, Africa and Iran, where precancerous mucosal lesions are found in most adults (Crespi, Muñoz, Grassi, Aramash, Amiri, Mojtabai & Casale, 1979), this would appear less likely for most cancers in this group and alternative aetiological explanations must be sought.

On one hand, it has been suggested that such cancers largely reflect additive exposures to multiple mutagens or carcinogens within the general environment predominantly of industrial origin (Epstein, 1978). Others believe that most of these cancers are predominantly related to exogenous and/or endogenous factors inherent in life-style modulating cells already initiated by undetermined stimuli, and are not dependent on exogenous initiators alone (Higginson & Oettlé, 1960; Wynder & Gori, 1977), as suggested by much recent experimental work (Slaga, 1980).

The role of ambient environmental pollution

The effects of ambient environmental pollution on several diseases, especially respiratory, are recognized. The fact that exposure to high doses of certain chemicals causes cancer in man and the synergism existing between several toxic agents have led to concern about the significance of multiple low exposures to chemicals in the general environment. Chemicals of natural and synthetic origin with biological activity have been and are ubiquitous in the environment in all countries. They include carcinogens and mutagens, promoters, enhancers, etc. Polycyclic aromatic hydrocarbons, nitrosamines, mycotoxins, flavonoids and other suspected carcinogens have been demonstrated in air, water (Kraybill, 1978; Wilkins, Reiches & Kruse, 1979), food, and alcoholic beverages (Rose, 1977). Many individuals have been and are exposed to many of the animal carcinogens reviewed in the IARC Monographs Series (Supplement 1: IARC Working Group 1979a). The problem of their evaluation at very low levels of exposure is illustrated by the nitrosamines which are not only widely distributed in the environment, but may be demonstrated in body tissues and fluids (Walker, Castegnaro, Gričič & Lyle, 1978; Yamamoto, Yamada & Tanimura, 1980). While many are carcinogenic to several animal species, to date there is no firm evidence of carcinogenic activity in man (Tannenbaum & Young, 1980).

Attempts to evaluate the specific carcinogenic potential to man of an individual chemical at low dose among the myriad chemicals present in the environment poses almost insoluble logistic and technical problems for the epidemiologist, especially in the presence of powerful confounding variables such as cigarettes. The difficulties are even greater than those of determining the effects of low doses of ionizing radiation, a recognized human and animal carcinogen where controversy still exists (Land, 1980), although in this case the "target cell" dose can be estimated.

The value of animal experiments in identifying potential human carcinogens is well recognized, but there is a tendency to concentrate on the statistical limitations of epidemiological methods rather than

the equally great limitations of biological extrapolation between species. Such difficulties cannot be overcome simply by theoretical mathematical models extrapolating from animals since many of the pertinent parameters relating to carcinogen metabolism, inhibition, etc., cannot be measured (Coulston, 1979), nor can the dose at the target cell, although newer technology, i.e. measurement of DNA adducts, may permit this possibility in the future (Rajewsky, 1979).

Within limits, however, epidemiology, by comparing cancer patterns in different environments, may help to evaluate the additional impact of the overall burden of chemicals in a specific environment, i.e. the sum of total increased risks. Such an approach has been attempted (Higginson, 1979; Lyon *et al.* 1980; Royal Society Study Group, 1978; Wynder & Gori, 1977) and further details may be obtained from these reports. In brief, no consistent relationships have been observed between total cancer patterns or between organ sites, and indices of probable ambient environmental pollution such as industrialization and urbanization. The most intensive studies have been in relation to air pollution, where no significant effect can be demonstrated if correction is made for such variables as cigarette smoking and occupational exposures (Cederlöf, Doll, Fowler, Friberg, Nelson & Vouk, 1978; Goldsmith, 1980; Haenszel, Loveland & Sirken, 1962; Haenszel & Taeuber, 1964; Hammond & Garfinkel, 1980). Such studies do, however, permit partial evaluation of the additive effects of occupational exposures in both urban and non-urban environments (Hammond & Garfinkel, 1980). The above observations are supported in a recent report indicating no significant differences in cancer incidence between urban and rural Mormons (Lyon *et al.* 1980). Thus it appears that only a very small part of the total cancer burden can be directly related to industrialization in a general sense, and alternative aetiological explanations must be considered for the majority of tumours of environmental origin. These observations also have obvious relevance to evaluating the existence of 'no-effect' exposure levels for identified carcinogens or reversibility in carcinogenesis.

Lifestyle

In addition to such clearly defined habits as cigarette smoking, alcohol ingestion, sunbathing and occupation, this term covers the total cultural, behavioural and dietary environment i.e. all exogenous factors inherent in daily life. Lifestyle includes such carcinogenic risk factors as behavioural patterns, e.g. age at first marriage and pregnancy; such physiological parameters as age at menarche or menopause; and such dietary patterns as quantity and quality of dietary fat, fibre etc. While the recognition of 'lifestyle' factors in cancer is not recent (Clemmesen, 1950; Higginson & Oettlé, 1960; Willis, 1948; Wynder & Gori, 1977), it has been neglected due to its poorly defined nature.

Diet should be considered as a highly complex chemical mixture. It not only includes preformed carcinogens and carcinogen precursors but also enhancing and inhibiting factors (Wattenberg, 1979; Wynder, Hoffmann, McCoy, Cohen & Reddy, 1978). Further, while diet might be directly related to stomach or

large intestine cancer through exogenous carcinogens or endogenous carcinogen formation e.g. nitrosamines (Tannenbaum & Young, 1980), it may also affect tumour development of the breast for example in a non-specific manner through variations in fat and calorie intake (Miller, 1980; Newberne & McConnell, 1980; Wynder & Gori, 1977). Further, diet is a determinant in menarche, height, obesity, etc., risk factors for cancers of the breast and endometrium (Hill, Wynder, Barbaczewski, Helman, Hill, Sporangisa & Huskisson, 1980). The nature of such non-specific effects is uncertain but they may operate through the hypophyseal axis. Moreover, numerous studies confirm the role of diet in modulating the induction of enzymes of potential significance in carcinogenesis (CIBA Foundation Symposium 76, 1980; Conney, Pantuck, Pantuck, Buening, Jerina, Fortner, Alvares, Anderson & Kappas, 1978; Kalamegham, Krishnamurthy & Bhargava, 1979; Newberne & McConnell, 1980; Vesell, 1980). Calorie and protein reduction have been associated with inhibition of tumours of the breast, intestine and skin in animals and a high-fat diet has been associated with increased cancer in humans and animals possibly due to late-stage effects (Gori, 1978; Hirayama, 1979; Hoehn & Carroll, 1979; Newberne, Weigert & Kula, 1979). The type of carbohydrate has been shown to be important under certain experimental conditions, but in man so far only for fibre is the evidence reasonably strong, notably for cancers of the colon and rectum. While the role of vitamin A deficiency has yet to be demonstrated in man, some studies report that increased vitamin A protects against lung cancer (Wald, Idle, Boreham & Bailey, 1980). Earlier reports that malnutrition was a major factor in liver cancer in Africa have not stood up to further examination (Higginson, 1963). Further, diet also modifies the nature and presence of mutagens in the faeces, some of which have been causally associated with cancer of the large intestine (Dion, Bright-See, Furrer, Eng & Bruce, 1980). Mutagens may arise from normal nutrients during cooking (Kawachi, Nagao, Yahagi, Takahashi, Sugimura, Takayama, Kosuge & Shudo, 1979). Reports relating gastric cancer to nitrate ingestion leading to endogenous formation of nitrosamines remain to be confirmed (Tannenbaum & Young, 1980).

Considerable data have accumulated, indicating that risk factors arising from behaviour and diet may also be associated with biochemical and metabolic variations in the host, especially hormonal variations which have possible relevance to human carcinogenesis (de Waard, 1979; Ernster, Sacks, Selvin & Petrakis, 1979; Gusberg, 1980; Moolgavkar, Day & Stevens, 1980; Ross, Paganini-Hill, Gerkins, Mack, Pfeffer, Arthur & Henderson, 1980; Trichopoulos, Cole, Brown, Goldman & MacMahon, 1980; Vesell, 1980). The promoting role of exogenous oestrogens in endometrial cancer (IARC Working Group, 1979b) and the inhibitory effect of certain contraceptives in endometrial and ovarian cancers also support the possibility that endogenous hormonal factors may be implicated (Cole, 1980; Hulka, Fowler, Kaufman, Grimson, Greenberg, Hogue, Berger & Pulliam, 1980; Smith, Prentice, Thompson & Herrmann, 1975).

Cancer incidence has been shown to be modified by

socio-economic gradients (Office of Population Censuses and Surveys, 1978) which are closely associated with diet, behaviour and cultural variations (Morris, 1979). Further, while epidemiological studies on cancer and occupation are classically associated only with the identification of discrete carcinogens, they may be equally informative in evaluating lifestyle and socio-economic factors (Morris, 1979; Office of Population Censuses and Surveys, 1978). Since individual occupations are recruited from specific segments of the community, the health patterns in such occupations reflect the local community environment and socio-economic background. Fox & Adelstein (1978) calculated that most of the differences (88%) in cancer patterns between occupational groups are probably due to lifestyle variations and not to workplace exposures. This is further illustrated by studies on breast and cervical cancers (Devesa & Diamond, 1980; Pell, O'Berg & Karrh, 1978; Trichopoulos, MacMahon & Brown, 1980). Davies, Edmundson, Raffonelli, Cassadi & Morgrade (1972) report that storage of chlorinated hydrocarbons also correlates with socio-economic gradient. Distinction should be made accordingly between tumours due to industrialization *per se* and cancers occurring within a highly developed society.

In contrast to the delay in accepting the role of lifestyle factors in cancer, the concept was accepted more rapidly in the case of cardiovascular disease, and led to considerable publicity on the benefits of dietary and behavioural changes.

Significance of time trends

The process of establishing the relative importance of ambient pollution and lifestyle factors has been complicated by conflicting interpretations of cancer-time trends. The evaluation of such trends is hindered by registration artefacts resulting from variations in population boundaries, census, diagnostic criteria, etc., as well as confounding variables such as cigarette smoking and social habits.

The morbidity data in the US from 1947 to 1970 have been analysed by Devesa & Silverman (1978) who showed that apart from the increase in tobacco and alcohol-related cancers, other cancers were tending to decrease, trends supported by the mortality data.

Changes since 1970 are especially difficult to interpret (Pollack & Horn, 1980), due to registration artefacts, etc., which have often been ignored. However, the available morbidity and mortality data are in general consistent with earlier observations, and do not suggest real increases in cancers apart from those associated with cultural habits, e.g. tobacco and sunlight. The modest increases in cancers of the breast, prostate and bladder are probably artefactual, e.g. increased histological diagnosis, or changes in lifestyle factors. The latter are also probably responsible for the decrease in gastric and cervical cancer. These interpretations do not imply that localized or general chemical exposures have been completely without effect on general cancer patterns. However, they provide no support for the existence of a new general cancer 'epidemic' apart from the effects of cigarette smoking, alcoholism, and to a much lesser degree asbestos in certain limited population groups.

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It has been argued that the increased production of industrial organic chemicals is too recent to permit evaluation of the long-term effects. However, considerable quantities were already in fact produced by 1950 (Davis & Magee, 1979). It should be emphasized that increased production, however, cannot automatically be equated with a comparable increase in carcinogen exposures in man (Lenihan & Fletcher, 1976; Morris, 1975) since public health and industrial controls affecting both ambient and point-source exposures have been implemented in many countries. From the microbiological angle it would be interesting to know if there has been any increase in background mutational rates which could be attributed to variations in the chemical environment over the last 30 yr. The existence of localized areas of unusual cancer incidence, or marked changes in temporal trends requires the continual support of adequate surveillance and monitoring mechanisms.

While attention has been concentrated on changes in chemical exposures, it is frequently forgotten that comparable changes in both dietary and behavioural patterns have occurred in many countries, largely as a result of efforts to control cardiovascular disease, and the effects of these changes on cancer patterns remain to be determined. In the United States, for example, between 1963 and 1977, there was a fall in the per capita consumption of tobacco of 29%, of eggs 15%, of milk and cream 23%, of butter 36% and of animal fats and oils 47%, whereas consumption of vegetable fats and oils increased by 58% (McQuade, 1980). Furthermore, between 1910 and 1976, the consumption of carbohydrates fell by 21%, of which the contribution from starches fell by 45% (Brewster & Jacobson, 1978). If such changes are as important in the modification of human cancer patterns as migrant and geographical pathology studies suggest, their effects should be observed during the next few decades, and appropriate action initiated. However, the literature on diet in animal and human cancer indicates many inconsistencies in establishing clear-cut relationships (Higginson & Muir, 1979).

In conclusion, although there is a widespread belief in the importance of dietary factors, and while their role in promotion, enzyme induction, etc., is well-established in experimental animals, their role in man is much less clear. The difficulties of evaluating the significance of modest dietary changes as seen in man in the context of other modulating factors compared with the very marked changes seen in isolated animal experimentation increases the difficulty of extrapolation. This is well illustrated by Hoehn & Carroll (1979)

who demonstrated the effects of simple carbohydrate changes on DMBA-induced tumours in rats.

Cancers of unknown aetiology

Only a few discrete causal factors have been determined for most tumours of children, bone and soft tissues and haemopoietic system, the aetiology of the majority being unknown. Most tumours show very modest geographical and temporal variations in incidence, and their relationship to environmental factors cannot accordingly be easily evaluated. It is tempting to regard such cancers as representing a background incidence in man similar to 'spontaneous' tumours in animals which, however, are often related to inherited factors or endogenous viruses.

The implications of epidemiology to the study of carcinogenic mechanisms

The multifactorial nature of human cancer has long been recognized. As the influence of other modulating factors in the early and late stages of carcinogenesis is increasingly recognized (Berenblum, 1979; Higginson, 1980; Miller & Miller, 1979; Wynder *et al.* 1978), it seems less appropriate to consider carcinogenesis in man only in terms of the classical two-stage skin model.

Contrary to recent reports, human cancer has not been considered in terms of single causes by most epidemiologists (Table 1). Kennaway (1950) emphasized the modifying effect of diet. The interplay of several factors was strongly supported by numerous studies such as on alcohol, asbestos and cigarette smoking (Hammond & Selikoff, 1973; Tuyns, 1978; Wynder & Gori, 1977), hepatitis virus in liver cancer (Higginson, 1963), and the interaction of multiple carcinogenic risk factors in cancers of the endocrine-dependent systems (Miller, A. B., 1978). Adenocarcinoma of the vagina due to diethylstilboestrol is also suspected to involve a second factor. Further, the fact that latent carcinoma of the prostate is equally prevalent in most races, whereas the incidence of invasive carcinoma varies widely, strongly suggests an interplay of initiating and promoting factors (Breslow, Chan, Dhom, Drury, Franks, Gelici, Lee, Lundberg, Sparke, Sternby & Tulinius, 1977).

Figures 1 to 3 are diagrammatic representations of the carcinogenic process. While division into early, initiation, and late phases is probably an over-simplification in view of the probable overlaps, the diagram provides a useful base for discussion. It does illustrate moreover that clinical cancer does not represent a single mechanism but a series of events, only a few

Table 1. Human cancers with evidence of multistage and multifactorial origin

Site/tumour type	Aetiological factors
Lung	Smoking, asbestos, ionizing radiation
Oesophagus	Alcohol, smoking
Liver	Hepatitis B, mycotoxins
Stomach and large intestine	Endogenous carcinogen formation, non-specific dietary factors
Breast and endometrium	Behaviour, diet, hormonal promotion
Burkitt's lymphoma	EBV, malaria, factor 'X'
Nasopharyngeal carcinoma	Host factor, EBV, factor 'X'

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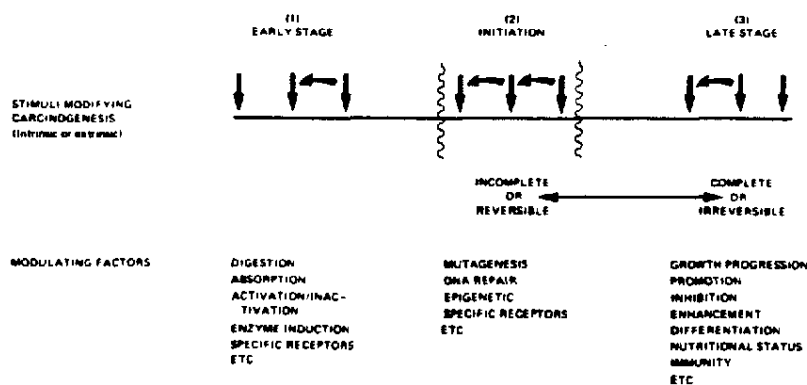


Fig. 1. A simplified diagram showing the hypothetical stages in carcinogenesis and some of the exogenous and endogenous factors possibly involved.

of which have adequately been studied and measured (Vesell, 1980). Although order is essential to the concept of cancer development and progression, there is good experimental evidence that many steps are at least partially reversible (Farber & Cameron, 1980; Singer, 1979).

It seems a reasonable possibility that individual susceptibility and the contribution of lifestyle as indicated by carcinogenic risk factors may eventually be explicable in terms of reversible and irreversible biochemical events and feedback mechanisms operating in both early and late-stage carcinogenesis. Further, there may be both qualitative and quantitative differences between the effects of modulating factors in cases where discrete carcinogens providing a genotoxic stimulus have been identified (Fig. 2), and 'lifestyle' cancers where both extrinsic and intrinsic carcinogenic initiating stimuli may be less important (Fig. 3). The latter illustrates the difficulty of extrapolation from experimental systems, especially for cancers where the modulating factors have yet to be clarified.

The importance of enzyme activation and inactivation of procarcinogens and carcinogens (CIBA Foundation, 1980) is widely recognized. Further, variations in enzyme induction, whether genetically or environmentally determined, may affect both individual susceptibility to carcinogens (Vesell, 1980), and the incidence of certain endocrine-dependent tumours in which subtle variations in hormonal metabolism may be important (Cole, Brown & MacMahon, 1976). Increased extra-glandular formation of oestrogens occurs largely in fat tissues and may explain the relationship between obesity, overeating and endo-

metrial cancer. Endogenous carcinogen formation from carcinogen precursors may be controlled to a considerable degree by environmental factors, by mechanisms including altered enzyme metabolism. Epidemiological studies are only now being developed and will require much more intensive laboratory backup.

Initiating events are easier to explain in terms of genotoxic than non-genotoxic stimuli, since the latter are poorly understood. They may involve gene unmasking, modification of DNA repair, epigenetic changes, etc. (Kroes, 1979), all of which can be environmentally modified and have important implications in terms of the completeness and the reversibility of initiation.

It is thus possible to assume that in man initiation is common but often incomplete or lethal, cancer development being dependent on events in the later stages as well as during initiation (Rajewsky, 1979). This is obviously difficult to demonstrate, but strains of rats with a high frequency of spontaneous hepatomas are regarded as being more susceptible to the promoting action of phenobarbitone than low-incidence strains (Peráino, Staffeldt, Haugen, Lombard, Stevens & Fry, 1980). Similarly, it has been found that initiated but not transformed cells are present in high-skin-cancer strains of mice but not in those of low incidence (S. Yuspa, U. Lichei, D. Morgan & H. Hennings, unpublished data, 1980). In man, the possibility of incomplete initiation is supported by pathological studies on the prostate and breast (Farber & Cameron, 1980), etc. Whether such initiation is due to endogenous agents alone is a matter of speculation. Archer (1980) has suggested that background radi-

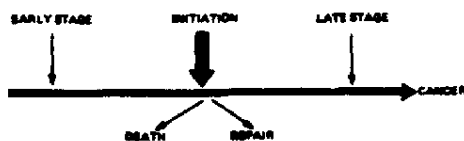


Fig. 2. The predominant effect of a strong discrete initiator (e.g. an alkylating agent) is illustrated in comparison with the slight effects of modulating factors.

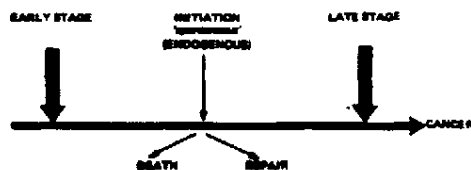


Fig. 3. The relatively important role of 'lifestyle' modulating factors is illustrated in a situation where the role of a direct initiating stimulus may be weak.

ation is a factor of significance in many cancers, but the failure to demonstrate any relation with ambient chemical pollution makes it more difficult to implicate the latter although obviously it cannot be excluded.

The many factors that may be involved in late-stage carcinogenesis have been reviewed (Slaga, 1980). But even for such extensively studied carcinogenic agents in man as cigarettes, the relative importance of initiation and promotion is not understood. However, the fact that the incidence of lung cancer 'freezes' after cessation of smoking suggests that promoting factors are important in later development. In addition to reactions at the target cell, changes in the host may also be pertinent, e.g. immunological factors (Kinlen, Eastwood, Kerr, Moorhead, Oliver, Robinson, de Wardener & Wing, 1980). Conney *et al.* (1978) have demonstrated the role of diet in carcinogen metabolism in man and animals. The slow increases in breast and prostatic cancers in migrants (Haenszel & Kurihara, 1968) show that environmental differences may take several generations to become apparent, and the possibility of sex-linked enzyme imprinting in such cancers in humans should not be ignored. Such a mechanism has been postulated for breast cancers in rats (Mori, Nagasawa & Bern, 1979).

Individual susceptibility

It is not easy to differentiate between environmentally and genetically determined individual susceptibility (Vesell, 1980). Previous attempts to relate lung cancer susceptibility to the induction of aryl hydrocarbon hydroxylase have not been confirmed.

Apart from such clear-cut hereditary syndromes as xeroderma pigmentosum and ataxia-telangiectasia, etc., few cancers can be directly attributed to genetic factors. On the other hand, genetic polymorphism may have considerable significance both in relation to enzyme induction and host reactions to exogenous modulating factors (del Villano, Miller, Schacter & Tischfield, 1980; Schull, 1979). Migrant studies, however, emphasize the overwhelming impact of environmental changes.

Conclusions

For nearly 50% of environmentally influenced cancers among men in North America and Europe the main aetiological factors have been identified but the percentage is much lower for women. Many of the causes had already been suggested in the fifties. Since then, much laboratory and epidemiological research has been directed towards investigating the nature of and the mechanisms involved in cancer in man. Increasingly sophisticated technology has been used in attempts to identify the initiation stimuli and the many modulating factors involved. Studies of the progression of cancer and the multi-stage nature of carcinogenesis make it increasingly evident that data from each species are complementary and that an interdisciplinary approach is important. Clearly exposure to high levels of carcinogens must be controlled, but the view that multiple exposure to very low levels of mutagens and carcinogens (general environmental pollution) may have relatively little effect on the total cancer burden indicates the importance of alternative approaches to cancer prevention and control.

The implication of a discrete chemical carcinogen or of a defined cultural habit allows a relatively straightforward approach to cancer prevention. However, the situation has not been so simple in the case of cancers thought to be related to lifestyle, where there have been only very general indications of the factors involved and understanding of the underlying biological mechanisms has been imperfect. It is becoming increasingly clear that combinations of modulating endogenous and exogenous factors may explain the influence of lifestyle based on identifiable biochemical mechanisms. This may eventually allow the possibility of tumour prevention by interference with other stages of carcinogenesis through chemoprevention (Sporn & Newton, 1979), use of cancer inhibitors and dietary changes. The process of attributing many cancers to lifestyle factors has the benefits of directing research attention to factors beyond discrete carcinogens and of arousing intellectual curiosity. Unfortunately, at present it does not provide a basis for the immediate and effective control of many cancers of environmental origin.

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Carcinogenicity of Epoxides, Lactones, and Peroxy Compounds^{1,2}

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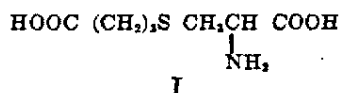
SUMMARY

Attention is focused on epoxides, lactones, hydroperoxides, and peroxides as carcinogens, as possible carcinogenic intermediates in the metabolism of aromatic hydrocarbons and as environmental carcinogens. Available information about the carcinogenicity of these compounds is reviewed. To ascertain their carcinogenic activity, 14 compounds in acetone or benzene solutions were tested by skin painting on

mice. Five epoxides, styrene oxide, 1-ethyleneoxy-3,4-epoxycyclohexane, 1,2-epoxybutene-3, and *dl*- and *meso*-1,2,3,4-diepoxybutane and one hydroperoxide, 1-hydroperoxy-1-vinylcyclohexene-3 showed carcinogenic activity. Some aspects of the relationship between structure and carcinogenic activity are discussed.—*J Nat Cancer Inst* 31: 41-55, 1963.

MUCH ATTENTION has been devoted to the study of the carcinogenicity, metabolism, and mode of action of many aromatic hydrocarbons, heterocyclics, amines, and azo dyes. Several other types of compounds, mostly nonaromatic in structure, have been investigated to a limited extent, some of which showed carcinogenic activity, *e.g.*, β -propiolactone (1, 2), 1,2,3,4-diepoxybutane (3, 4), and 6- β -hydroperoxy- Δ^4 cholestene-3-one (5). Several features of these compounds suggested that further inquiry into their carcinogenic activity was merited.

Chemical reactivity.—These compounds are all highly reactive chemically, in contrast to the relatively unreactive aromatic hydrocarbon carcinogens, and probably undergo a variety of chemical reactions with tissue constituents. Thus the crosslinking abilities of epoxides are well known (6); β -lactones react with amines and other functional groups such as the sulfhydryl group of cysteine. Thus, S-2-carboxyethylcysteine, I, is obtained (7) by reaction of cysteine with β -propiolactone.



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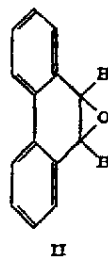
This compound has been prepared earlier by other methods (8, 9). Hydroperoxides and peroxides can be expected readily to bring about oxidation reactions.

The epoxides, like the various nitrogen and sulfur mustards, exhibit radiomimetic properties, *e.g.*, growth-inhibitory, mutagenic, and cytotoxic activity (10-13). These radiomimetic agents all have one property in common: They are alkylating agents that undergo reactions with suitable nucleophilic groups via carbonium ions (14). The types of reactions

which the carbonium ions can undergo with anions, *e.g.*, $\text{O-P}^{\ominus}$, $\text{Cl}^{\ominus}$, etc.,

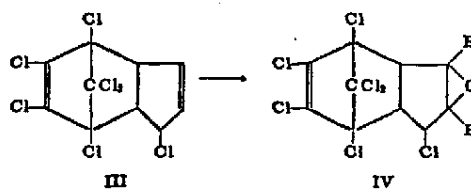
and bases, *e.g.*, RNH_2 , R_2S , etc., have been summarized and discussed by Ross (14). The effects of a number of alkylating agents in biological systems have been investigated (12, 15-17) extensively.

Relationship to aromatic hydrocarbon metabolism.—An understanding of the necessary structural features required for carcinogenicity of the nonaromatic materials may be enlightening with respect to the mechanism of chemical carcinogenesis by aromatic hydrocarbons. For example, Boyland (18) has suggested that a phenanthrene-epoxide, II, may be an intermediate in the metabolism of phenanthrene, and Haddow has suggested that epoxides may be the proximal carcinogens (10).



Epoxides of this type have not yet been synthesized, but if they were obtainable would probably be highly reactive.

Although unrelated in its biological activity, the insecticide heptachlor, III, is metabolized to the epoxide, IV, in dogs and rats (19). The epoxide,



IV, appears to be one of the first reported metabolites containing the epoxide function. The formation of hydroperoxides as intermediates in the metabolism of aromatic hydrocarbons has also been suggested (20). Such compounds, however, have not been identified in the metabolism of aromatic hydrocarbons. Allsopp (21) found that the alkali

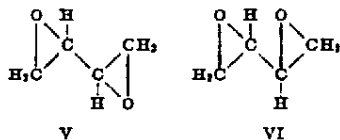
extractable portion of photooxidized benzo[a]pyrene is carcinogenic to mouse skin. The products of the photooxidation were not identified, but they probably contained peroxides, phenols, and quinones (22).

Significance as environmental carcinogens.—Kotin (23) has suggested that epoxides and peroxy compounds, formed by the oxidation of olefins, may be carcinogenic air pollutants and has produced skin tumors in mice with atmospheric extracts free of aromatic hydrocarbons. It was suggested that the activity of this material was due to the presence of epoxides, but their presence was not confirmed experimentally.

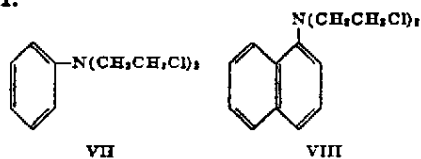
We have suggested earlier that compounds such as diketene (methylene- β -propiolactone), hydroperoxides, and peroxides may be components of cigarette smoke (24). Diketene, for example, can conceivably be formed by dimerization of ketene, itself a pyrolysis product of acetone. Also, hydroperoxides and peroxides can be formed by autoxidation of olefinic hydrocarbons in cigarette smoke. The search for, and identification of, environmental carcinogens of these types will become worth while after their biological activity has been examined more extensively.

The carcinogenicity of hydroperoxides, peroxides, and epoxides known to be present (25, 26) in autoxidized fats and oils are of obvious importance because of extensive use of such materials for human consumption.

Stereochemical considerations.—Some metabolites of aromatic hydrocarbons have been isolated in optically active forms (27, 28). But the stereochemistry of carcinogens has received little or no attention, though in some instances mixtures of stereoisomers were subjected to biological testing (3). The biological testing of pure isomers thus becomes of considerable importance, *e.g.*, the *racemic*- and *meso*-1,2,3,4-diepoxybutanes, V and VI.

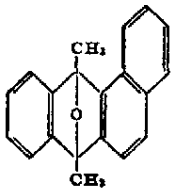
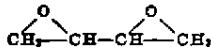
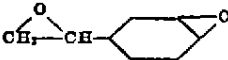
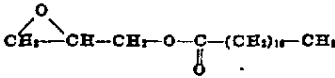
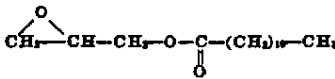
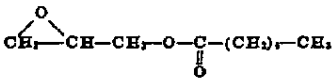
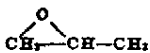
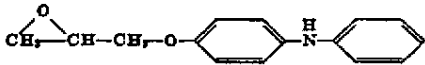


Biological testing.—Tumors were produced (10, 29) in rats, mice, and hamsters with bifunctional aliphatic and aromatic nitrogen mustards, *e.g.*, VII and VIII.



Walpole and co-workers (3, 13, 30) showed that some bifunctional epoxides exhibiting radiomimetic properties, like the nitrogen mustards, are also carcinogenic. Since then, several other compounds of these and related types have shown carcinogenic activity. These experiments and the results on other known carcinogenic epoxides, lactones, and peroxy compounds are listed in tables 1 and 2.

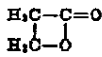
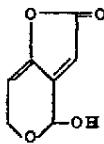
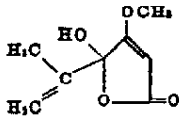
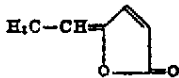
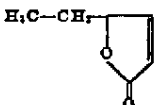
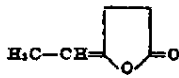
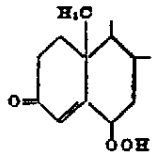
TABLE 1.—Known carcinogenic epoxides

Compound	Method of testing	References
7,12-Dimethyl-7,12-epoxybenz[a]anthracene, X	Mouse skin	(31)
		
1,2,3,4-Diepoxybutane, XI	Rats, intraperitoneal injection	(3)
		
1-Ethylenoxy-3,4-epoxycyclohexane, XII	Rats and mice, skin and intraperitoneal injection	(3, 20)
		
1,2-Epoxy-3-stearoyloxypropane, XIII	Rats, subcutaneous injection	(13)
		
1,2-Epoxy-3-lauroyloxypropane, XIV	Rats, subcutaneous injection	(13)
		
1,2-Epoxy-3-caproyloxypropane, XV	Rats, subcutaneous injection	(13)
		
1,2-Epoxypropane, XVI	Rats, subcutaneous injection	(13)
		
p-Hydroxydiphenylamineglycidyl ether, XVII	Rats, subcutaneous injection	(13)
		

Dickens and Jones (7) reported a low and possibly questionable order of carcinogenicity for α -carboxy- β -phenyl- β -propiolactone, α,α -diphenyl- β -propiolactone, and S-2-carboxyethyl-L-cysteine. These materials were tested subcutaneously in rats. Hine and co-workers (33) produced sarcomas in rats and skin cancer in mice with certain epoxy resins.

The following materials were tested by various methods and gave negative results: 1,2-epoxy-3-oleyloxypropane (13); 1,2-epoxy-3-(2,4-dinitrophenyloxy)propane (13); ethylene oxide (13); γ -valerolactone (34); 2,4-cholestadiene peroxide (35); and 7,12-dimethyl-7,12-epoxybenz[a]anthracene (36). Biological testing was in some instances carried out by

TABLE 2.—Known carcinogenic lactones and hydroperoxides

Compound	Method of testing	Reference
δ -Propiolactone, XVIII 	Mouse skin Rats, subcutaneous injection	(1, 2, 38) (7)
Patulin, XIX 	Rats, subcutaneous injection	(7)
Penicillic acid, XX 	Rats, subcutaneous injection	(7)
Methyl protoanemonin, XXI 	Rats, subcutaneous injection	(7)
4-Hex-2-enolactone, XXII 	Rats, subcutaneous injection	(7)
4-Hex-4-enolactone, XXIII 	Rats, subcutaneous injection	(7)
66-Hydroperoxy- Δ^4 -cholestene-3-one, XXIV 	Mice, subcutaneous injection	(8)

subcutaneous injection in arachis oil solutions in rats. Walpole (13) points out that this method is unsatisfactory because arachis oil itself gave rise to late tumors in rats by subcutaneous injection. Walpole also observed that impure preparations of 1-ethylenoxy-3,4-epoxycyclohexane gave higher tumor yields than purified preparations (13).

The paucity of information that could serve to establish relationships between structure and carcinogenicity indicated the need for biological

testing of pure compounds and, where necessary, of known stereochemical configuration.

In the present work we attempted to select for biological testing, from available information, a variety of compounds of the types under investigation. Commercially available compounds were subjected to careful purification, and purity was checked by the usual procedures including infrared spectroscopy; volatile materials were also checked by vapor phase chromatography. Compounds not commercially available were synthesized in this laboratory. The results obtained in the initial phases of this work are presented here.

MATERIALS AND METHODS

Preparation and Purification of Compounds for Biological Testing

Hexapoxysqualene.—The synthesis, purification, and characterization have been described elsewhere (37).

9,10-Epoxystearic acid.—Oleic acid was oxidized with perbenzoic acid (38) and purified by crystallization from acetone, mp 59° [reported mp 59.5–59.8 (38)].

9,10,12,13-Diepoxystearic acid.—Linoleic acid was oxidized with peracetic acid (39) and purified by crystallization from acetone, mp 77° [reported mp 78° (39)].

Styrene oxide.—Commercial quality (Matheson, Coleman & Bell, East Rutherford, N.J.) styrene oxide was purified by vacuum distillation, bp 105°/1 mm [reported bp 87–88°/23 mm; n_D^{25} 1.5319, reported n_D^{25} 1.5331 (40)]. The homogeneity of the material was determined by vapor phase chromatography; only one peak was observed with a 2 m polypropylene glycol column at 175° and 20 psi; retention time 13.6 minutes.

1-Ethylenoxy-3,4-epoxycyclohexane.—Commercial quality (K & K Laboratories, Jamaica, N.Y.) material was purified by vacuum distillation, bp 134 to 135°/19 mm [reported bp 110–113°/20 mm (41)], n_D^{25} 1.4747. The vapor phase chromatogram on a 2 m silicone column, 130°, 5 psi, showed a peak with retention time 10.5 minutes. Two minor impurities, retention time 9.0 and 11.9 minutes, could not be removed by redistillation.

1,2-Epoxybutene-3.—Commercial quality (K & K Laboratories) 1,2-epoxybutene-3 was purified by distillation, bp 63.5 to 64.0° at atmospheric pressure [reported bp 65–65.8°/739 mm (42)]. The vapor phase chromatogram on a 2 m polypropyleneglycol column, 100°, 20 psi, showed one peak only, retention time 2.4 minutes.

dl-1,2,3,4-Dieporybutane.—*cis*-2-Butene-1,4-diol (General Aniline and Film Corp., New York, N.Y.), purified by chromatography on acid-washed alumina followed by distillation, was brominated (43) to give 1,4-dihydroxy-2,3-dibromobutane; this product was purified by crystallization from benzene, mp 87.5 to 88° [reported mp 87° (44)], yield 74 percent. This compound was converted to the epoxide by treatment

with potassium hydroxide in ether (45) to give the *dl*-epoxide, yield 75 percent. The product was purified by chromatography on activated alumina and eluted with petroleum ether (bp 30–60°)-ether (1:1) to remove halogen-containing impurities. The epoxide was distilled, bp 144°/760 mm, n_D^{25} 1.4289 [reported bp 140°/760 mm (46)]. The vapor phase chromatogram on a 2 m polypropyleneglycol column, 125°, 20 psi gave one peak only, retention time 14.0 minutes. Infrared absorption (liquid film): 3070, 3010, 2942 (C–H), 1255, 1245 (doublet), 917, and 840 cm^{-1} (). Bands at 1449, 1198, 970, and 780 cm^{-1} were characteristic of

this isomer and were absent in the infrared spectrum of the *meso*-isomer.

To confirm the identification of the *dl*-epoxide, *dl*-1,4-dipiperidino-2,3-dihydroxybutane hydrochloride was prepared from it (45), mp 263.5 to 264.5° [reported mp 258–259° (45)].

Analysis: Calculated for $\text{C}_{14}\text{H}_{25}\text{Cl}_2\text{N}_2\text{O}_2$: C, 51.11; H, 9.19. Found: C, 51.03; H, 9.15.

The free base was liberated from the hydrochloride and crystallized from ether, mp 62 to 63° [reported mp 62° (45)].

meso-1,2,3,4-Diepoxybutane.—Erythritol was converted to *meso*-1,4-dibromo-2,3-dihydroxybutane by treatment with 47 percent hydrobromic acid; the dibromide was crystallized from chloroform, mp 137°, yield 10 percent. The dibromide was converted to *meso*-1,2,3,4-diepoxybutane by treatment with potassium hydroxide in ether (45); yield 65 percent, bp 140°/760 mm, n_D^{25} 1.4272 [reported bp 37°/11 mm (47)]. The purity of the material was established by vapor phase chromatography. It showed, under the conditions specified for the *dl*-isomer, only one peak with a retention time of 12.7 minutes. Infrared absorption (liquid film): 3070, 3005, 2930 (C–H), 1260, 1250 (doublet), 915, and 845 cm^{-1} (.

Bands at 1030, 1000, and 731 cm^{-1} were characteristic of this isomer and were absent in the infrared spectrum of the *dl*-isomer.

The *meso*-diepoxy was also prepared, in higher over-all yield, from butadiene via *trans*-1,4-dibromo-2-butene (48), *trans*-1,4-diacetoxy-2-butene (47), *trans*-1,4-dihydroxy-2-butene (47), and *meso*-1,4-dihydroxy-2,3-dibromobutane (47).

The derivative *meso*-1,4-dipiperidino-2,3-dihydroxybutane was prepared as previously described (45), mp 108° [reported mp 106° (45)].

Analysis: Calculated for $\text{C}_{14}\text{H}_{25}\text{N}_2\text{O}_2$: C, 65.68; H, 11.03. Found: C, 65.38; H, 10.97.

Diketene.—Commercial quality diketene (K & K Laboratories) was purified by distillation, bp 126°/760 mm [reported bp 125–128°/760 mm (49)]. The vapor phase chromatogram on a 2 m silicone column, 40°, 2.9 psi, showed a single peak with a retention time of 9.7 minutes.

γ -Butyrolactone.—Commercial quality γ -butyrolactone (K & K Laboratories) was purified by vacuum distillation, bp 37°/1.0 mm [reported bp 89°/12 mm (50); n_D^{25} 1.4328, reported n_D^{25} 1.4348 (51)]. The vapor phase chromatogram on a 2 m polypropyleneglycol column, 175°, 20 psi, showed a single peak with a retention time of 9.6 minutes.

1-Vinylcyclohexene-3.—Commercial quality (K & K Laboratories) material was purified by removal of autoxidation products with aqueous ferrous sulfate followed by distillation in a nitrogen atmosphere, bp 128°/760 mm, n_D^{25} 1.4593 [reported n_D^{25} 1.4629 (52)]. The material gave one peak in the vapor phase chromatogram, 2 m silicone column, 100°, 5 psi, retention time 3.17 minutes.

1-Hydroperoxy-1-vinylcyclohexene-3.—This material was prepared by the previously described procedure (53) as a 0.5 percent solution in 1-vinylcyclohexene-3.

Lauroyl peroxide.—Commercial quality (Thompson Chemical Co., Pawtucket, R.I.) material was purified by repeated crystallization from chloroform-petroleum ether (bp 30 to 60°) at 4°, mp 49° [reported mp 49° (54)].

Benzoyl peroxide.—Commercial quality (Matheson, Coleman & Bell) material was purified by repeated crystallization from chloroform-petroleum ether (bp 30 to 60°) at 4°, mp 106.6 to 107.6° [reported mp 104–106° (55)].

Solvents.—Reagent grade benzene and acetone were used as solvents.

Biological testing methods.—Male Swiss-Millerton mice, 30 to a group, were used. The animals were approximately 8 weeks old at the beginning of the experiments and were painted 3 times weekly with an artist's watercolor brush, which delivered approximately 100 mg of solution per application. The few exceptions to these conditions are noted in tables 3 and 4. The entire backs of the animals were painted and the hair clipped when necessary. The concentrations and solvents used were determined by the toxicity and solubility of the materials. Four types of control groups were included in the test: 1) groups that received 3 paintings per week of 100 mg of benzene only; 2) groups that received 3 paintings per week of 100 mg of acetone only; 3) positive control groups that received 100 mg of a benzo[*a*]pyrene solution in benzene or acetone containing 100 ppm (0.01%) of the carcinogen; 4) groups receiving no treatment. All tumors were excised at death and confirmed microscopically.

RESULTS

The tumor responses obtained with 14 compounds are given in tables 3 and 4. The median survival time shown in the tables gives an indication of longevity of the group. The total tumor and malignant tumor indexes are values for relative tumorigenic activity defined as 10,000 times the reciprocals of the computed time in days to produce tumors in 50 percent of the mice. The nominal or computed times to produce tumors in 50 percent of the mice were calculated by a "life-table" analysis which takes into account both the incidence of tumors and survival of the mice; this procedure is explained in an earlier publication (1). As an example, if the computed time to the presence of tumors, benign or malignant, in

TABLE 3.—Mouse skin painted with epoxides*

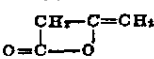
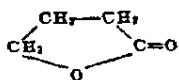
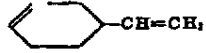
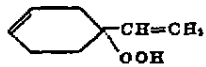
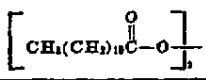
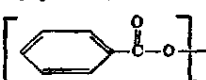
Material	Concentration and solvent	Median survival time	Cumulative No. of mice		Tumor index†	
			Tumor	Cancer	Total	Malignant
Hexapoxysqualene, XXV	1% Acetone	253	1	0	<10	<10
9,10-Epoxystearic acid, XXVI	1% Acetone	165	3	0	23	<10
9,10,12,13-Diepoxystearic acid, XXVII	1% Acetone	196	4	0	20	<10
Styrene oxide, XXVIII	10% Benzene	431	3	1	14	<10
1-Ethyleneoxy-3,4-epoxycyclohexane, XXIX	10% Benzene	325	14	9	24	22
1,2-Epoxybutane-3, XXX	No solvent	237	3	1	10	<10
dl-1,2,3,4-Diepoxypentane, V	10% Acetone	78	2	1	26	16
meso-1,2,3,4-Diepoxypentane, VI	10% Acetone	154	6	4	29	23

*Thirty male Swiss-Miller mice painted 3 times weekly, 100 mg per painting.
†Calculated from estimated time for total tumor induction or/and cancer induction in 50 percent of animals adjusted for observed survival (see text).

50 percent of the mice were 100 days and cancers in 50 percent of the mice were 200 days, this substance would have a "total tumor index" of 100 and a "malignant tumor index" of 50. Thus with substances of low tumorigenic activity, the computed time to 50 percent incidence will be longer than the lifetime of the mice.

At the concentration levels of 1-vinylcyclohexene-3 and its hydroperoxide used there was extensive skin damage and slight to moderate skin damage with lauroyl peroxide, *dl*-1,2,3,4-diepoxypentane, and styrene oxide, but none with any of the other compounds. Both *dl*- and *meso*-1,2,3,4-diepoxypentane showed pronounced toxicity. The notable toxicity of these materials in acetone has been reported by earlier workers (3, 13) and the high mortality may account for the negative carcinogenicity re-

TABLE 4.—Mouse skin painted with lactones, hydroperoxides, and peroxides*

Material	Concentration and solvent	Median survival time	Cumulative No. of mice		Tumor index†	
			Tumor	Cancer	Total	Malignant
LACTONES						
Diketene, ‡ XXXI 	10% Acetone	561	0	0	<10	<10
γ-Butyrolactone, XXXII 	10% Benzene	292	2	1	<10	<10
HYDROPEROXIDES						
1-Vinylcyclohexene-3 (control), § XXXIII 	50% Benzene	875	6	1	13	10
1-Hydroperoxy-1-vinylcyclohexene-3, XXXIV Same	0.5% Benzene-1-vinylcyclohexene-3 (1:1) Same	165 403	8 3	2 2	28 17	15 <10
						
PEROXIDES						
Lauroyl peroxide, XXXV 	5% Benzene	437	1	0	<10	<10
Benzoyl peroxide, XXXVI 	5% Benzene	292	1	0	<10	<10

*Thirty male Swiss-Müller mice painted 3 times weekly, 100 mg per painting, except where otherwise indicated.

†Calculated from estimated time for total tumor induction and/or cancer induction in 50 percent of animals adjusted for observed survival (see text).

‡Fifty CAF₁, Jackson Laboratory female mice.

§Forty-five mg per painting.

|| Nineteen animals.

sults obtained in the mouse skin experiments by Hendry and co-workers (3). Moreover, as 10 percent solutions in benzene the materials were very toxic, and a 50 percent or higher mortality resulted within hours of painting. But 10 percent solutions in acetone were less toxic; in a group of 30 animals receiving a 10 percent solution of the *dl*-isomer 3 times weekly the median survival time was 78 days.

The results obtained in the control experiments are shown in table 5.

DISCUSSION

During the period in which the compounds listed in tables 3 and 4 were tested, an extensive series of control groups were maintained. The results of these experiments (table 5) merit discussion. Of the 150 ani-

TABLE 5.—Painting of mouse skin, controls

Treatment †	Number of animals ‡	Median survival time	Cumulative No. of mice		Tumor index*	
			Tumor	Cancer	Total	Malignant
Benzene	30	264	2	0	10	<10
	30	262	5	0	19	<10
	30	412	2	0	<10	<10
	60	292	2	1§	<10	<10
Acetone	30	240	2	0	10	<10
	30	652	0	0	<10	<10
	30	330	4	0	14	<10
	30	134	2	0	18	<10
Benzo[a]pyrene, 100 ppm, acetone	30	211	16	7	50	32
	30	378	24	20	34	29
	30	240	25	11	45	27
	30	259	18	11	45	36
Benzo[a]pyrene, 100 ppm, benzene	30	351	16	7	42	27
	30	348	10	6	24	21
	30	370	23	13	36	26
	30	175	2	1§	14	<10
No treatment	30	342	0	0	<10	<10
	30	730	0	0	<10	<10
	30	624	0	0	<10	<10
	30	217	5	0	20	<10
	28	112	1	0	<10	<10
	29	253	4	0	<10	<10
	60	345	1	0	<10	<10

* Calculated from estimated time for total tumor induction and cancer induction in 50 percent of animals adjusted for observed survival (see text).
† Three times weekly, 100 mg per painting.
‡ Male, Swiss-Müllerton mice, except where indicated otherwise.
§ See text.
|| Jackson Laboratory, Bar Harbor, Maine, female CAF₁ mice.

mals treated with benzene, 6.7 percent developed tumors. One of 11 tumors¹ was a cancer; the remainder were papillomas. The cancer appeared on the left front leg, remote from the site of application, in an animal from a group in which much biting occurred. The tumor appeared in the area of the bites. The 120 acetone-control animals showed a 6 percent incidence of papillomas and no cancers. In the 3 positive control groups, in which benzo[a]pyrene with benzene as solvent was used, 49 of 90 animals developed tumors—26 were cancers. In the 4 positive control groups in which acetone was used as solvent, 83 of 120 animals developed tumors—49 were cancers.

It should be noted that there were considerable variations in gross tumor incidence and survival time for the positive controls. Nevertheless the tumor indexes, both total and malignant, showed good agreement, e.g., in the benzopyrene (100 ppm, benzene) group the number of animals with cancer ranged from 6 to 13 in groups of 30 animals. The malignant tumor indexes in these groups were 21 to 27.

In the untreated control series 267 animals were used; five percent developed tumors, 1 of which was a squamous cell cancer. The median survival time ranged from 112 to 643 days.

¹ In all these experiments the papillomas were of the benign squamous cell type and the cancers were predominantly squamous cell carcinomas. In this "Discussion," tumors refer to total tumors, i.e., including benign papillomas and squamous cell cancers. Many animals bore multiple tumors.

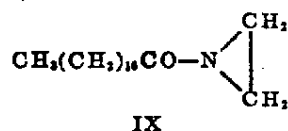
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During part of the test period ectromelia was present in the colony, in experimental and control groups. Subsequently all mice were successfully vaccinated against this disease.

During the past year the background incidence of tumors in the untreated control groups in which Swiss-Millerton male mice were used was 1.4 percent. These tumors were all squamous papillomas. Tumors were not observed, however, in the Jackson Laboratory CAF₁ control female mice nor in 1 experimental group painted with diketene (table 4). A 2 percent benign skin-tumor incidence was reported by Jackson Laboratories for the similar strain C3H mice (56).

In the present work 1-ethyleneoxy-3,4-epoxycyclohexane exhibited pronounced activity, and this result confirms the findings of Walpole (13). Tests on the two isomers of 1,2,3,4-diepoxybutane were unsatisfactory because of poor survival of the mice. One cancer was observed in the group receiving *dl*-isomer. Only 4 animals survived for longer than 8 months and after 12 months there were no survivors. In the group receiving *meso*-isomer 10 animals survived more than 8 months; 4 cancers were observed. New tests are currently under way with these 2 compounds.

Included in the series of epoxides tested in the present work are 3 monofunctional epoxides, 1 of which, 1,2-epoxybutene-3, showed a low order of activity. Five diepoxy compounds and hexaepoxysqualene were examined and 3 of these 6 showed activity. From these and earlier results (3, 13, 30) it appears that a vicinal diepoxide structure, such as occurs in the isomers of diepoxybutane, is not required for carcinogenicity in this series; in the most potent compound, 1-ethyleneoxy-3,4-epoxycyclohexane, the two epoxide functions are separated from each other by ring carbon atoms. It does appear, however, that specific structural features in addition to one or more epoxide functions are required for carcinogenicity, and this structural specificity may be associated with physical properties as well as chemical reactivity. Although the present work has been restricted to date to oxygenated functions, carcinogenicity has been observed earlier in the related ethyleneimines (13), *e.g.*, steaoylethyleneimine, IX,



In this series, also, bifunctionality was not a prerequisite for carcinogenicity.

From the results obtained in this work and those summarized in tables 1 and 2, it can be concluded tentatively that strained lactone rings may favor carcinogenicity. γ -Butyrolactone, the most stable lactone in the series, has a low order of activity. Dickens and Jones (7) found no activity for this material by subcutaneous injection in rats. In the strained ring systems, β -propiolactone and the unsaturated 5-membered-ring lactones

(table 1), more pronounced activity is observed. Reactivity and ring strain obviously cannot be the only requirements for carcinogenicity, as evidenced by the complete lack of activity observed for diketene, a highly reactive compound.

Fieser's reported carcinogenicity for 6-hydroperoxy- Δ^4 -cholestene-3-one (5) prompted an examination of other hydroperoxides. The hydroperoxide of butadiene dimer (1-vinylcyclohexene-3) showed definite carcinogenic activity in 2 experiments. The hydrocarbon itself, in which the hydroperoxide was dissolved, gave 1 cancer on prolonged painting. The possibility was not excluded that the hydrocarbon contained a minute amount of the hydroperoxide formed by autoxidation, especially since the hydrocarbon is readily autoxidized in air (53). This possibility is currently being examined in a new test that utilizes the hydrocarbon freed from autoxidation products by treatment with ferrous sulfate before use.

Neither of the 2 peroxides showed carcinogenic activity; 2,4-cholestadiene peroxide (55) and 7,12-dimethyl-7,12-peroxybenz[*a*]anthracene, (51, 56) also did not show any activity when tested subcutaneously in mice. But the corresponding epoxide 7,12-dimethyl-7,12-epoxybenz[*a*]anthracene did elicit skin tumors in mice [(51), table 1].

The present work has been extended to include many structures containing the functional groups of interest. These results may facilitate a re-examination of the structural types of importance and a more detailed discussion is deferred until then.

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Cancer, mice

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Carcinogenicity of Epoxides, Lactones, and Peroxy Compounds. IV. Tumor Response in Epithelial and Connective Tissue in Mice and Rats^{1,2}

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SUMMARY—The carcinogenic potencies of a series of alkylating and peroxy compounds were compared by the use of various routes of administration in mice and rats. Twelve of these compounds, earlier tested on mouse skin, were tested by one or more of the following routes: subcutaneous injection in mice and rats and intragastric feeding in rats. These experiments showed that those compounds tumorigenic to mouse skin are also active by other routes. These compounds are: β -propiolactone, β -butyrolactone, *d,l*-diepoxybutane, glycidaldehyde, and 1,2,5,6-diepoxyhexane. Those compounds inactive on mouse skin are weakly active or inactive by other routes. These compounds are: cumene hydroperoxide, 9,10-epoxystearic acid, diketene, 2,2,4-trimethylhydroxypentenoic acid- β -lactone, cyclohexenehydroperoxide, and lauroyl peroxide. β -Propiolactone and β -butyrolactone are mouse-skin carcinogens and induce gastric cancers when fed repeatedly to rats; however, 3 epoxides carcinogenic to mouse skin (glycidaldehyde, *d,l*-diepoxybutane, and 1-ethyleneoxy-3,4-epoxycyclohexane) do not induce gastric cancers in rats. This finding is related to the rapid acid-catalyzed hydrolysis of epoxides in the rat stomach. The compounds tested induced tumors mainly at the site of application and did not give any significant incidence of tumors at sites distant from the site of application. These experiments indicate that connective tissue of rats is not unusually sensitive to tumor induction by these compounds and that subcutaneous injection in the rat is a useful system for the evaluation of potential carcinogens.—*J Nat Cancer Inst* 37: 825-838, 1966.

CARCINOGENIC ALKYLATING agents of the epoxide and β -lactone types have a low order of potency when compared to the classical aromatic hydrocarbon and heterocyclic carcinogens, and tumorigenic dosages of these alkylating agents are frequently close to their toxic dosages (1, 2). With a few exceptions, the assay of these compounds has

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been carried out by skin application in mice, and species specificity and tissue specificity have not been explored. Also, tumors distant from the site of application have usually not been observed.

In view of our continuing interest in the carcinogenicity and mechanism of action of epoxides, lactones, and peroxy compounds, a selected group of materials, including some that are carcinogenic and others that are inactive on mouse skin, were tested. The compounds were tested by subcutaneous injection in mice and rats at various dosages and by single and repeated intragastric feeding in rats. The results of these tests are described in the present report and are compared with earlier results dealing with bioassay of these materials by skin application in mice and other routes of administration in mice and rats. The usefulness of these various bioassay procedures is discussed here, particularly since considerable discussion has been related to the reliability of subcutaneous injection, especially in rats, for bioassay of potential carcinogens.

MATERIALS AND METHODS

Chemicals.—The preparation and purification of all compounds used for bioassay in this study were described in detail in earlier papers in this series (1, 2).

Animals.—The rats were female Eastern Sprague-Dawley (Blue Spruce Farms, Altamont, N.Y.); they were at least 6 weeks old and weighed 120–125 g when tested. The mice were of an ICR/Ha Swiss strain (Millerton Research Farms, Millerton, N.Y.); 8-week-old females were used throughout. All mice were vaccinated against ectromelia at age 6 weeks. Mice and rats were fed Purina Laboratory Chow and water *ad libitum*. The animal rooms were maintained at 22–24.5 C. All compounds were in solution in tricaprylin (Eastman Kodak Company, Rochester, N.Y., except where noted in tables). Where there were solubility limitations, solutions were warmed to 37 C; otherwise, they were administered at room temperature. Animals were given subcutaneous injections once weekly in the left axillary area; a $\frac{3}{8}$ -inch, 26-gauge needle was used for injections in mice and a $\frac{1}{2}$ -inch, 23-gauge needle for rats. The compounds were dissolved in 0.05 ml tricaprylin for mice and 0.1 ml

for rats. Materials for gastric intubation in rats were in solution in tricaprylin and administered with a #8 rubber catheter and syringe. Some materials were tested by single intubations and others by repeated weekly intubations. Animals were examined for palpable tumors at regular intervals, and their condition was recorded once a month. They were treated and observed for their lifespan, or until the predetermined end of the experiment as noted under Results; those having massive tumors were killed. Whenever possible, animals were completely autopsied at death and their tumors excised. All diagnoses were confirmed histologically. Control groups included animals treated with tricaprylin only, as well as groups receiving no treatment.

RESULTS

Tables 1, 2, and 3 show the results of a series of tests in which mice and rats received subcutaneous injections, at various dosages and intragastric feeding, of 12 compounds earlier tested on mouse skin (1, 2). The duration of all experiments is indicated in the tables; the times to first tumor appearance are given in table 1. These are the approximate times to the appearance of palpable masses.

Subcutaneous Injection in Mice

In the first series of experiments (table 1), mice, 50 to a group, were given weekly subcutaneous injections of 0.1 mg of the compounds. The median cumulative dose per animal ranged between 6.5 and 7.8 mg and the median survival with most test compounds was 15 months or more. A few benign tumors were observed. Animals treated with tricaprylin only bore local injection granulomas.

The tumor response of the mice to the carcinogen was low in most cases. *d,l*-Diepoxybutane was the most active and its injection resulted in 5 animals with fibrosarcomas and 2 with adenocarcinomas at the site of injection. Lauroyl peroxide showed some activity, causing 3 local fibrosarcomas. β -Butyrolactone induced 4 local malignant tumors (2 fibrosarcomas and 2 anaplastic sarcomas). All other compounds in this group induced 1–3 local malignant tumors in each group.

TABLE 1.—Subcutaneous injection in mice

Compound	Dose, mg in 0.05 ml tricaprylin	Number of animals	Median survival time (days)	Duration of test (days)	Months to first tumor	Animals with tumors at injection site	
						Benign	Malignant*
4,4'-Diepoxylutane	0.1	50	436	589	12	None	5 Fibrosarcomas 2 Adenocarcinomas
	1.1	30	328	401	8	None	5 Fibrosarcomas
1,2,5,6-Diepoxyhexane	0.1	50	534	590	18	None	1 Fibrosarcoma 1 Anaplastic sarcoma
	1.1	30	287	533	16	None	2 Fibrosarcomas 1 Squamous cell carcinoma
9,10-Epoxyoctadecanoic acid	0.1	50	325	590	—	None	None
	10	30	301	530	—	None	None
Glycidaldehyde	0.1	50	593	595	16	None	2 Fibrosarcomas 1 Squamous cell carcinoma
	3.3	30	472	536	16	1 Papillary tumor	3 Fibrosarcomas 1 Squamous cell carcinoma 1 Undifferentiated sarcoma 2 Adenocarcinomas
β -Butyrolactone	0.1	50	483	595	14	None	2 Anaplastic sarcomas 2 Fibrosarcomas
	10	30	265	490	8	None	15 Fibrosarcomas 2 Squamous cell carcinomas 1 Adenocarcinoma
2,2,4-Trimethylhydroxy- pentenoic acid- β -lactone	10	30	474	534	—	None	None
Lauroyl peroxide	0.1	50	324	375	12	None	3 Fibrosarcomas
	10 (0.1 ml vehicle)	30	331	333	—	None	None
Cyclohexanediylperoxide	0.1	50	573	580	—	None	None
	3.3	30	376	533	16	None	1 Fibrosarcoma
β -Propiolactone	0.73	30	279	505	7	3 Squamous papillomas	9 Fibrosarcomas 3 Adenocarcinomas 6 Squamous cell carcinomas
Cumene hydroperoxide	3.3	30	472	533	16	None	1 Fibrosarcoma
Diketene	1.1	30	383	540	—	None	None
7,12-Dimethylbenz[a] 4-anthracene	0.1	50	141	158	4	None	28 Fibrosarcomas 4 Squamous cell carcinomas
	0.01	30	184	251	6	None	20 Fibrosarcomas† 1 Squamous carcinoma
	0.001	30	332	530	12	None	4 Fibrosarcomas 4 Undifferentiated sarcomas 1 Reticulum cell sarcoma
Tricaprylin	0.05 ml	50	535	551	—	None	None
	0.05 ml	30	368	534	—	None	None
	0.05 ml	30	484	532	—	None	None
No treatment	—	50	415	519	—	None	None
No treatment	—	30	431	599	—	None	None

*All adenocarcinomas were of breast origin.

†One animal had metastasis to the liver (fig. 6).

Some of these same compounds were subsequently tested in mice at doses from 10–100 times those used in the first series. These dosages were as high as possible and were limited only by toxicity and skin effects. In this second series, also given in table 1, 30 mice per group received from 1.1–10 mg/injection; the exact doses are given in table 1. Tumorigenic activity increased at higher dosages with most of the compounds which showed activity at lower dosages. Several compounds which were weakly active or inactive at lower dosages were markedly active at higher

dosages. For example, β -butyrolactone administered at 0.1 mg/injection resulted in an 8% tumor response, but at the 10 mg/injection level exhibited a 60% incidence of local malignant tumors. Similarly, glycidaldehyde, which gave a 6% tumor response at the 0.1 mg/injection level, showed a 23% incidence of local tumors at the 3.3 mg/injection level. A local squamous cell carcinoma from this group is shown in figure 1.

In the lauroyl peroxide-treated group, 3 of 50 animals had malignant tumors at a low dose, but no tumors were induced at the higher dose

level, possibly due to the toxicity and hence lower survival at this dosage, as shown in table 1.

With β -propiolactone, 3.3 mg/animal was first used but severe chronic skin damage at the site of injection necessitated a decreased dose. A satisfactory dose was obtained at 0.73 mg/mouse/injection. At this level 18 local malignant tumors were induced.

Cumene hydroperoxide induced 1 fibrosarcoma at the injection site; cyclohexenehydroperoxide, diketene, 2,2,4-trimethylhydroxypentenoic acid- β -lactone, and the vehicle control group did not cause any tumors, benign or malignant, at the injection site.

As a positive control experiment, 7,12-dimethylbenz[*a*]anthracene (DMBA) was subcutaneously injected in mice and tested at 3 dosage levels to establish a level which gave a tumor response corresponding to that obtained with the weakly active epoxides, lactones, etc. The results (table 1) indicate that a suitable dose in mice is 1 μ g/injection, once weekly.

Subcutaneous Injection in Rats

In a companion experiment, the same series of compounds were tested at a weekly dose of 1 mg/animal by subcutaneous injection in rats.

The results of these tests are given in table 2. At this level, significant activity was observed in animals receiving *d,l*-diepoxybutane, in which 10 local malignant tumors were induced, with a median cumulative dose of 67 mg. This compound is currently on test at a dose of 2 mg/injection. After 1 year of treatment, 7 of the 20 rats bore malignant tumors; the first tumor was recorded after 10 months of testing.

1,2,5,6-Diepoxyhexane, 2,2,4-trimethylhydroxypentenoic acid- β -lactone, and glycidaldehyde each induced 1 malignant tumor at the injection site whereas 9,10-epoxystearic acid, β -butyrolactone and cyclohexenehydroperoxide did not induce any β -Butyrolactone is currently on test at a dose of 100 mg/injection in 20 rats. At this dose, 7 local malignant tumors, one of which is shown in figure 2, have been confirmed to date. These preliminary data indicate that β -butyrolactone is sarcomagenic at higher dosages in rats. In the nontreatment control group, 1 animal had fibroadenoma of the breast. In the group receiving only tricaprylin, 1 animal had an adenocarcinoma of the breast. Four rats in treatment groups bore local benign tumors, skin fibroadenomas; 3 of the tumor-bearing animals were treated with glycidaldehyde and 1 was treated with 2,2,4-trimethylhydroxypentenoic acid- β -lactone.

TABLE 2.—Subcutaneous injection in rats*

Compound	Median survival time (days)	Duration of test (days)	Animals with malignant tumors at injection site†
<i>d,l</i> -Diepoxybutane.....	471	550	1 Adenocarcinoma 9 Fibrosarcomas
1,2,5,6-Diepoxyhexane.....	>552	552	1 Fibrosarcoma
9,10-Epoxystearic acid.....	>551	551	None
Glycidaldehyde.....	545	558	1 Fibrosarcoma
β -Butyrolactone.....	>559	559	None
2,2,4-Trimethylhydroxypentenoic acid- β -lactone.....	>564	564	1 Adenocarcinoma
Cyclohexenehydroperoxide.....	548	565	None
7,12-Dimethylbenz[<i>a</i>]anthracene.....	>158	158	20 Fibrosarcomas‡ 6 Rhabdomyosarcomas§ 2 Undifferentiated sarcomas 1 Reticulum cell sarcoma 1 Adenocarcinoma 1 Adenocarcinoma
Tricaprylin.....	>565	565	1 Adenocarcinoma
No treatment.....	554	563	None

*Sprague-Dawley female rats, 80 per group, were given weekly injections of 1 mg of compound in 0.1 ml tricaprylin.

†All adenocarcinomas were of breast origin.

‡Two animals had metastasis to the lung; 1 animal had a fibroadenoma also.

§One animal had metastasis to the lung.

TABLE 3.—Gastric feeding in rats*

Compound	Number of animals	Dose, mg/ml, tricapylin	Median survival time (days)	Duration of test (days)	Animals with gastric tumors	Animals with other tumors
β -Propiolactone.....	5	10/0.5, once weekly.	426	487	3 Squamous cell carcinomas of stomach.	None
<i>d,l</i> -Diepoxybutane....	5	5/0.5, once weekly.	342	363	None.....	None
β -Butyrolactone.....	5	100/0.5, once weekly.	426	492	3 Squamous cell carcinomas of stomach†.	None
Glycidaldehyde.....	5	33/0.5, once weekly.	329	492	None.....	None
1-Ethyleneoxy-3,4-epoxycyclohexane.	5	100/0.5, once weekly.	500	538	None.....	None
7,12-Dimethylbenz[<i>a</i>]-anthracene‡.	6	50/1, single treatment.	357	385	None.....	2 Fibroadenomas 2 Adenocarcinomas of breast 1 Lymphoma
Tricaprylin§.....	5	0.5, once weekly...	525	537	None.....	None

*Female Sprague-Dawley rats, age 51-57 days at beginning of treatment, were used except where noted.

†One of these animals also had metastasis to the liver.

‡Animals were 100-125 days old at time of first treatment.

§Obtained from E & K Laboratories, Jamaica, N. Y.

In the results listed in tables 1 and 2, the median survival times were good except in those experiments where high tumor yields or toxicity influenced the survival time.

Intragastric Feeding in Rats

A selected series of compounds were tested in rats by single or repeated gastric feedings. The results of some of these experiments are listed in table 3.

DMBA was included in this series as a positive control; after a single feeding it induced several mammary tumors (table 3) but no stomach tumors.

The other 5 compounds listed in table 3 were tested by repeated gastric feedings of rats beginning at 51-57 days of age, 5 animals per group. Of these, β -propiolactone and β -butyrolactone induced malignant stomach tumors. Of the 5 rats fed β -propiolactone, 3 had squamous carcinomas of the stomach after about 1 year. Of the 5 rats fed β -butyrolactone, 3 developed squamous cell carcinomas of the forestomach with marked basal cell hyperplasia and moderate atypia of the squamous epithelium of the stomach (fig. 3). One of these animals also had isolated metastasis to the liver (fig. 4) which was found when the rat was killed at 385 days. *d,l*-Diepoxybutane, glycidaldehyde,

and 1-ethyleneoxy-3, 4-epoxycyclohexane did not induce tumors in any animals.

Tumors in Control Groups

The incidence of tumors distant from the site of weekly injections of tricapylin in mice and rats is given in table 4. Also included in this table is the tumor incidence in no-treatment control groups for both mice and rats. These are the same control groups listed in detail in tables 1 and 2. Both groups of mice had a substantial number of lymphomas, lung adenomas, and some reticulum cell sarcomas. Several tumors were also noted in the rat control groups. An unusual type of tumor observed in the no-treatment group of rats was an adenocarcinoma of the uterus.

Tumors at Distant Sites in Test Groups

Mice.—In experiments in which mice were given subcutaneous injections, tumors were observed at sites other than those of injection. Various such tumor types were observed, most likely due to the longevity of the test and control groups. In mouse test groups, lymphomas, lung adenomas, and reticulum cell sarcomas of lymph nodes occurred as frequently as in the control groups listed in table 4.

TABLE 4.—Distant tumors in tricapylin-control groups and tumors in no-treatment groups

	Treatment	Duration of test (days)	Number of animals	Number of animals with tumors
Mice.....	Tricaprylin.....	532	110	1 Leukemia 8 Lung adenomas 7 Lymphomas 1 Breast adenosis 2 Reticulum cell sarcomas of lymph nodes.
Mice.....	No treatment.....	519-599	89	8 Lymphomas 3 Lung adenomas 3 Reticulum cell sarcomas of lymph nodes. 1 Malignant hemangiopericytoma
Rats.....	Tricaprylin.....	565	50	1 Reticulum cell sarcoma of lung 1 Lymphoma 1 Fibroadenoma
Rats.....	No treatment.....	563	50	1 Adenocarcinoma of uterus 1 Lymphoma 1 Fibroadenoma

In addition, other tumor types were observed. Thus injection of glycidaldehyde resulted in 1 animal with an adenocarcinoma of the breast; ⁴ in the group given epoxystearic acid, 1 mouse bore an anaplastic sarcoma on the right-side flank; in the group given 2,2,4-trimethylhydroxypentenoic acid- β -lactone, 1 mouse bore a hepatoma and 1 an adenocarcinoma of the breast; in the group given lauroyl peroxide, 1 animal had an intraabdominal sarcoma with metastasis to the pancreas and stomach. The cyclohexenehydroperoxide-treated group included 1 animal with breast tumors, an adenocarcinoma and a fibroadenoma; in the cumene hydroperoxide-treated group, 1 mouse had an adenocarcinoma of the breast.

Rats.—In rats treated subcutaneously with the various compounds listed in table 2, some tumors appeared at sites distant from the application. The tumor types and frequency of incidence were not significantly different from those noticed in untreated and tricapylin control groups (table 4). The occurrence of spontaneous mammary tumors in Sprague-Dawley female rats has been reported earlier (3).

⁴ Breast tumors on the side of injection were classified as local tumors. Breast tumors on the opposite side were included under distant tumors.

DISCUSSION

The malignant tumor incidence in rats and mice treated with a series of epoxides, lactones, hydroperoxides, and peroxides by various routes of administration is compared in table 5 and discussed below.

Comparison of Various Routes of Administration in Mice and Rats

The reliability of testing of potential carcinogens by subcutaneous injection in mice and particularly in rats has been discussed considerably in recent years. Clayson (5), for example, concluded that sarcomas in the rat by themselves cannot be regarded as evidence for the chemical induction of cancer, since substances normally occurring in the body, such as sodium chloride and glucose, have been associated with sarcomas on subcutaneous injection in the rat. The Food Protection Committee of the Food and Nutrition Board, National Academy of Sciences (U.S.), raises the same issue and points out that trauma and regeneration are produced by each injection and may be significant if repeated injections are necessary to produce the sarcomas (6). The same group concluded that this test is of limited

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TABLE 5.—Comparison of number of animals with malignant tumors with the use of different test systems

Compound	Skin application in mice*	Subcutaneous injection in mice†		Subcutaneous injection in rats†	Intragastric feeding in rats†
		Low dose	High dose		
β -Propiolactone.....	11/30†	—	18/30	13/20§	3/5
d,l-Diepoxybutane.....	6/30	7/50	5/30	10/50	0/5
Glycidaldehyde.....	8/30	3/50	7/30	7/20§	0/5
β -Butyrolactone.....	21/30	4/50	18/30	1/50	3/5
				0/50	
				(Low dose)	
				7/20	
				(High dose)§	
1,2,5,6-Diepoxyhexane.....	0/30	2/50	3/30	1/50	—
	(4 Papillomas)				
1-Ethyleneoxy-3,4-epoxycyclohexane....	9/30	—	—	—	0/5
Cyclohexenehydroperoxide.....	1/30	1/50	0/30	0/50	—
Lauroyl peroxide.....	0/30	3/50	0/30	—	0/5
	(1 Papilloma)				
Cumene hydroperoxide.....	0/30	—	1/30	—	—
9,10-Epoxystearic acid.....	0/30	0/50	0/30	0/50	—
	(3 Papillomas)				
2,2,4-Trimethylhydroxypentenoic acid- β -lactone.....	0/30	—	0/30	1/50	—
Diketene.....	0/50	—	0/30	—	—

*References (1) and (2) except where noted.

†This study.

‡Reference (4).

§Tests currently under way.

value and dubious interpretation. The opposite view is held by Hueper and Conway, who regard such subcutaneous tests valid and significant (7). It is therefore important to examine the present findings against this background of conflicting viewpoints. In the tests carried out in this laboratory, a series of compounds of known and constant purity were examined by the various routes under discussion, and adequate solvent and no-treatment controls were included. In these experiments no local benign or malignant tumors were observed in any of the 110 mice of the control groups treated once weekly with tricaprylin. In the group of 100 rats, treated similarly, 1 animal bore an adenocarcinoma of the breast which may be ascribed to the spontaneous background incidence of tumors in rats. These findings suggest that trauma and regeneration associated with the injections were not effective in inducing tumors under the conditions used in our laboratory in either mice or rats, contrary to some of the viewpoints cited above.

A similar conclusion was drawn by Dickens and Jones (8) who also used both mice and rats, although in small groups. In their work the

medium was arachis oil. Walpole *et al.* (9) earlier had observed tumor induction with arachis oil (peanut oil) alone, but the possibility of contamination of arachis oil by the recently discovered aflatoxin cannot be excluded (10). Hieger (11) found that, in control tests with 1,122 C57 mice given subcutaneous injections of a variety of vegetable oils, only 5 animals bore sarcomas.

Sodium chloride, glucose, and related sugars, when given repeatedly at high concentration by subcutaneous injections in mice or rats, induce sarcomas at the injection site (12-14). These experiments are frequently quoted to indicate that the subcutaneous tissue of the rat is too sensitive for carcinogenicity assay. An examination of the results indicates that daily injections with 25% glucose for 250 days resulted in 5 of 18 mice with sarcomas (13). In rats given daily injections of 25% sodium chloride for 150-250 days, 2 of 39 animals bore sarcomas (14). In another experiment using the same regimen with 25% glucose, 11 of 46 rats bore sarcomas (12). These results do not support the contention that rat connective tissue is more sensitive than that of the mouse (6). Further, these data indicate

clearly that repeated injections of hypertonic solutions in mice and rats are sarcomagenic and that this phenomenon may well be one of physical rather than chemical carcinogenesis. This distinction should be made clear when conclusions are drawn about the findings of these workers. The induction of local sarcomas by hypertonic solutions is of interest, and, surprisingly, has not been reexamined since the findings of the Japanese workers almost 30 years ago (12-14).

Of the 12 compounds tested in mice and rats by various routes, 6 induced malignant tumors on mouse skin (1, 2), 8 caused malignant tumors in mice by subcutaneous injection, and 6 induced one or more malignant tumors in rats by subcutaneous injection. The connective tissue of rats, at least the strain used in this work, does not appear to be remarkably sensitive to repeated injections. These animals, however, gave the expected high tumor response in the positive control group treated with DMBA.

When the results of the various test methods are compared for each compound, clearly the various test methods correlate well. Compounds inactive on mouse skin, *viz.*, cumene hydroperoxide, 9,10-epoxystearic acid, diketene, and 2,2,4-trimethyl-3-hydroxypentenoic acid- β -lactone, induced 0-1 tumor by subcutaneous injection into mice and rats. The first 5 compounds listed in table 5 caused tumors on mouse skin (1, 2) and subcutaneous tumors in mice and rats. Of these, 1,2,5,6-diepoxyhexane induced only benign tumors on mouse skin (2). All other tumors obtained with 1,2,5,6-diepoxyhexane and with 4 other compounds (β -propiolactone, *d,l*-diepoxybutane, glycidaldehyde, and β -butyrolactone) were malignant. That malignant skin tumors were not induced by 1,2,5,6-diepoxyhexane on mouse skin may be related to the severe corrosive effects of this compound on skin and to its toxicity.

In the discussion of these results, there is some question as to the validity of a test in which only 1 or 2 of 50 animals bore malignant tumors at the site of application. These tumor responses are below the accepted percentages if one uses a procedure outlined earlier (6). On the other hand, these results should probably not be disregarded in view of the absence of local sarcomas in most control groups treated with tricapylin

only and even in several groups treated with chemicals in tricapylin. Thus the single fibrosarcomas obtained with 1,2,5,6-diepoxyhexane and glycidaldehyde by subcutaneous injection in 50 rats may be significant, since these materials also were tumorigenic in mice by skin application and subcutaneous injection. On the basis of these results, the carcinogenic activity of the 12 compounds tested by various routes can be ordered as follows: moderately active— β -propiolactone, *d,l*-diepoxybutane, β -butyrolactone, and glycidaldehyde; weakly active—1-ethylenoxy-3,4-epoxycyclohexane, 1,2,5,6-diepoxyhexane, cyclohexenehydroperoxide, lauroyl peroxide, 2,2,4-trimethylhydroxypentenoic acid- β -lactone, and cumene hydroperoxide; and inactive—9,10-epoxystearic acid and diketene.

Sites of Tumor Induction

A significant feature of tumor induction with the compounds under discussion is that tumors are usually induced by these compounds at the site of application, whether this be skin, connective tissue, or stomach tissue. Malignant tumors at distant sites were induced in only a few instances. The incidence of local malignant tumors is particularly noticeable with β -propiolactone and β -butyrolactone, both of which are highly reactive chemically; nevertheless, both gave a 60% yield of malignant stomach tumors when fed to rats. By contrast, aromatic hydrocarbons such as 3-methylcholanthrene, when fed repeatedly to rats, did not induce gastric tumors (15); in our experiment a single feeding with DMBA caused 3 mammary tumors in a group of 6 rats, but no stomach tumors. The induction of mammary tumors in rats fed DMBA was described recently by Huggins and Yang (16). Hueper and Conway (7) indicate that carcinogenesis in the stomach is related to the degree of absorption of the chemical by the gastric tissues; metabolic patterns are also significant.

The induction of stomach cancers by the β -lactones is of interest, since *d,l*-diepoxybutane 1-ethylenoxy-3,4-epoxycyclohexane, and glycidaldehyde are carcinogenic by other routes and like the β -lactones, are alkylating agents which are expected to act locally, but they gave no gastric

cancers. This is probably due to the difference in the acid-catalyzed hydrolysis of lactones and epoxides. Ross (17) has shown that the hydrolysis of epoxides is catalyzed by acid or base. The 3 epoxides, *d,l*-diepoxybutane, 1-ethylenoxy-3,4-epoxycyclohexane, and glycidaldehyde, are therefore expected to be hydrolyzed rapidly in the acid pH environment of the rat stomach (18, 19). On the other hand, the hydrolysis of β -propiolactone and β -butyrolactone, although relatively fast, is independent of pH in the range of pH 1-7 (20, 21) so that these 2 materials may remain in the stomach long enough to exert a carcinogenic effect.

Other alkylating agents such as *N*-nitroso-*N*-alkylurethans (22) also induce malignant stomach tumors when fed to rats. The aflatoxins of moldy peanuts are also implicated in the induction of adenocarcinomas of the glandular stomach when fed to rats (23).

Dickens and Jones (8) and Walpole *et al.* (9) reported earlier on sarcoma induction in mice and rats by subcutaneous injection of β -propiolactone; Hendry *et al.* described the carcinogenicity of diepoxybutane (stereochemistry not specified) when injected intraperitoneally in rats (24).

In our study, subcutaneous injections into mice of *d,l*-diepoxybutane, 1,2,5,6-diepoxyhexane, β -propiolactone, and β -butyrolactone all induced squamous cell carcinomas in one or more animals, and fibrosarcomas in larger numbers of animals. This probably results from spreading of material injected subcutaneously into the skin. With β -propiolactone, the ratio of squamous carcinomas to fibrosarcomas was particularly high.

In a recent report from this laboratory, the chemical reactivities of a series of these compounds were compared with their biological activities (25). Further studies are under way on the mode of action, chemical reactivity, and carcinogenic activity of the compound types used in this work.

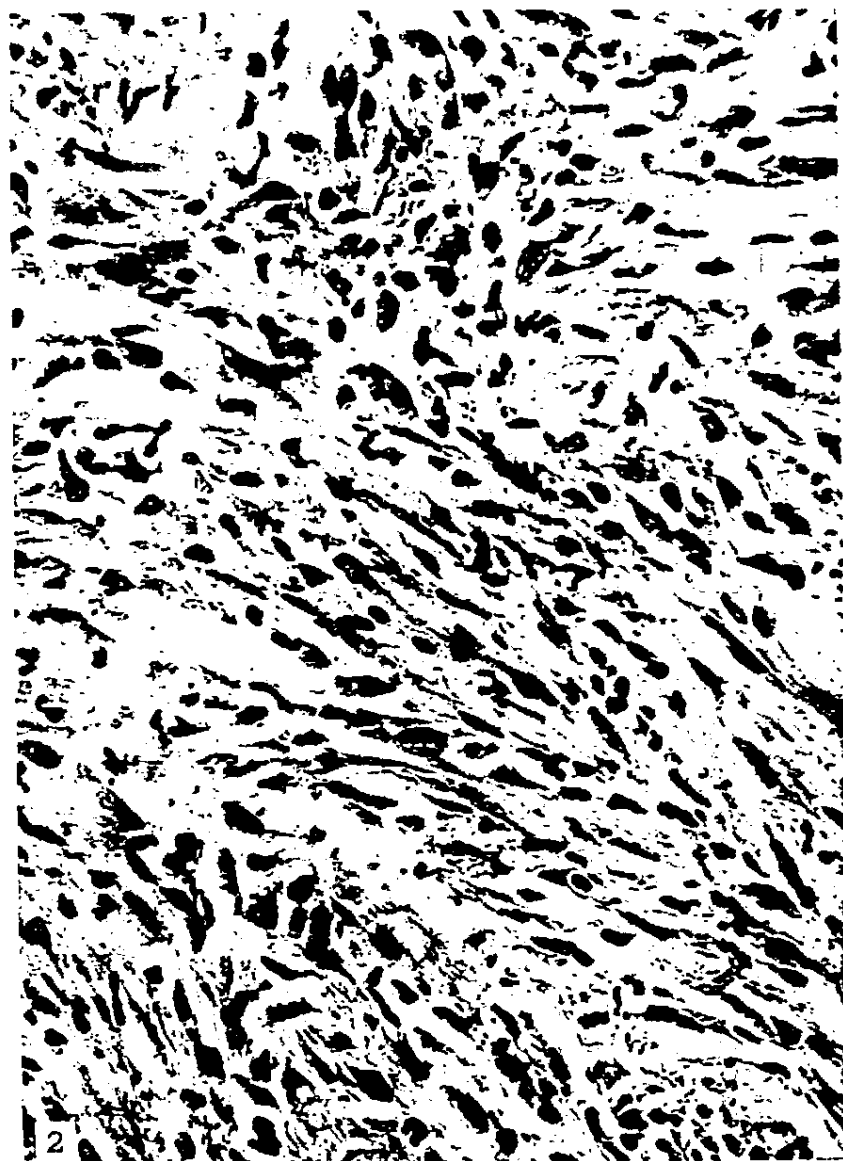
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FIGURE 1.—Squamous cell carcinoma of mouse skin at site of subcutaneous injection of glycidaldehyde given once weekly. $\times 400$



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FIGURE 2.—Fibrosarcoma in a rat at the site of subcutaneous injection of β -butyrolactone given once weekly.
 X 300



FIGURE 3a AND b.—Squamous cell carcinoma of rat stomach after gastric intubation of β -butyrolactone given once weekly. Tumor infiltrating entire thickness of forestomach wall; lumen at *top*. (a) $\times 30$, (b) $\times 400$

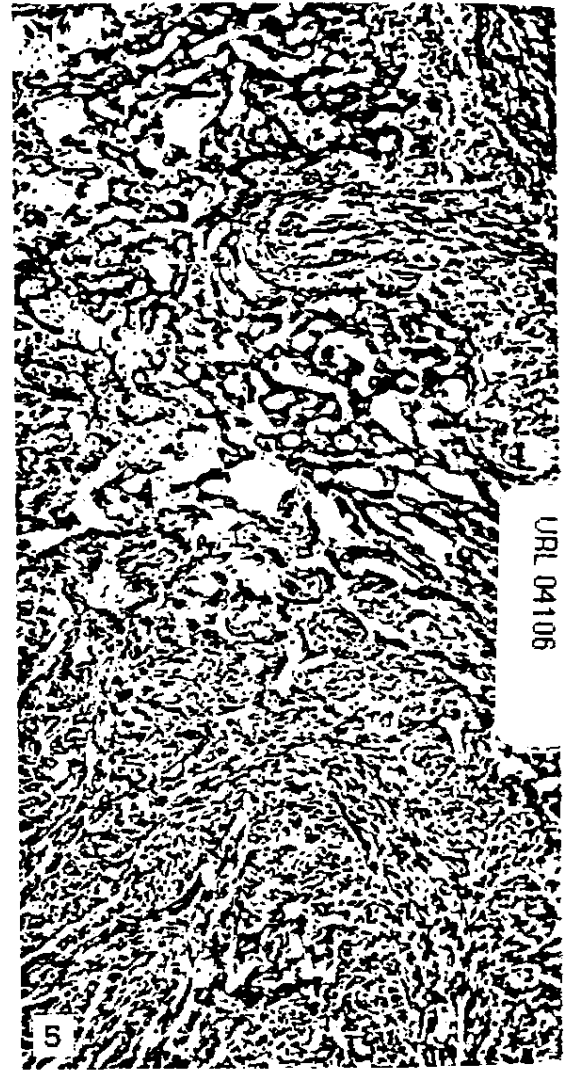


FIGURE 4.—Liver metastasis from squamous cell carcinoma of rat stomach shown in figure 3. $\times 150$
 FIGURE 5.—Metastasis to the liver from a local fibrosarcoma induced by subcutaneous injection of 7,12-dimethylbenz[*a*]anthracene given once weekly. $\times 100$

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RADIATION RESEARCH SUPPLEMENT 3, 193-211 (1963)

Organic Peroxides, Hydrogen Peroxide, Epoxides, and Neoplasia¹

PAUL KOTIN AND HANS L. FALK²

University of Southern California School of Medicine, Los Angeles, California

INTRODUCTION

In the more than the half-century since the recognition of the carcinogenic properties of ionizing radiation (I-R), there have been numerous suggestions as to the mechanisms concerned with the induction of neoplasia. Currently there is no universally accepted concept; however, the physical properties of I-R, particularly in relation to the reactions occurring in both water and tissues, suggest a possible avenue by which the carcinogenic effect may be mediated. The production of ions (H^+ , OH^-) and inorganic radicals (OH , HO_2) with hydrogen peroxide formation in aqueous systems in response to I-R is well known. A similar reaction sequence within the aqueous milieu of the cell apparently occurs. Controversy exists, however, as to which of the reaction products, if indeed any, is responsible for the radiation effects. Organic peroxides have been considered by some to be responsible for the cellular effect seen after radiation, whereas others, of whom perhaps Warburg is the most resolute, maintain that hydrogen peroxide is the active intracellular product. Warburg (1) has recently stated that the direct action of X-rays on cell metabolism, in this instance cancer cells, was due to the hydrogen peroxide produced rather than to organic peroxides, intermediate hydrogen atoms, or OH radicals.

On the assumption that organic peroxides are produced after exposure to I-R, a preliminary study was undertaken to determine the carcinogenicity of organic peroxides. This report presents data obtained at the unsophisticated level of routine bioassay of a broad spectrum of these compounds. We wished to determine whether information and research leads could be obtained in a manner analogous to that

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which has served so brilliantly the studies of mechanisms of action of carcinogenic hydrocarbons, azo dyes, and aromatic amines. The utilization of bioassay data served as a basis for the studies of Pullman and Pullman (2), Daudel and Daudel (3), and Daudel *et al.* (4) aimed at relating chemical structure to carcinogenic properties and potency. Of additional concern to us is the growing general environmental occurrence of the class of compounds of primary interest to this symposium. Urban air pollution, food preparation, processing, and storage, and the increasing production and use of synthetic polymers are all potential sources of human exposure to peroxides.

The established carcinogenic properties of I-R are of particular interest in that peroxide formation suggests an *in vivo* mode of action. In a discussion of carcinogenesis by I-R, Furth and Lorenz (5) concluded: "The induction of neoplasms by ionizing irradiation is a fascinating chapter in the history of science. It is full of problems of increasing practical importance awaiting solution. As a tool in cancer research, it is unequaled." Pertinent to this are the observations that similarities exist between the biochemistry of the response to I-R and the biochemistry of aging (6-8). Both biological situations have additionally in common an increased incidence of neoplastic disease. The studies of Harman (9) have been particularly pertinent, and his experimental data describe a prolongation of the normal life span and inhibition of spontaneous cancer in mice by antioxidants. Finally, environmental carcinogenic stimuli have been shown to be capable of acting in a combined manner. Experimentally, it has been demonstrated that I-R has an enhancing effect on the leukemogenic action of methylcholanthrene (10), a cocarcinogenic effect on the production of skin cancer by benzo(a)pyrene (11) and benzo(a)pyrene and dibenz(a,h)anthracene (12), a synergistic action in association with estrogenic hormones in the induction of lymphosarcomas in mice (13), and an additive effect in the carcinogenic response after the application of cigarette tar to the skin of mice (14). Prior to discussion of certain factors concerned with the *in vivo* formation and action of peroxides, a general assessment of their environmental status and role in carcinogenesis will be undertaken.

ENVIRONMENTAL SOURCES

Gastrointestinal Environment

Among the earliest studies on the causative factors in human cancer were those on heated fats in an effort to explain the pathogenesis and high incidence of cancer of the gastrointestinal tract. The chemical relationship between sterols and bile acids on one hand and 3-methylcholanthrene on the other was recognized at the outset of these studies, and a diligent search was instituted for 3-methylcholanthrene and associated compounds, possibly resulting from the application of heat to the sterols present in fatty materials. Although many investigators produced sarcomas after the injection of subfractions of different heated fats, none succeeded

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in isolating the proximate carcinogenic agent. Hieger (15), who has performed perhaps the most detailed of these studies particularly utilizing cholesterol and its derivatives, ultimately had to concede that the proximate carcinogenic compound or compounds were most elusive. During the heating of lipids, dehydrogenation occurs with the production of polycyclic aromatic hydrocarbons (PAH) proceeding at high temperatures. However, the yield of carcinogenic hydrocarbons under usual circumstances is at best minimal, and those theoretically capable of formation are primarily of weak potency, e.g., chrysene (16). Davies and Wilms-hurst (17) have reported the formation of benzo(a)pyrene from starch at 370° to 390°C. The low temperature of formation is of pertinence here. Fieser (18) and Bischoff (19) investigated the production of peroxides from cholesterol and more unsaturated sterols with these serving as the sources of the proximate carcinogenic compounds. Fieser (18) advanced the concept that with the *in vivo* peroxidation of cholesterol an endogenously initiated carcinogenic mechanism of considerable significance existed. It is wholly conceivable that the elusive compound or compounds of Hieger which disappear on successive fractionations are peroxides. Data from numerous studies are either inconclusive or negative. Studies are now in progress on epoxy derivatives of corticosteroids, and evidence of weak carcinogenicity is being obtained. The role of heated fats and allied compounds as carcinogens has been reviewed by Arffmann (20).

A ubiquitous source of entry of peroxidic compounds into the intact host is through dietary fats. Further, there is ample evidence that polyunsaturated fatty acids in the depot fats of animals can peroxidize *in vivo*. Peroxidation can be demonstrated by analytical studies on the fat and by the typical yellow-brown discoloration which has led to the term "yellow fat disease" (21). Although perhaps superficially unrelated to carcinogenesis, it has been suggested that *in vivo* peroxidation of lipids, particularly in nonadipose tissue, may result in symptoms of vitamin E deficiency. The antioxidant effect of vitamin E will be discussed. Antioxidants have the capability of preventing *in vivo* peroxidation, and tocopherols serve as an excellent example of these compounds. Of major concern to us is the observation that this effect is not limited exclusively to fatty tissue but also to the lipid-containing units within the cells, as, for example, mitochondria. It is reasonable to assume that other lipid structures, including those occurring in the membranes of the cell walls, the microsomes, and the lysosomes, are susceptible to dietary influence, and these structures also, as a result, may undergo peroxidation (22). In addition to *in vivo* peroxidation, the lipids in dietary substances may also undergo peroxidation. This exogenous process may be by far the most significant source of lipoperoxides. Heating in the presence of air, as well as the radiation process, is capable of forming peroxides in fats. On a practical level has been the report that highly peroxidized oils and fats present in fish meal induce hepatomas when fed to trout (23). The toxicity and carcinogenic effect of lipoperoxides

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TABLE I
TUMORIGENIC RESPONSE AFTER EXPOSURE TO LIPOPEROXIDES

Compound	Total dose (mM)	No. of mice and strain	Appearance of first tumor		Tumor-bearing animals					
			Time (months)	No. of survivors	Subcutaneous sarcomas		Malignant lymphomas		Pulmonary adenomas	
					No.	%	No.	%	No.	%
Epoxydated soya oil	0.5	50 C57Bl	24	20	1	5	2	10	2	10
Butyl 9, 10-epoxy-stearate	1.7	50 C57Bl	17	31	3	10	2	6	1	3
Allyl 9, 10-epoxy-stearate	1.0	30 C57Bl	—	—	—	—	—	—	—	—

are in need of intensive study, particularly as the data obtained may well also apply to peroxides formed after exposure to I-R. The studies of Glavind and Tryding (24) are pertinent to this. Their findings indicate that peroxidized lipids when ingested are hydrolyzed and absorbed through the intestine and that after absorption the peroxidic nature of the compound is lost, indicating perhaps that the peroxide is destroyed in the intestinal mucosa. The relation of the destruction of the lipoxy material to development of neoplasms of the gastrointestinal tract is unknown. Lipoperoxides may undergo further reaction leading to epoxidated fats with potential carcinogenic properties (Table I). Bioassay data in this and subsequent tables frequently report findings from experiments still in progress, and quantitative interpretation of all the data is impossible at the time of this symposium. The method of application is given on the table for each experiment, and except where noted C57 Black mice were used in all studies. A dose of 0.5 mM was used for the testing of pure compounds except when otherwise designated. Epidemiologic studies on groups with both high and low gastrointestinal cancer rates, particularly in relation to dietary lipids, have been unrewarding. The real and progressive decrease in gastric cancer being reported from other than high endemic areas is resulting in an increased effort to identify variations in dietary characteristics.

Although this phase of the symposium is concerned primarily with the carcinogenic properties of peroxides, data from bioassay experiments with epoxides are also being reported. The theoretical possibility that the carcinogenic properties of peroxides may be mediated through the formation of epoxides must be considered, since epoxides are known to be readily formed from peroxides, hydrogen peroxide, and peracids. Inasmuch as considerably more information is available relative to the mode of action and carcinogenic properties of epoxides, the findings would appear to be pertinent.

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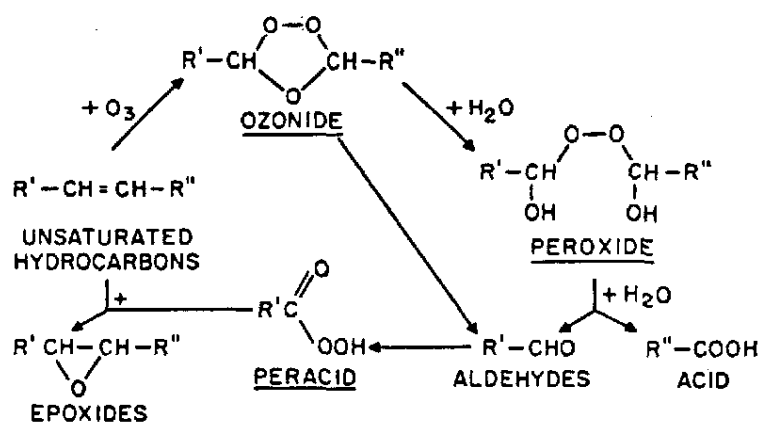


Fig. 1. Postulated photochemical reactions of carcinogenic significance occurring in polluted air

Respiratory Environment

Peroxides have been identified in the polluted atmosphere of several large American cities, most particularly, however, in Los Angeles. The photochemical reactions between the oxides of nitrogen and the unsaturated hydrocarbons resulting in the formation of peroxides have been well established (Fig. 1). The exhaust products of gasoline engines and industrial effluents have been shown to be the primary source of the oxides of nitrogen and unsaturated hydrocarbons taking part in this reaction. Samples of Los Angeles atmosphere collected in liquid nitrogen traps have been shown to be carcinogenic when either painted on the skin or injected subcutaneously in mice (Table II and Fig. 2). Aerosols of ozonized gasoline synthetically reproduced in inhalation chambers have resulted in the produc-

TABLE II
PRODUCTION OF SKIN TUMORS IN MICE WITH OXIDATION PRODUCTS OF ALIPHATIC
HYDROCARBONS AFTER PAINTING THREE TIMES A WEEK

Compound	No. of mice and strain	Appearance of first tumor		Tumor-bearing animals					
		Time (months)	No. of survivors	Skin		Malignant lymphomas		Pulmonary adenomas	
				No.	%	No.	%	No.	%
Aerosol condensate, Los Angeles atmosphere	50 C57Bl	14	35	7	20	—	—	—	—



FIG. 2. Photomicrograph of carcinoma in mouse induced by skin painting with polycyclic aromatic hydrocarbon-free condensate of smog.

tion of pulmonary neoplasms in strain A and C57 Black mice. Strain A mice are characterized by a high spontaneous incidence of pulmonary neoplasms and a marked susceptibility to tumor induction by carcinogenic agents. This property permits the implementation of a modified time-tumor-dose study as shown in Figs. 3 and 4. C57 Black mice, in contrast, have a less than 1% spontaneous incidence rate of pulmonary neoplasms and extreme resistance to pulmonary tumor induction. The results of inhalation experiments with this strain are shown in Table III.

The end state of the complex reaction in the atmosphere is the formation of aldehydes and organic acids. The aldehydes in particular resulting from partial combustion of gasoline have been found to be responsible for the major proportion of the irritant effect of Los Angeles smog. In the presence of atomic oxygen these aldehydes can give rise to hydroperoxides when moisture is available either exogenously or endogenously. Another group of compounds formed from the reaction products of vehicular exhausts are epoxides which, though far less irritating than aldehydes, have been shown to be carcinogenic (25, 26). The formation of epoxides in the atmosphere may or may not require the intermediate formation of

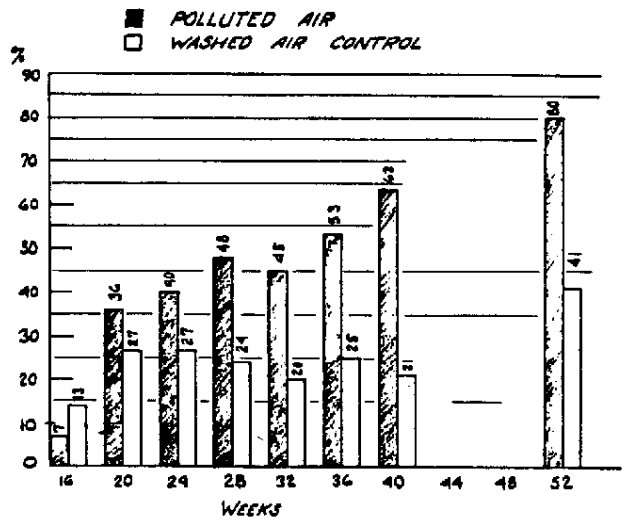


FIG. 3. Percentage of pulmonary tumor-bearing mice (strain A) removed from synthetic smog chambers at planned intervals.

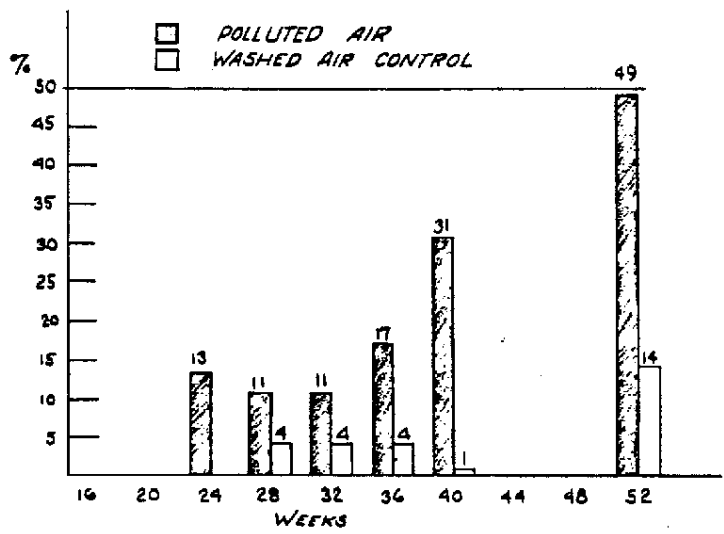


FIG. 4. Percentage of multiple pulmonary tumor-bearing mice (strain A) removed from synthetic smog chambers at planned intervals.

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TABLE III
RATE OF INDUCTION OF PULMONARY TUMORS IN C57 BLACK MICE AFTER
EXPOSURE TO AN ATMOSPHERE OF OZONIZED GASOLINE

	Washed air chamber	Smog chamber
Total number of tumor-bearing animals	6	15
Number of survivors at time of first tumor	367	155
Per cent tumor-bearing animals	1.6	9.6

TABLE IV
EPOXIDES CURRENTLY BEING BIOASSAYED FOR CARCINOGENICITY:
SIGNIFICANCE OF COMPOUNDS RELATES TO THEORETICAL FORMATION
IN URBAN POLLUTED AIR

1,2-Epoxy-3-methoxypropane
1,2-Epoxy-3-ethoxypropane
1,2-Epoxy-3-propoxypropane
1,2-Epoxy-3-(1-methylethoxy)propane
1,2-Epoxy-3-butoxypropane
1,2-Epoxy-3-(2-methylpropoxy)propane
1,2-Diisobutylene oxide
Triisobutylene oxide
Vinylcyclohexene monoxide

peracids; however, chromatographic analysis of smog has indicated that peracids are indeed present in addition to a large variety of other peroxides (P. Kotin, H. L. Falk, and N. Hellinger, unpublished data). A listing of epoxides theoretically present in polluted air and currently under bioassay is given in Table IV. Subsequent reaction between aldehydes and peroxides may result in the formation of the potent chemical agent bishydroxymethyl peroxide (Fig. 5). The formation of related compounds may begin with the lower aldehydes and ozone or other oxidants with the bishydroxymethyl peroxide having been shown to possess carcinolytic properties. Although likely, it is yet to be determined whether this carcinolytic agent in common with numerous others possesses carcinogenic potency as well.

Ozone, a major constituent (35 to 40%) of the oxidant material in the urban atmosphere in the Los Angeles area (27), has now been classified as a radiomimetic gas (28, 29). It would therefore appear to be of double significance, first as an integral reagent in the photochemical reactivity in the Los Angeles atmosphere, and second as a mutagenic, radiomimetic agent on its own. The toxicity of this agent has heretofore precluded any detailed carcinogenic studies.

The expanding synthesis and commercial use of polymers have created in a small group of workers a theoretical occupational hazard incidental to the use of benzoyl peroxide, for example, as a major compound in the polymerization of monomers.

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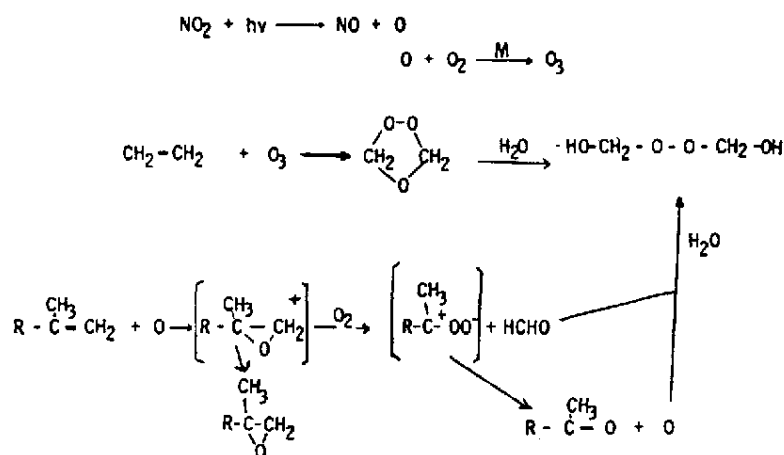


FIG. 5. Anticipated reactions leading to the formation of bishydroxymethyl peroxide in polluted air.

TABLE V
TUMORIGENIC RESPONSE AFTER EXPOSURE TO EPOXIDES OF INDUSTRIAL SIGNIFICANCE

Compound	Total dose (μM)	No. of mice strain	Appearance of first tumor		Tumor-bearing animals					
			Time (months)	No. of survivors	Skin		Malignant lymphomas		Pulmonary adenomas	
					No.	%	No.	%	No.	%
Cyclohexene epoxide	20	30 C ₃ H	7	22	—	—	—	—	1	5
Styrene epoxide	20	30 C ₃ H	11	19	—	—	3	16	—	—
Octylene epoxide	20	30 C ₃ H	7	26	—	—	6	23	—	—
Dodecene epoxide	20	30 C ₃ H	10	21	—	—	4	19	—	—
Hexadecene epoxide	25	30 C ₃ H	9	30	—	—	7	23	1	3
Epichlorohydrin	5	30 C ₃ H	3	30	1	3	3	10	1	3
Condensation product of epichlorohydrin with 4,4'-isopropylidene diphenol (8.1% epoxy O)	900	50 C57B1	8	46	1	2	3	6	—	—

Epoxides similarly have broad industrial use, and certain ones of these have been bioassayed for carcinogenicity (Table V).

As stated previously, our interest in the carcinogenic response has caused us to consider the possibility that the carcinogenic effect of peroxides might be mediated

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TABLE VI
TUMORIGENIC RESPONSE AFTER EXPOSURE TO SELECTED DIEPOXIDES

Compound	Total dose (mM)	No. of mice and strain	Appearance of first tumor		Tumor-bearing animals					
			Time (months)	No. of survivors	Skin		Malignant lymphomas		Pulmonary adenomas	
					No.	%	No.	%	No.	%
1,2,3,4-Diepoxybutane	1	20 C57Bl	5	20	7	35	2	10	—	—
1,2,3,4-Diepoxybutane	1	20 C57Bl	5	20	5	25	1	5	—	—
1,2,3,4-Diepoxy-2-methylbutane	0.2	10 C57Bl	10	10	—	—	1	10	—	—
1,2,4,5-Diepoxypentane	0.2	10 C57Bl	—	—	—	—	—	—	—	—
1,2,5,6-Diepoxyhexane	1	20 C57Bl	12	20	—	—	2	14	—	—
Diepoxyimonene	1	20 C57Bl	18	17	1	6	6	35	—	—
Vinyl cyclohexene diepoxide	0.5	20 C57Bl	14	16	1	6	4	25	—	—
Di(2,3-epoxy)propyl ether	0.75	20 C57Bl	5	10	4	40	—	—	—	—
Di(2,3-epoxy)propyl ether	0.25	20 C57Bl	—	—	1	8	—	—	—	—
Diepoxydihydromyrcene	0.75	10 C57Bl	6	8	1	12	1	12	—	—
Methyl diepoxydiallylacetate	0.5	10 C57Bl	12	5	1	20	1	20	—	—
1,2,7,8-Diepoxyoctane	0.75	10 C57Bl	—	—	—	—	—	—	—	—
1,2,8,9-Diepoxyonane	0.6	10 C57Bl	9	6	1	16	1	16	—	—
1,2,9,10-Diepoxydecane	0.75	10 C57Bl	18	7	—	—	1	14	—	—
1,3-Bis(2,3-epoxypropoxy)benzene	0.75	20 C57Bl	8	14	1	7	—	—	—	—
1,3-Bis(2,3-epoxypropoxy)benzene	0.25	20 C57Bl	—	—	—	—	—	—	—	—
1,4-Bis(2,3-epoxypropoxy)benzene	1	20 C57Bl	—	—	—	—	—	—	—	—

through the formation of epoxides. Because of the plethora of knowledge relating to the carcinogenic mechanisms of epoxides—e.g., crosslinking, mutagenicity—we are intentionally including data from experiments devoted to bioassay of epoxides. The carcinogenicity of certain selected diepoxides is given in Table VI.

FACTORS MODIFYING THE PEROXIDE EFFECT

The assessment of host resistance factors is crucial to the study of mechanisms of carcinogenesis. Compounds possessing an active SH group somewhere in the molecule may act as protective agents against the potential adverse effects of peroxides by sparing the essential SH groups in the cells for physiologic activity.

Vitamin E, on the basis of its antioxidant properties, has also been found to be a significant protective agent. Particularly important is the observation that a protein-bound vitamin E complex, presumably responsible for protection, exists (30). It is also of interest that different tissues require different lengths of time to acquire protection against radiation after vitamin E has been restored to the

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diet, and fluctuations in the supply of the vitamin may enhance susceptibility to injury.

Iron and many iron-containing compounds are of great importance, since they may exert a protective effect under circumstances when the complexity of an organic peroxide precludes the protective action of catalase and peroxidase. Hemoglobin and cytochromes are active, although when compared to catalase their destructive action on simple peroxides is insignificant. In the case of complex peroxides, when catalase is inactive, the action of iron-containing compounds assumes great significance.

The action of siderophilin and chelating agents is related to the availability of iron, the local oxygen tension being the controlling factor in the intensity of the effect (31, 32).

RADIOMIMETIC ACTION OF PEROXIDES

The biological significance of hydrogen peroxide which is presumably formed incidental to the action of I-R on cells is as yet not fully determined. A limited role is strongly suggested when one recognizes that the yield is insignificant when contrasted with the far greater amounts produced incidental to the action of certain riboflavin-containing enzymes (33). Furthermore, of all peroxides, hydrogen peroxide is the most efficiently destroyed, particularly by catalase and formic acid oxidase action. The destructive effect of catalase on various peroxides is shown in Table VII. The relation of stability in the presence of catalase to carcinogenesis is unknown; however, we feel it merits further study.

Current concepts suggest a possible primary role for organic peroxides in mechanisms of carcinogenesis by radiation. Peroxides formed have been shown to produce cell injury at a level consistent with cellular survival. Further, the growing acceptance that cancerization of the cell requires alteration in the genetic material

TABLE VII
SUSCEPTIBILITY TO DESTRUCTION BY CATALASE*

Compound	Destruction
Hydrogen peroxide	Yes
Carbamine peroxide	Yes
Histidine peroxide	Yes
Methyl hydroperoxide	Yes
Ethyl hydroperoxide	Yes
1-Hydroxyethyl hydroperoxide	Yes
Bishydroxymethylperoxide	No
tert-Butyl peroxide	No
Caprylic peracid	No

* From Weitzel *et al.* (34) and Maehly (35).

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is compatible with the mutagenic action described for certain organic peroxides (36). Dickey *et al.* (37) induced mutation in *Neurospora* with organic peroxides and suggested that in the mixture of formaldehyde and hydrogen peroxide the active agent might be bishydroxymethyl peroxide. Altenburg (38) used *tert*-butyl hydroperoxide for the production of mutations in *Drosophila*. Chromosome breakage was described by Loveless (39), also using *tert*-butyl peroxide. The mutagenic effects of organic peroxides are critical to any discussion of the mechanism of the carcinogenic action of I-R.

A strict parallelism between carcinogenicity and mutagenicity limited specifically to I-R has been emphasized by Latarjet (36). The studies on the mutagenicity of peroxides referred to previously suggest a species-specific effect. The data of Chevallier and Luzatti (40) additionally suggest a site-specific effect within the genetic material. Data obtained from bioassay of certain organic peroxides are shown in Table VIII.

We have bioassayed numerous mutagenic agents for carcinogenic potency, and in our hands there is an inconstant relationship between the two properties. Mutagenic action of such compounds as formaldehyde, ethylene oxide, and propylene oxide has not been found to be associated with carcinogenicity, but technical limitations incidental to the study of these compounds are obvious. Of interest is the mutagenicity of the organic peroxides listed in Table IX. Data on associated

TABLE VIII
TUMORIGENIC RESPONSE AFTER EXPOSURE TO SELECTED ORGANIC PEROXIDES

Compound	Dose (μ M)	No. of mice and strain	Appearance of first tumor		Tumor-bearing animals					
			Time (months)	No. of survivors	Subcutaneous sarcomas		Malignant lymphomas		Pulmonary adenomas	
					No.	%	No.	%	No.	%
Acetyl peroxide	60	50 C57Bl	12	38	—	—	4	10	1	3
Lauroyl peroxide	40	50 C57Bl	12	43	—	—	2	5	—	—
Sodium peracetate	80	50 C57Bl	14	37	—	—	3	8	—	—
Diisopropyl benzene hydroperoxide	50	50 C57Bl	10	46	—	—	2	4	3	6
<i>p</i> -Menthane hydroperoxide	90	50 C57Bl	11	46	—	—	6	13	2	4
Cumene hydroperoxide	50	50 C57Bl	14	38	1	3	11	29	—	—
<i>m</i> - and <i>p</i> - <i>tert</i> -Butyl isopropyl benzene hydroperoxide	50	50 C57Bl	13	44	—	—	22	50	3	7
Methyl ethyl ketone peroxide	40	50 C57Bl	15	34	1	3	3	9	1	3
Di- <i>tert</i> -butyl peroxide	100	50 C57Bl	19	35	—	—	7	20	1	3
<i>tert</i> -Butyl perbenzoate	40	50 C57Bl	13	47	—	—	10	21	—	—

TABLE IX
ORGANIC PEROXIDES WITH MUTAGENIC PROPERTIES

Compound	Ref.
Hydrogen peroxide	(37, 42)
Bishydroxymethyl peroxide	(31, 37)
Hydroxymethyl <i>tert</i> -butyl peroxide	(37)
Diisopropyl ether peroxide	(37)
<i>tert</i> -Butyl peroxide	(37-39)
Succinic peroxide	(40)
Cumene peroxide	(40)
Thymine peroxide	(40)
Peroxy fatty acids	(43)
Ethylene oxide	(39)

mutagenicity and carcinogenicity are least satisfactory in relation to carcinogenic hydrocarbons. It is true that 3-methylcholanthrene, dibenz(*a,h*)anthracene, and 7,12-dimethylbenz(*a*)anthracene were tested by Barratt and Tatum (41) and found to be mutagenic for *Neurospora*; however, their mutagenic capability was at a significantly lower level than that observed for nitrogen mustard or radiation.

Although slightly remote from the immediate interests of this symposium, certain parallelisms between carcinogenicity and mutagenicity are worthy of denotation. Allylthiocyanate has been shown to be mutagenic (44). We are currently reporting two cases of lung cancer in workers exposed to Fiberglas manufacture and synthesis (45). β -Propiolactone was shown to be carcinogenic by Roe and Glendenning (46) and by us (47). The mutagenicity of this compound for *Neurospora* was reported by Smith and Srb (48). Extensive data exist describing the mutagenicity of mustard gas and nitrogen mustards. Alkylating agents, particularly the mono- and diepoxides, similarly show mutagenicity and carcinogenicity (49). In summary, we do not feel that the correlation as originally claimed for PAH but later questioned by Demerec (50) necessarily precludes a combination of mutagenicity and carcinogenicity for these compounds. Rather our own data suggest that, in the instance of PAH, factors affecting biologic availability may be critical in elucidating mutagenicity. These factors include the vehicle of the carcinogen, its stability, sites of elective anatomic localization, and local and hepatic metabolic degradation. Similar reasoning may be applied to the lack of data suggesting mutagenicity for carcinogens belonging to the group of azo dyes and aromatic amine carcinogens.

SUMMARY AND CONCLUSIONS

The preliminary data being reported support the suspicion that organic peroxides may possess carcinogenic properties. This suspicion in large measure relates to the acknowledged mutagenicity of certain peroxides.

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As a possible mechanism of action it is suggested that peroxides may act by catalyzing the depolymerization of deoxyribonucleic (DNA) and ribonucleic acid (RNA). This radiomimetic property may provide a fundamental bridge between the carcinogenicity of I-R and that of organic peroxides when used in pure form.

Emphasis has also been directed to the carcinogenic properties of epoxides. Pertinence to the topic of the symposium exists in two areas. First, organic peroxides may lead to the formation of epoxides. Second, the established carcinogenicity of epoxides has resulted in the accumulation of a large body of information relating to the mechanism of their action. Of particular significance in this area is their capability of acting as crosslinking agents.

The possibility of utilizing the established carcinolytic properties of certain carcinogenic agents must be considered in the study of the carcinogenic properties of organic peroxides. Bishydroxymethyl peroxide is an example of a compound possessing carcinolytic activity (34) in need of bioassay for carcinogenicity.

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DISCUSSION

LATARJET: How many animals on that slide? (Kotin and Falk, Table VIII)

KOTIN: Fifty animals to an experiment, fifty C-57 black mice.

EPSTEIN: Do you have aerosol experiments?

KOTIN: Yes. The only thing we have been able to produce with aerosols thus far has been squamous metaplasia of the tracheobronchial epithelium and an increased tumor yield in A strain mice. No effect at all on C-57 black mice.

C. F. DECKER: What age animals do you use?

KOTIN: The animals are exposed at 6 weeks of age.

MILAS: Were any animals tested with more than one dose?

KOTIN: No, all at one dose. The concentration of total oxidant was always 0.5 to 0.75 ppm, measured as oxidant.

A VOICE: Did you pick these substances on the basis of their mutagenicity?

KOTIN: Actually they are classified on another table on the basis of mutagenicity. We attempted to analyze the data in terms of going back to the literature of Dr. Sobels and the like, and to see whether they were related to the mutagenic compounds at all. We find no constant correlation between mutagenicity and carcinogenicity, although more of the carcinogenic compounds have been reported as being mutagenic than not. That is all that we can say. There is no statistical analysis of the data.

This is a crude bioassay, and I think I should indicate that my invitation to be here is presumably predicated on the fact that we are the only ones foolish enough to waste this much time bioassaying these compounds. (Laughter)

WALLING: Do you have comparable data on a series like aldehydes, ketones, etc.?

KOTIN: I should mention that methyl ethyl ketone peroxide is another peroxide that has just produced its first tumor in 11 months. We have bioassayed formaldehyde, acetaldehyde, and propionaldehyde, with no tumors, in incomplete experiments. The reason I say that some of these experiments are not finished is that we do not report any carcinogenicity without doing the experiment twice.

MILAS: Would you care to comment on the early work of Koch and Maisin,¹ who found that peroxides cure cancer?

¹ *Cumpr. rend. soc. biol.* **120**, 106 (1935); see also Magat, *Z. Krebsforsch.* **27**, 378 (1928).

KOTIN: Yes. I think one of the truisms in carcinogenesis is that at a given concentration a carcinolytic agent may be carcinogenic, and a carcinogenic agent may be carcinolytic. There is nothing incompatible with those findings. Rather, I think this demonstration of carcinogenicity is not incompatible with this effect. Along a different line, we assayed carcinogenic compounds for 2 years by attempting to see how much carcinolytic activity they had. This was a blind alley, very regrettably.

HARRIS: I should like to extend the comments you made on heated fats: I think it was Roffo² who first reported a carcinogenic effect of heated fats. People forget that Roffo heated his fats to about 300°C, which would crack the fats. This is not the way fats are treated when used for human food. The temperature is 350°F. Studies done with fats that have been heated at 350°F have not shown this carcinogenic effect.

About 12 years ago, because of this work, we did some studies with heated fats. We found that if we actually abused a fat by heating it for quite a long time, and then fed it in the diets of rats, in about 3 weeks they were dead. We became quite concerned about this. We analyzed the diet that had been fed during this period, and we found that essentially all the fat-soluble factors had been destroyed while standing in the refrigerator during this period. All the tocopherol, all the vitamin A, all the vitamin K, and some fair amount of some of the B complex factors were destroyed. We repeated this work with a diet newly mixed every day, and the "toxicity" was much milder; the animals developed a mild diarrhea but recovered quite well.

One of the effects of heated fats is the destruction of nutrients in the food with which it is mixed. Since heated fats may be quite well peroxidized, we wondered if part of the toxicity we had observed with heated fats was due to peroxides that were absorbed into the tissues and that destroyed nutrients in the tissues.

As I mentioned yesterday, this was why we did a study on vitamin A-deficient diets with animals. We found that rats on a vitamin A-deficient diet developed the deficiency about 25 to 30% sooner with heated fat than with ordinary fat that had not been heated. An analysis of the tissue showed that much of the vitamin A had been destroyed in the tissues. This then made us wonder whether the peroxide caused this destruction. As I mentioned yesterday, the evidence indicates that apparently the peroxides are not absorbed. Thus it was some chemically active material in the heated fat which was absorbed, that caused the destruction in the tissues.

You will notice that people who are connected with academic institutions often publish papers on heated fats that are quite alarming, whereas those connected with the food industry publish papers that appear to indicate that heated fats are innocent. I think the truth is somewhere in between. In academic studies, fats have been overtreated and abused in order to bring out an academic point, whereas in the food industry studies are designed to see what happens in the practical use of normally processed fats.

This problem is not so alarming as we think. It seems that the only important problem in heated or oxidized fats is the destruction of tocopherol in the fat before it reaches the palate of the consumer. For instance, tocopherol is destroyed more rapidly than the man-made antioxidants, BHA and BHT, that are added to fats that are to be used in frying.

In studies that we have made, maybe as much as 95% of the tocopherol was destroyed in the ordinary heating of these food fats, whereas the BHA and BHT survived. Because the fats do not become oxidized, the food industry feels it has solved the problem. But it should be realized that none of the man-made antioxidants completely replaces vitamin E in the tissues. Vitamin E has the ability to get into tissue cells and function as an antioxidant. These other antioxidants do this incompletely, if at all. A fat that has been treated this way is not protected by antioxidants in terms of human tissues. Therefore the stress from a greater use of these oxidized fats may cause deficiency. The compounds in fats that cause toxicity do not form urea adducts. Ap-

² *Bol. Inst. Med. Exptl. Estud. Cancer* 15, 539 (1938).

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parently they are branched-chain compounds, and most of them are peroxidic. Their importance is mostly related to the side reactions which occur in contact with food.

KRAYBILL: In agreement with what Dr. Harris has just said, I should like to report again the work that Da Costa and Levenson did some years ago³ in which they produced sterility in rats. This is primarily due, we think, to the radiation end products which have an effect on vitamin E. It can be corrected by the administration of vitamin E. This was found later also in subsequent experiments in which dogs were fed various types of irradiated foods. So it has become quite essential to give the fat-soluble vitamins, E, A, and K, orally to the rats, not simultaneously with the ration. If one leaves the vitamin mixture in contact with the irradiated ration, in a couple of days the vitamin is destroyed.

Later, about 1958, it was also noted that in feeding irradiated foods, like beef, there was a destruction of vitamin K. Particularly if one leaves menadione out of the diet, with certain types of diets one produces hemorrhagic diathesis in rats. The effect is more marked as the radiation dose used for processing food is increased.

In the case of vitamin K destruction and hemorrhagic diathesis, there is also a vitamin A-vitamin E relationship, and an influence here of estrogens and certain bile acids. If you administer a ten- or twelve-carbon aldehyde to the animal, you will get a hemorrhagic syndrome confirming the influence of carbonyls. If you take soybean oil or cod liver oil and autoxidize them, you can similarly get an effect on vitamin K.

Again in agreement with what was said, Dr. Mead at California found⁴ that fats with peroxide values of 200 were somewhat toxic, and at 300 or 350 they were not only toxic but lethal in effect.

Last fall, in a meeting in Brussels, Dr. Lang from Germany reported⁵ that autoxidized fats would produce certain pigmented lesions in the liver of the rat. There are thus many effects of oxidation of vitamins and formation of these degradative products on physiological action.

EPSTEIN: I should like to ask Dr. Harris what evidence there is that BHA and BHT are not taken up by cells.

HARRIS: BHA and BHT are among those compounds that are absorbed into the blood stream and exert an antioxidant effect extracellularly. They do not completely replace vitamin E. I think the reason is that they are rejected in part, or entirely, by certain cells, so that intracellularly they are not effective.

EPSTEIN: What evidence is there that they are not taken up by cells?

HARRIS: Because they do not replace vitamin E.

EPSTEIN: That is not the answer to the question. Surely if they were taken up by cells in the absence of vitamin E, that would mean that the uptake would exert an opportunity for protection.

HARRIS: I don't know the answer to that. I am saying that they do not replace vitamin E in the diets of animals, which indicates that there has been a rejection somewhere.

EPSTEIN: They may be exerting a different kind of antioxidant effect.

HARRIS: Yes, but you get the vitamin E syndrome pathology even in their presence.

EPSTEIN: Dr. Kotin, is there any evidence that these aliphatic oxidation products are obtained in tobacco distillates? That is the first question. The second question: Is there any evidence that oxidation products of polycyclic hydrocarbons are found in the atmosphere, and, if they are found, are they carcinogenic?

KOTIN: In answer to the first question, I know of none. Secondly, what we did was to expose

³ U. S. Army Med. Nutr. Lab. Rept. 89 (1951).

⁴ Federation Proc. 15, 918 (1956).

⁵ FAO/UN Technical Meeting on the Evaluation of the Wholesomeness of Irradiated Foods (Brussels, October 1961).

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to an oxidative atmosphere the crystalline carcinogens, 3,4-benzopyrene and 1,2,5,6-dibenzanthracene, both in crystalline form and adsorbed on soot, and bioassayed their carcinogenicity, and the property was attenuated.

G. COHEN: Would you comment on the problem of carcinogenesis in man versus carcinogenesis in the mouse? You gave one or two examples of workers exposed to peroxide who developed cancer subsequently, with the implication that this was perhaps the cause. Have there been any statistical studies of people exposed over a long period of time, and would you include in your answer a comment about the incidence of cancer in smog-bound Los Angeles as compared to a smog-free city?

KORIN: I have a habit of answering the last question first. According to the best estimates, the latent period for the induction of pulmonary neoplasms is one and one-half to two decades, based on population exposed to cigarette smoke. Here you do have a restricted population, and the basis for a carcinogenic effect can be properly anticipated. Such an effect would not be readily manifest in a migratory population such as Los Angeles represents.

I might say, though, that a five-year study supported by the American Cancer Society and the State Department of Health is now in its third year, and the findings in Los Angeles indicate an increased risk on the part of residents over and above that one would normally anticipate by virtue of their living in an urban area. Living in an urban area gives you an increased risk. Apparently there is an enhancement of this risk, but you do have this catch: How many of these people came from breathing the polluted atmosphere of Chicago or New York at the time of the induction of the neoplasm? I don't know.

Secondly, it was not a peroxide, it was Plexiglas and isothiocyanate that we are reporting. All that we are reporting is that we have the induction of two pulmonary neoplasms in persons occupationally exposed to these products. The product is present in the neoplasm. The two most readily available pathogenic environmental factors have been ruled out. So in the manner of the original cases of asbestosis which were reported as the appearance of lung cancer in persons with asbestos bodies, we are reporting these as two cases of human lung cancer, Plexiglas fibers, and history of exposure to Fiberglas.

G. COHEN: You also suggested benzoyl peroxides in industrial sites. Is there any evidence on this?

KORIN: I put a good healthy "theoretically" in front of that. The only evidence is that, all other things being equal, industrial populations have a higher incidence of lung cancer than nonindustrial. I should say industrial policy holders according to the work of the Metropolitan Life Insurance Company. They have a significantly greater liability to the development of lung cancer than ordinary policy holders.

BERNHEIM: Has anybody bioassayed haze? As I understand it, haze is due to the distillation of oils and waxes from the leaves of trees by the hot sun, and once in the atmosphere these become highly peroxidized.

KORIN: To my knowledge, it hasn't.

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Behaviour of plastics and rubber foams in fire

Keith Paul

The behaviour of plastics and rubber foams when burned depends on many factors including the type of material used, its form, any interactions with other materials used and the design of the product. It also depends on the many environmental and human factors affecting the actual fire in which the product is burned.

The majority of plastics and rubbers can be prepared as foams while many individual polymeric foams can be prepared with a range of properties. Polyurethane foams are well known for their wide range of properties, examples of which are indicated below:

- low and medium density, flexible foams for upholstery and bedding, etc;
- medium and high density flexible foams for packaging and impact-absorbing applications;
- low density, rigid foams for insulation purposes;
- medium density, rigid foams for insulation and structural purposes;
- high density foams for structural purposes;
- integral skin, medium density, semi-flexible materials for impact-absorbing uses e.g. crash pads;
- integral skin, medium-high density rigid materials for structural mouldings;
- micro-cellular and non-foamed materials are also available.

The complexity becomes even more apparent when the foams are combined with additives ranging from glass fibre reinforcements to flame retardants. With the exception of the integral skin foams, few foams are used on their own but are invariably used in combination with other materials. Examples include laminated, foam core building panels and fabric-covered, foam-filled upholstered furniture. Other applications include *in situ* generated foams which may be injected into cavities or which may be sprayed onto structures and then coated. Polyurethanes probably have the widest range of uses but other foamed polymers with a range of properties and applications can also be produced.

Many foams are chemically similar to their unfoamed polymer bases but differ markedly in density and consequently in thermal properties. It is proposed to consider the effect of these physical changes on the burning behaviour of polymers on a general basis and then to consider the burning behaviour of typical foam-based products selected to illustrate the many types of product and behaviour encountered. In some products, residual blowing agents or the breakdown products from blowing agents may be present although the quantities are likely to be insignificant.

Effect of cellular structure on fire performance

Temperature/time curves are given for test walls without insulation and for walls insulated with 25mm of polyurethane foam and 50mm expanded polystyrene flame-retarded additive, both protected with 12.5mm of plasterboard (Fig 1). The plasterboard did not fail and at no time did the foams become involved in the fires. The increased temperatures and faster burn rates were due to the insulation preventing heat loss from the fire. Thomas and Bullen¹ have studied this theoretically and have shown that:

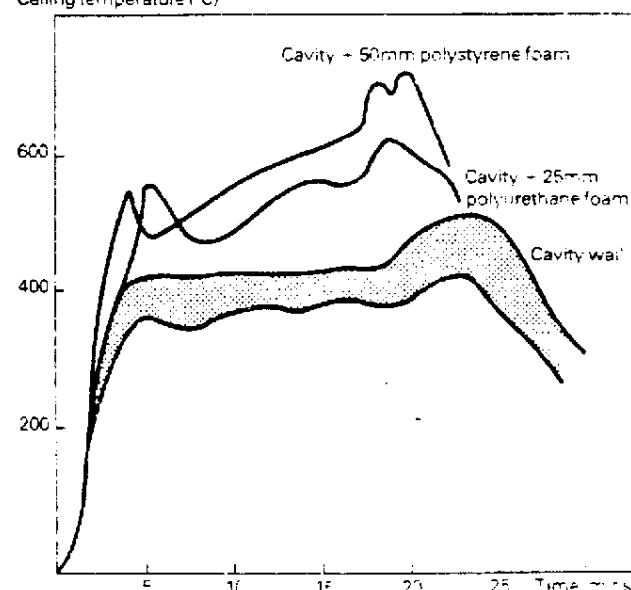
$$t_g \propto (KqC)^m$$

where t_g = fire growth time; K = thermal conductivity; q = specific gravity; C = specific heat capacity. The index m is typically 0.25 to 0.125. This implies that a fire which takes 20-30 minutes to full involvement in a room of brick/plaster construction would take about one third of that time in one insulated with a non-combustible lining of low conductivity. Note that the type of insulation does not matter. Typical values of KqC are given in Table 1 for various wall lining materials.

It has been shown that some polymeric materials have an exponential rise in burning rate with temperature and that for such very rapid fires, the effect of KqC becomes of secondary importance.² A major consequence of this is the higher temperatures reached in fires and the shorter times to reach flash-over conditions. The latter have been defined by various authors but are typically ceiling temperatures of 400-600°C corresponding to a heat flux of 2W/cm² on the floor.²

The rate of burning of a material increases with increasing

Fig 1 Effect of wall insulation on ceiling temperature: corner test.
Ceiling temperature (°C)



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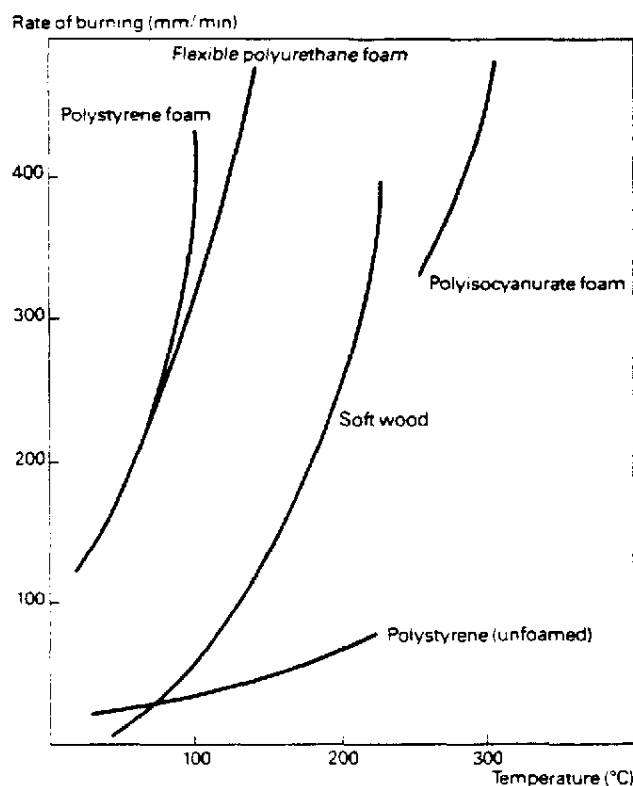


Fig 2 Relationship between rate of burning and temperature (small specimens, vertical downwards test)¹

temperature and the rate of flame spread on a continuous surface approaches infinity as the surface temperature approaches the ignition temperature of the surface material (Fig 2).¹ The effect of insulation on fire growth is therefore considerable even when the insulating material is not actually burning. With foamed materials, the insulation effect is actually built into the polymer system and consequently heat is concentrated at the polymer surface. The net result is that cellular materials are more easily ignited and burn more rapidly than similar non-foamed materials. The 'edge effect' of the thin foam walls may also be significant (see Table 2).

Rigid polymer foams are used for insulation and structural applications because of their very low density and conductivity or because of their light weight per unit volume. Cellular upholstery materials attain their desirable seating properties because of their cellular nature. Consequently, there is no possible way of reducing the insulation effect of foams and it is necessary to consider carefully all aspects of material design and end-use hazard to ensure their proper and safe use.

Fire and causes of fire casualties

Fire is the oxidative destruction of a material and is characterised by the evolution of heat and light. When heated, an organic solid will break down to produce a gaseous fuel which mixes with air (oxygen) and burns as a flame or glow. Heat from the combustion causes further breakdown of the material and the process continues providing that fuel, heat and oxygen are present. Removal of one or more components causes the flame or glow to cease.

In 1976, a major research project was begun in the Strathclyde area of Scotland⁴ to determine the physical causes of death in fire and the circumstances leading to the fatal fire.

Attention was also paid to factors such as the existence of cardiovascular and lung diseases, age, drugs, alcohol, suicide, homicide, etc. The study showed that more than half of the fatalities (54 per cent) had received a fatal exposure to carbon monoxide and that the majority of the remainder involved carbon monoxide in conjunction with burns or cardiovascular or lung diseases etc, with a minor proportion (about 10 per cent) involving other unidentified factors.

Fire gases are the major contributor to fire deaths and it is tempting to relate fire deaths directly to the toxic gases produced by a burning material. However, toxicity involves concentration, exposure time and temperature, all of which vary during a fire. Carbon monoxide, which is produced by all burning organic materials, is the main toxic component of the smoke and alone accounts for more than half of the fire deaths. Oxygen depletion can also be significant.

People will not willingly inhale fire gases and it is therefore logical to infer that they do so either because they are trapped by the fire itself or become incapacitated or disorientated and thence unable to escape. Such effects may be due to smoke and fire gases causing loss of visibility, irritancy or narcotic effects. The role of minor concentrations of other gases at sub-lethal levels is receiving considerable study to determine possible incapacitating effects but the main problem is the loss of visibility and disorientation preventing escape. Trapped persons may subsequently be overcome by toxic gases. This is indicated by the number of persons who are overcome by smoke and have to be rescued by the fire brigade.

Concern has been expressed in the UK by various fire and legislative authorities about the increasing number and changing pattern of fires. Suspicion has been growing, especially among firemen, that modern, upholstered furniture and plastics foams are relatively easy to ignite and once burning will produce intense fires and large quantities of dense smoke. This concern resulted in a number of major research programmes to investigate fire hazards.^{3,5} The results of these programmes were in general agreement and provided detailed and comprehensive background information about the fire behaviour of modern materials.

The UK Home Office combined these data with surveys of opinions of fire officers and analyses of fire statistics in its recent report about the fire risks of new materials.⁶ Furniture manufacturers were recommended to improve the fire performance of their products, giving top priority to ignition resistance to small sources, to the rate of fire

Table 1 Typical physical properties of some common materials at 20°C²

Material	Thermal conductivity (W/m·K)	Density (kg/m ³)	Specific heat capacity (J/kg·K)	Thermal inertia (W/m ² ·K) ^{1/2} sec
Brick	0.8	2600	800	1700 × 10 ³
Chipboard	0.15	800	1250	150 × 10 ³
Plasterboard	0.16	950	840	130 × 10 ³
Fibre insulation board	0.053	240	1250	15 × 10 ³
Polystyrene foam	0.034	20	1500	1 × 10 ³
Air	0.024	1.3	1000	0.03 × 10 ³

Table 2 Effect of density on rate of burning to BS4735 for standard polyether foams¹

Density of material (kg/m ³)	Rate of burning (mm/min)
15	3.7
22	2.5
32	1.6
10 ³ (solid)	0*

*Did not ignite in the test.

growth, and to reducing the rate of production of smoke and toxic products. These recommendations are perfectly logical because without ignition there can be no fire while a slow burning fire would produce lower concentrations of smoke etc, thereby giving greater time for escape and for first aid fire-fighting. This situation applies to items ignited in the early stages of a fire but once the fire is fully developed, the situation changes and the hot smoke and toxic gases produced become more dependent on the total materials involved and the ventilation, building design, use, etc.

Punderson⁷ has described how the use of toxicity tests to rank or rate materials with respect to fire safety has not proved to be a fruitful concept because of the fundamental nature of fire. He concludes that it is impossible to specify materials of zero or acceptable fire toxicity because all burning organic materials produce toxic gases and the degree of toxicity, whether lethal or harmless depends on concentration, and duration of exposure. The wide range of rates of burning recorded for materials greatly exceeds the maximum differences of the specific toxicity of their fire gases and control of ignition and burn rate offers the best means of reducing toxic hazards.

Smoke and gases from fires

Although many of the chemical compounds found in fire smoke are undeniably highly toxic, there are a number of factors which must be taken into account when considering toxicity: firstly, the nature of the compound in question; secondly, the concentration of the compound; thirdly, the duration of exposure; and fourthly, other compounds present as well as heat and oxygen depletion.

Factors 1 and 4 are directly related to the materials burned and to oxygen availability. Factor 2 depends largely on the rate of burning, the smoke catchment volume, degree of ventilation and the amount of material present. The duration of exposure (factor 3) depends on the possibilities of escape which are partly determined by human and environmental factors and partly by the density and irritancy of the smoke restricting visibility, factors which relate to the type of material and the rate of burning.

Hydrocarbon polymers containing only hydrogen and carbon will burn to completion to produce carbon dioxide and water. Polymers containing sulphur will also produce sulphur dioxide and those containing nitrogen, nitrogen oxides. Halides will be released as hydrogen halides. Complete combustion rarely occurs in natural fires and the products of combustion normally comprise a mixture of pyrolysis, partial oxidation and complete

Table 3 Typical analysis of smoke from polyurethane foam*

Gases produced	Approximate percentage by weight	Comments
Carbon monoxide	12-30	Mainly above 300°C, increased without oxygen
Carbon dioxide	60-80	Mainly above 300°C
Hydrogen cyanide	1-3	Mainly above 600°C
Isocyanates		Mainly 200-300°C Decompose at 800°C
Acetonitrile		
Acrylonitrile		
Benzonitrile	1	Mainly during pyrolysis at 700-1000°C
Pyridene		
Ammonia		Mainly 400-600°C
Nitrogen oxides		Mainly above 800°C
Aliphatic hydrocarbons	1	Above 300°C
Aromatic hydrocarbons	1	Above 300°C, increases with higher temperature

Table 4 Hydrogen cyanide from polymers at 500°C¹⁰

Material	Hydrogen cyanide (mg/g)
Polyurethane flexible foam	0.1- 9.0
Polyurethane flame-retarded flexible foam	1.7
Polyurethane rigid foam	11.5-17.1
Polyurethane flame-retarded rigid foam	4.3- 8.0
Polyisocyanurate foam	9.1-20.7
Nylon	1.6
Polyacrylonitrile	20.4
Wool	67.7
Dried sardines	5.3

oxidation products. Low temperature pyrolysis (less than 400°C) gives a number of complex degradation products which directly relate to the basic polymer structure. Higher temperatures (400-700°C) produce a complex mixture of degradation and partial oxidation products including carbon monoxide (in large quantities), aldehydes etc. High temperatures (>800°C) combined with high oxygen availability tend to produce the simple products of complete combustion. In actual fires, products from each of these three zones are present, their relative concentrations depending on both material and fire parameters.⁸ Typical breakdown products from polyurethane foams are shown in Table 3.⁹

Polyurethane foams in fire are invariably linked to the production of hydrogen cyanide but the assumption that removing polyurethane from fires would eliminate hydrogen cyanide is wrong as it is produced by many other materials (see Table 4).¹⁰ Despite the prevalence of hydrogen cyanide in smoke, however, the main toxic hazard in fires is carbon monoxide which is produced in all fires.⁴ Animal toxicity tests using pyrolysis products from polyurethane foams have shown that hydrogen cyanide gave a statistically significant increase in toxicity over that due to carbon monoxide but that the increase in actual hazard was only marginal.¹¹

Much has been said about super-toxicants but the only proven instance of a super-toxicant is related to the use of trimethylol propane polyols in polyisocyanurate (PIR) and polyurethane (PUR) foams. The formation of trimethylol propane phosphate in very small concentrations was demonstrated under laboratory conditions rather than in actual fires and the result of this work was the reformulation of rigid foams eliminating the use of trimethyl propane and thereby the possibility of its phosphate occurring in fire.¹²

Behaviour of plastics and rubber foams in fire

Rigid foams, building and insulation applications

A number of rigid foams can be formulated to give a relatively high performance in specified standard tests, for example, polyisocyanurate and phenolic foam boards will give a Class 1 spread of flame rating to BS476, Part 7. As with all organic materials, polymer-based foams will burn if the fire conditions are sufficiently severe. In actual fires,

Table 5 Corner tests, unprotected foam - 75kg crib¹

Parameter	Polyisocyanurate foam	Phenolic foam
Time to flashover (mins)	1.5	—
Time to maximum temperature (mins)	3	14
Maximum temperature (°C)	600	637
Maximum smoke density (OD/m)	>4.5	1.4
Smoke volume (m ³)	>4000	1100
Ceiling temperature* (°C)	875	780
Smoke density* (OD/m)	1.6	0.6
Carbon monoxide* (per cent)	0.33	0.16
Carbon dioxide* (per cent)	19	16
Oxygen* (per cent)	6.7	7.9

* After 10 mins

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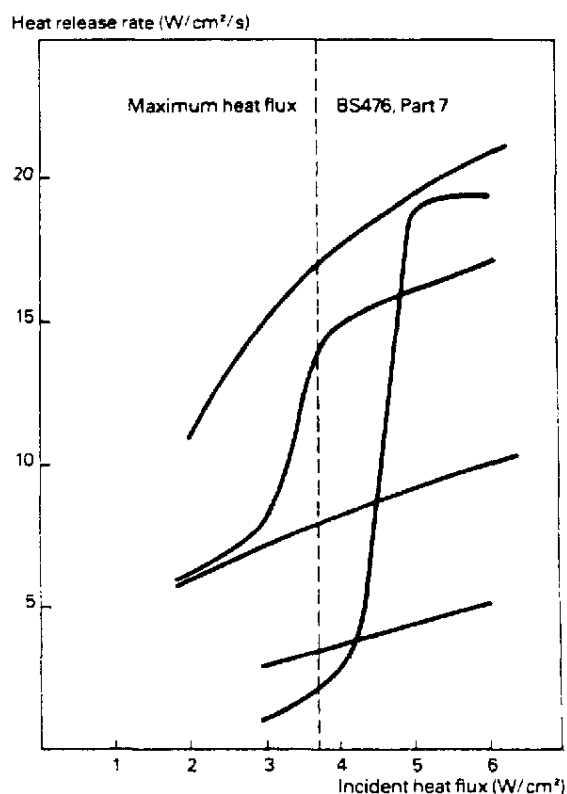


Fig 3 Relationship between heat release rate and incident heat flux for polyurethane foams¹⁴

heat flux values of more than 6-10W/cm² can occur whereas the maximum heat flux in BS476, Part 7 is only 3.7W/cm². BS476, Part 6, the fire propagation box, exposes samples to 5W/cm².¹³

Figure 3 shows that an increase in heat flux can completely alter the ranking and behaviour of materials.¹⁴ In RAPRA corner tests, a Class 1 polyisocyanurate foam burned rapidly when exposed to a heat flux of more than 7W/cm² whereas a phenolic foam, also Class 1, merely charred under similar conditions (Table 5). Corner tests¹⁵ with unprotected insulating foam with a 2.5m corner and a

14kg wood crib produced rapid flame spread with polyisocyanurate foam, conditions approaching flashover. Polystyrene (PS), phenolic foams (PF) and furane foams (FF) gave progressively less severe fires with animal mortality data indicating that phenolic foam gave the worst result (Table 6), possibly due to a smouldering reaction which gave high amounts of carbon monoxide.

A comparison of bulk materials is given in Table 7 which shows the effect of different material types and of different material foams. As expected, foamed polyurethanes and polystyrene produce hot smoky fires with phenolic foams, wood sticks and wood wool producing similar fire temperatures but less rapid burning and less smoke.¹⁶ Although important from a storage point of view, these differences are less important in practice because foam insulation is rarely used in its natural state but is almost invariably protected from mechanical damage. The fire behaviour then becomes intimately linked with that of the composite.

Large fire tests with stacks of crosslinked polyethylene and ethylene-vinyl acetate foams produced lower smoke than other plastics foams. Fire gases comprised carbon monoxide and carbon dioxide and a wide range of hydrocarbons which produced an irritant effect. A major hazard was the formation of a pool of burning polymer melt.²³ The latter effect has also been observed with other thermoplastics materials.

Fire performance of polymeric foams in building applications

A major part of this section deals with panels comprising surfaced polyurethane boards but the principles indicated also apply to boards based on other foams. Ignition tests using sources such as matches, crumpled newspaper and fibreboard cribs failed to cause sustained flaming of any of the board surfaces tested. Although these sources are minor, they represent ignition sources likely to occur on building sites etc, and show that the boards concerned are not likely to be ignited accidentally. Tests to BS476, Part 6 indicate the different performance when the panels were exposed to a more intense heat source (Table 8).

Intermediate scale tests involved the use of a 1m²

Table 6 Corner tests, unprotected foams - 14kg crib¹⁵

Parameter	Soft wood	PIR	PF	PS	FF
Flame spread (m)	1	2.5	0.5-1	1.5-2.5	1
Maximum temperature (°C)	220	560	340	230	300
Time to reach OD m=0.26 (mins)	∞	4	8	5	∞
Maximum carbon monoxide (per cent)	0.1	1.6	1.2	0.4	0.2
Maximum carbon dioxide (per cent)	5.8	5.9	7.3	6.1	5.6
Minimum oxygen (per cent)	12.8	10.5	11.8	11.4	13.2
Maximum hydrocarbons (ppm)	<100	1900	1000	400	<100
Maximum hydrogen cyanide (ppm)	0	380	50	0	130
Maximum nitrogen oxides (ppm)	5	45	12	0	130
Rat mortality (rank order A = worst)	E	B	A	C	D

Table 7 Burning characteristics of materials - small room test¹⁶

Material	Form	Maximum temperature (°C)	Time to maximum temperature (mins)	Rate of temperature rise (°C/min)	Maximum smoke density (OD/m)	Maximum smoke rate (m ³ /min of OD/m = 1)	Total smoke (m ³ of OD/m = 1)
Newsprint	Folded	237	4	187	0.4	0.8	8
Wood - pine	Sticks	748	7	187	0.8	5.5	38
Wood - oak	Sticks	130	7	30	—	—	—
Wood	Wool	729	4	760	2.7	0.8	38
Phenolic foam	Granules	82	7.5	13	—	—	—
Phenolic foam	Board	770	7.5	148	1.2	3.6	9
Polystyrene foam	Blocks	1100	6.5	458	18.7	139	1452
Flame-retarded polyurethane foam	Blocks	880	4.5	419	15.7	225	503
Flame-retarded polyurethane foam	Blocks	850	3.5	644	15.2	165	482
Polyisocyanurate foam	Blocks	771	5.5	482	15.5	130	322

Table 8 Fire propagation indices of panels containing polyurethane foams¹

Surface	Core	Indices BS476, Part 6	
		i	l
None	PUR	24	32
None	PIR	11	19
Acrylic paint, aluminium foil	PUR	1.6	4.1
Hardboard	PUR	7.9	29.0
Plywood	PUR	6.0	25.2
Plasterboard	PUR	3.9	8.5
Plasterboard	PIR	4.2	9.0

ventilated steel box.¹ Measurements of smoke, temperature, carbon monoxide and hydrogen cyanide were made during a series of tests in which foam-based panels were exposed to a 3kg, fast burning crib on the floor of the box. All panels consisted of a 25mm foam slab (except the penultimate which was 16mm thick) bonded to the surface shown. Fire retarded polyurethane foam was used for all the tests except the ultimate which used polyisocyanurate foam.

Table 9 shows that when the polyurethane and polyisocyanurate foam boards were protected by 9.5mm plasterboard, the concentration of carbon monoxide was similar to that of the ignition source alone and that hydrogen cyanide concentrations remained less than 20ppm. In contrast, carbon monoxide concentrations of 2.5-5.5 per cent and hydrogen cyanide concentrations of 150-250ppm were recorded when the polyurethane foam was used behind flammable coverings. The painted aluminium foil did not thermally insulate the foam and actually melted during the test, thereby enabling the foam to burn, producing similar atmosphere to that with the wood/foam panels.¹

Corner tests with a 16kg crib placed 50mm from the test walls showed that 12.5mm plaster offered considerable protection to the 25mm extruded polystyrene foam boards.¹⁷ Even when the plaster was partly removed and the foam directly exposed to the fire source, the resultant fire was limited in area and went out when the fire source was extinguished. The use of 9.5mm plasterboard offered less protection than the 12mm plaster but even so the protection was considerable. Tests showed non-burning adhesives should be used to minimise the extent of burning behind the plaster. In tests using a much larger crib (50kg), the plaster again offered considerable protection but collapsed as the thermoplastic foam melted. In these tests, the plasterboard was bonded to the foam which was in turn bonded to the wall. The foam melted leaving the plaster unsupported which then collapsed giving rapid flashover and a very smoky fire of brief duration. The latter was due to the low

Table 10 Penetration of polyurethane foam ceilings, crib test¹

Type of ceiling	Penetration of ceiling	Penetration of adjacent room	Smoke (OD/m = 1.4 in adjacent room)
Plasterboard (12mm) + 50mm glass fibre	29.5	None	38
Fibre insulating board	21.5	32.5	26
Polyurethane	6.75	8.5	10
Polyisocyanurate	11	14.75	15

Table 11 Penetration of polyurethane foam ceilings, high severity fire test¹⁸

Type of ceiling	Failure of ceiling (mins)	Full fire in loft (mins)	Penetration of adjacent room (mins)
Plasterboard (9mm) + skin coat	21	21	None
Polyisocyanurate (12mm) + 12mm plasterboard + ceiling finish	25	25.25	None
Polyisocyanurate (12mm) + ceiling finish + 50mm mineral wool inadequate laying	10.5	11.25	13
Polyisocyanurate (12mm) + ceiling finish + 50mm mineral wool good laying	20	16	None

density of the foam and the small mass of material actually present. A combination of mechanical fixing of the plaster to the wall with adhesive bonding should increase the time to failure.¹⁷ Corner tests involving a 50kg crib with plasterboard (9mm) covered with polyisocyanurate and polystyrene foams have been discussed earlier and their temperature/time profiles are shown in Fig 1. In these tests, the plasterboard was mechanically fixed to the wall (Fig 1).¹

The use of polyurethane foam insulation panels in ceilings has been questioned following fires in residential buildings. Fire investigations and large-scale tests at the Fire Research Station¹⁸ concluded that the use of polyurethane ceilings could lead to increased fire hazard because of easier penetration of the roof space and adjacent rooms. The rate of burning of the roof itself was independent of the ceiling, being related to the roof timbers and sarking (the weather barrier between the tiles and the roof - often of bitumenised paper). Ceiling penetration was not observed with plasterboard ceilings and although penetration occurred with fibre insulation board ceilings, it was only after 30 minutes compared to 8.5-15 minutes for polyurethane and polyisocyanurate boards (Tables 10 and 11).

With hindsight, it is easy to point out the poorer perfor-

Table 9 Burning characteristics of panels containing polyurethane foam¹

Test no.	Panel surface	Maximum core temperature (°C)	Maximum surface temperature (°C)	Maximum smoke	Maximum CO (per cent)	Maximum CO ₂ (per cent)	Minimum O ₂ (per cent)	Foam maximum HCN (ppm)
1	None	—	725	0.66	0.4	6.0	11.4	12
2	Acrylic painted	—	2.5	3	3	6	3.5	3.5
	Aluminum foil	780	725	>1.7	1.0	8.0	12.0	150
3	Hardboard	6	4	8	6.25	7	6	12
		900	775	>1.7	2.5	15.6	2.75	150
4	Plywood	15	8	10	8.5	9.5	8.5	6.5
		800	800	>1.7	5.6	15.6	1.25	250
5	Plasterboard (9mm)	12	11	10	11	12	12	11
		90	325	0.71	0.4	12.8	7.0	5
6	Plasterboard (12mm)	3	3	3	7	5	5	6
		100	600	0.83	1.4	7.5	13.5	20
		4	3	3	3	3.5	5	4.5

Table 12 Polystyrene foam ceilings in fire¹³

Adhesive	Thickness (mm)	Finish	Fire growth
Five dabs	9	None	Rapid fire spread, burning debris
All over	9	None	Limited spread, no burning debris
All over	9	Emulsion paint	Limited spread, no burning debris
All over	9	Gloss paint	Rapid fire, burning debris
All over	9	Ceiling paper	Rapid fire, burning debris
All over	9	Plaster	Very limited, rate determined by failure of plaster

mance of the synthetic materials but this type of application is controlled by building regulations which specify tests and performance levels for the products used in buildings. In the case of ceilings, fire penetration tests are not required if the space above is not occupied. In addition, polyisocyanurate foam boards can comply with the Class 1 spread of flame rating (BS476, Part 7). This is a good example of the development of new materials and products outstripping that of tests and test specifications. Although these shortcomings could be and were shown by *ad hoc* tests, the existence of regulations would tend to discourage this type of research.

The performance of other materials would be one of pure speculation in the absence of experimental data. Polystyrene foam would be expected to melt causing rapid penetration and while other foams such as phenolic materials would not melt, shrinkage cracks may occur. In the absence of experimental data, the only satisfactory method of decreasing the penetration risk of foam ceilings is by protecting the foam by the use of materials such as gypsum plaster (Table 11).

Polystyrene foam is widely used for ceiling insulation, as insulation boards, in suspended level ceilings and as 'do-it-yourself' tiles. The total amount of polystyrene present in a given room is relatively low because of the low density of the tiles and even if burned to completion, the smoke and toxic gases produced would be relatively low. A large number of tests based on simulated fat frying fires (using a 6.5kg wood

Table 13 Comparison of traditional and steel/polyurethane foam panel buildings¹⁴

Parameter	Traditional mineral wool A	Steel/PUR panels B	Steel/PUR + cavity C
Minimum visibility (m)	3.5	2.5	2.5
Maximum carbon monoxide (ppm)	200	100	100
Maximum hydrogen cyanide (ppm)	Trace	<5 but peaks of 8 & 17	<5 but peaks of 8 & 17
Nitrogen oxides (ppm)	8	8	8

Table 14 Comparison of structural foams (SPI/UL study)¹⁵

	A	B	C	D	E
UL94	VO	VI	VO	VO	VO
	SV	SV	SV	SV	SV
Oxygen index (per cent)	38	29	27	27	33
Ignition temperature (flash) (°C)	425	414	395	370	422
Ignition temperature (self) (°C)	560	475	470	400	560
Ignition temperature (OSU) (°C)	370	1310	2480	2870	530
Smoke density - ASTM E662 (D _m)	448	732	750	752	389
Smoke obscuration - ASTM D2843 (per cent)	82	346	791	577	86
Rank order	79	94	96	95	69
Full-scale room test	1	2	3	4	5
Smoke production	A	E	B	C	D
Carbon monoxide	A	E	D	B	C

Table 15 Ignition characteristics of structural plastics foams and traditional materials used in furniture applications¹

Material	Minimum heat flux* (W/cm ²)	Ignition time (mins) at specified heat flux		
		2.5W/cm ²	3.75W/cm ²	5W/cm ²
<i>Pilot flame ignition</i>				
Chipboard	3.0	—	6.2	4.5
Plywood	2.2	7.6	4.8	3.8
Hardboard	1.9	7.6	4.8	3.5
Polyurethane foam	1.6	3.9	2.5	2.2
Flame-retarded polyurethane foam	1.5	7.5	3.2	2.0
Polystyrene foam	1.6	5.4	3.4	2.7
Polypropylene	1.1	4.3	3.3	3.0
<i>Self-ignition</i>				
Chipboard	2.9	—	6.0	3.9
Plywood	2.9	—	6.4	4.2
Hardboard	2.2	11.0	5.7	4.1
Polyurethane foam	2.5	14.0	3.2	2.6
Flame-retarded polyurethane foam	2.2	16.5	3.9	2.8
Polystyrene foam	2.2	9.2	4.3	2.9
Polypropylene	1.4	6.3	3.5	2.7

* Min heat flux to cause sustained burning

crib) and a full-scale room burn (200kg wood crib), proved that the amount of foam burned depended largely on the method of fixing of the tiles and boards¹⁹ (see Table 12).

The use of 50mm foam boards instead of 9mm tiles produced a rain of burning molten polymer. Flame retarding additives did not significantly affect the results of 9mm thick tiles but resulted in a non-burning rain of molten polymer with the thicker (50mm) materials. With suspended level ceilings in which sheets of material softening below 120°C to BS2782, Part 102C are loosely supported on an inverted T-frame, flame-retarded polystyrene foam slabs fell to the floor without igniting. Plaster offered considerable protection to the polystyrene foam but could only be effective as long as it remained in position. As polystyrene foam is a thermoplastic, the plaster should be mechanically fixed e.g. by nailing to the ceiling structure and should remain intact during the fire if it is to be fully effective.

Steel/PU foam building panels. Full-scale tests of buildings constructed from polyurethane foam-filled steel panels and of traditional buildings, were carried out in buildings measuring 30ft x 15ft with a sloping room 17.5ft x 15ft high.²⁰ The traditional building was insulated with mineral wool covered with fibre insulating board treated to give Class 1 spread of flame (BS476, Part 7) performance. The fire source consisted of timber cribs of 635kg giving a maximum temperature of 740°C.

The roof of the traditional building burned fiercely, causing burning bitumen to spread fire, but although there was damage, this did not occur with the polyurethane foam building. Fire did not penetrate the walls of the panels in which the steel facings were in direct contact with both sides of the foam. In contrast, fire spread along the wall comprising 25mm polyurethane foam with a 25mm cavity. It was concluded by the Fire Research Station that the use of polyurethane foam cored panels did not add to the severity of the fire and did not produce any serious toxic hazard within the building (Table 13).

The Fire Offices Committee accepted that steel-faced panels, completely filled with polyurethane foam did not increase the risk above that of conventional steel cladding and should not attract increased premiums.²⁰ The data show that polymeric foams do not constitute a hazard provided

Table 16 Burning behaviour of three-piece suites upholstered with flexible polyether foam with different structures

Cover	Structure	Maximum smoke temperature (°C)	Maximum smoke density (OD/m)	Total smoke volume (OD/m = 1 m ³ × 10 ³)	Maximum carbon monoxide (per cent)	Maximum carbon dioxide (per cent)
Cellulosic	Wood	540-790	1.8-1.9	5-9	.33-.35	17-20
	Polystyrene foam	590-640	2.9->3	>24->30	.85-1.7	15-18
	Polyurethane flexible foam	500-700	1.9-2.5	7-11	.35-.4	13-15
Plasticised PVC	Wood	480	2.9	17	0.37	8
	Polyurethane rigid foam	530	>3	>29	1.3	12

they are adequately protected from fire. The protection must obviously remain in position and must resist the heat flux values imposed by a real fire. A major problem exists for specifiers in that no standard test exists which imposes heat flux values greater than 5W/cm² and *ad hoc* or full-scale tests are often needed for critical applications. Corner tests are increasingly used to assess the performance of wall linings, building panels etc, and are now being considered for standardisation.

Fire performance of structural or integral skin foams

Structural foam consists of a low-density, closed-cell, cellular core contained within an integral polymer skin. The resulting structure gives a high stiffness to weight ratio. Structural foams can be produced using chemical blowing agents, or by gases such as nitrogen, pentane, fluorinated compounds etc. They may be single thermoplastic materials, layered thermoplastic materials made using the sandwich process or, alternatively, may be a crosslinked material made using a casting or reaction moulding process. The latter is usually a polyurethane but thermoplastic foams may be prepared from many polymers including polyacetal, ABS (ter-polymer of acrylonitrile, butadiene and styrene), polyamide, polycarbonate, polyester, polyethylene, modified polyphenylene oxide, polypropylene and polystyrene.

The burning behaviour of structural foams from these polymers and especially the smoke and toxic gases produced will obviously vary enormously. Little published information is available concerning their burning characteristics but theoretical considerations would indicate that they would be expected to burn faster than the unfoamed polymer bases.

Structural foams are increasingly used as replacements for metal cases for business machines, computers etc. A major project was carried out by the Society of the Plastics Industry and the Underwriters Laboratory in the USA to determine the behaviour of structural foams in fire using standard, small test methods.²¹ An important aspect of the work was the use of 'internal' gas ignition sources representing burning electronic components inside the cabinets. The tests concentrated on ignition and burning but

also measured smoke and carbon monoxide. Five structural foam materials were used but these were not identified except by their performance in standard tests. It was concluded that small-scale tests can identify structural foam materials which ignite readily (UL 94, Setchkin), flame spread (radiant panel, OSU calorimeter) and to the rank order of smoke release (XP2, NBS and especially the OSU calorimeter). An important conclusion was that structural foam materials could be prepared which resisted the internal ignition sources (see Table 14).

Structural foam furniture. A comparison of the ignition resistance of structural foam materials used in furniture is shown in Table 14 and 15.¹ Two main structural furniture applications exist: first, as the main frame or shell of upholstered furniture and, second, as simulated wood in cabinet furniture. The latter application is similar to that of materials-handling and machinery covers mentioned above, but, in contrast, plastics foams are used to replace wood rather than metal.

Upholstered furniture. When used as chair shells, plastics foams are not structural foams within the definition given above but are, strictly speaking, foams used for structural purposes. They do not have an integral skin and correspond more closely to higher density versions of the insulation foams described previously.

When used as a chair shell, polyurethane and polystyrene foams permit the use of complex, curved designs without the complicated construction needed to produce a similar design with traditional materials. The ignition and early burning characteristics of upholstered shell chairs will be largely determined by the covering fabric and soft upholstery which are discussed in the next section. The foam core would not be expected to burn until the later stages of a fire. If ignited, both polyurethane and polystyrene foam shells would be expected to make a major contribution to the heat, smoke and toxic fume hazards, but this would depend on the ignition resistance of the foam used. A polystyrene foam shell would melt while the polyurethane shell would probably not. Both shell materials would be expected to produce a greater fire hazard than the alternative wooden structure although it would be unlikely to be involved in the very early stages of the fire (Table 16).

Full-scale tests with structural foam. Ignition tests on moulded and rigid foam furniture showed that it was easier to ignite and burned more readily than traditional furniture producing burning molten drips and dense smoke (Table 17). Certain items were ignited by a match while other items, particularly the flame-retarded polyurethane foams proved relatively difficult to ignite. In a full-scale fire test, an 'all

Table 17 Comparison of fully furnished office burns²

Parameter	Wood-based office	Structural foam-based office
Maximum temperature (°C)	600	775
Time to maximum temperature (mins)	123	11
Time to OD/m (mins)	5	3.5
Time to OD/m (mins)	8.5	3.5
Maximum CO (per cent)	0.05	3
Maximum CO ₂ (per cent)	2.25	12.5
Minimum O ₂ (per cent)	20	0.5
Maximum NO _x (ppm)	40	50
Maximum HCN (ppm)	3	300

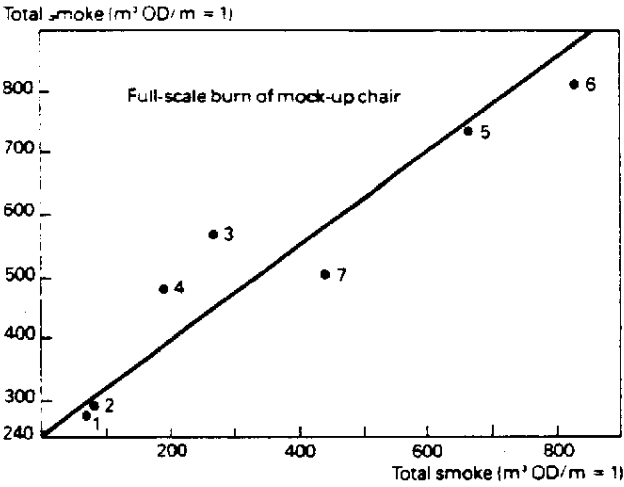


Fig 4a Correlation between smoke formation from full-scale tests and results calculated from NBS (flaming) data (1 = cotton/standard polyether; 2 = acrylic/standard polyether; 3 = polypropylene/standard polyether; 4 = polypropylene/standard polyether; 5 = flame-retarded PVC standard polyether; 6 = flame-retarded PVC/standard polyether; 7 = PVC/standard polyether)¹

plastics' office suite including polyurethane, polystyrene, and PVC structural foams gave a fast burning fire with large quantities of smoke. The smoke temperature did not remain high for long, but the emission of dense smoke continued from small floor fires after the temperature had dropped. In contrast, a traditional 'wooden' office generated more heat but burned slowly over a long period with less smoke especially towards the end of the fire.³

A true assessment cannot be made, especially in view of the very wide range of improved materials now available, but the limited test data indicate the poor performance obtained from ill-chosen materials. It is now possible to obtain structural foams which will resist the No. 7 crib of DoE/PSA/FR10 and there is an obvious need to investigate the fire performance of structural foam materials in order to avoid the fire situation illustrated by the office test discussed above.

In contrast to the many thermoplastic structural foams, rigid polyurethane foams do not melt and as such come closer to the Home Office recommendation⁶ that structural foam furniture should behave in a similar manner to wood. The criteria cannot be applied in detail because of the

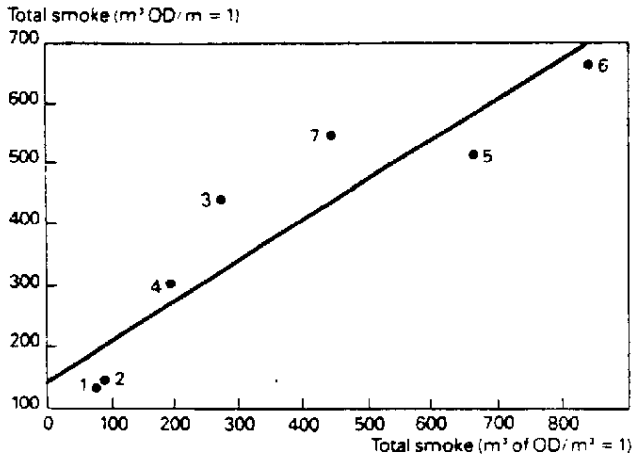


Fig 4b Correlation between smoke formation from full-scale tests and results calculated from XP2 (modified ASTM D2834) data¹

inherent differences between wood and plastics but the idea is that the ignition resistance of structural furniture should be increased and the burn rate, smoke and toxic gas formation of current products should be reduced. Many timber products and panel furniture are resistant to fairly large ignition sources and the Home Office suggestion⁶ that large areas of panel furniture should comply with flame spread ratings may be obtainable with carefully formulated structural foams.

Fire performance of polymeric foams in upholstered furniture and bedding

The burning characteristics of some modern upholstered furniture have been identified as relatively easy ignition (estimated as 90 per cent by match flame, 20 per cent by cigarette) and rapid burning, the latter causing dense smoke, intense heat and toxic combustion products. An extreme case is represented by a chair being ignited by a match and smoke filling an open room (34m³) within 4-5 mins with heat and toxic fumes reaching lethal levels shortly afterwards.³

These characteristics are often considered to be directly due to the introduction of polyurethane foam and while it is an important factor, it is the combustion of cover and core which is important (Table 18a). If many of the traditional fabrics such as woven wool and wool moquettes were used with polyurethane foam, the incidence of match and

Table 18 Effect of alternative fabrics and alternative fillings¹ - model upholstered chair test

Material	Ignitability*	Maximum temperature (°C)	Time to maximum temperature (mins)	Time (OD/m = 0.2 mins)	Maximum smoke density (OD·m)	Total volume smoke (m ³)
Table 18a Standard polyether (SPE) foam with different fabrics						
1 Acrylic/cotton SPE	1P	180	4	1.25	0.7	307
2 Polypropylene/SPE	1P	283	3	1.25	0.5	114
3 Viscose/SPE	1P	190	8	8.5	0.3	71
Table 18b Traditional fabrics						
4 Wool/SPE	2P	125	4	3.5	0.4	132
5 Wool/cotton wadd/SPE	3/4P	38	3.5	—	0.1	4
6 PVC/cotton/SPE	6P	280	9	2.5	1.9	500
Table 18c Viscose fabric with alternative fillings						
7 SPE	1F	190	8	8.5	0.3	71
8 Flame-retarded polyurethane foam†	1P	170	7	16	0.5	107
9 Polyester fibre	1P	250	4.5	3	1.2	177
10 Latex foam†	1F	170	12	3	>1.3	>200
11 Cotton wadd. SPE	1F	324	11.5	6.5	1.1	314
12 FR cotton int. SPE	1F	280	6.5	8.5	0.3	75

* To BS 5852 P = pass cigarette; F = fail cigarette; number equals flame source to fail
† Estimated value

Table 19 Comparison of heat, smoke and gas data for fully furnished room tests

Parameter	Wool/cotton* fibre and wadding	Cotton/wool nylon latex	Cotton/ standard polyurethane	Flame-retarded PVC/standard polyurethane	Flame-retarded PVC/flame- retarded polyurethane	Flame-retarded cotton flame retarded polyurethane
Maximum temperature (°C)	620	710-790	775	735	750	680
Time to maximum temperature (mins)	25-56	20-38	3-7.5	47	12	117
Time to 0.32 OD/m (mins)	15-16	1-5	1.75-3	6	6	10
Time to 0.64 OD/m (mins)	24-40	2.25-6	2.5-5.5	8.5	13	47
Maximum CO (per cent)	0.4-0.7	0.3-0.7	0.2-1.8	33	0.5	2.5
Maximum CO ₂ (per cent)	5-10	11-16	7.5-18	15	8	11
Minimum O ₂ (per cent)	8-13	2.5-7.5	4.5-12.5	1	11.5	2.5
Maximum NO _x (ppm)	0-40	0-10	60	40	60	100
Maximum HCN (ppm)	8-50	0-10	40-80	100	500	56

* Fabric and main upholstery

cigarette ignition would largely be eliminated (Table 18b). The frequently suggested solution of banning polyurethane foam or of using flame-retarded polyurethane foam will not and cannot be effective in preventing ignition, because it is the combination of modern easily ignitable fabrics with foams which causes the problem. The effect of alternative cores with a viscose fabric is shown in Table 18c. All composites will ignite with a match and all except 8, 9 and 12 with a cigarette. In terms of increasing escape time and low smoke production, clearly only 8 and 12 show any improvement. Non-urethane alternatives 9 and 10 show a major increase in smoke production. The purpose of this table is to illustrate that removal of polyurethane foam cannot alone solve the fire problem as it can only be solved by setting meaningful standards in terms of identified hazards i.e. ignition, heat/temperature rise, smoke and toxic fumes. Table 18b shows data of chairs containing polyurethane which pass both cigarette and match test (BS5852, Part 1) and are considerable improvements on many current fabric/foam combinations.

Current furniture controls are aimed at increasing the ignition resistance of composites which indirectly may or may not reduce the rate of burning. A significant decrease in burn rate should, providing care is taken with material selection, reduce the rate of production of heat, smoke and toxic fumes and thereby increase the possibility of escape and of fire-fighting.

By careful selection of materials, it is possible to produce furniture of similar burning characteristics to traditional furniture. At present there is not a wide variety of flame resistant materials but this should improve. An important recent development is the fire retarded barrier foams based on polyurethanes and polychloroprenes. These foams, which can individually attain a Class 1 spread of flame to BS476, Part 7 are used as barriers around standard polyether foams to impart increased ignition resistance (often greater than the 126g. No. 7 wood crib of BS5852, Part 2) and to reduce significantly the rate of burning and smoke and toxic gas production (Table 19).

It has been shown that the maximum amount of smoke produced from upholstered composites can be approximated from the sum of the smoke produced by the individual components. Good correlations have been obtained between full-scale and model furniture tests using the XP2 and the NBS smoke tests, the latter being operated in the flaming mode (see Figs 4a and 4b). The use of highly flame-retarded fabrics and barrier foams can restrict the amount of foam burned²² and in certain fires, the theoretical amount of smoke and toxic gas may not be produced. While small-scale tests can, in this case, be used to indicate the maximum amount of smoke produced, they are not able to indicate the rate of burning.

A similar situation exists with mattresses and beds where once again, the role of the cover, in this case the blankets, is all important. A direct change from a polyurethane foam mattress to a rubber latex mattress in a fully furnished room test resulted in complete destruction of the room furniture compared to destruction of only the bed in the polyurethane mattress test. Table 20 shows that it is possible to obtain more satisfactory characteristics by the careful selection of polyurethane foams, covers etc.³ An important advantage of polyurethane foams and especially the high resilience of fire retarded polyurethane foams is that they do not readily smoulder. In contrast, many traditional materials are very prone to smouldering. It is natural to compare the performance of new with traditional products but in devising a safer environment, it is necessary to recognise the shortcomings of existing products and to overcome both traditional and new hazards.

Fire performance of polymeric foams in transport

Fire requirements vary between applications but the US test FMVSS 302 is widely used in the automobile and road transport industries and is applied to internally used materials. A strip of a material or a component is subjected to a small flame and the horizontal rate of burning measured. Specifications exist for maximum rates of burning of 100mm/min, 80mm/min and non-burning in the test. Many of the applications for foams in transport are similar to those discussed previously; they include sound insulation, rigid structural foams, flexible structural foam crash pads and upholstered seating.

Discussion and conclusion

In its Report on the Fire Risks of New Materials,⁶ the Home Office Fire Department Technical Sub-Committee said 'There is no case at present for a general ban on the use of any particular material'. This statement should be applied to polymeric foams for two reasons. First, a ban is not effective as it merely encourages the use of alternative materials, some of which may have even worse fire behaviour than the material banned. Secondly, foams are rarely used on their own but are almost invariably part of a composite and it is the fire performance of the composite which is important. Providing the selection of materials and product design are properly carried out, then products containing polymeric foams can be produced with satisfactory fire performances.

The only way to reduce the fire hazard of a product is by design based on identifying the risks, and the critical factors in product performance. All product designs are compromises in which the designer has to determine and trade-off the various advantages and disadvantages of his product. Many factors are involved e.g. mechanical,

Table 20 Comparison of burning characteristics of furnished bedrooms, showing effect of blanket and mattress' Blanket type

Mattress type	Acrylic	Wool	Wool	Wool	Acrylic	Acrylic
	Polyurethane	Polyurethane	Interior spring	Flame-retarded polyurethane	Flame-retarded polyurethane	Latex
Maximum temperature (°C)	690	570	100	445	500	730
Time to maximum temperature (mins)	6.5	6.5	80	24	8	27
Time to 50 per cent obscuration (mins)	5	4	8	23	2.5	5
Time to 100 per cent obscuration (mins)	6.5	8.25	58	25	8.25	6.5
Maximum CO (per cent)	0.14	0.13	0.03	0.03	0.13	0.2
Maximum CO ₂ (per cent)	6.0	3.2	2.0	2.4	3.2	6.3
Maximum HCN (ppm)	30	20	1	<30	<30	40
Maximum NO _x (ppm)	50	14	20	17	15	80

comfort, aesthetic etc, as well as fire and it is only by meeting a properly evolved design standard which considers all aspects of the use and potential hazard that a satisfactory product will be produced. Fire tests and test data are only two of a number of factors to be considered in assessing fire hazard which is discussed in BSDD64, 'Guidelines for the development and presentation of fire tests and their use in hazard assessment'.

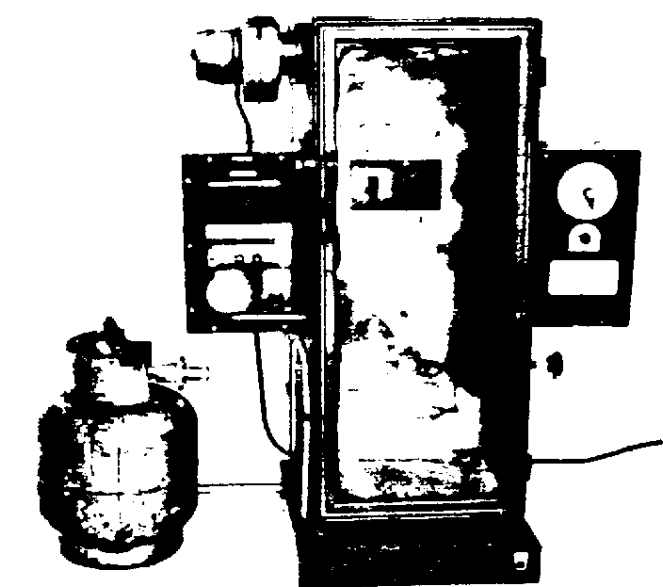
The fire performance of polymeric foams varies with material type and composition. Polymeric foams do not necessarily constitute a hazard in buildings and furniture provided proper consideration is given to materials selection, design, construction, and fire protection of such products. This statement is applicable to all products whether or not they contain polymeric foams and a minimum, acceptable level of hazard can only be obtained by correctly identifying the hazard and by hazard-based performance specifications.

Effective control of fire hazard is achieved primarily by increasing ignition resistance (without ignition there can be no fire) and by decreasing burn rate. The latter has a direct influence on heat, smoke and toxicity and attempts to control smoke and toxicity cannot succeed without control of burn rate.

Cellular plastics are, by definition, low density products based on polymers and because of their insulation characteristics will influence a fire even if they are not burned. Cellular plastics are, from a theoretical point of view, easier to ignite and will burn faster than unfoamed products, but as foams frequently form part of a composite for reasons of wear resistance or aesthetics, it is essential to design the

composite to meet relevant safety standards. Ignition and initial burning characteristics frequently depend on the outer covering material. Structural foams are rarely covered but the indications are that material developments can reduce the fire hazards of these materials, but little information has been published. Plastics and rubber foams offer an extremely wide range of very useful and desirable properties and products and, provided all aspects of material selection, design and end-use hazard are carefully assessed, products based on these foams should not present an unacceptable hazard.

Fig 5 BS5111: Determination of smoke generation characteristics of cellular plastics and cellular rubber materials

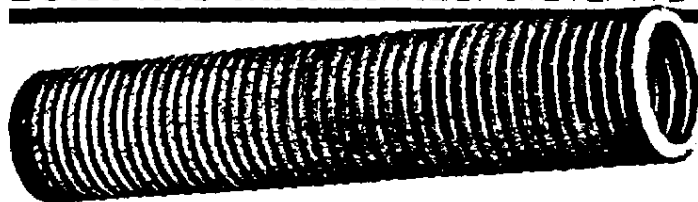


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THE DUBIOUS WAR

BUILDING MATERIALS/STEVEN FLAX



■ Fire horrifies. Leaping flames and billowing smoke, victims carried choking on stretchers or lifeless in body

bags—the sights arouse a deep-seated urge to control the menace. Major disasters like the hotel fire below can also be a marketing windfall for companies seeking to fight competing products by playing on the fear of fire.

This is the story of a company that has done so with an extraordinary outpouring of half-truths and misinformation. Its target: plastic pipe. Allied Tube & Conduit Corp. of Harvey, Illinois, is the largest U.S. producer of rigid metal pipe used to contain and protect electrical wiring. Plastic conduit, mainly made of polyvinyl chloride (PVC), has made heavy inroads, partly because it's easier to install. According to Predicasts, a market-research firm, its share of the conduit market widened from an estimated 9% in the late 1960s to 54% in 1980. Meanwhile, steel conduit's share fell, from nearly 50% to 32% of a business worth hundreds of millions a year. But Allied, a privately owned company with 1982 sales of about \$300 million, has not been content to fight back with conventional salesmanship.

Since the late Seventies, Allied has run a campaign to publicize the supposedly unusual fire hazards of PVC, pitching it at consumers, contractors, legislators, and officials who write building codes. To give its effort the appearance of impartiality, Allied has set up a not-for-profit organization, the Foundation for Fire Safety. Supposedly the foundation, with offices in Rosslyn, Virginia, is dedicated to disseminating impartial scientific information. But with about 75% of its \$750,000 budget coming from Allied, it has often served as a vehicle for anti-PVC propaganda. The foundation's officials frequently travel the country citing plastics as contributing to some of the deaths in prominent fires, even though there's no proof that their combustion products were responsible.

Allied has also lobbied heavily against the inclusion in the National Electrical Code, which serves as a guide for local codes, of a new, flexible type of PVC conduit. The new

conduit—that's a sample above—is even cheaper to install. Allied's efforts have prompted the Carlon division of New York-based Indian Head Inc.—in turn controlled by Curaçao-based Thyssen-Bornemisza N.V.—to sue it for restraint of trade. The Society of the Plastics Industry, which has long retained the public relations firm of Hill & Knowlton, has also redoubled its efforts to present its side of the controversy. Carlon, with 1982 sales of \$150 million, introduced the flexible conduit in 1980; it's also the leading maker of rigid PVC conduit. Other makers include CertainTeed Corp. of Valley Forge, Pennsylvania, and Robintech Inc. of Fort Worth, both publicly owned. Leading suppliers of PVC are B.F. Goodrich, Tencoco, and Du Pont.

ALLIED SAYS IT HAS "a moral and legal responsibility" to oppose products that constitute "an inherent, immediate, and substantial danger to the public." Asserts Theodore H. Krengel, 57, Allied's founder, president, and controlling shareholder: "The plastics produced now kill." Allied's awareness that plastics are hazardous goes back to 1972, he says. "We began to hear more and more about the problems of toxicity, flammability, and smoke of plastics." Buildings, warns Krengel, are becoming "plastic bombs—they go up in a matter of seconds." Allied's general counsel, John Lison, adds ominously: "Any company that knowingly puts a harmful product into the stream of commerce is liable for punitive damages."

Allied is not the only campaigner against plastic pipe. Carol Bellamy, New York City's RESEARCH ASSOCIATE *Ford S. Worthy*

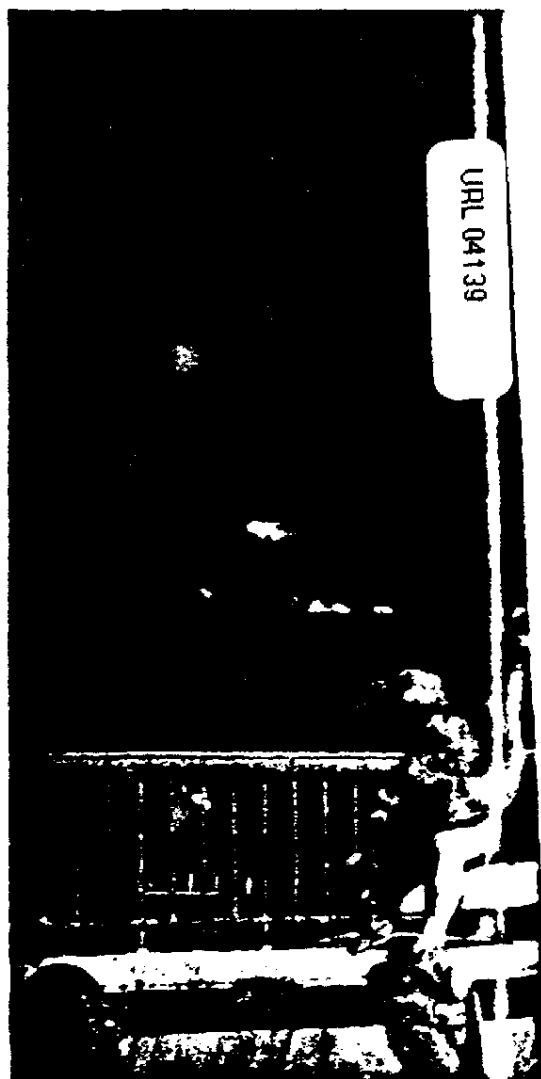


PHOTO BY GARY THOMPSON

IRON PLASTIC PIPE

politically ambitious city council president, made a fuss last year about new PVC conduit installed in subways. At her insistence \$2 million is being spent to rip out some and replace it with metal, though most of the subway system's PVC will be left where it is. Plastics' growing use in plumbing, primarily for water and drain pipes, has also come in for criticism. Allied's crusade against PVC has been especially vehement, however. A significant proportion of its sales are threatened. The last straw may have been Carlon's flexible conduit, aimed at a \$190-million-a-year market that steel had all to itself.

"I wouldn't deny we have a commercial in-

terest," says Allied's President Krengel. "It's a big market. If you include cable, conduit, ducts, and pipe, you're talking in the multibillions of dollars." If the competition gets much worse, Krengel adds, Allied might switch to plastics—"if we could find one that is proven safe." Krengel goes on to declare: "I'm not going to make any Three Mile Islands or any Love Canals. I'm not going to make anything where I can't sleep nights because we've made a product that's unsafe. I don't want that on my conscience."

Krengel could be speaking his convictions, of course. But doubt is fanned by a curious fact recently discovered by FORTUNE: Allied

itself has marketed PVC conduit on and off in recent years. In fact, it's still selling PVC-coated products.

PVC is hazardous when it burns, as the plastics industry admits. So is everything else that burns. PVC may give off, among other things, hydrogen chloride, a corrosive gas that is lethal in high concentrations. But burning natural materials also give off a host of dangerous substances, such as carbon monoxide and acrolein. When wool carpeting or upholstery catches fire, it can produce deadly cyanide gas. Furthermore, a government-sponsored study has shown that Douglas fir, widely used in construction, is every bit as hazardous when it burns as PVC.

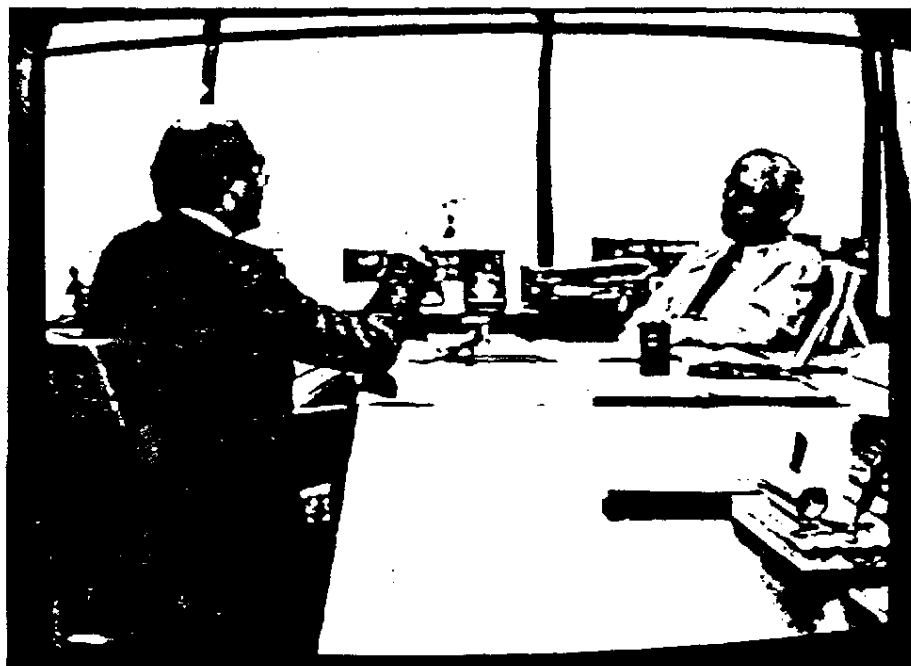
Still, wouldn't it make sense to ban plastics and use just noncombustible metal for conduit? The answer, according to experts not financed substantially by either side in this dispute, is a surprising no. There is much more to fire hazards than the toxicity of a material's combustion products. "You may be making the situation worse by having metal conduits," says Dr. Edward Radford of the University of Pittsburgh's Graduate School of Public Health. "Many fires result from electrical short circuits. One common type occurs as a result of improperly grounded metal conduits." Adds Radford, a leading authority on what kills in fires: "There is no evidence that PVC plays a major role in whether an individual dies in a fire."

Since no material is hazard free, the real issue is whether PVC is more hazardous than others. Here again the answer, according to impartial authorities, seems to be no. According to Jack Snell, director of the Center for Fire Research at the National Bureau of Standards, PVC conduit would probably be among the least of the worries in most fires. "Plastics would not create a significant additional hazard to life," says Snell. "Typically it's the contents of a building—in contrast to construction, plumbing, and electrical materials—that represent the largest fuel load. You would need a large fire before the conduit became involved, and by that time the burning contents of the room would



Neptune stood helpless as flames ravaged the MGM Grand Hotel in Las Vegas on November 21, 1980, leaving 85 dead. Allied Tube & Conduit has cited this and other fires to publicize the supposedly extraordinary hazards of the lethal products given off when plastic pipe catches fire. The final report on the fire, however, did not blame plastics for any of the deaths.

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Caught with his funding showing, Michael Olsen (right), then managing director of the Allied-supported Foundation for Fire Safety, admitted in an interview for TV that the bulk of his support came from the metal industry. At left on this April 1982 broadcast on Houston's KHOU-TV is reporter Roger Lindberg, interviewing Olsen at the foundation's headquarters.

have caused the occupants to succumb."

Snell's opinion is tersely endorsed by Irwin Benjamin, an eminent fire researcher and now a private consultant after many years at the Bureau of Standards. Says Benjamin, "If the public has been terrified about plastic conduit, that's completely ridiculous."

Surprisingly, these opinions are echoed even by Merritt Birky, director of research at the Allied-supported Foundation for Fire Safety. "Plastic conduits play little role in an ordinary hotel fire," Birky conceded in a recent interview. "It is unlikely," he adds, "that plastic conduit played any role in, for example, the fire at the Westchase Hilton in Houston." He refers to a March 1982 fire that took 12 lives.

A toxicologist with a Ph.D. in chemistry from the University of Virginia, Birky until January 1982 headed combustion toxicity studies at the National Bureau of Standards. He has generally been careful in his public utterances. Yet some of them, both before and after he changed jobs, have been used by Allied to give spurious scientific credibility to its campaign against PVC.

One of Allied's chief exhibits is the November 1980 fire at the MGM Grand Hotel in Las Vegas, which killed 85 people and injured hundreds of others. The cause of the fire, it should be noted, was a short circuit in the hotel's metal conduit. But burning PVC plumbing, Allied asserts, has been implicated

in some of the deaths. The company has seized on a statement made by Birky while he was still with the Bureau of Standards and involved in the investigation of the blaze. The carbon monoxide levels in the victims' blood, Birky speculated at that time, were too low to have caused all the deaths.

This implied that something else caused them, and Allied has repeatedly pointed to plastics ever since. In a September 1982 news release headlined "Plastics in Construction Add to Fire's Tombstones," the company's public affairs vice president, Laurence Zoeller, declared: "There is mounting evidence that decomposing plastics contributed to the vast majority of fatalities in such recent tragic fires as at the MGM Grand Hotel."

This release did not mention that in November 1981 the National Fire Protection Association, an investigative and code-writing body, had issued its final report on the fire. It said that 79 out of the 85 victims died primarily from a combination of smoke inhalation and carbon monoxide or smoke inhalation only. Burns and smoke inhalation killed three more, while the remaining three each died of a different cause: burns, skull fracture, and heart failure. As Birky emphasizes, the report's findings are vague. But they are a far cry from implicating plastics.

Birky may nevertheless have done a bit of out-and-out propagandizing, though he vigor-

ously denies it. He had taken up his post at the Foundation for Fire Safety when he was interviewed by reporter Rolando Santos of KPIX-TV in San Francisco. Santos reported last March 23: "The leading researcher in the study of the deaths at the MGM, Dr. Merritt Birky, told me in these cases the toxic gases had to come from the plastics in the room, probably cyanide." Birky claims that Santos misquoted him. Santos says that he and Birky had a detailed conversation prior to the broadcast, and that Birky said exactly what was reported.

Whatever Birky may have said about cyanide, Allied brandished this scare word before the final report on the MGM fire was out. In one May 1981 advertisement in the trade press, the company said: "The Clark County Coroner reported that five victims, who were among the first autopsied, showed evidence of cyanide in their bodies. Cyanide is produced from burning plastic pipe frequently used for drain, vent, and waste disposal, as well as plastic that is commonly used for decorative wall coverings." Allied omitted to say that cyanide is also given off when other synthetic materials burn. In any event, the presence of cyanide is no proof that it killed anyone. The final investigation report on the MGM fire never mentioned synthetic materials—plastic pipe or whatever—as the primary cause of any death.

One of the Foundation for Fire Safety's officials has resorted to innuendo in discussing the Westchase Hilton fire in Houston. Birky, as noted, says it's unlikely that plastic conduit caused any deaths there. Yet Michael Olsen, then the managing director of the foundation, got on KHOU-TV in Houston a month after the fire, offering his "preliminary report" that "toxic gases in addition to carbon monoxide must be considered as causes of death." The TV reporter told his audience, "The Fire Safety Foundation believes some of those deadly gases can come from plastics."

AS A RULE, the foundation's spokesmen have not volunteered the source of their funds during such interviews. Unfortunately for Olsen, KHOU-TV's reporter Roger Lindberg was one of the few to ask him where the money came from. Olsen admitted, listeners were told by another reporter, that "he was funded largely by the metal industry." Olsen insisted that his report was unbiased. KHOU-TV then presented Dale Everitt of the Houston Fire Department, who dismissed Olsen's views. Said Everitt, "When you have a group like this, I think they're going to be interested in keeping those funds coming in." Olsen has since

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left the foundation. Conveniently for Allied, some professional firefighters have strong opinions against plastics and will state them publicly. Shortly after the Westchase Hilton fire, Andrew Casper, then chief of the San Francisco Fire Department, appeared on KPIX-TV. Casper sketched a simple causal sequence in the MGM Grand fire: "More plastics, more fires, more deaths caused by the inhalation of toxic fumes." Later he went on the payroll of the Foundation for Fire Safety, which he has since left.

WHILE AN EX-FIRE CHIEF has credentials to speak out, this can hardly be said for another PVC opponent financed by Allied. Deborah Wallace is president of the Public Interest Scientific Consulting Service, another organization mainly backed by the company, to the tune of \$17,000 a quarter. With a Ph.D. in environmental physiology, Wallace has little expertise in deaths from fires. Yet she travels the country preaching the dangers of PVC and calling herself "an expert on fire toxicology."

She also made a pretrial deposition in 1980 as an "expert witness" in the litigation arising from a May 1977 fire at the Beverly Hills Supper Club in Southgate, Kentucky, in which 165 died. Later she testified that autopsy reports, hospital admission records of survivors, and questionnaires strongly suggested that PVC was responsible for the deaths and injuries at the fire. On this point she was disputed during the trial by the medical examiner of St. Louis County.

The Foundation for Fire Safety has lobbied repeatedly. Several states have been considering bills that would require that all materials be tested for their combustion toxicity before they can be considered for approval in building codes. Since metal conduit can't burn, it would pass such tests; PVC might not. Unfortunately, satisfactory testing procedures don't yet exist. Yet on May 6, 1982, Birky appeared before the fire safety subcommittee of the New York State senate finance committee and declared that they do. An inhalation test protocol that he helped develop at the National Bureau of Standards, he said, is able to prove that one material is more toxic than another.

One reputable authority disputes Birky. "There's no correlation between the test method and what happens in a real fire," says Irving Einhorn, an adjunct professor at the University of Utah, who has published 100 papers on combustion toxicology. "Yet Birky is going around the country trying to ban materials based on incomplete tests." The Bureau of Standards adds that even if its test



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protocol were ready to be used, which it isn't, it alone would not justify banning some materials from building codes.

The plastics industry and Carlon have charged Allied with using questionable tactics within the National Fire Protection Association. Prior to an NFPA vote in May 1980 that would have recommended the inclusion of Carlon's flexible PVC conduit in the National Electrical Code, they say, Allied and other companies and trade organizations in the steel industry purchased scores of (and perhaps as many as 100) \$50 memberships in the organization. When the vote came up, these new members helped to defeat Carlon's conduit by 394 to 390.

SEVERAL NFPA MEMBERS were outraged by the way the outcome was achieved. One, Nathaniel Addleman, a registered fire protection engineer with Boeing Co., was particularly incensed when he addressed the group after the vote: "I had occasion to have lunch with a gentleman who is a salesman for one of the steel companies and he didn't know why he was here. He was going to go to a meeting at 12:30 to find out why he was here and be told how to vote. This is what's happening here today. We cannot let the NFPA degenerate to that type of thing."

Before and after Allied helped vote Carlon's conduit out of the electrical code, it was selling PVC products. Even though Allied President Krengel claims that he was aware of plastics' hazards as far back as 1972, his company sold PVC conduit during the mid-Seventies through distributors. Asked how he could sell a product that he considers hazardous, Krengel says, "Maybe we began to realize how bad it was later. Anyway, as soon as we began to realize how bad it was, we got out of the business."

Allied's general counsel, John Lison, says Allied got out of the PVC business for a different reason: it lost its supplier. He adds, "Our suppliers could not keep us price competitive. There wasn't enough profit margin in 1975." Allied got back into the PVC market during the summer of 1980. Krengel and other Allied officers worked out a deal in which Robintech would produce rigid plastic conduit for Allied on a private label basis. Krengel now says he wasn't aware that Allied was in the PVC business a second time. Asked if subordinates had put Allied back into PVC without his knowing about it, he responds: "Look, it was a nothing kind of thing. It was incidental and momentary. When we knew what was going on we backed off."

Actually Allied sold PVC conduit from about September 1980 to March 1981. It did so, moreover, without warning users of the

fire hazards it professes to be concerned about. According to Lison, Krengel got out of the business because this time he *really* found the material hazardous. "Ted was amazed by the horrors of the MGM fire, and then the others," says Lison.

Allied's suppliers remember a completely different story. When Allied concluded its relationship with Robintech, Pat Madormo, Robintech's executive vice president, met Bernie Auerbach, Allied's product development manager, and asked why Allied was calling it quits. According to Madormo, Auerbach told him that Allied felt that PVC was a different market from those it was used to. "The PVC market got pretty tough around then," says Madormo, "and there wasn't enough margin for them to buy from Robintech and resell."

Allied returned to the PVC business for a third time in November 1981. It acquired Elcen Metal Products Co. of Franklin Park, Illinois, one of whose product lines is PVC coated. A current product, called Strut, is a hanging PVC-coated pipe holder. If the big hotel fires convinced Krengel he should get out of the business, why is Allied selling something like this? "Look," says Lison, "we don't know why people die from fires in this country." But wouldn't PVC be dangerous in this sort of application? "We don't have a corner on the wisdom of the world," Lison says, "and Factory Mutual [a testing organization that does inspections for the insurance industry] kept telling us that this stuff is fine, that it'll be included in all the codes. You know, for lots of applications PVC is better than our stuff."

Despite this strange admission, Allied's war on PVC has continued without letup. Last September the newsletter of the American Council on Science and Health, a consumer education group, published an article headlined "The Merchandising of Fear" on some less than savory aspects of Allied's campaign. Zoeller's quick reply was morally lofty: "Although Allied obviously has an interest in this issue, we had every opportunity to enter the PVC market and chose not to on safety grounds. We believe this issue concerns human life and safety, and therefore transcends the commercial interest of any company or industry. We believe our record shows this concern."

■ Allied's record is something rather different—a rare well-documented example of unfair tactics in the marketplace. Companies battling new products often play rough, with high-pressure salesmanship, aggressive pricing, antagonistic ads. But a few go further, in ways that sidestep notice. Allied has called attention to itself by overreaching. **E**

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