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MEDICAL PROGRESS

CHEMICAL CARCINOGENESIS

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THE perception of cancer as a disease primarily related to the environment has been progressively strengthened since the publication of a Medical Progress article on chemical carcinogenesis in 1971.[1] By the mid-1960s, the view of the cause and pathogenesis of cancer had already begun to change radically from that of previous decades, during which no clear perception of the possible role of the environment in the genesis of most of the major forms of cancer was evident. Although the relation of cancer to environmental hazards in the work place and to a few unusual cultural patterns around the world was established, the examples were considered exceptional.

The general acceptance of cigarette smoking as a major factor in the development of lung cancer and the increasing number of examples of major shifts in the organ or tissue distribution of primary cancers with migration of distinctive ethnic groups from one geographic location to another were important in changing the whole perception of the genesis of cancer.[2,3]

This radical change has encouraged increasing attention to the nature of the environmental influences. The available epidemiologic evidence indicates the likelihood of several (if not many) components in this environmental outlook. Diet, carcinogenic chemicals, radiation, and viruses are among the major factors that appear to be involved. Of these, chemicals as carcinogens have been receiving increasing attention as having the greatest role in the genesis of cancer. How justified is this perception of the causation of cancer?

About one third of the cancer in North America and Europe is related to the use of cigarettes or other tobacco products. There is incontrovertible evidence that chemicals related to the work place or occupation are responsible for a segment of cancer in human beings (Table 1). To these must be added a growing list of naturally occurring chemicals and of drugs used in medicine. All these known agents, together with ultraviolet light as the single most common cause of cancer, can easily account for over 50 per cent of the cases

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Table 1. Chemicals Known or Strongly Suspected to be Carcinogenic for Human Beings.

CHEMICAL	SITE OF CANCER
Occupational Contacts	
Aromatic amines	Bladder
Arsenic	Skin and bronchus
Asbestos	Bronchus, pleura, and peritoneum
Benzene	Marrow
Bis(chloromethyl) ether	Bronchus
Bis(chloroethyl)sulfide	Respiratory tract
Cadmium	Prostate
Chrome ores	Bronchus
Coke ovens	Bronchus
Nickel ores	Bronchus and nasal sinuses
Soots, tars, and oils	Skin and lungs
Wood dust	Nasal sinuses
Vinyl chloride	Liver
Medical Agents	
Alkylating agents (e.g., melphalan and cyclophosphamide)	Bladder Hematopoietic tissue
Anabolic steroids	Liver
Arsenic	Skin
Chlornaphazine	Bladder
Diethylstilbestrol	Vagina
Immunosuppressive drugs	Lymphoid tissue
Phenacetin (acetophenetidin)	Renal pelvis
Others	
Aflatoxins	Liver
Tobacco smoke	Bronchus, mouth, pharynx, larynx, esophagus, bladder, and pancreas
Oral contraceptives	Liver
Betel nut and lime	Mouth

seen in the United States, Canada, and several other Western countries.

What about the remaining cancers — those in the colon, breast, uterus, hematopoietic-lymphoid system, prostate, and other sites, including a substantial proportion of those in the urinary bladder and pancreas? Can these be closely related to the chemical contamination of the air, water, and food that has been occurring in the industrialized countries? [4,5] No definitive answer is available. Evidence can be offered in favor of such a view or against it.

On the positive side are the following considerations: the known relation, mentioned above, between many different types of cancer and exposure to chemicals in tobacco and in the work place; the widespread presence of chemical mutagens and potential carcinogens in the environment in industrialized countries, as monitored by short-term in vitro tests in prokaryotes or eukaryotes; the observations that some religious groups in Utah and elsewhere, who have different patterns of tobacco and alcohol use and different codes of behavior, appear to have a lower incidence of some forms of cancer in the respiratory, gastrointestinal, and genitourinary systems[6]; the increasing evidence that cancers of many systems, resembling cancers in human beings, can be induced by chemical carcinogens in one or more laboratory animals; and the observations that cancers of the esophagus or liver have an unusually high incidence in some geographic locations in China, and that domestic animals in close contact with human beings in these locations frequently have similar cancers in the same anatomic sites.[7-10]

On the negative side, no clear-cut clinical experience or epidemiologic evidence has been presented to implicate environmental chemicals as causative agents for the wide variety of cancers seen clinically. According to German[11] and Cairns,[12] patients with xeroderma pigmentosum, who have a defect in their fibroblasts in the repair of DNA damage by some chemicals as well as by ultraviolet light, have no elevated risk of cancer in organs or tissues other than the skin. If the general environmental exposure to chemicals were effective in the causation of cancer, such patients could be expected to have elevated incidences of cancer not related to ultraviolet light. In some cancers, such as leukemia and cancers of the stomach and cervix, there were decreases in age-adjusted incidence and in mortality from 1969 to 1971 and from 1973 to 1976 among white persons in the United States, while there were only small increases in most of the other tumors, as compared with cancers of the lung and with melanoma.[13] No increase comparable to that of lung cancer has been seen in the past two decades in the patterns of cancer incidence in the United States.

None of the above considerations are by any means conclusive. Most of the arguments on either side have enough contingencies to leave the question in major doubt. The close monitoring of cancer occurrence over the next two decades may allow a more definitive position to be taken. In the meantime, a greater understanding of how chemicals are involved in cancer development would seem important in helping us to arrive at a conclusive position.

In addition, the large increase in the use of chemical carcinogens in the development of new experimental models for the study of the possible cause, development (pathogenesis), or treatment of cancers in specific organ or tissue sites in human beings further emphasizes the importance of the study of chemical carcinogenesis. Such studies should generate a much more critical understanding of the relative roles of exogenous and endogenous factors, including diet and hormones, in cancer development and behavior.

The orientation in this presentation will be mechanistic, not descriptive, and will attempt to relate fundamental concepts and understanding derived from experimentation in animals and from other sources to the causation, prevention, and (to some degree) therapy of cancer in human beings. For this reason, little coverage will be given to the many different types of chemical carcinogens themselves or to their distribution.[14]

CANCER AS A CHRONIC MULTISTAGE PROCESS

It is now appreciated that the development of cancer in human beings in various sites takes many years and that progressive tissue and cellular changes

can be seen during the long so-called latent period. Often, new cell populations appear that probably represent stages or steps in the cellular evolution from normal cells through initiated, preneoplastic, and premalignant cells to highly malignant neoplastic cells.[15-17] Research in laboratory animals has concentrated heavily on the early events and has suggested the existence of common patterns of early preneoplastic changes in several organ systems and in several species.[17] Research in human beings has concentrated largely on the later events and has confirmed the occurrence of atypical hyperplasias, dysplasia, and carcinoma in situ as probable precancerous steps in several organs late in the process.[17-19] However, the critical properties of each different cell population that relate to the possible roles of these cells in cancer development and to the manner in which the two aspects of carcinogenesis (the experimental and the human) fit together into some biologically meaningful pattern remain as challenges.[17]

The conceptual advances made in the late 1930s and 1940s concerning the first two early steps in chemical carcinogenesis, initiation and promotion, are well known.[15-17,20] However, their mechanistic bases are only now unfolding, and these will be topics for review. The basic validity of these concepts for cancer development in human beings has been established in a few instances and as such is reassuring both for the physician and for the scientist. For example, a relatively brief exposure to diethylstilbestrol during pregnancy may be associated with the development of vaginal neoplasia some 15 to 25 years later in the exposed person's daughters.[21] Parenthetically, it should be mentioned that it is not known whether diethylstilbestrol acts in this situation as a chemical carcinogen or whether the effect is predominantly hormonal. Again, a limited exposure for only a few months to a known chemical hazard, such as vinyl chloride, may lead many years later to the appearance of angiosarcoma of the liver.[22] Nonneoplastic cellular and tissue changes are seen during the apparent latent period.

Initiators and Initiation

Initiators

Until fairly recently, one of the most puzzling and confusing aspects of chemical carcinogenesis was the diversity in the chemical structure of carcinogens. This problem has now been largely resolved by the discovery of metabolic conversion of many carcinogens to highly reactive metabolites.[20] The best-known form of reactive moieties, generated from several different types of carcinogens, is the "electrophilic reactant," which is a positively charged molecule that reacts well with sites of electron densities in many different cellular components, including DNA, RNA, protein, glutathione, and probably also polysaccharides.[20] A minority of chemical carcinogens, such as some nitrosamides (e.g., alkyl nitrosourea), bis(chloromethyl) ether, and nitrogen mustard, are active by themselves and do not seem to require any metabolic conversion to more reactive metabolites.

Activation and Metabolism of Procarcinogens

The first major type of metabolic activation discovered was the conversion of an aromatic amine, 2-acetylaminofluorene, by *N*-hydroxylation to an N–OH derivative.[20] This type of metabolic product is seen with several aromatic amines, including the human bladder carcinogen 2-naphthylamine (β-naphthylamine).

More recently, much effort has been spent on the clarification of the activation of benzo(*a*)pyrene and the many other polycyclic aromatic hydrocarbons. Many of them are easily generated by burning (pyrolysis), are widely distributed in our environment, and are thought to be partly responsible for the carcinogenicity of coal-tar products, oils, and tars. These compounds undergo epoxidation to form reactive epoxides, some forms of which are considered to be ultimate carcinogens.[23] In the case of benzo(*a*)pyrene (and probably other polycyclic aromatic hydrocarbons), the initial site of epoxidation may undergo hydration to form a dihydrodiol in a reaction catalyzed by epoxide hydrolase. This inactive derivative, in turn, can be converted to another epoxide at a second site to form a dihydrodiol epoxide. These are considered to be the most likely ultimate carcinogens for at least some polycyclic aromatic hydrocarbons. This series of reactions for benzo(*a*)pyrene is illustrated in Figure 1. Another potentially important carcinogen for human beings that is subject to activation through epoxidation is aflatoxin B_1.[20] This substance undergoes oxidation at its 2,3-position, and this derivative, the 2,3-oxide, appears to be one form of ultimate carcinogen of this mycotoxin. Vinyl chloride is also activated through epoxidation.[20]

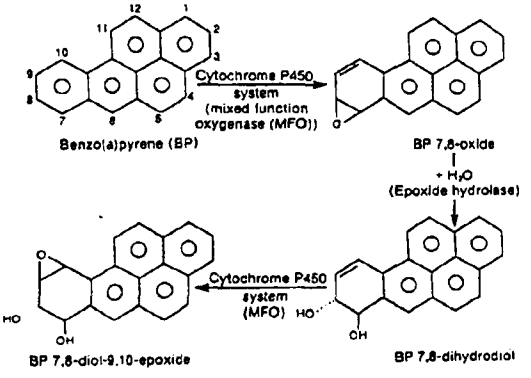

Figure 1. Current View of the Activation of Benzo(a)pyrene (BP) to Benzo(a)pyrene 7,8-Dihydrodiol,9,10 Epoxide through the Mixed-Function Oxygenase (MFO) System (Cytochrome P-450 System) and Epoxide Hydrolase.

Other types of metabolic conversions are also considered important in carcinogenesis. Nitrosamines are converted to highly reactive alkylating moieties, probably through oxidation. Aromatic or heterocyclic nitro-compounds, such as nitrofurans and 4-nitroquinoline-*N*-oxide, probably undergo initial reduction to hydroxy-amino-derivatives. In the case of 4-nitroquinoline-*N*-oxide, this form is considered to be an ultimate carcinogen.

For the majority of known conversions, the system most active in the cell is the "mixed-function oxygenase" system, consisting of several cytochromes P-450, reduced nicotinamide-adenine dinucleotide phosphate cytochrome reductase, and lipid.[24] This inducible system is located predominantly in the microsomal fraction of the cell (endoplasmic reticulum), although recent work increasingly points to a second comparable system in the nucleus.[25] In addition to these oxidative systems, reducing systems also exist for some procarcinogens, such as the aromatic or heterocyclic nitro-compounds. Diaphorase or other reduced nicotinamide-adenine dinucleotide phosphate or reduced nicotinamide-adenine dinucleotide reductases are widely distributed among different cells and are effective in reducing the nitro-group to the hydroxy-amino-derivative.

The liver is by far the most active and most versatile organ in the metabolism of procarcinogens and of xenobiotic agents generally. It and other organs have an ability to detoxify potential carcinogens as well as activate them, and the ultimate fate of a chemical depends largely on the balance between activation and inactivation — a balance that is easily modulated in major ways by drugs and other chemicals, age, nutrition, and hormones, as well as genetics.[26-31] For example, the carcinogenicity of several aromatic amines for the liver can be completely prevented by simultaneous exposure to phenobarbital or 3-methylcholanthrene — agents that induce many liver enzymes.[32,33] This phenomenon of resistance of the induced liver to some carcinogens may be of great practical importance to human beings. Virtually all people in the Western world have levels of several xenobiotic agents, such as chlorophenothane, dieldrin, aldrin, polychlorinated biphenyls, and dioxins, in their adipose and other tissues. These agents as a group are effective enzyme inducers in the liver. In addition, many drugs induce various microsomal or other enzymes in the liver or other tissues. Are these inducers making tissues more or less susceptible to the acute toxic or carcinogenic effects of other environmental agents?

The organ or tissue distribution of the carcinogen-metabolizing enzymes may be involved in the organotropism of carcinogens. Since there is an almost certain requirement for activation, and since the metabolic patterns affecting carcinogens and other xenobiotic agents are so variable from cell to cell or from organ to organ, the absolute activities of the various enzymes and especially their relative balance may have key roles in determining which organ will be a target for a particular carcinogen. An interesting example of modulation at this level is the liver-kidney axis with dimethylnitrosamine. This potent carcinogen normally induces liver cancer when taken through the gastrointestinal tract. However, if the animal is placed on a low-protein diet, the hepatic activation and metabolism fall off. This pattern allows more of the carcinogen to be available for action on the kidney and changes the dominant cancer pattern from the liver to the kidney.[34]

Clearly, the pathologic consequences of exposure to any potentially toxic xenobiotic are related not only to the pathways available for its metabolism but also to the physiologic state at the time of exposure. Thus, the presence of a known mutagen or carcinogen in the environment is by no means synonymous with a mutagenic or carcinogenic response by the exposed person.

Possible Molecular Targets for Ultimate Carcinogens

The molecular targets for the electrophilic reactants are many. To date, the bulk of the effort has been concentrated on DNA, with some interest in RNA and protein. In principle, many other molecular targets exist at the macromolecular and "micromolecular" levels. Because of the ease of relating changes in DNA to a permanent change in the biologic behavior of cells, DNA has received the lion's share of attention. Since the first discovery of the chemical nature of an adduct of a carcinogen with nucleic acids in a target organ in vivo,[35] there has been an increasing interest in establishing the chemical nature and site of interaction with several different carcinogens.[36] As shown in Figure 2, virtually every potential site for interaction with DNA bases (as well as with phosphates) reacts with one or more carcinogens. In attempts to narrow down the possibilities for relevance to cancer, sites that are more likely to be involved in hydrogen bonding between bases (purines and pyrimidines) in DNA, and thus to be involved in possible miscoding, are often considered most relevant. This presupposes that the major or only biochemical lesion in DNA that is relevant to cancer initiation is a miscoding lesion. This judgment may not be justified, since the evidence is circumstantial and inconclusive. Conceivably, other types of alterations in DNA besides miscoding, such as recombinations and gaps, may be important to the early events in cancer development.[12,36-39] In fact, Cairns has suggested that mutagenesis may be of only minor importance in the initial events in chemical carcinogenesis, and that genetic transposition including relatively large regions of the genome may be more relevant to this process.[12] The application of this newer technology to appropriate "clean" cell populations during the development of cancer is to be awaited with interest.

Another important aspect is the site of interaction of the ultimate carcinogen within the long DNA molecules. With several different approaches, such as

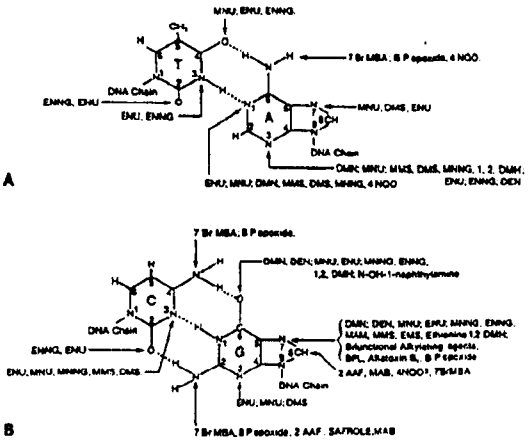

Figure 2. Diagrammatic Representation of Sites of Interactions of Activated Forms of Carcinogens with the Four Bases of DNA.

T denotes thymine; A, adenine; C, cytosine; G, guanine; MNU, *N*-methyl-*N*-nitrosourea; ENU, *N*-ethyl-*N*-nitrosourea; ENNG, *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine; 7 Br MBA, 7-bromothyl-benzo(a)anthracene; BP epoxide, benzo(a)pyrene 7,8-dihydrodiol,0,10 epoxide; 4NQO, 4-nitroquinoline-*N*-oxide; DMS, dimethylsulfate; DMN, dimethylnitrosamine; MMS, methyl methanesulfonate; MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; 1,2,DMH, 1,2-dimethylhydrazine; DEN, diethylnitrosamine; N-OH-1-naphthylamine, *N*-hydroxy-1-naphthylamine; 2 AAF, 2-acetylaminofluorene; MAM, methylazoxymethanol; EMS, ethyl methane sulfonate; BPL, β-propiolactone; and MAB, 4-methylaminoazobenzene.

studies of susceptibility to nucleases, separation of transcriptionally active from inactive DNA, or isolation of linker and nucleosome regions in chromatin, some of the interactions of carcinogens with DNA have been found to be nonrandom. However, no single region of high affinity has been found.[36] The use of DNA cloning for specific genes in biologically well-defined preneoplastic and early neoplastic populations should be profitable.

DNA Repair

In view of the essentially irreversible nature of initiation with chemicals and the apparent focal nature of the initiation process, major emphasis at the molecular level is given to DNA as a probable target in initiation. However, the evidence is largely circumstantial.

The bulk of evidence comes from studies in patients with xeroderma pigmentosum, who have a high incidence of skin cancers. The etiologic agent is ultraviolet light, and the vast majority of patients have a defect in their ability to repair the damage inflicted on DNA by this form of radiation. Such patients can be protected from skin cancer by careful avoidance of exposure to ultraviolet light.[40] Fibroblast cultures from these patients have increased sensitivity not only to ultraviolet light but also to some chemical carcinogens and mutagens.[41] Other diseases (e.g., ataxia-telangiectasia and Fanconi's anemia) also involve deficiencies in DNA repair as well as an increased risk of neoplasia.[42]

Experimentally, there is a correlation under some conditions between the formation of specific DNA adducts (such as O^6-methylguanine in the brain), persistence of this biochemical lesion, and the ultimate appearance of gliomas.[43] Other studies indicate that the genesis and persistence of O^6-methylguanine may be important but is insufficient to account for cancer production with some nitrosamines or nitrosamides[44] or 1,2-dimethylhydrazine.[45]

The major protection against cancer may reside in the efficiency with which we repair DNA that has been damaged by mutagens and carcinogens. As pointed out by German[11] and by Cairns,[12] since fibroblasts from patients with xeroderma pigmentosum show a defect in the repair of DNA damage by some chemicals as well as by ultraviolet light, such patients should have an elevated risk for cancer in organs or tissues other than the skin if chemical carcinogens are important in the genesis of many forms of cancer. Yet the available data show no apparent increase in the risk of cancers other than those of the skin in patients with xeroderma pigmentosum.[12] Patients with Bloom's syndrome do have an elevated risk. Such observations are interpreted as evidence against a role for the many environmental chemical hazards in the causation of human cancers generally, except in well-established instances such as respiratory-tract cancer in smokers and in persons with occupational exposures.[12] An alternative hypothesis must be entertained: that the role of repair may be quite different in the dynamics of cancer induction by chemicals, as compared with induction by ultraviolet light.

The past 10 years have seen a large expansion of studies of DNA repair, largely in vitro. These studies should lead to a much clearer delineation of the types of repair that may occur in carcinogenesis. What does happen remains poorly understood. Processes of repair by base excision, by "long-patch versus short-patch" removal, by possible recombination or other forms of post-replication repair, by removal of interstrand cross-links or other more complex forms of damage, and by other mechanisms are slowly being studied in many different systems in vitro and to a much lesser degree in vivo.[36,41] The enzymology, although difficult, is also slowly becoming clear. In the case of methylating carcinogens, an interesting recent finding is the discovery of an enzyme in bacteria that removes the methyl group from the O^6 position of guanine in DNA by transmethylation with its transfer to a sulfur-containing moiety in protein.[46] This raises the question of whether some of the enzymes involved in repair of DNA damage perform physiologic functions other than repair that subserve normal cellular requirements.

Obvious failings in many of the attempts to correlate patterns of DNA repair with carcinogenicity include the wide gap between the chemistry and the bi-

ology and the primitive state of the art of identifying and quantitating discrete biologic steps in the carcinogenic process.

Carcinogen Metabolism in Human Tissues

A gratifying aspect of recent studies of the early chemical and biochemical events in carcinogenesis is the overall similarity in patterns of activation and types of adducts seen in tissues from human beings and in those from laboratory animals. Studies with some nitrosamines, benzo(*a*)pyrene, aflatoxin B_1, and other carcinogens[47-53] have shown that human tissues in general resemble one or more animal tissues in their patterns of activation and in the type of carcinogen-DNA adducts formed.

An interesting development that may help to clarify some aspects of this important phase of activation is the use of radioimmunoassays for specific adducts in DNA, especially with highly sensitive new approaches to radioimmunoassay.[54-56] Such detection of minute quantities of carcinogen-induced adducts in DNA opens up the possibility of studying exposure patterns to carcinogens. This technique, coupled with adequate medical examination and appropriate epidemiologic studies, might well offer new approaches to the ultimate problem of risk assessment. Although it is far too early to discuss such possibilities except in general terms, adduct formation is an index of exposure, not initiation, and persistence of a chemical lesion in DNA is by no means synonymous with cancer development. As discussed below, initiation of the carcinogenic process is not an inevitable consequence of adduct formation.

Human cells resisted in vitro transformation with chemicals for several years. However, the past three years have seen positive results in some human-cell systems.[57-61]

Initiation

In the classical system of skin carcinogenesis in mice and rabbits, a single or brief exposure to a carcinogen induces some change in the tissues, called initiation, such that focal proliferations called papillomas can be made to appear by application of croton oil or other agents called promoting agents, which by themselves are noncarcinogenic or only weakly carcinogenic. More recently, this phenomenon was also found in several other organs, such as the liver, brain, mammary gland, colon, and urinary bladder.[16,17] Such focal proliferations (papillomas, polyps, or hyperplastic nodules), in turn, are sites for further evolution to neoplasia. The term neoplastic refers to a cell that can proliferate without the need for an added or known stimulus for growth — i.e., a cell that has acquired some degree of autonomy. Initiation at the skin, liver, and mammary gland, and probably at most sites, is generally permanent. This suggests that the very early carcinogen-induced altered cells are not recognized by the host in a manner that leads to their destruction, and it raises serious doubts about the validity of the concept of immune surveillance at an early stage in carcinogenesis,[62] as has been proposed for host control of cancer development.[63]

These observations are most easily interpreted as reflecting some permanent change in the DNA in the rare cell affected during initiation in vivo. Whether the change is a mutation in a structural or regulatory segment of DNA[64-66] or whether it involves more complex rearrangements of larger segments of DNA, as in transpositions, remains unknown. Pertinent are the observations on transformation initiated with x-rays or with polycyclic aromatic hydrocarbons, in which some change or changes other than a mutation in a rare cell appear to be involved as an early step in initiation in vitro with a highly selected cell type.[67,68]

One cannot equate activation of carcinogens leading to DNA-adduct formation with initiation. For example, many carcinogens, such as benzo(*a*)pyrene and 7,12-dimethylbenz(*a*)anthracene, can be activated by the liver to form adducts without initiating or inducing liver cancer in adult animals. However, if coupled with one round of cell proliferation, many can initiate[69-72] and induce liver cancer.[73] In vitro, with different cell systems, cell proliferation early in the carcinogenic process is required for transformation with x-rays,[74] some viruses,[75] and some chemicals.[76] Thus, as shown in Figure 3, initiation is at least a two-step process: a biochemical step that is reparable, followed by a round of cell proliferation that "fixes" some change so as to make it essentially permanent. The cell proliferation can be generated by some primary mitogenic stimulus, such as partial hepatectomy, by a chemical mitogen,[69] or as a response to cell death.[77] The mechanistic role of cell proliferation in initiation is not understood. A popular hypothesis involves DNA synthesis or replication as somehow fixing damage in a daughter strand.

The probable requirement for cell proliferation for initiation has important implications for cancer development in many sites. In the nonproliferating tissues, such as the pancreas, salivary gland, liver, kidney, urinary bladder, and brain, an important rate-limiting step for beginning the carcinogenic process may be cell proliferation. Thus, concomitant cell ne-

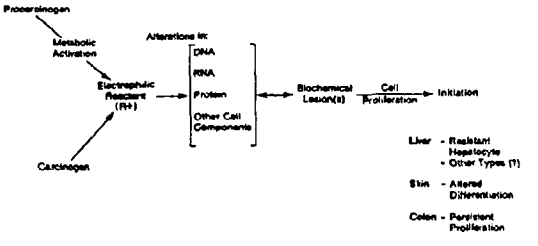

Figure 3. Steps in the Initiation of Carcinogenesis with Chemicals.

The listings under "Initiation" refer to some properties either used in the next phase, promotion (liver), or associated with the initiated tissue (skin or colon).

crosis induced by viruses, toxic agents, parasites, or dietary deficiencies, followed by cell regeneration, could be a major determinant of cancer initiation in many sites. In addition, the presence of many proliferating cells is probably one basis for the susceptibility of the fetus and neonate to many chemical carcinogens[78] and could account for the peak in cancer incidence in the first decade of life. The human fetus, unlike the rodent fetus, acquires the capability of activation of some carcinogens early in development[79,80] and thus may be at greater risk than some laboratory animals for cancer development with chemicals.

A most important question about initiation concerns the essential biologic nature of the initiated cells. What properties have they acquired that allows them, as a group, to be precursors for the ultimate development of cancer? The available evidence is against any conclusion that the initiated cells have acquired any autonomy of growth.[15,17]

In most systems, the properties critical to initiation remain unknown. In two continuously proliferating tissues, the skin[81] and the colon,[82] an early property of carcinogen-altered cells may be some disturbance in programming or control, such that the cells do not show the normal progression of differentiated properties. In the liver, the cell with acquired resistance to the inhibitory effects of carcinogens on cell proliferation has been shown to be one type of initiated cell.[17,83]

Although a majority of chemical carcinogens fall well within the current paradigm in which initiating effects are related to some form of DNA damage, there are known carcinogens that appear to be exceptions. A growing list of hypolipidemic agents[84] and several pesticides, herbicides, and other xenobiotics[85] have not been shown to generate mutagenicity or other DNA-damaging effects. Is this merely a reflection of deficiencies in our technology, or are there pathways to cancer that do not involve DNA damage of exogenous origin as essential early steps in the process?

Promoters and Promotion

The term "promotion" is often used for the process whereby neoplastic development, tumor formation, or cancer development is accelerated or encouraged in a tissue that has been exposed to an initiating dose or doses of a carcinogen.

Promoters

Early in the recent history of chemical carcinogenesis it was found that a noncarcinogen, croton oil, could stimulate tumor formation in the skin after a brief initial exposure to a carcinogen. This naturally encouraged a chemical "attack" on croton oil Hecker[86] and Van Duuren[87] discovered that esters of the diterpene phorbol, isolated from croton oil, have potent promoting effects on mouse skin. Many biochemical, metabolic, and biologic effects are induced in many different normal cells by the phorbol esters and related compounds; in the vast majority of cases, the intensity of the particular effect closely parallels the efficacy of the compounds as promoters of skin papillomas in mice after exposure to a single dose of dimethylbenzanthracene or a similar carcinogen.[88,89] These effects include changes in morphology, microtubule polymerization, cell proliferation, enzyme induction, polyamine synthesis, phospholipid synthesis, membrane structure and function, ATPase, release of prostaglandins, inhibition and sometimes stimulation of differentiation, and many others in a wide variety of normal cells and cell lines. Many of the effects are considered pleiotropic.

Recently, with the use of a less active phorbol ester, ^{3}H-phorbol dibutyrate, membranes of fibroblast cultures and mouse epidermal cells were found to contain a high-affinity receptor for many phorbol esters, including one of the most active, 12-*O*-tetradecanoylphorbol-13-acetate.[88,90] The "natural" metabolites for the receptors have not been identified. Given the wide diversity and large number of effects seen in treated cells, it may be that more than one type of receptor is involved.

At present it is impossible to relate the findings in vitro to chemical carcinogenesis in vivo. Given the array of phenomena induced by the phorbol esters, how does one select relevance to promotion?

A major problem concerns the matching of the target cells in vivo and in vitro. In the intact animal, an active promoter does not induce focal proliferations (such as papillomas) in the normal skin, but does so only after initiation. However, many skin promoters do induce general hyperplasia of the epidermis in animals in which initiation has not taken place. Studies in several laboratories have shown that a general stimulation of cell proliferation is insufficient for the selective or differential stimulation of initiated skin to form papillomas.[91]

Promotion

A question that requires an early answer is whether the differential effect of a promoting environment on initiated tissues is directed primarily to the initiated cells or to the surrounding cells.[92] There is considerable circumstantial evidence to suggest that the first major phenomenon in promotion is the selection of an appropriately altered cell to produce a focal proliferation, and that promoting environments create differential effects on the initiated cells and on the surrounding cells.[17,83,92] Cancer frequently arises in an atrophic tissue or organ, not a hypertrophic one. Is this the consequence of a change in the local environment, favoring growth of the rare initiated cell?

As observed in vivo in experimental models in common use today, cell proliferation is an essential phenomenon in promotion. It is therefore to be anticipated that many biochemical changes that are seen in the different phases of the cell cycle,[93] such as an increase in ornithine decarboxylase activity, will be seen in promotion. Determining whether one or more of such changes in enzymes, the cell membrane, DNA organization, or other factors will have a special role in pro-

motion over and above that related to the cell cycle itself remains an interesting challenge.

There is a growing belief that one of the major factors in determining cancer development is the presence and intensity of promoting environments.[94] In addition to some specific agents such as phenobarbital, butylated hydroxytoluene, and polychlorinated biphenyls for the liver,[95] prolactin for the mammary gland,[96] and bile acids for the colon,[97,98] diet has been found to influence carcinogenesis in several systems.[2,3] However, the specific roles of individual dietary components and of their balances remain to be established in most instances.

There is also a suspicion on the part of some investigators that tissues or organs may create a physiologic promoting or selecting environment. The most provocative evidence relates to carcinogenesis in the colon[99,100] and the urinary tract.[101,102] In each of these locations, diversion of the normal movement of contents has been found to lead to a decreased incidence of neoplasia with known carcinogens.

There are several examples in which synergism or promotion may be operating in human beings. For example, exposure to asbestos, nickel, or uranium may be associated with only a relatively low risk of lung cancer. However, when coupled with cigarette smoking, the risk becomes extremely large — much larger than that associated with smoking alone.

Subsequent Steps

Progression

Focal proliferative lesions resulting from a promoting environment, such as papillomas, nodules, or polyps, undergo a number of further changes before malignant behavior is expressed.

In many organs, including the bronchial tree in the smoker, one consistently sees several types of putative precancerous lesions, such as atypical hyperplasias, dysplasias, and carcinoma in situ.[18,19] How these lesions relate to each other and to altered biologic behavior of cells transformed in vitro (e.g., atypical growth, growth in soft agar, or tumorigenicity) is not clear and remains a potentially fruitful area for study.

An important property of many of these changes is reversibility. There is considerable evidence in experimental models and in human beings that some focal proliferative lesions, such as hyperplastic nodules in the liver (and their probable human equivalent, liver "adenomas," which are seen rarely in women using oral contraceptives), polyps in the colon, and papillomas in the skin, can undergo regression or remodeling with normal differentiation.[17,103] This area is becoming of particular interest in view of the reported efficacy of vitamin A analogues, such as *cis*-retinoic acid, in preventing or delaying cancer development initiated with chemical carcinogens[104] in several sites, such as the bronchus and the urinary bladder.

Transfection

An interesting recent development is the induction of transformation by the transfer of DNA or chromatin to susceptible cell lines. DNA from several types of cells transformed in vitro or in vivo with different carcinogens was shown to induce transformation.[105,106] Of great interest is the observation that DNA from normal cells was also effective.[106] These new approaches to chemical carcinogenesis open up many areas of exploration, including the question of whether the information contributions of the DNA are positive (i.e., they code for an identifiable component that is important in cell transformation) or negative (i.e., they disorganize the host genome in an appropriate manner).

Diet and Carcinogenesis

The composition of the diet may influence the incidence of cancer in some sites, such as the colon, breast, and endometrium.[107-109] The mechanism or mechanisms through which diets exert their influences are not well understood. In some instances, carcinogens occur in the food as natural substances, as contaminants (e.g., aflatoxins), or as products of food-preparation methods (e.g., pyrolysis). Dietary alterations also modulate the activation of carcinogens.

In other instances, micronutrients have a role in the endogenous formation of carcinogens. The past 10 years or so have seen a revival of the concept of the endogenous formation of carcinogens. This hypothesis was proposed in the 1930s in relation to cholesterol or other sterols, but it was progressively abandoned as the evidence failed to materialize. The resurrection of this idea assumes a new form in the genesis of nitroso-compounds from nitrite and secondary or tertiary amines.[110] Dietary amines or drugs such as oxytetracycline of chlorpromazine can react with nitrous acid, generated in the stomach from nitrite, to form nitroso-compounds. Some of these substances are carcinogenic in laboratory animals. The formation of such nitroso-compounds can be effectively inhibited by dietary ascorbic acid[111] and by vitamin E.

This general model is being explored in colon cancer in human beings.[112] The hypothesis states that carcinogens can be generated in the feces and that the levels of the carcinogens, as measured by mutagenic action, are influenced by the dietary composition, including vitamins C and E and fiber content.

Chemotherapy and Carcinogenesis

Some of the most effective chemotherapeutic agents for cancer are carcinogenic. For the treatment of cancer in patients above 50 or 60 years old, the importance of the risk of carcinogenicity is clearly minimal, given the long latent period for cancer development. However, in children and young adults, the development of second cancers years after the effective treatment of the primary tumor is now becoming a recognizable problem.[113,114] Over 25 drugs used in chemotherapy have been found to be carcinogenic in animals, and some have also been implicated in human beings.

The magnitude of the risk associated with the many treatment regimens is not fully known. However, for

some patients the risk of second primary tumors varies from 7 to 12 per cent.[115] These figures include some risk factors aside from the therapy, that are possibly genetic and favor second or multiple primary tumors. However, there is an additional risk from the chemotherapy itself. This risk will increase in volume as current types of chemotherapy for cancer become more successful. However, as mechanisms by which chemicals induce cancer and kill cancer cells are clarified, ways will be found to prevent the carcinogenic effects without interfering with the drugs' efficacy in treating cancer.

A common problem in cancer chemotherapy is the appearance of resistant cancer cells. Since resistant cells are also produced by carcinogens during carcinogenesis, and since such resistance constitutes one form of initiation,[17,83] it is conceivable that the induction of resistance to therapy and that of cancer are related.

New Bioassays for Carcinogens and Promoters

The basic knowledge that has been generated during the past 20 years or so has spawned a large array of short-term tests (mostly in vitro) for potential carcinogens, and more recently for promoters.[116] These tests generally fall into two broad groups: those that use some correlate of DNA damage or chromosomal damage as end points and those that use cell transformation as the end point.[116-118] The majority require the inclusion of an appropriate activation system.

This development is an essential requirement for rapid monitoring of our environment. In addition, these tests are being used increasingly as rapid assays for some more basic aspects of chemical carcinogenesis, such as patterns of activation in tissues and the endogenous production of carcinogens or mutagens, to name but two of many examples.

Conclusions

The past decade has produced new insights into the need for metabolic activation, the nature of the active molecules, the enzymology of activation, and the array of products generated, including major ultimate carcinogens. The interactions of such derivatives with DNA and other cell constituents and the possible toxicities and other effects on cells are becoming clearer. There is a general belief that DNA damage is involved in cancer initiation in most instances. The basic studies have generated over 100 short-term assays for possible carcinogens, with many of the assays reflecting damage to DNA or chromosomes.

The importance of the modulation of metabolic activation by enzyme induction with hormones, diet, drugs, and chemicals (including many environmental contaminants such as pesticides, herbicides, and polychlorinated biphenyls) is appreciated. How this ubiquitous exposure may influence cancer development in human beings is not known.

The repair of lesions in DNA is receiving increasing emphasis. The majority of such studies are in relatively simple in vitro systems and are generating insights into what may happen. The study of what does happen remains a major challenge. It may be aided by the development of ultrasensitive enzymatic radioimmunoassays for carcinogen-DNA adducts.

There is an increasing realization that many post-initiation phenomena, including promotion, are of major importance in cancer development. Alterations in diet, hormones, drugs, and xenobiotic agents have major influences through effects on promotion and other later steps. The reversibility of many of the precancerous steps is being studied with vitamin A and its analogues and with other dietary components.

Despite the major advances, the study of the later phases of chemical carcinogenesis lags far behind that of the early biochemical events. A nagging uncertainty in this field in general concerns the role of chemical carcinogens of either exogenous or endogenous origin in the causation and pathogenesis of some of the major forms of cancer. There is a general agreement that cigarette smoking and ultraviolet light are two major factors in the genesis of several important types of cancers. There is also incontrovertible evidence that many chemicals to which persons are exposed in the work place or its environs cause cancer in several organ systems. However, the relative quantitative importance of these considerations in the majority of patients with cancer not related to smoking is unclear. The resolution of this problem, although no doubt difficult, will almost certainly have a major impact on our approaches to cancer prevention in coming decades.

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MEDICAL INTELLIGENCE

PULMONARY ALVEOLAR PROTEINOSIS IN FOUR SIBLINGS

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PULMONARY alveolar proteinosis (PAP) is a distinctive pathologic lesion in which alveoli are filled with periodic acid–Schiff-positive proteinaceous material. It is usually idiopathic, but it has been reported in association with a variety of other conditions.[1-8] PAP is usually seen in adults, although the first description of cases included a young child.[9] Since then, there have been several reports of PAP in infants and children, and the condition has been found at birth.[8]

This report describes four siblings in whom PAP developed during infancy. Our review of the literature disclosed several other affected sibships, yet the familial aspect of PAP has been only briefly considered.[10] Analysis of the foregoing observations suggests that some cases of PAP may have a genetic basis, with a possible autosomal-recessive inheritance.

CASE REPORTS

The patients were the first four children of a healthy black couple. The parents are second cousins (Fig. 1). The mother was in her teens during all four pregnancies and had preeclampsia in the third trimester of each. Despite normal gestational periods as determined by history, the birth weights of the infants ranged from 1540 to 2320 g. The parents subsequently had two additional healthy offspring who had normal birth weights. These pregnancies were not accompanied by preeclampsia. We identified no environmental exposure affecting the parents or their children. The parents were not aware of illnesses in other close family members.

Patient 1

This girl seemed well until she was seven months old, when she was admitted to the University of Virginia Hospital because of vomiting and fever. Respiratory distress developed, and chest radiographs showed pneumonitis. The white-cell count was 29,100 per cubic millimeter. Cultures of blood, urine, stool, and cerebrospinal fluid were negative. The patient was treated with penicillin and discharged. She was readmitted three weeks later because of cough, vomiting, and diarrhea. A skin test with purified protein derivative of tuberculin (PPD) and a throat culture were negative. Respiratory distress worsened, and the patient died on the seventh hospital day. An autopsy was not performed.

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Patient 2

This six-month-old girl was admitted to the University of Virginia Hospital with anemia and cough. The chest was normal by physical examination, but the respiration rate was 60 per minute. Radiographs showed diffuse, stringy pulmonary densities. The white-cell count was 24,300 per cubic millimeter. Blood transfusions, iron, and antibiotics led to clinical improvement, but the radiographic appearance did not change. Cultures of blood, urine, stool, and cerebrospinal fluid were negative, as were skin tests with PPD and fungal antigens. Serum protein electrophoresis gave normal results. The patient was discharged, but respiratory distress necessitated readmission. Her respiratory status worsened, and she died on the fifth hospital day. An autopsy was performed.

Patient 3

This 16-month-old boy was admitted to the University of Virginia Hospital for failure to thrive. He appeared cachectic, and there was clubbing of the fingers. Chest radiographs showed bilateral pulmonary infiltrates with hilar prominence. Negative laboratory studies included a sweat test, skin tests with PPD and fungal antigens, and serum protein electrophoresis. The hematocrit was 36 per cent, and the white-cell count 14,500 per cubic millimeter. An open-lung biopsy was performed. During the next 14½ years, the radiographic findings showed progression, with fibrosis and honeycombing.

When the patient was 16 years old, he was again admitted to the University of Virginia Hospital because of acute pneumonitis. An open-lung biopsy was performed. The patient was treated with antibiotics and discharged. During the next 26 months he had moderate intolerance to exercise and required treatment for recurrent respiratory-tract infections.

The patient was readmitted when he was 19 years old because of extreme dyspnea. Radiographs showed a large area of consolida-

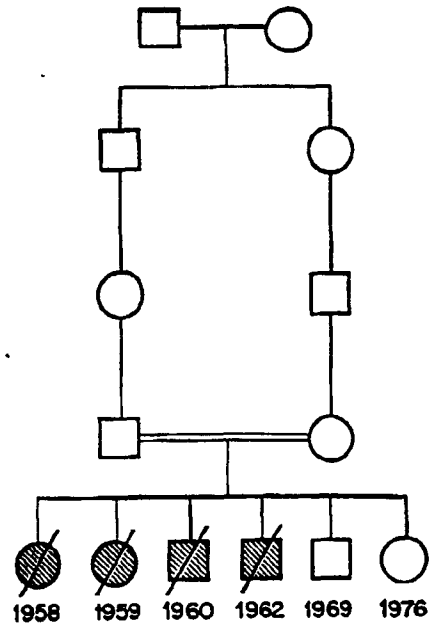

Figure 1. Simplified Pedigree of Family with Pulmonary Alveolar Proteinosis.
Squares denote males, circles females, and shaded symbols affected children. The year of birth of each sibling is indicated.

Use of Serum Bile Acids in the Identification of Vinyl Chloride Hepatotoxicity

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Most previous studies proposing serum bile acids as indicators of hepatic function have been performed in hospitalized patients in whom overt symptomatic liver disease was present. The ability of fasting levels of serum bile acids to identify mild, clinically inapparent chemical liver injury in an occupational setting was compared with that of indocyanine green clearance and routine biochemical liver tests in 67 asymptomatic chemical workers in whom liver biopsies had been performed for medical indications. Histologically, 15 were found to have chemical liver injury, 27 had nonchemical liver disease, and 25 were normal. Two serum bile acids, cholylglycine and conjugates of cholic acid, were determined by radioimmunoassay, using 466 "normal" males from the same worker cohort as a reference range. The geometric mean concentrations of cholylglycine in patients with chemical liver injury, patients with nonchemical liver disease, and normal subjects were 47.9, 19.1, and 20.0 μg/dl, respectively ($p = 0.036$ by analysis of variance). Conjugates of cholic acid showed similar differences ($p = 0.027$), as did indocyanine green clearance with mean half-life of 4.2, 3.2, and 3.3 minutes in the three biopsy subgroups, respectively ($p = 0.043$). Such differences were not observed for biochemical liver tests. The fasting level of serum bile acids provided high specificity but lower sensitivity in the detection of all types of liver disease. However, serum bile acids and indocyanine green clearance provided a higher specificity and sensitivity for chemical liver injury than for nonchemical liver disease. An index of average exposure to vinyl chloride was significantly greater in the subgroup with chemical liver injury than in the other two groups, further supporting the association of chemical type injury with impaired anion uptake. These data identify the fasting level of serum bile acids as a clinically usable indicator of early chemical injury in chemically exposed asymptomatic worker populations with liver dysfunction. Further investigation is needed in other occupational hepatotoxic environments to determine if this association is limited to vinyl monomer type injury.

Occupational and nonoccupational exposure to hepatotoxic agents can lead to acute, subacute, or chronic liver injury. The decrease in acute occupational hepatic injury in recent decades has focused concern that chronic hepatic disease, including malignant neoplasms, may develop as a result of prolonged low-level industrial exposure [1,2]. Currently, there are no specific clinical means to identify hepatic injury from chronic low-level exposure to chemical hepatotoxins.

The inability of standard biochemical enzyme studies to identify the early phases or progression of liver injury has been shown [3–10].

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Serum enzyme studies are of limited value because they primarily reflect acute disruption of cell membrane integrity (liver cell "leaking") rather than the uptake, metabolism, storage, or excretion functions of the liver cell [8]. In subacute, chronic, and end-stage liver disease, the enzymes often return to normal levels after initial elevations [9], and fail to reflect the decreased functional capacity of the remaining parenchyma. Enzyme levels can also be increased in nonhepatic diseases. In severe hepatic injury (fulminant hepatitis or severe toxic necrosis), decreasing enzyme levels may reflect worsening disease [8].

At present, measuring clearance rates of substances primarily or solely removed from circulation by the liver provides the most sensitive and specific indicator of liver function [5]. Such substances include exogenous anionic dyes (bromosulphthalein or indocyanine green) and endogenous metabolites (serum bile acids). However, few studies have examined the ability of these tests to identify early subclinical liver disease in asymptomatic populations.

The discovery of vinyl chloride-related hepatic angiosarcoma and nonmalignant hepatocellular injury in 1974 [11] provided an opportunity to study chronic occupational chemical liver injury. Recent studies [4,10] have shown that indocyanine green clearance provides the best combined sensitivity and specificity for detecting persons with subclinical hepatic disease. However, this test has certain limitations. It is invasive, involves intravenous injection of a synthetic dye, and requires multiple blood samplings. Further, blood samples are best analyzed the same day and not stored for prolonged periods of time due to indocyanine green degradation.

Serum bile acids, which have been proposed as a clinical test of hepatic function, are natural substances, cleared only by the liver. Bile acid studies have been conducted in the fasting [12–16] and postprandial [12,13,17,18] states and following exogenous loading, either by the intravenous [19–22] or, more recently, the oral [23,24] route. Most of these studies, however, have been conducted in persons with overt disease with liver damage of mixed origins. The objectives of this study were to determine if serum bile acid levels could identify chemical workers who had had chemical liver injury after exposure to vinyl chloride and other vinyl monomers, and to compare the capability of bile acid levels with those of indocyanine green dye clearance and conventional biochemical tests.

PATIENTS AND METHODS

The study population consisted of 1,200 employees of a vinyl chloride/synthetic rubber manufacturing plant. The occupational surveillance program that monitored these vinyl monomer workers for a seven-year period provided medical data including annual history, physical examination, 45 biochemical and hematologic parameters, urine analysis, chest radiography, liver/spleen scanning, indocyanine green dye clearance tests, and serologic studies for viral infection. Fasting serum samples were obtained from all employees who participated in the screening program. Criteria for participation in this bile acid study included male employees who had worked for one or more years, had undergone the conventional biochemical screening tests, had indocyanine green clearance test performed at the low-dose (0.5 mg/kg) level, and had fasting serum samples available for bile acid determination. The latter two tests had to have been performed on the same day.

A total of 589 employees met these inclusion criteria. At the beginning of the study, prior to any serum bile acid determinations, these employees were divided into three groups on the basis of the results of medical examination, liver/spleen scanning, and enzyme studies. Employees who had undergone cholecystectomy were excluded. None had had bowel resection. Group I included 466 employees who had no clinical, radiologic, serologic, or biochemical evidence of liver disease; Group II included 56 workers who had repeated biochemical test abnormalities consistent with liver injury but in whom no liver biopsies had been performed; and Group III consisted of 67 employees who had liver biopsies performed, 30 because of biochemical abnormalities, 24 because of radiologic abnormalities, and 13 incidental biopsies performed during non-hepatobiliary abdominal surgery. Group III forms the basis for this report.

Serum bile acids, cholylglycine and conjugates of cholic acid, were determined by radioimmunoassay. Radioimmunoassay method used had the same selectivity for cholylglycine as the present-day radioimmunoassay (Diagnostic Kits, Abbott Laboratories, Chicago, Illinois). All assays were performed at one time. In the healthy workers (Group I), the normal range [25] for cholylglycine was 0 to 48 μg/dl, and 0 to 74 μg/dl for conjugates of cholic acid. The indocyanine green clearance, expressed as the half-time, was calculated following intravenous injection of 0.5 mg/kg indocyanine green as previously described [26,27]. The normal range in the same standard population is 1.8 to 3.6 minutes [28]. The following biochemical tests were performed in serum using standard methods: alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transpeptidase. The normal ranges used were also those previously described in this population [28]. All biochemical data from Group I did not significantly differ from generally published norms of similar occupational and general populations [5,6,8].

Biopsy results were interpreted in a double-blind duplicative fashion as previously described [29]. All were classified, a priori into one of three categories: (1) chemical liver injury, (2) nonchemical liver disease, or (3) normal biopsy results, on the basis of known histologic features of vinyl chloride-associated chemical liver injury [3,29–31]. These include focal hepatocellular hyperplasia, mixed focal hyperplasia with focal increased reticulum and/or collagen, and focal sinusoidal dilatation and vascular changes.

All indocyanine green clearance and serum bile acid de-

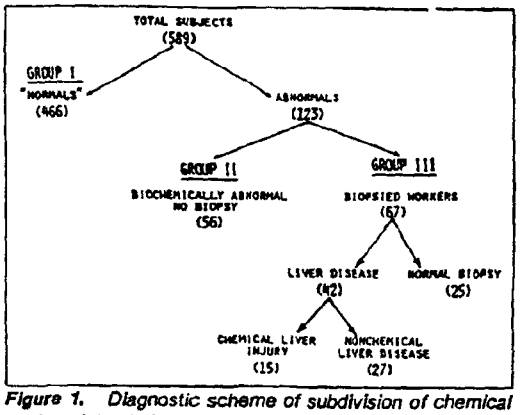

Figure 1. Diagnostic scheme of subdivision of chemical workers into study groups.

terminations in a person were from blood samples drawn on the same day. The biochemical blood studies were not performed at the same time as indocyanine green clearance and serum bile acid tests in all cases. In 44 (66.7 percent) of 66 patients, serum samples for indocyanine green clearance, bile acid determination, and biochemical studies were obtained from blood samples drawn on the same day. The remainder were obtained from blood samples drawn within six weeks. The date of biochemical tests chosen for analysis was that closest to the serum bile acids/indocyanine green clearance test date. To assess if this had any effect, the results for those persons with versus those without simultaneous data were compared for each biopsy subgroup. The time interval between liver biopsy and serum bile acid determinations varied, but the median interval was similar in the three biopsy subgroups: six months in patients with chemical liver injury, nine months in patients with nonchemical liver disease, and nine months in normal subjects.

Chemical exposure was assessed through an exposure ranking system [32], which consists of a work and job classification based on an ordered, six-level exposure rating regarding the intensity of exposure to 22 potentially hepatotoxic chemicals, including vinyl chloride. Cumulative exposure rank months and average exposure indexes were determined for each employee.

For analysis, logarithmic transformation was performed when skewed distributions were encountered (serum bile acids, biochemical data). Differences among the three biopsy groups were assessed by analysis of variance on the log-transformed data. The differences in exposure indexes between biopsy groups were assessed as described by Mantel and Haenszel [33] and confidence limits for the odds ratio were calculated with the program of Rothman and Boice [34]. A test for linear trend in proportions of liver function abnormalities with cumulative exposure categories was assessed by the method of Fleiss [35]. The Kruskal-Wallis test was used to compare ages between biopsy subgroups. The Wilcoxon rank-sum test was used to compare results between simultaneously and nonsimultaneously obtained data.

RESULTS

The derivation of the study group from cohort is summarized in **Figure 1.** Group I laboratory values were used as the reference group for "normal values range" since all tests were performed in an identical manner in the same laboratory. The 67 workers who underwent biopsy (Group III) are further subdivided into 25 with no liver disease (normal biopsy results) and 42 with liver disease on biopsy. This latter subgroup was further subdivided into 15 with chemical liver injury and 27 with nonchemical liver disease, usually of viral origin or alcohol-induced. Biopsy findings in the subgroup with nonchemical liver disease consisted of mild or moderate steatosis and/or portal fibrosis or portal triaditis. Only two patients were described as having "beginning cirrhosis" with steatosis or portal fibrosis. The ages of the workers in the three biopsy subgroups were similar: 45.6 ± 2.7 (mean ± SEM) in patients with chemical liver injury; 46.9 ± 1.9 in patients with nonchemical liver disease; 45.9 ± 1.9 in normal subjects ($p > 0.8$ by Kruskal-Wallis test).

An analysis of the levels of the four enzymes, indocyanine green clearance, and both serum bile acid values revealed no significant differences in results between persons in whom the determinations had been made simultaneously and those in whom it had not. This was observed for all three biopsy subgroups ($p > 0.1$ for all comparisons). The data were thus pooled in further analyses.

Serum bile acid levels differed among the three biopsy subgroups of Group III. Analysis of variance on the log-transformed data revealed significant differences in bile acid levels ($p < 0.05$) among groups. Moreover, considering a priori contrasts, patients with chemical liver injury had the highest mean value, which was significantly greater than that in normal subjects, and therefore in patients with nonchemical liver disease ($p < 0.05$). However, the mean cholylglycine level in patients with liver disease as a whole (chemical liver injury and nonchemical liver disease pooled) was not significantly different from that in normal subjects. Geometric mean level of cholylglycine was 47.9 μg/dl in patients with chemical liver injury, 19.1 in patients with nonchemical liver disease, and 20.0 in normal subjects. There were similar findings for conjugates of cholic acid (**Table I**).

The sensitivity and specificity were determined by examining individual serum bile acid values in relation to the normal ranges described earlier. For cholylglycine, the sensitivity in chemical liver injury was 40 percent and in nonchemical liver disease was 14.8 percent; the specificity was 80 percent based on the normal group. For conjugates of cholic acid, the sensitivity in chemical liver injury was 26.7 percent and in

TABLE I Mean Values for Serum Bile Acids and Indocyanine Green Dye Clearance, by Biopsy Group

Test (normal range)	Biopsy Subgroup: Chemical Liver Injury (15)	Biopsy Subgroup: Nonchemical Liver Disease (27)	Biopsy Subgroup: Normal Subjects (25)	Analysis of Variance: F	Analysis of Variance: p
Cholyglycine (0–48 μg/dl)					
Geometric mean	47.9	19.1	20.0		
Log_{10}, mean ± SEM	1.68 ± 0.14	1.28 ± 0.09	1.30 ± 0.17	3.52	0.036
Conjugates of cholic acid (0–74 μg/dl)					
Geometric mean	45.7	17.4	19.1		
Log_{10}, mean ± SEM	1.66 ± 0.13	1.24 ± 0.08	1.2 ± 0.11	3.83	0.027
Indocyanine green clearance (1.8–3.6 minutes)					
Mean ± SEM	4.2 ± 0.6	3.2 ± 0.2	3.3 ± 0.2	3.35	0.043

nonchemical liver disease, 7.4 percent; specificity was 92 percent.

The indocyanine green clearance half-times revealed similar significant differences among biopsy groups ($p < 0.05$). A priori contrasts again showed that mean indocyanine green clearance was highest in the subgroup with chemical liver injury, significantly greater ($p < 0.05$) than in the normal subgroups or the subgroup with nonchemical liver disease whereas the mean value in patients with liver disease (chemical liver injury and nonchemical liver disease pooled) was not significantly different from that in normal subjects (Table I). The sensitivity of indocyanine green clearance was found to be 77.8 percent in chemical liver injury and 26.1 percent in nonchemical liver disease (40.6 percent for all liver disease). Specificity was determined to be 90.5 percent.

Analysis of the biochemical data (Table II) revealed significant differences among the three biopsy subgroups ($p < 0.05$ by analysis of variance) for only one enzyme, aspartate aminotransferase. In this case, the mean value was greatest in the subgroup with nonchemical liver disease, the opposite of the situation found with serum bile acids and indocyanine green clearance.

The relative sensitivity and specificity of serum bile acids (cholylglycine and conjugates of cholic acid) versus standard biochemical tests and indocyanine green clearance in detecting chemical liver injury, nonchemical liver disease, and all liver disease are shown in Figure 2. The top panel shows the sensitivity for identifying all types of liver disease; cholylglycine and conjugates of cholic acid provided relatively lower sensitivity whereas gamma-glutamyl transpeptidase had

TABLE II Mean Values for Biochemical Studies, by Biopsy Subgroup

Test (normal range)	Biopsy Subgroup: Chemical Liver Injury (15)	Biopsy Subgroup: Nonchemical Liver Disease (27)	Biopsy Subgroup: Normal Subjects (25)	Analysis of Variance: F	Analysis of Variance: p
Alkaline phosphatase (42–109 units/liter)					
Geometric mean	87.1	77.6	66.1		
Log_{10}, mean ± SEM	1.94 ± 0.04	1.89 ± 0.04	1.82 ± 0.03	2.45	0.094
Aspartate aminotransferase (11–32 units/liter)					
Geometric mean	24.0	31.6	20.9		
Log_{10}, mean ± SEM	1.38 ± 0.11	1.50 ± 0.06	1.32 ± 0.04	3.42	0.039
Alanine aminotransferase (1–36 units/liter)					
Geometric mean	17.0	25.7	13.8		
Log_{10}, mean ± SEM	1.23 ± 0.11	1.41 ± 0.09	1.14 ± 0.07	2.89	0.063
Gamma-glutamyl transpeptidase (10–41 mU/liter)					
Geometric mean	44.7	43.7	25.1		
Log_{10}, mean ± SEM	1.65 ± 0.16	1.64 ± 0.08	1.40 ± 0.07	2.55	0.087

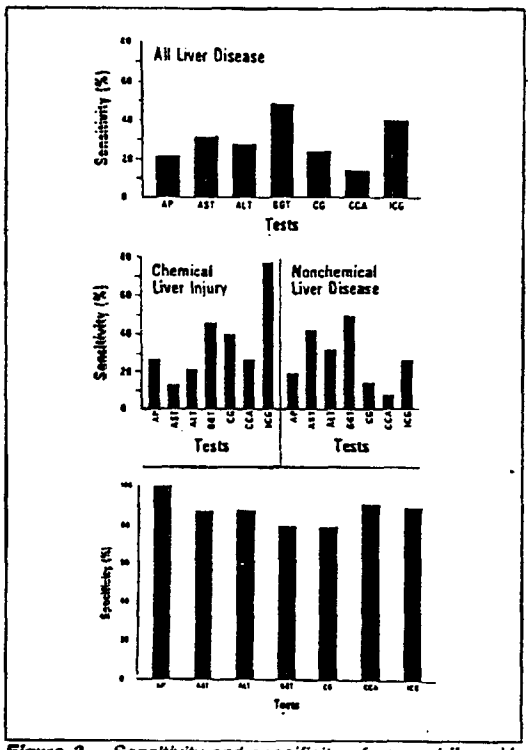

Figure 2. Sensitivity and specificity of serum bile acids (cholylglycine and conjugates of cholic acid) versus standard biochemical tests and indocyanine green clearances in detecting chemical liver injury, nonchemical liver injury, and all liver disease. AP = alkaline phosphatase; AST = aspartate aminotransferase; ALT = alanine aminotransferase; GGT = gamma-glutamyl transpeptidase; CG = cholylglycine; CCA = conjugates of cholic acid; ICG = indocyanine green.

the highest, followed by indocyanine green clearance. The middle panel shows the sensitivity for chemical liver injury and nonchemical liver disease; indocyanine green clearance was by far the most sensitive in detecting chemical liver injury, followed by gamma-glutamyl transpeptidase and cholylglycine. The bottom panel illustrates the specificity of the tests; specificity increased, in turn, with gamma-glutamyl transpeptidase, cholylglycine, alanine aminotransferase, aspartate aminotransferase, indocyanine green, conjugates of cholic acid, and alkaline phosphatase, the latter showing 100 percent specificity.

The ratio of frequency of positivity of these test results in chemical liver injury relative to nonchemical liver disease was determined (Figure 3). Levels of the parenchymal enzymes (aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transpeptidase) were more frequently elevated in nonchemical liver disease, whereas results of tests that reflected overall hepatic clearance (serum bile acids, indocyanine green) were far more often positive in chemical liver injury.

Examining average exposure indexes in the different biopsy subgroups revealed that the highest exposure rankings (4 or greater) were found in seven (50 percent) of 14 patients with chemical liver injury but in only 11 (21.6 percent) of 51 without chemical liver injury, five (19.2 percent) of 26 with nonchemical liver disease, and six (24 percent) of 25 normal subjects, supporting the relationship between average and cumulative exposure indexes and the specificity of the histologic findings on liver biopsy. The association of chemical liver injury histologic characteristics with high exposure ranking was statistically significant (chi-square 4.4; odds ratio 3.64; 95 percent confidence interval 1.08, 12.2).

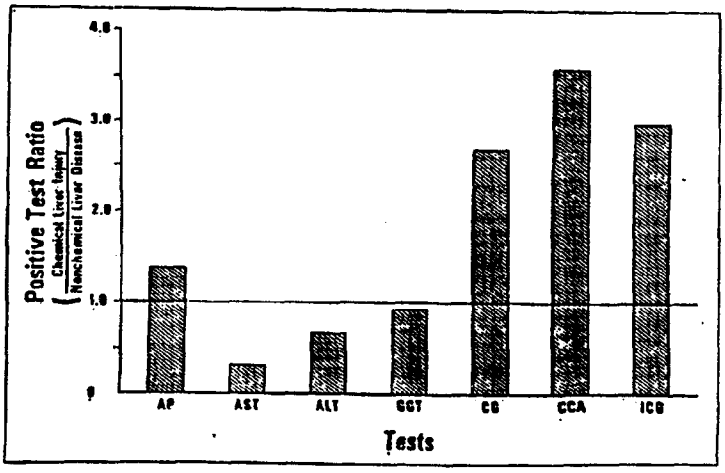

Figure 3. The ratio of frequency of positivity in chemical liver injury versus nonchemical liver disease. Abbreviations as in Figure 2.

TABLE III Frequency of Abnormality of Liver Function Test Results in Entire Population by Cumulative Vinyl Chloride Exposure Category

Vinyl Chloride Exposure Category	Test		
	Cholylglycine	Conjugates of Cholic Acid	Indocyanine Green Clearance
20–500	22/238 (9.2%)	15/238 (6.3%)	4/190 (2.1%)
500–900	6/123 (4.9%)	2/123 (1.6%)	4/115 (3.5%)
900–1,200	5/62 (8.1%)	5/62 (8.1%)	3/50 (6%)
1,200–1,600	5/47 (10.6%)	4/47 (8.5%)	1/57 (1.8%)
>1,600	4/25 (16%)	3/25 (12%)	2/18 (11.1%)

Finally, the frequency of abnormalities of liver function in the entire population determined for the various cumulative exposure categories is shown in Table III. A trend is suggested by the increasing frequency of abnormality with increasing exposure category (dose-response relationship) for both serum bile acids and indocyanine green clearance. The trend was statistically significant ($0.01 < p < 0.025$) for indocyanine green clearance, but did not quite reach statistical significance for cholylglycine and conjugates of cholic acid.

COMMENTS

This study has illustrated that mean serum bile acid levels are significantly greater in asymptomatic exposed chemical workers with histologically proved chemical liver injury than in those workers with nonchemical liver disease or with normal findings on biopsy. A similar relationship is seen with indocyanine green clearance values but not with the conventional biochemical tests. In severe or massive hepatic injury, all these tests have high sensitivity. This capability, however, is clinically irrelevant when a test's suitability for the identification of early chemical injury among asymptomatic workers is concerned. Therefore, the clinical significance of these results is related to the increasing need for means of identifying the causal agent in chemically exposed persons who are discovered to have persistent liver enzyme abnormalities. Determinations of indocyanine green clearance and bile acids provide a better means of differentiating the underlying causative agent(s) for such abnormalities. Liver enzyme abnormalities in the presence of normal results of bile acid studies are more characteristic of asymptomatic persons with histologically acute-type cellular injury. In contrast, persistent enzyme abnormalities with elevated levels of serum bile acids are shown to be more indicative of persistent subclinical injury associated with increased collagen deposition and vascular changes due to low-grade chemical exposure.

The predictive values of such tests are greatly dependent upon the prevalence of the disease they are to detect. We can provide an approximation of their predictive value only by using a hypothetic model since the actual prevalence of chemical liver injury cannot be determined without obtaining a liver biopsy specimen in all exposed workers with biochemical abnormalities. If the assumption is made that our biopsy group has identified *all* cases of chemical liver injury (the lowest prevalence possible), then the prevalence rate would be 13 per 1,000 exposed workers. If our biopsy group reflects the distribution of disease throughout the cohort and the biochemical screening tests have identified all cases (reasonable assumption since we have three to seven-year follow-up on 90 percent of all workers), then the highest prevalence rate for chemical liver injury would be 30 per 1,000 exposed workers. The positive predictive values for alanine aminotransferase, gamma-glutamyl transpeptidase, conjugates of cholic acid, cholylglycine, and indocyanine green clearance were 5, 7, 9, 6, and 21 percent, respectively, in a working population with the aforementioned assumption regarding prevalence of chemical liver injury. It can be seen that conjugates of cholic acid bile acid test and indocyanine green clearance have better predictive value than the most sensitive (gamma-glutamyl transpeptidase) and most specific (alanine aminotransferase) of enzyme tests.

The positive predictive values of these tests are lower than is usually accepted for clinical use. It must be kept in mind, however, that we were screening for latent subclinical liver injury in an asymptomatic population at high risk of exposure. These persons were not the customary symptomatic patients with clinically overt liver disease seen in the hospital.

This is an important difference. Physicians are often called upon to rule out occupationally related hepatic injury in an asymptomatic person incidentally discovered to have persistent enzyme abnormalities. A positive screening result will either direct the patient to further medical workup for nonoccupational disease or indicate a need to remove the patient from a toxic environment until the exact nature of the hepatic dysfunction is determined. Serum bile acids, especially

conjugates of cholic acid, provide a suitable substitute for indocyanine green clearance as a means of differentiating these types of causes.

Essentially all previous studies examining serum bile acids considered hospitalized or ill patients or at least persons being assessed for overt clinical liver disease. Moreover, comparisons between these studies are limited by the heterogeneous types of hepatic disease considered and the methods of serum bile acid determination (enzymatic and gas-liquid chromatography) that preceded radioimmunoassay.

Although it is not certain that vinyl chloride monomer exposure alone is accountable for all the differences observed, several pieces of evidence suggest it. First, the subgroups were formed on the basis of "a priori" identification of pathologic features shown to be characteristic of vinyl chloride-type chemical liver injury. Second, greater mean cumulative and average exposure ratings for vinyl chloride (but not for any other of 22 chemicals assessed) were observed in the group with chemical liver injury. Third, in this analysis, tissue documentation was used as a criterion. The alcohol-related type injury was diagnosed in over half the patients with nonchemical liver disease and in none of the subgroups with chemical liver injury. Thus, alcohol is not a likely explanation for the observed differences. Fourth, there is a strong suggestion of a dose-response relationship between the frequency of abnormality of serum bile acid and indocyanine green clearance test results and increasing cumulative exposure category. However, we have observed but do not yet have a definitive explanation for the relatively high proportion of abnormalities in the low exposure category in the overall worker population. Future sequential studies of these abnormalities may provide an explanation.

The differences in the frequency with which results of clearance tests versus enzyme tests were positive in the two groups with liver disease (chemical liver injury/nonchemical liver disease ratio less than 1) probably reflect the more cytotoxic nature of the cellular injury seen in nonchemical liver disease, due to alcohol, drugs, and/or viral hepatitis. On the other hand, long-term, low-level chemical injury to the liver tends to have less observable cytotoxicity and more evidence of chronic injury with fibrotic repair. The less frequent enzyme elevations and more frequently abnormal serum bile acid levels and indocyanine green clearance (chemical liver injury/nonchemical liver disease ratio greater than 1) better reflect the type of overall functional impairment of the hepatocytes.

Although there were no significant differences in the biochemical studies between the subgroup with nonchemical liver disease and the normal subgroup, the mean value for all tests was greater in the subgroup with nonchemical liver disease than in the normal subgroup. The absence of significance is not unusual considering the mild nature of the liver injury in the subgroup with nonchemical liver disease and the variability in enzyme test values. It is not unexpected that the sensitivity of serum bile acids found in our present study is below that found in various studies in the literature [12]. The subjects in those studies were, for the most part, symptomatic hospitalized patients with more cytotoxically severe and acute disease. A few studies have attempted to look primarily at anicteric subjects or those with minor hepatic dysfunction. In 16 anicteric subjects with chronic liver disease (chronic active hepatitis, alcoholic cirrhosis, and primary biliary cirrhosis) studied by Gilmore and Thompson [24], fasting concentrations of bile acids by the enzymatic method were abnormal in 10 (63 percent). Kobayashi et al [36] investigated 70 patients with histologic evidence of liver disease but with normal results of routine liver function tests. They found a sensitivity of 24 percent for fasting serum bile acid concentrations (enzymatic method), which compares closely with that in our study. Their patients included 10 with minimal methotrexate-induced liver injury, 13 with hepatic sarcoidosis, seven with resolving viral hepatitis with bridging necrosis, 12 with fatty infiltration of unknown cause, and 28 with miscellaneous hepatic lesions. Douglas et al [20] examined fasting and postprandial levels of serum bile acids by radioimmunoassay in 20 patients with anicteric liver disease who had only minor abnormalities on conventional liver function tests. The diagnoses included alcoholic hepatitis in six, alcoholic cirrhosis in eight, and one case each of nonspecific reactive hepatitis, alcoholic hemosiderosis, cirrhosis with hemosiderosis, alpha$_1$-antitrypsin deficiency cirrhosis, and mild alcoholic liver disease. Bilirubin level was abnormal in nine of 20, alanine aminotransferase level abnormal in four of 20, aspartate aminotransferase level abnormal in seven of 20, alkaline phosphatase level abnormal in four of 20, and gamma-glutamyl transpeptidase level abnormal in 10 of 20. The authors found abnormal fasting cholate levels in seven of 20 and abnormal postprandial cholate levels in 10 of 20. These studies illustrate the higher frequency of liver disease detection by functional type tests (bilirubin and bile acids) when the disease process is mild in activity and/or chronic and cumulative in its severity. Comparisons between studies, however, are limited because of the heterogeneity in the types of liver injury and the various pathologic stages. Consistent with our findings is Berk et al's [37] observation of abnormal cholylglycine clearance as the sole liver dysfunction present in a case of persistent vinyl chloride-induced liver injury.

A potential limitation to this prospective study might

be the time relationship between the performance of the liver biopsy, biochemical studies, and the serum bile acid determinations and the indocyanine green clearances. Three factors mitigate against the time differences influencing the results. First, there is the nonprogressive nature of these early histologic lesions associated with vinyl monomer chemical injury. Second, the microcirculatory rather than the hepatocellular nature of the injury, which has already been described [8,9], is highly correlated with increased collagen deposition and appears to be persistent. Third, the permanent nature of the histologic lesion has been documented by serial biopsy in the same person [28,29]. Finally, the results for each test were similar in each biopsy subgroup between simultaneous and nonsimultaneous determinations as shown before.

In conclusion, fasting serum bile acid levels, especially conjugates of cholic acid, provided a high degree of specificity (i.e., very few false-positive results) in persons with normal biopsy findings—a highly desirable characteristic for a diagnostic test to be used in asymptomatic populations. Fasting serum bile acid levels, because of their lower sensitivity in detecting acute but mild cytotoxic disease, are not appropriate for use as a screening test by themselves. They do, however, when used in combination and in the proper sequence, identify those among the exposed with a very high probability of having low-grade, chronic, or progressive chemical liver injury and needing more extensive clinical investigation. The low sensitivity may be improved by use of an exogenous load, preferably by the oral route [23,24], but this remains to be demonstrated.

Finally, the serum bile acid determinations provide a clearance-type liver test with greater acceptability with regard to time, cost, storage of blood samples, and ease of performance than does indocyanine green clearance. This diagnostic use of bile acids should continue to undergo further validation in other distinct types of hepatotoxic liver injury.

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PUBLIC-HEALTH ROUNDS AT THE HARVARD SCHOOL OF PUBLIC HEALTH

Edited by Peter Braun, M.D., and Eleanor Druckman, M.S.

Vinyl Chloride: Can the Worker Be Protected?

Introduction

*David H. Wegman, M.D.**

OVER 80 million American men and women spend one quarter of their lives in a workplace outside the home. The deterioration in health quality that results from exposure to hazards in their workplace is not widely known. The annual toll of job-related injuries is estimated at 2.2 million, and these injuries result in 14,000 deaths.[1] The National Health Survey has estimated that the average worker experiences six days of absence and more than 16 days of restricted activity because of some type of job-related disability per year and that one out of eight workers will receive a job-related injury each year. The incidence of occupational disease was recently estimated at 390,000 new cases and as many as 100,000 deaths per year. Job-related disabilities account for 10 times as many lost man days as from strikes.[1]

This human cost can, in large part, be assigned to well known hazards of the workplace or to sporadic human error. The first, at least, is subject to control if we are prepared to accept an increment in price or inconvenience.

In a technologically innovative society, the worker is subject to an additional risk. Morbidity or death may also result from exposure to new processes or agents with unanticipated potential for injury.

The quality of a society should be judged in part on its awareness of the potential for injury in innovative technology. There must be procedures for evaluating the level of danger to which workers are exposed, and for responding to demonstrated dangers. The following account illustrates how we have dealt with one specific problem.

Perspectives

John M. Peters, M.D.†

Most of the known occupational diseases are chronic, untreatable and fatal. Despite the fact that the prevention of occupational diseases is highly desirable, they are not subject to systematic surveillance in the United States today by industry, unions, the government or universities. In addition, physicians frequently fail to recognize occupational disease. In this context the discovery of a new occupational disease most commonly results from an unusual group of clinical manifestations or disease occurring in an industrial cluster, as was true of vinyl chloride.

Vinyl chloride is a gas used to make polyvinyl chloride, the basis of widely used plastic products such as phonograph records, meat wrappers, upholstery covering, plastic containers, toys and pipe for water supplies. The production of vinyl chloride and polyvinyl chloride is a post World War II industry that has been growing for the last 20 to 25 years.

In January, 1974, in Louisville, Kentucky, three cases of a rare neoplasm, angiosarcoma of the liver, were reported in workers exposed to vinyl chloride.[2] If these had not been such unusual tumors, they would have gone unnoticed. If cancer of the lung, bladder or bowel had developed in these workers, we would probably not know about the connection between vinyl chloride and cancer. These tumors occurred in workers who had been exposed for roughly 20 years. It is probable that we are just beginning to see a larger epidemic.

It is instructive to examine the accumulation, over time, of information about the biologic effects of vinyl chloride. In 1938 there was a report describing acute toxicity from vinyl chloride to guinea pigs, mice and dogs.[3] In 1949 a report of cases of liver damage from vinyl chloride in human beings was published.[4] In 1961, studies of chronic toxicity on rats, rabbits, guinea pigs and dogs revealed that liver damage occurred at levels above 50 parts per million.[5] In 1966 another very unusual disease associated with vinyl chloride, acroosteolysis, was described.[6] This condition, characterized by the dissolution of the bones of the fingers and toes, was clearly related to vinyl chloride exposure. In 1970 a study showing the development of cancer in animals appeared.[7] Since announcement in January, 1974, of the first three patients with angiosarcoma of the liver an additional 26 such cases have been identified.[8] Several epidemiologic studies[9-11] have consistently found an association of vinyl chloride exposure with tumors in sites other than the liver. One study indicates a time trend with increasing rates of all cancers in the exposed population.[9]

It is hard to escape the conclusion that, well before 1974, there was enough evidence to support the presumption of a serious occupational hazard. What was, and is, lacking is a national policy of screening new materials for potential hazard before their dissemination in the workplace, a policy that might have prevented widespread exposure to vinyl chloride. Should we have a policy for screening chemicals? Could we implement such a policy if we had one?

Dr. Rudolph J. Jaeger will tell us what we might have learned from animal studies.

Toxicology

Rudolph J. Jaeger, Ph.D.‡

The central problem in toxicology is extrapolation to man of the toxic responses observed in the laboratory animal. Individual species vary widely in their susceptibility to injury from a given compound, and none meet the requirements of equivalency to man in biologic response, short latency, low cost and convenience across the spectrum of agents to be

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tested. Despite these shortcomings inherent in toxicologic studies as now conducted, experiments in traditional laboratory animals could have supported a stronger and earlier presumption of the dangers of vinyl chloride.

Under normal environmental conditions vinyl chloride is a colorless, nearly odorless gas that is highly explosive at modest concentrations in the air — i.e., 4 per cent and above. At 8 per cent and above, it is an anesthetic — a use that was suggested in the 1930's[12] but abandoned owing, in part, to flammability and myocardial sensitization.[13]

What tests of toxicity might be performed on an agent like vinyl chloride? As a first step, experiments might be conducted in which the gas is inhaled by a test species at a given concentration — e.g., 100; 1000; 10,000; 100,000 parts per million (ppm) — for a single acute period comparable to a normal work day of eight hours or less. Except for death due to central-nervous-system depression, vinyl chloride is without immediate lethal action.[14] After a single subacute exposure, the animals will recover without residual damage and live without apparent ill effects for a normal life-span. This lack of acute toxic effect in rats is reflected in the experience of workers who have been rendered unconscious by high concentrations of vinyl chloride and then moved to other jobs with little or no subsequent exposure. They have had no apparent sequelae.

A second test of toxicity in animals involves repeated inhalation exposure for days, weeks, or months. Such experiments are comparable to short-term work situations. In experiments such as these, vinyl chloride produced no dramatic effect on repeated exposure at concentrations up to 20,000 ppm for 92 days.[15] In a study done in 1961 by the Toxicology Laboratory of the Dow Chemical Company, rats, rabbits, guinea pigs, and dogs were exposed to vinyl chloride for seven hours a day, five days a week for periods up to six months at concentrations of 500, 200, 100 and 50 ppm of vinyl chloride. In this study the animals were killed at the end of the exposure period and autopsied.[5] Growth rates and hematologic indexes were within normal limits. Slight liver enlargement was noted after exposures at concentrations of 100 ppm or more. Minor pathologic changes were also detected in other species at doses higher than 100 ppm. Fifty ppm, a concentration without effect on liver weight, was suggested by Dow as a maximum time-weighted concentration for eight-hour exposure of workers, on the grounds that these conditions of exposure represented levels that did not affect rats exposed for six months.

A somewhat different approach to the study of toxicity is represented by the studies of Maltoni[16] and Viola,[7] which showed that exposure of rats to 30,000 ppm of vinyl chloride four hours a day, five days a week, for a year, caused malignant neoplasms in a variety of sites, including the liver, brain, kidneys and vascular tissue. In these studies the pathologic findings were sought after the animals' lifetime rather than at an earlier experimental sacrifice point. This was a crucial change of experimental design for the identification of potent carcinogenicity of vinyl chloride.

Maltoni found cancers at exposure concentrations as low as 250 ppm in rats.[16] Furthermore, his data on tumor rate with decreasing dose suggest that there is risk of cancer at concentrations of vinyl chloride below 50 ppm. Thus, a compound with little acute lethal action and negligible short-term toxicity is a potent carcinogen in rodents under worklike exposure conditions of 50 ppm and less.

What can be learned from this series of events? It required two decades for the development of malignant tumors in human beings, when 12 months of experimentation in lower vertebrates could have established the carcinogenicity of vinyl chloride at exposures comparable to those in the working environment. Although a negative experimental result in animals cannot exclude potential carcinogenicity in man, a positive result under these circumstances is an unambiguous warning. The events described here suggest that useful toxicologic screening can be carried out in locations other than the workplace, and in species other than man.

Engineering

*William A. Burgess, S.M.**

The manner in which workers are exposed to vinyl chloride varies greatly, depending on their specific roles in the process of production. The industry can be divided into three manufacturing stages — the production of vinyl chloride monomer (VCM), the reaction of this material to form the solid polyvinyl chloride (PVC), and the fabrication of plastic products from polyvinyl chloride.

Over 350,000 workers are exposed to potential hazard at various stages of this industrial sequence. Vinyl chloride monomer is manufactured by a number of different processes in the United States in 15 plants with a total work force of about 1000. In a common process ethylene is combined with chlorine to form ethylene dichloride, which is pyrolyzed to form vinyl chloride. Although this continuous operation is enclosed, leaks from various valves, pumps, and fittings result in exposure of the worker to airborne vinyl chloride gas. Maintenance of process equipment is a major problem in this industry.

Limited data on air concentration are available from the monomer production plants. In normal operations the concentration may be at the level of 1 ppm. Occasional spills, however, will result in peak exposures of hundreds of parts per million.

Fortunately, effective methods of control are available in some parts of the industry. Maintenance obviously must be improved to reduce leaks into the workplace. New procedures must be developed to permit transfer of the monomer and sampling of the product so that the worker need not be exposed during these operations.

In the second industrial stage, vinyl chloride monomer is reacted to form the solid polymer, polyvinyl chloride (PVC). Polymer production is carried out in this country in 36 plants with about 5000 employees. The major exposures to vinyl chloride monomer occur in this part of the industry, and most of the cases of angiosarcoma of the liver have come out of these plants.

Polyvinyl chloride is produced in a batch operation in a glass-lined reactor or pressure vessel. Vinyl chloride monomer is introduced into the reactor with an initiator, a catalyst, and water. The temperature is raised to 60°C, the pres-

*Kresge Center for Environmental Health.

sure is increased, and the material polymerizes over a period of 10 to 16 hours. At the end of this interval, the solid granular polyvinyl chloride is formed, drops to the bottom of the reactor, and is then dried. Some unreacted vinyl chloride monomer remains in the polymer and presents an exposure problem to fabricators of end products. Still, fabrication offers fewer hazards than polymer production. The most common exposure occurs when workers periodically crawl into the reactor to clean the inside surface, a situation in which exposures may exceed 1000 ppm. How can this exposure be reduced? The industry would like to automate the cleaning, but this step has not yet been implemented widely.

Is control of vinyl chloride feasible in this part of the industry? This question sets the stage for the major debate. In testimony before the Occupational Safety and Health Administration, the spokesman for the Society of the Plastics Industry had stated that it is not economically feasible to reduce the levels below 25 ppm. A consulting firm retained by the Department of Labor had stated that it is feasible to bring the concentration down to 2 to 5 ppm in this part of the industry. Other authorities said that air concentrations of vinyl chloride monomer of 1 ppm can be achieved.*

In the third stage, the fabrication sector of the industry, the granular polyvinyl chloride is made into useful commercial and consumer products. The bulk polymer is compounded with various plasticizers, stabilizers, and other additives depending on the ultimate application. The products made from the polyvinyl chloride include intermediate industrial materials such as tubing, rod, bar and sheet, and a host of consumer products.

There are 7500 fabrication plants with nearly 350,000 workers. The exposure to vinyl chloride monomer in these plants is due to the release of the residual monomer from the solid material. The residual monomer in many polymers is in the range of 1000 to 5000 ppm by weight. The hazard in the fabrication industry could be eliminated if the polymer manufacturers reduced the residual amount to 1 ppm.

In summary, although there should be no disagreement that controls must be implemented promptly, there has been much dispute over the levels of control for vinyl chloride that can be achieved, and over their costs.

Economics

Leslie I. Boden, B.S.[†]

On the list of the 50 most common industrial chemicals, vinyl chloride ranks 23d. Annual United States production of this gas is over 2.3 billion kg. By far the most important use of vinyl chloride is in the production of polyvinyl chloride, of which we also produce about 2.3 billion kg a year at a cost of over $1 billion. United States production of this important plastic has been growing steadily since 1939 at an average annual rate of 12 per cent. If the polyvinyl chloride products industry were to be shut down before substitutes could be found and their manufacture begun, perhaps two million jobs and 75 billion dollars worth of production would be lost.[17]

The basic institutions of the economy, firms competing for profits, have been well suited to increase rapidly the availability of goods and services. Through the struggle for profits, business has played a central part in producing the innovations that have raised the standard of living in this country to levels that most people formerly believed impossible.

On the other hand, there are several things for which these industrial organizations are poorly suited. They have few incentives to educate the population, lower unemployment, promote income equality, protect the environment, or help improve the health of the public. Often, these goals come into direct conflict with the profit motive. Concern for workers' health, for example, might require the individual company to conduct expensive research, and cause it to delay the introduction of new products and processes. Both responses would place it at a competitive disadvantage.

This situation has led to government intervention in the private market to diminish the impact of occupational disease. There are at present two types of laws designed to make business more concerned about the health and safety of workers. Worker's compensation laws have created state-regulated programs ensuring that a company pays for medical expenses and for part of lost income if an occupational disease develops in a worker. Unfortunately, these laws do not provide the companies with adequate incentives to protect the health of their workers. For example, the worker's compensation costs for medical expenses and support payments to the families of the men who died in Louisville, Kentucky, average about $64,000. If 1000 workers a year died, the cost of paying this level of compensation would amount to approximately $0.01 per pound of polyvinyl chloride production, or less than 3 per cent of the cost of its manufacture. This proportion is substantially below the costs of rigid manufacturing controls.

In 1970, Congress passed the Federal Occupational Safety and Health Act.[18] This law attempts to prevent disease by compelling companies to conform to standards that are designed to prevent illness due to toxic exposure in the workplace. The enforcement of this act has, however, been weak, and its effectiveness questionable.

How, then, might the government stimulate adequate testing of the potential toxicity of chemicals that enter the industrial environment? One way to achieve this goal would be the implementation of an occupational health act similar to the Pure Food and Drug Act — that is, new industrial chemicals with definite potential for human exposure would have to be demonstrated to be nontoxic before being approved for use. This step would compel industry to test these substances before they were widely used.

Laws like those just mentioned can help to protect the health of workers, but at a cost. This cost might be expressed in a slower rate of technologic innovation, and thus lower production of some goods and services. It might also be felt in increased prices for goods. These increases result from the added costs of experimentation and changes in the

*Official Report of Proceedings before the Occupational Safety and Health Administration of the U.S. Department of Labor in the matter of proposed permanent standards for occupational exposure to vinyl chloride. Washington, D.C., June - July, 1974 (Available from H R Company, Official Reporters, 320 Massachusetts Avenue, N.E., Washington, DC 20002).

†Occupational Health Program.

production process that help to protect workers' health. For example, a spokesman for Firestone Plastics has stated that the costs of lowering vinyl chloride exposure from the 1973 standard to 1 ppm would increase the cost of polyvinyl chloride by 50 per cent.*

The problem is further complicated by the fact that the United States produces less than 30 per cent of the world supply of polyvinyl chloride. If other countries do not implement strict standards, what is to prevent the multinational corporations that produce polyvinyl chloride from building new factories overseas, "exporting" both jobs and disease?

Regulation

David H. Wegman, M.D.†

In my position with the Massachusetts Division of Occupational Hygiene, my first contact with vinyl chloride came in early 1973 with an attempt to evaluate the problem of acro-osteolysis induced by vinyl chloride in Massachusetts workers. The attempt was short lived because other occupational health problems had higher priority. The issue of vinyl chloride, however, was brought back to me with the reports of the cases of angiosarcoma of the liver in January, 1974. As happened elsewhere, we immediately gave the vinyl chloride problem a high priority and began to investigate the four local firms manufacturing polyvinyl chloride from vinyl chloride. Although we were easily able to evaluate current exposure levels in these plants, our efforts to evaluate possible chronic disease foundered because of the small numbers of employees and our inability to locate those who had left employment.

At the same time that we were attempting to determine the magnitude of the problem in Massachusetts, the Federal Occupational Safety and Health Administration (OSHA) was faced with the more difficult problem of establishing national standards.

In July, 1971, OSHA established its first standard on asbestos as a result of heavy pressure from labor.[19] The carcinogenic effect of asbestos was ignored, and the standard was set solely with asbestosis in mind. The next standard promulgated referred to 14 carcinogens but set no "safe" exposure level.[20] Then the vinyl chloride story broke publicly, and the resulting widespread interest forced OSHA to deal directly with a single cancer-causing agent. The agency knew that vinyl chloride was carcinogenic at exposure levels yet to be determined, and its use was widespread, and that control of exposure was difficult. The first step was to set an emergency standard at 50 ppm, the level that the Dow Chemical Company had recommended as an upper limit in 1961.[3] The agency then had six months, by law, to promulgate the final standard. This period included development of both economic and environmental impact statements and the announcement of three days of public hearings. The response to the announcement was so great that hearings continued for three weeks.

As a medical consultant to OSHA, I was asked to address the problem of the human health effects, and how well substantiated they were. The federal government, through the Food and Drug Administration (FDA), had indicated before the hearings had begun that exposure to vinyl chloride was a serious human health problem that needed immediate attention. On February 21, 1974, the FDA received a petition to withdraw vinyl chloride as a propellent in aerosol products (including hair sprays, aerosolized household products and pesticides), and in only six weeks such an order was issued. Information on how widespread the use of these products had been was unavailable.

It was clear that more than the identification of cases of a rare tumor would be required if a recommendation of any exposure level below 50 ppm were to be made. Generally, standards are set on the basis of acute effects of a toxic material. Vinyl chloride was not known to cause acute problems below the lower explosive limit, and since provisions had been made to prevent explosions, concentrations that could cause acute disease were unlikely.

The chronic effects, however, were difficult to evaluate. "Slap-in-the-face epidemiology" had succeeded in bringing the problem to our attention. It was "quick-and-dirty" epidemiology that gave an initial idea of the size of the problem. Although several attempts to conduct mortality surveys had been made, only a few were sufficiently well designed and carried out to provide useful data. Even they fell short of allowing specific estimates of risk. They did tell the unfortunate story of cancer of the liver as well as lung, brain, skin, and lymphatic system in vinyl chloride workers. Three other studies of current workers showed that vinyl chloride and polyvinyl chloride were also associated with chronic disease of liver and spleen, bleeding conditions, skin and bone disease, and chronic lung disease.[21-23]

On the basis of these findings, my first recommendation was for a standard of 1 ppm as the maximum exposure acceptable on medical grounds. The response of the director of OSHA to this recommendation was most interesting. Although initially he had asked for a medical evaluation of the potential health effects, his final request was for a legal defense of the levels. He believed that regardless of what level he set, he would be taken to court for setting too lenient (by labor) or too stringent (by industry) a standard.

This raised the core question: How well-supported do health effects need to be to be acted upon? For vinyl chloride it seemed reasonable to assume that human beings are at least as sensitive as laboratory animals and that the standard would have to be below 50 ppm. But how far below 50 ppm can a number be selected and defended? The most convincing argument for the figure of 1 ppm was the unusual behavior of vinyl chloride. It would be difficult to imagine a material with more potential for human damage, for it is associated not only with cancer at multiple sites but also with non-neoplastic disease of at least six different organs or organ systems.

Epilogue

On October 4, 1974, the Federal Occupational standard for vinyl chloride was issued.[24] Although it accepted in principle 1 ppm as the maximum possible exposure, work practices temporarily permitted exposures of up to 25 ppm with-

*Proceedings before the Occupational Safety and Health Administration.

†Mass. Division of Occupational Hygiene and Occupational Health Program.

out respiratory protection. This standard was immediately challenged by the Society of the Plastics Industry and various companies when they petitioned the federal courts for a stay in the implementation of the standards.[25] The petition was denied, and the United States Supreme Court refused to review the decision.[26] Since the promulgation of the standard, apparently only one small plant has closed, and four new plants are nearing completion.[27]

Polyvinyl chloride was briefly used to make containers for alcoholic beverages. The discovery that migration of detectable amounts of vinyl chloride from the containers to the beverage led to its removal from this use. Definite evidence on the migration of vinyl chloride from food wraps or pipes for drinking water does not exist. Inadequate study has been performed to date to determine whether there is a hazard of vinyl chloride exposure to consumers using nonfood-related polyvinyl chloride products.

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