Occupational Carcinogenesis: The Louisville Experience with Vinyl Chloride-Associated Hepatic Angiosarcoma

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Hepatic angiosarcoma in man was first associated with exposure to vinyl chloride in Louisville, Kentucky, where it was identified in 10 persons from a single vinyl chloride polymerization plant; clinical manifestations are summarized herein. Following prolonged exposure to vinyl chloride, the onset of this disease is insidious and the clinical picture is that of nonspecific hepatic injury with mildly abnormal biochemical liver test results. Carcinoembryonic antigen and alpha fetoprotein are undetectable. Radionuclide and angiographic studies of liver show characteristic but nondiagnostic abnormalities. A definite diagnosis is usually made only by open liver biopsy. Treatment is unsatisfactory but chemotherapy seems to prolong survival. Average survival from diagnosis is about 12 months. Overt liver failure usually occurs only as a preterminal event and was the major cause of death in all of our patients. Preventive measures are now in effect in the plant. This experience illustrates the importance of the clinician in occupationally-related cancer.

Most cancers in man result from exposure to environmental factors. The occupational environment may account for as many as 10 percent of these malignancies [1]. We present the course of events and clinical findings of an industrial epidemic of hepatic angiosarcoma, a rare tumor considered to originate from the vascular lining cells of the liver [2,3]. This experience demonstrates the importance of the clinician in recognizing and controlling occupationally-induced cancer.

In 1974, several patients in Louisville, Kentucky, were found to have angiosarcoma of the liver [4]. All of these patients were men who were chemical workers at a local industrial plant that polymerizes vinyl chloride. Subsequently, additional cases in which the patients had a history of prolonged exposure to vinyl chloride were reported in Louisville [5,6] and elsewhere [7–11]. Various aspects of this malignancy including histology [11], angiographic and radionuclide characteristics [12], urine [13] and tissue [14] glycosaminoglycan patterns and epidemiologic studies [15] have been reported from Louisville. In addition, interest in this disease has propagated elsewhere, both nationally and internationally [16–18]. However, the total experience of vinyl chloride-associated angiosarcoma in the Louisville area has never been told. With a cohesive summary one begins to appreciate how the initial clinical observation of a patient with hepatic angiosarcoma led to the realization of a possible etiologic association. This, in turn, has cast a profound influence on the health of those working in a major worldwide industry involving the production of polyvinyl chloride.

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This concentration of cases of hepatic angiosarcoma gave us the opportunity to study the clinical manifestations of this unusual cancer. In view of concern regarding its increasing incidence [7,19-21], we believed a summary of this information would be valuable in detecting and managing future patients. In this paper we present the natural course, diagnosis and treatment of this disease as well as possible preventive measures that we have learned from study of the patients discovered in the Louisville area. Characteristic features of the disease, including a long period of asymptomatic laboratory abnormalities, the difficulty in diagnosis and the poor response to treatment, are demonstrated by the following illustrative case.

CASE REPORT

The patient, a 51 year old man who entered the vinyl chloride industry 28 years ago as a vat cleaner, worked in the vinyl chloride polymerization area for 18 years. During medical screening of plant employees in April 1974, he was found to have mildly abnormal serum biochemical liver test results. Liver-spleen scan disclosed no abnormalities, and he was asymptomatic.

Because of persistently abnormal biochemical liver tests, he was evaluated further in December 1974. He remained asymptomatic, and findings on physical examination were within normal limits. A hepatic angiogram showed multiple distended sinusoids throughout the liver with pooling of contrast material within these lesions. Transjugular liver biopsy specimens were histologically interpreted as showing chronic inflammatory reaction and fibrosis, attributable to the patient's previously known liver involvement with malaria. He was removed from the vinyl chloride processing area and continued active employment without symptoms.

His liver profile continued to deteriorate; by September 1975, he began to complain of tiredness and easy fatigability. The findings on physical examination remained within normal limits. Liver scan now demonstrated hepatomegaly with multiple defects throughout the liver. Biopsy specimens demonstrated sinusoidal dilatation and Kupffer cell proliferation in some of the specimens, but the diagnosis of angiosarcoma could not be made.

The patient did not return to work. His fatigue increased over the next two months and vague abdominal pain developed. In January 1976, gross hepatosplenomegaly with a bruit over the liver and signs of high output cardiac decompensation were detected. Liver-spleen scan and angiogram showed further enlargement of the previously identified lesions. An increase in arterial-venous shunting was demonstrated. At abdominal operation, direct examination revealed that the veins of the portal system were somewhat distended and that the surface of the liver was slightly nodular with some areas of bluish discoloration which were somewhat serpentine in nature. There were multiple areas on the liver in which the tissue was slightly elevated with a central depressed area of a somewhat cyanotic appearance. A strong thrill could be felt when palpating the liver. The hepatic arteries were ligated, and there was complete disappearance of the thrill.

Wedge biopsy of the liver demonstrated areas of angiosarcoma among areas of fibrosis and sinusoidal dilatation with hypertrophy and hyperplasia of sinusoidal lining cells.

Postoperatively, the cardiac decompensation was corrected and the spleen size was reduced to normal, but the liver size remained the same. Chemotherapy with doxorubicin, cyclophosphamide and methotrexate was started, and the liver was reduced to its normal size but the biochemical liver test results remained abnormal. The reduction in liver size lasted four months during which the patient was unable to carry on normal activity without fatigue. Over the next four months there was a gradual increase in the size of the liver with the development of ascites and abdominal pain with deteriorating biochemical liver test results. The patient died in December 1976 with hepatic encephalopathy and gastrointestinal bleeding. Autopsy revealed an enlarged liver with multiple small nodules of angiosarcoma throughout. The tumor was metastatic to the spleen and lungs.

BACKGROUND AND METHOD OF STUDY

Vinyl chloride is the raw material with which the common plastic polyvinyl chloride is made. Vinyl chloride monomer (CH_2CHCl) is a gas made by the reaction of ethylene and chlorine, which, when processed commercially, is polymerized in large vats under increased temperature and pressure to form a white solid. Workers are exposed to gaseous vinyl chloride by leakage from the manufacturing vats during processing, upon opening the vats after the processing is complete, or, most intensely, in the cleaning process when the worker enters the vat and chips residue off the interior wall releasing pockets of gas. Vinyl chloride was initially thought to be completely harmless, and there was no limit to the exposure workers received in the 1940s and early 1950s, however, a voluntary restriction of 50 parts per million was eventually adopted. The gas was considered to be so harmless that it was even used as a propellant in aerosol sprays [22] and a general anesthetic [23]. It gradually became apparent that prolonged exposure could induce injury in many tissues including skin, blood vessels and the reticuloendothelial system [24-26]. Evidence of liver injury was soon found [27,28], which was associated histologically with changes in the hepatocytes, fibrosis and sinusoidal dilatation [29,30]. In animal experiments, prolonged inhalation of vinyl chloride was shown to induce cancer in rats [31].

Soon thereafter an employee of the Louisville vinyl chloride polymerization plant died of hepatic angiosarcoma, but the significance of this was not appreciated until nearly two years later when a second employee also died of this rare tumor. Since these workers were cared for by different physicians at different hospitals, this might still have gone unnoticed were it not for the plant physician, whose system of requesting reports of illnesses and deaths of employees brought the diagnoses to his attention. His memory of the earlier patient with the disease led him to initiate a search for other cases among former employees and to institute a medical surveillance program of all present employees which was later supported by the National Cancer Institute and the University of Louisville. This program involved testing nearly 1,200 employees with a standard automated biochemical profile and radionuclide liver-spleen scan. Workers with persistent biochemical liver abnormalities or with abnormalities of their radionuclide scan were then examined by selective hepatic arteriography.

hepatic venography and transjugular hepatic biopsy [20,32,33].

Patients with hepatic angiosarcoma seen by the staff of the Divisions of Hematology-Oncology and Digestive Diseases and Nutrition, University of Louisville, from January 1, 1974, to January 3, 1977, include those accrued through the Vinyl Chloride Project as well as patients referred by physicians in the Louisville area and elsewhere, and a few patients found in a search of autopsy records and death certificates. A total of 11 patients have been studied, 10 of whom have had heavy industrial exposure to vinyl chloride. The other patient was a woman, referred from Florida, who had a history of prolonged use of hair spray containing vinyl chloride as a propellant. Only the 10 patients with occupational exposure to vinyl chloride are included in this report. In addition, data are derived from employee records of the Louisville vinyl chloride polymerization plant during its 35 year operation to the date of this report.

RESULTS

Epidemiology. The cohort for this analysis are the employees of the Louisville plant from its opening in 1942 to the end of 1976. The employee population has been extremely stable and during this 35 year period a total of 1,855 persons have been employed. Of these, 1,184 are current employees and 671 are past employees.

During the 35 year period, 167 members of the cohort died. Sixty-six of these died of heart disease and stroke, 39 of cancer, 19 of accidents and, nine of other disease; in 34 the cause of death could not be determined. Primary brain cancer has been related to vinyl chloride exposure [34,35], and five of the 39 deaths from cancer were from this cause. The mortality rate in our population adjusted for age is approximately twice that expected [36].

Ten cases of hepatic angiosarcoma have been identified among our 1,855 employees during the 35 year period. All of these employees worked in areas of the plant with high vinyl chloride exposure. We divided these cases into a retrospective group—cases discovered by review of hospital and pathology records, and a surveillance group—cases discovered prospectively in the surveillance program. In the retrospective group, one case each was diagnosed in 1964, 1967, 1970, 1972, 1973 and 1974. In the surveillance group, three cases were identified in 1974 and one in 1976. No other cases of angiosarcoma have been identified in the Louisville area. The incidence over the 35 year period in our cohort is 0.54 percent, equivalent to 15.4 cases per 100,000 population per year. (Predicted annual incidence in the U.S. in 0.0014 cases per 100,000 population [37].) The mean age at the time of diagnosis was 47.6 years, with a range of 36 to 58 years. All patients were white men, reflecting employment patterns at the plant. The mean duration of exposure to vinyl chloride was 17.4 years, with a range of 12 to 28 years. The mean duration from first exposure to vinyl chloride to the time of diagnosis of hepatic angiosarcoma was 19.9 years, with a range from 12 to 28 years. No differences were noted between the retrospective group and the surveillance group. Three patients had known heavy exposure to alcohol, and none of the patients had previously had viral hepatitis or exposure to any other known hepatotoxins.

TABLE I Early Clinical Features Related to Hepatic Angiosarcoma in 10 Patients

Symptoms	Patients (no.)	Signs	Patients (no.)
Pain	5	Hepatomegaly	7
Fatigue	5	Splenomegaly	5
Weakness	4	Abdominal tenderness	3
Weight Loss	3	Hepatic bruit	1
Anorexia	3		
Indigestion	1		
Abdominal fullness	1		
Melena	1		

Clinical Features. The earliest clinical features which could be related to the angiosarcoma are presented in Table I. Pain was localized in the right upper abdominal quadrant or in the lower right hemithorax. In two of the patients the pain was acute, but in the others it ranged from two to six months in duration. Fatigue and weakness had been present from one month to one year. Weight loss was significant, averaging 25 pounds over four and a half months. Hepatomegaly was the most common physical sign. The liver measured from 15 to 24 cm in total vertical span at the right mid-clavicular line by percussion. When splenomegaly was present the spleen was easily palpable 3 to 7 cm below the costal margin. Two patients, both in the surveillance group, had no physical signs, and one of these patients was asymptomatic.

Laboratory Findings. Hematologic evaluation at diagnosis showed three patients with normal complete blood counts. Mild anemia was found in five patients, but reticulocyte counts were not determined. Two of the patients had abnormal erythrocyte morphology with target cells and schistocytes. In one patient the anemia was combined with leukocytosis and in three patients with thrombocytopenia. Each patient with thrombocytopenia had splenomegaly. Two other patients had leukocytosis as their only hematologic abnormality. In two patients the bone marrow was examined: one was normal; the other demonstrated erythroid hyperplasia.

No patient was without some abnormality in at least one of the standard biochemical liver tests. Most of the values were only minimally abnormal initially. There was no consistent abnormality of any single test result or apparent pattern of groups of tests, although the alkaline phosphatase level was most frequently elevated. Hepatitis B surface antigen and alpha fetoprotein were not found in four patients who were tested, and carcinoembryonic antigen was not present in two patients.

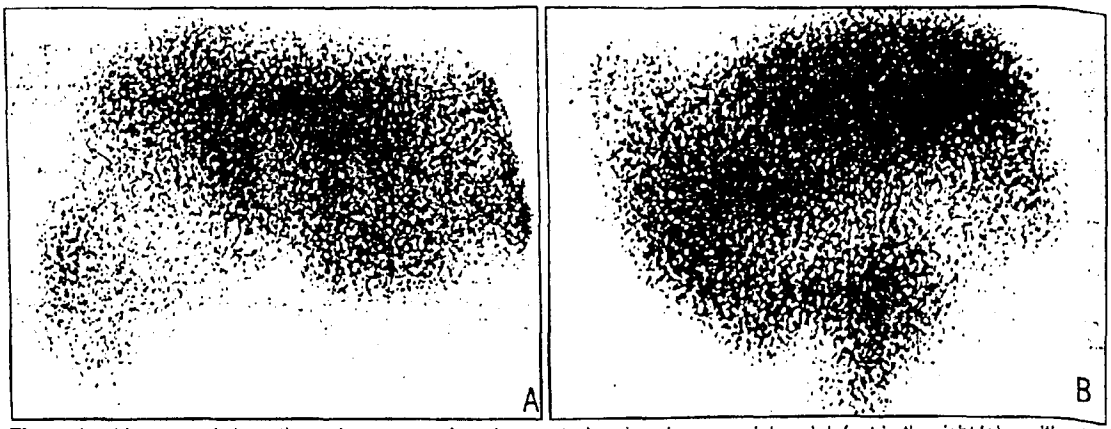

Figure 1. Liver scan in hepatic angiosarcoma. A, anteroposterior view. Large peripheral defect in the right lobe with other smaller defects. B, right lateral view.

Results of routine roentgenologic studies and gastrointestinal contrast studies were normal in most instances. However, special roentgenologic studies revealed many abnormalities. Radionuclide liver scan disclosed abnormalities in all patients; however, the abnormalities were not consistent. Six patients had hepatomegaly. In eight patients a mass was demonstrated which usally appeared as a single negative defect greater than 4 cm in diameter in the periphery of the liver. In four patients this was accompanied by one or more additional defects, and two patients had only evidence of diffuse hepatocellular disease. Typical abnormalities on liver scan are demonstrated in Figure 1. Splenomegaly was demonstrated in five patients by radionuclide scan. Hepatic arteriograms were obtained in six patients and demonstrated enlarged sinusoidal spaces in each of them, with a mass demonstrated in five patients. The masses were supplied by normal-sized hepatic arteries and were characterized by persistent peripheral tumor stains with some degree of central hypovascularity as shown in Figure 2. Hepatic ultrasound was performed in only one patient and demonstrated a solid mass.

Clinical Diagnosis. Methods: Although the signs and symptoms of our patients were fairly nonspecific, there was some finding in almost every patient that would direct one's attention to the liver in an effort to explain the clinical features. Biochemical tests of blood usually revealed nonspecific hepatic injury, but no pattern of abnormality could distinguish angiosarcoma.

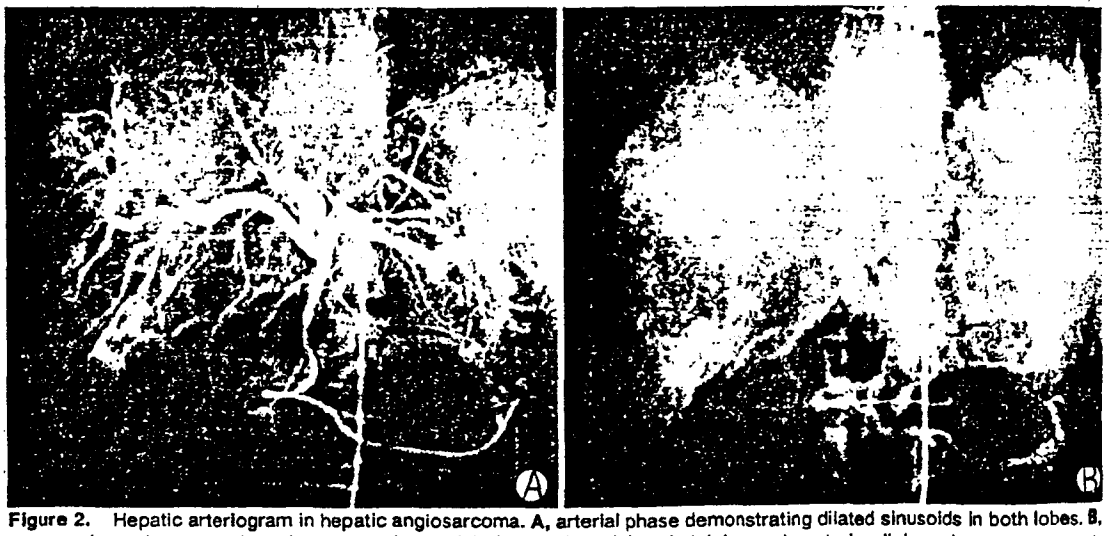

Figure 2. Hepatic arteriogram in hepatic angiosarcoma. A, arterial phase demonstrating dilated sinusoids in both lobes. B, venous phase demonstrating a large mass in the right lobe with peripheral staining and central radiolucent area.

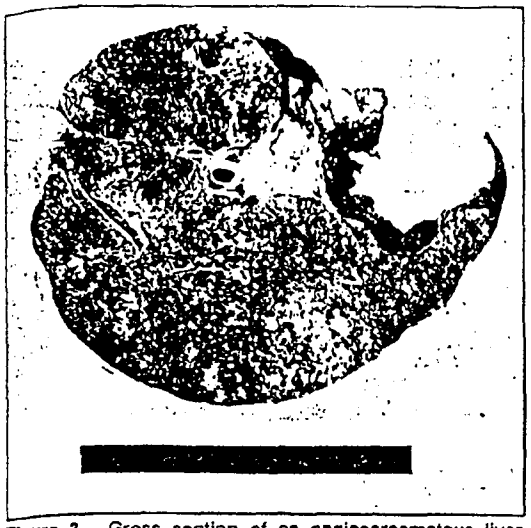

Figure 3. Gross section of an angiosarcomatous liver displaying multifocal solid and hemorrhagic cystic growth pattern with a large ruptured cyst.

The radionuclide liver scan suggested involvement of the liver by a malignant process because of the negative defect, however, the peripheral location could be mistaken for a rib impression, porta hepatis or other normal anatomic variation. The presence of multiple defects could give the impression of a malignancy metastatic to the liver. Although the liver scan frequently indicated malignancy in the liver, it was not specifically diagnostic of angiosarcoma.

The abnormal vascular pattern demonstrated by the hepatic arteriogram with the persistent peripheral tumor stain and central radiolucent area is strongly suggestive of hepatic angiosarcoma. Under unusual circumstances this appearance could mimic benign vascular lesions, liver cell adenomas or hepatic infarction. However, it is the most accurate clinical means of diagnosing hepatic angiosarcoma and should be obtained in all patients in whom the diagnosis is suspected. Further information regarding special roentgenologic studies has been detailed in the literature [12].

Extent of disease: At diagnosis the extent of disease was determined using clinical parameters and information obtained at exploratory laparotomy. In four of the patients disease was confined to one lobe of the liver and in six patients the disease involved more than one lobe of the liver. Direct extension involving the diaphragm was present in two patients and the stomach in one patient. Porta hepatis lymph nodes were involved in one patient. One patient had distant spread to the lung.

Pathology. Gross description: The liver is usually enlarged with blunt edges and a variegated brown-red-orange color. The surface may contain one or more purple-blue nodules with spongy consistency varying in size from 1 to 4 cm. In two cases these ruptured and caused hemoperitoneum. The cut surface is diffusely involved with cystic areas filled with dark blood with occasional cysts measuring up to 20 cm in diameter (Figure 3). In one case the tumor had a primarily solid appearance.

Definitive diagnosis: Tissue for histologic examination was obtained by percutaneous biopsy, transjugular biopsy, open liver biopsy and autopsy. Although six tissue specimens obtained by percutaneous and transjugular biopsy did identify other liver abnormalities, specimens were insufficient for accurate diagnosis of malignancy. All definitive diagnoses of angiosarcoma in this group of patients were made by tissue obtained at open liver biopsy (seven patients) and at atuopsy (three patients). In our experience, in a patient with biochemical evidence of hepatic injury with a history of prolonged exposure to vinyl chloride, open liver biopsy under direct vision is needed for diagnosis. We have no experience with laparoscopy and directed biopsy, which may be associated with less morbidity, but this approach should be tempered with caution in such a vascular tumor with major potential for hemorrhage.

Histology: All tumors were primary angiosarcoma of the liver with a wide range of patterns and differentiations seen between different tumors and within the same tumor (Figures 4 and 5). Areas of extensive hemorrhagic necrosis and areas of enlarged spindle-shaped endothelial cells lining irregular vascular channels appearing as a spongy network of sinusoids were present. Tumor cells occasionally projected into these channels. Large cavities lined by sarcomatous cells contained fresh or altered blood and, at times, organized clot. Three patients had histologic evidence of cirrhosis, two of whom had histories of heavy alcohol intake. One additional patient had fibrosis, and two patients had evidence of toxic hepatitis. The variable histology of angiosarcoma and the difficulties of diagnosis have been described previously [3,11,38].

Metastatic spread: Nine of the 10 patients have died, and eight of them have had postmortem examination. The single patient without a postmortem had an extensive clinical evaluation and exploratory laparotomy two weeks prior to his death. His data have been combined with the autopsy data regarding extent of disease at death. One patient with extensive hepatic fibrosis considered to be secondary to radiotherapy had microscopic angiosarcoma in the minimal remaining liver tissue with no other evidence of disease. Eight patients had extensive disease throughout the liver. Five of these eight patients had regional dissemination with involvement of diaphragm (three of five), lymph nodes (two of five), abdominal wall (two of five), gallbladder (one of five) and bowel (one of five). Four of these eight patients had distant spread with involvement of lung (four of four), bowel (two of four), adrenals (two of four), lymph nodes (one of four), brain (one of four), bone (one

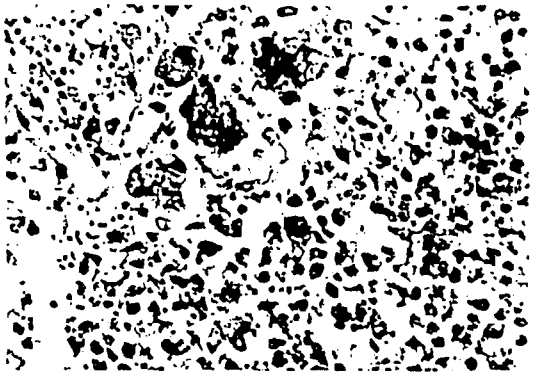

Figure 4. Undifferentiated angiosarcoma with tumor giant cells replacing the usual histologic pattern of liver tissue. Hematoxylin and eosin stain; magnification X 500, reduced 50 percent.

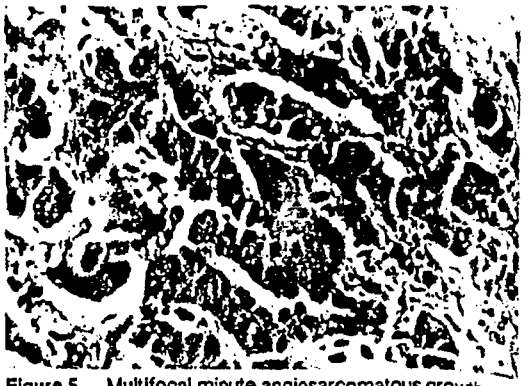

Figure 5. Multifocal minute angiosarcomatous growths are conspicuous among liver cell cords in locations distant from grossly visable tumors. Hematoxylin and eosin stain; magnification X 500, reduced 50 percent.

of four), spleen (one of four), pleura (one of four), pericardium (one of four), myocardium (one of four) and kidney (one of four). One patient had distant metastases without evidence of regional spread, and two patients had no evidence of regional or distant spread.

Therapy. In no patient was a surgical attempt at resection made. In those patients with disease confined to one lobe of the liver, fibrosis or cirrhosis in the remainder of the liver was thought to contraindicate surgery. One patient underwent hepatic artery ligation with no benefit. Radiotherapy to the liver was given to one patient early in his course with minimal tumor found at autopsy one year later, but extensive hepatic fibrosis led to his death. However, another patient, following tumor recurrence during chemotherapy, received radiotherapy seven months after diagnosis. He was found to have extensive angiosarcoma in the liver at autopsy two months later, with no apparent response to the radiotherapy. Both patients received 5,000 rads in five weeks to a major volume of the liver. Seven patients who received chemotherapy had some evidence of response by reduction in liver size or tumor size, and one patient continues to respond to chemotherapy 56 months from the time of diagnosis. Various drugs have been used individually and in combination including fluorouracil, vincristine, cyclophosphamide, doxorubicin and methotrexate.

Clinical Course. Major clinical events during the course of each patient's disease are listed in Table II. Congestive heart failure was preceded by a period of high output cardiac volume overload in patients with demonstrated arterial-venous shunts. Microangiopathic hemolytic anemia and platelet destruction in the peripheral blood were present without evidence of disseminated intravascular coagulopathy or sepsis, and were possibly due to cell damage in the abnormal tumor vasculature [39–42]. Symptoms of overt hepatic failure were unusual until very late in the clinical course.

The factors contributing to death in these patients are listed in Table III. The major cause of death was the sudden onset of irreversible rapidly progressive hepatic failure.

Survival. One patient remains alive 56 months from the time of diagnosis. Survival of the other nine patients from the time of diagnosis ranged from four months to

TABLE II Major Clinical Events Occurring Prior to Terminal Events During the Course of Angiosarcoma Seen in 10 Patients

Event	Patients (no.)
Pneumonia	1
Pleural effusion	1
Staphylococcal septicemia	1
Peripheral platelet destruction	2
Microangiopathic hemolytic anemia	1
Congestive heart failure	3
Renal failure	1
Hypertension	1
Gastrointestinal bleeding	1
Hepatic failure	2
Hemoperitoneum	2
Fever	1

TABLE III Contributing Causes of Death in Nine Patients with Angiosarcoma

Cause	Patients (no.)
Hepatic failure	9
Gastrointestinal bleeding	2
Ruptured tumor with hemoperitoneum	2
Cardiac tamponade	1
Hemothorax	1

29 months, with a mean survival of 11.7 months. When measured from the time of first symptoms, the range of survival was five months to 34 months, with a mean duration of 14.9 months. Survival from the time of diagnosis was distinctly longer in the six patients who received chemotherapy (mean 15 months) than in the three patients who did not (mean five months).

Preventive Measures. Shortly after the discovery of an epidemic of angiosarcoma in the Louisville vinyl chloride polymerization plant, new limitations of employee exposure to vinyl chloride were imposed at 1 ppm. Adherence by industry involved tighter seals on the polymerization vats and conduits which lessen leakage into the ambient atmosphere. The vat cleaning process is now performed by workers wearing protective respirators. A record keeping system has been initiated for all workers which relates length of employment and potential chemical exposure levels in each work environment to possible harm from vinyl chloride and other chemicals, so that a worker can be removed from exposure before he is likely to sustain injury. Since this process involved a change in behavior for all employees, and some jobs were more cumbersome and lengthy, an education program was designed and implemented in an effort to relieve anxiety related to work in a potentially carcinogenic environment and to promote understanding of good health maintenance. The program resulted in better cooperation with the surveillance and prevention programs. No new cases of angiosarcoma have been discovered in the Louisville plant since 1976 among 1,184 current employees and 504 past employees followed at regular intervals for four years.

COMMENTS

The Louisville hepatic angiosarcoma experience unfolds in many ways as a medical detective story. First, a fatal incident occurred. A diligent search for clues revealed vinyl chloride as a potential suspect. Further investigation and intimate cooperation among all those concerned quickly gathered sufficient evidence to incriminate vinyl chloride as the etiologic factor for hepatic angiosarcoma. The evidence included the occurrence of angiosarcoma in Louisville only in workers exposed to high concentrations of vinyl chloride over a long period of time, the discovery of similar patients in other plants producing vinyl chloride [8,9], and the production of similar hepatic lesions in experimental animals exposed to high concentrations of vinyl chloride [43,44]. The detective was an observant clinician who recognized the initial incident, realized its potential importance, instituted a systematic investigation and initiated a cancer control effort. This entire experience demonstrates the importance of the clinician in controlling occupationally-induced cancer.

The early fears of progressively increasing numbers of angiosarcoma cases related to vinyl chloride exposure [7,19-21] have not come to pass. The absence of new cases, however, should not be looked on as the end of the story. The Louisville plant is one of the oldest vinyl chloride polymerization facilities in the nation, and these workers have had the longest potential exposure to the carcinogen. In addition, because of the early lack of concern regarding exposure levels, they also have had the highest exposure. We have seen that the incubation period may range from 12 to 28 years, and it is difficult to imagine that the recently instituted preventive measures have resulted in the absence of new cases. More likely, this has resulted from the voluntary reduction in exposure levels or some other alteration in the work environment in the past. Moreover, we know that the level to which vinyl chloride exposure was voluntarily reduced is still carcinogenic [45]. We have probably seen a dose-response effect with a large number of cases occurring after exposure to a large dose. A smaller number of cases resulting from lower exposure levels will probably continue to develop in the ensuing years because of the long incubation period. This problem may become more pressing in other parts of the country in which workers in vinyl chloride polymerization plants, opened in the 1950s and early 1960s, are now nearing the mean incubation period for this disease. Mandatory restriction of atmospheric vinyl chloride levels to 1 ppm has only been in effect for five years, and it is not known whether lowering exposure levels reverses the possibility of cancer in those already exposed to higher levels. This must continue to be investigated. In the group of workers with high exposure to vinyl chloride, close surveillance should be continued indefinitely. Although the Louisville experience has revealed the close association between vinyl chloride exposure and hepatic angiosarcoma, the mechanism by which vinyl chloride triggers carcinogenesis remains to be studied through animal model experiments [46]. It is intriguing that angiosarcoma is so frequently associated with environmental factors, such as thorium dioxide [47-49], arsenic [50-52] and vinyl chloride, each of which results in stimulation of the hepatic sinusoidal lining cells [38]. Other associated factors include hemochromatosis [53-55], copper [56] and estrogens [57]. However, some cases discovered during early childhood are apparently congenital [58]. When faced with a patient with angiosarcoma without one of these recognized factors, a diligent search for other possible environmental factors should be pursued so that preventive measures can be undertaken.

Many new chemicals are introduced into our work and home environments each year. There is increasing concern that environmental factors are responsible for many cancers in man. Although sophisticated laboratory techniques and specialized knowledge are essential in cancer research, the Louisville experience in vinyl chloride-associated hepatic angiosarcoma demonstrates how a careful clinical observation followed by a well conceived clinical approach may reveal an environ-

mental etiology of neoplasia and result in profound benefit for all of us.

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Bacterial Tests for Potential Carcinogens

New short-term tests can identify environmental agents that cause damage to DNA, the primary event in chemical carcinogenesis. The tests are also valuable for clarifying the mechanism of DNA damage

by Raymond Devoret

The fact that physical and chemical agents in the environment enhance the incidence of cancer has become of great concern. It is clear that an adequate limitation of human exposure to carcinogens would save lives. To identify potential carcinogens in the environment is therefore an urgent task. In the case of chemical carcinogens that is no easy matter. It is estimated that more than 50,000 different man-made chemicals are currently in commercial and industrial use; between 500 and 1,000 new chemicals are put on the market every year. The standard animal tests for potential carcinogenicity take a long time and cost a great deal of money.

Fortunately there is an alternative to the classical animal tests. One can take advantage of the profound unity of living matter and resort to bacteria as the test organisms. A bacterial assay for carcinogenicity takes a few hours or days rather than the two or three years required for an animal test, and it costs far less. An effective bacterial test has been developed by Bruce N. Ames of the University of California at Berkeley, based on the ability of a chemical to cause mutations in bacteria. More recently my colleagues and I at the Centre National de la Recherche Scientifique in Gif-sur-Yvette have devised a group of tests based on a chemical's ability to induce the development of a dormant virus in bacteria. The Ames test and our tests not only provide means of identifying dangerous chemicals but also are powerful new tools for learning to understand the primary events of the carcinogenic process initiated by chemicals.

Cancer is a disease of highly evolved multicellular organisms such as human beings, whereas bacteria are minute single cells at the opposite end of the evolutionary scale. It may therefore seem paradoxical that bacteria can serve to identify substances that cause cancer. Actually, however, there is no paradox.

One tends to think of a cancer as a tumor that can spread through the body to form multiple tumor colonies (metastases). That is a clinical, macroscopic view of cancer at a multicellular stage, since just one gram of malignant tumor already contains a million cancer cells. Cancer begins at the level of the single cell. A cell in an adult tissue evolves in such a way that it departs from conformity with the strict physiological rules governing the set of identical cells that constitute a tissue; it becomes a unique and distinguishable defect in an otherwise monotonous structure. The cell begins to divide and a tumor grows. Some of the daughter cells may break the tissue barrier, invading adjacent tissues and usually metastasizing to distant sites. Cancer cells have a great selective advantage, since they escape the programmed fate of most normal cells: to age and die. For cancer cells the entire body is a culture medium in which they thrive, ultimately to die with the body they kill.

Physical and chemical agents in the environment cause cancer by damaging DNA, the cell's hereditary material. DNA damage initiates a complex cellular process that in mammalian cells can eventually lead to transformation into a cancerous state. Agents that damage DNA are therefore potential carcinogens. DNA is the hereditary material of all living cells, and both DNA lesions and the cellular processes that repair them are remarkably similar in bacteria and in human cells; what is detrimental to bacterial DNA is likely to harm human DNA. That is the theoretical justification for substituting bacteria for mammalian cells in tests to detect damage to DNA.

The theoretical justification is supported by experimental and practical results: bacterial tests distinguish with more than 90 percent reliability between known carcinogens and known noncarcinogens, and they have identified as potential carcinogens new chemicals that have subsequently been shown to be carcinogenic in animal tests. Of course, the manifestations of DNA damage are very different in bacteria from the transformation to the cancerous state that is observed in mammalian cells. In compensation the bacterial tests are so much faster and less expensive as to finally make comprehensive screening for potential carcinogens a feasible objective.

The carcinogenic potential of physical agents, and notably of ionizing radiations, is much better understood than that of chemical agents. Broad popular awareness that radiations can cause cancer has come only in the era of nuclear weapons and reactors, but the danger actually became apparent soon after the discovery of X rays by Wilhelm Konrad Röntgen in 1895. Only four years later it was reported that a technician who checked newly manufactured X-ray tubes by fluoroscoping his own hand was afflicted with a skin cancer; he eventually died of it. The warning was ignored, and most of the first generation of radiation therapists died of cancer. The carcinogenic effect of the ultravio-

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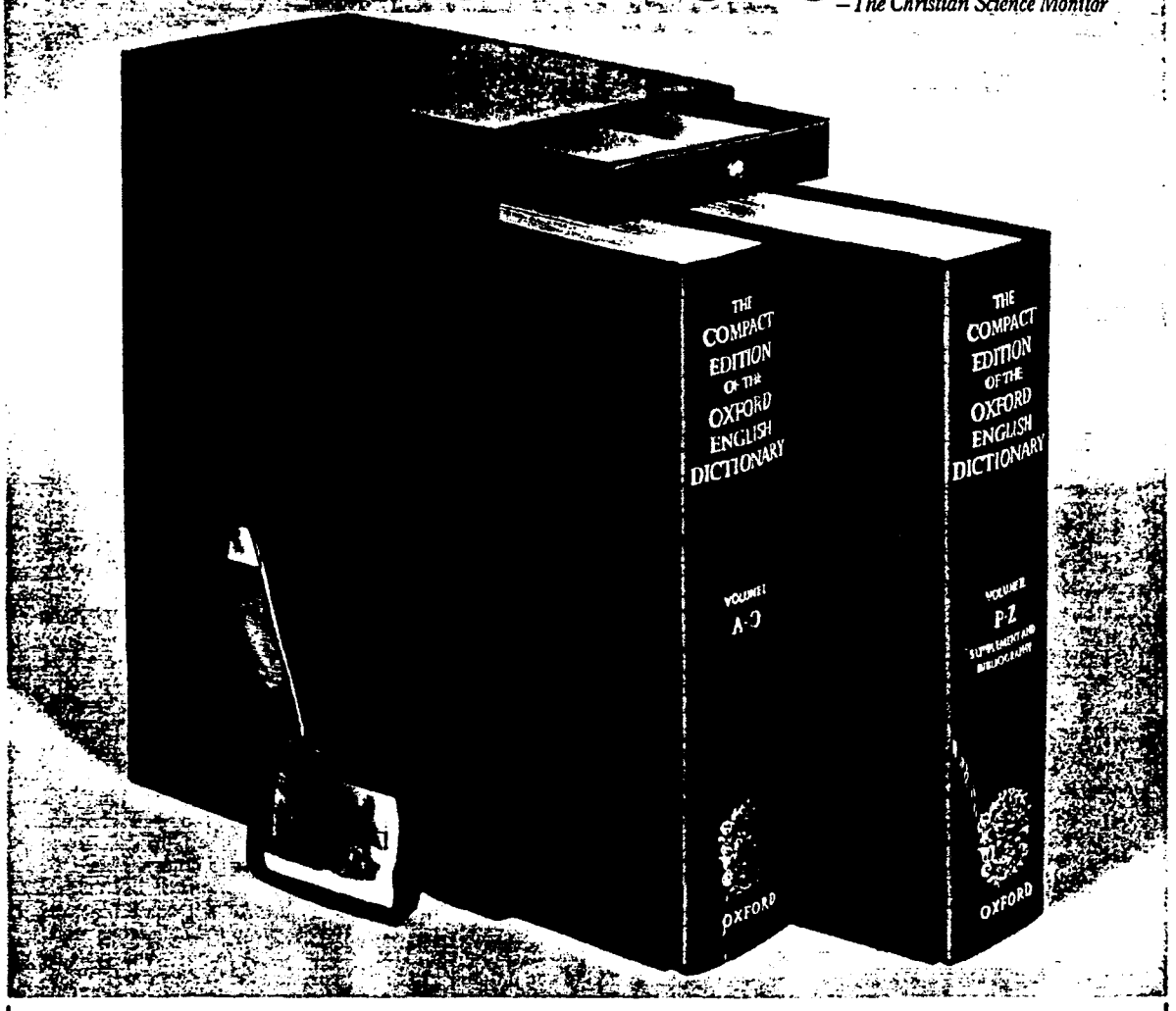

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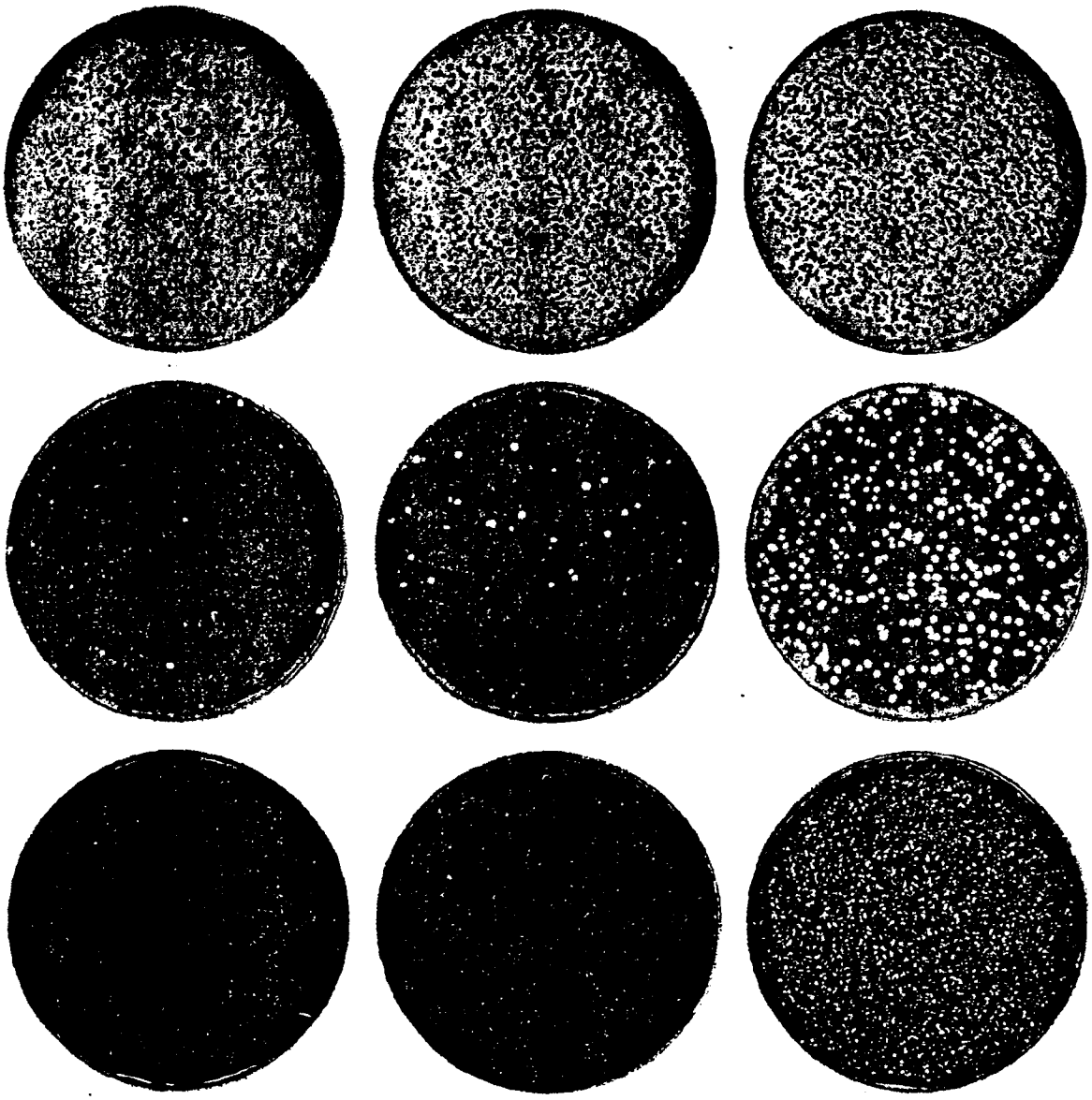
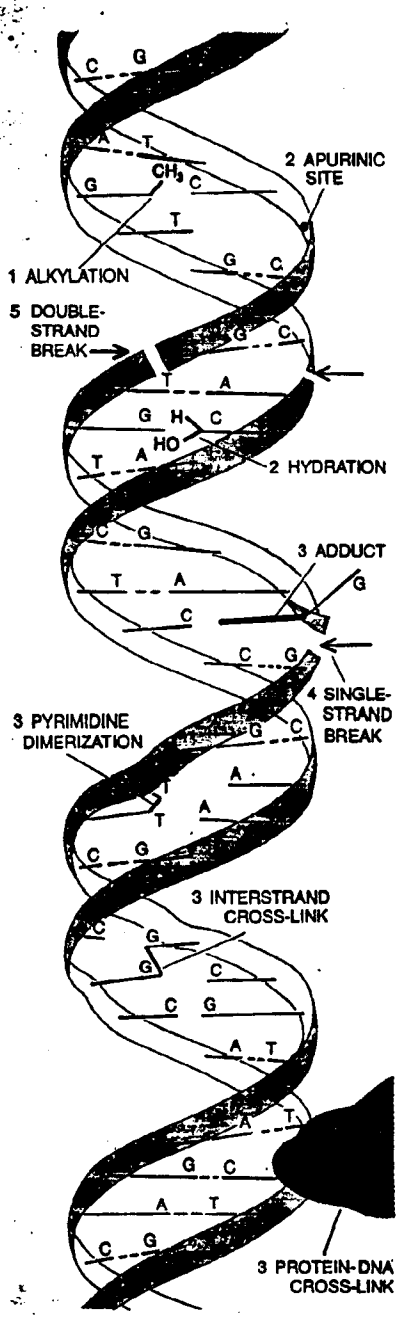
let radiation in sunlight has also been known for some time. As long ago as 1905 a French physician named Dubreuilh observed that skin cancers of the back of the neck were particularly prevalent among workers who were exposed to the sun as they tended the vineyards and harvested grapes in the Bordeaux region.

Although X rays, gamma rays and other radiations hit all the components of cells in a random manner, it was recognized quite early that the genetic material must be the most radiation-sensitive cellular target. DNA constitutes only a minor fraction of the chemical components of a cell, but direct or indirect damage to it has great impact on the cell's future, whereas damage to proteins and other cellular components has much less effect. That is because DNA is the memory of the cell. DNA replicates to beget DNA, and so errors in DNA are transmitted from cell generation to cell generation.

The double helix of DNA consists of two chains of sugar (deoxyribose) and



THREE BACTERIAL TESTS for potential carcinogens reveal DNA damage: the Ames test (*top*), the inductest (*middle*) and the lambda mutatest (*bottom*). In each case the culture plate at the left is an untreated control; tester bacteria on the center plate were treated with a moderate dose and those at the right with a higher dose. The Ames test shows the extent of reverse mutations in histidine-deficient *Salmonella typhimurium* that enable the revertant bacteria to proliferate. A background of spontaneous mutant colonies (*red stain*) is seen at the left. Many more colonies grow when tester bacteria are exposed to 250 nanograms (*center*) and 750 nanograms (*right*) of the potent mutagen (and carcinogen) nitrosoguanidine. In the inductest (*middle*) DNA damage is revealed by the induction of a prophage, a dormant bacterial virus integrated in the DNA of "lysogenic" *Escherichia coli;* mature phage particles burst out of the tester bacteria and create plaques on a lawn of indicator bacteria of strain *A* (*red stain*). Here the DNA damage was caused by the antitumor drug mitomycin C, 10 nanograms of it on the center plate, 200 nanograms at the right. In the mutatest (*bottom*) a modified form of the prophage makes plaques on a lawn of *E. coli* of strain *B* (on which nonmutated phage cannot form plaques) when it undergoes mutation in its "operator" regions and can no longer remain dormant. Again the treatment was 10 nanograms of mitomycin C (*center*) and 200 nanograms (*right*).



STRUCTURAL ALTERATIONS are imposed by radiations or by chemical agents on the double helix of DNA, two chains of sugar and phosphate groups (*helical bands*) linked by paired bases: either adenine (*A*) and thymine (*T*), or guanine (*G*) and cytosine (*C*). The alterations can be classified in five categories, examples of which are illustrated: negligible helix distortions (*1*), as by alkylation of one of the bases; minor distortions (*2*) caused by hydration or the absence of a base; major distortions (*3*) caused by insertion of an "adduct," linking of two bases to form a dimer, or cross-linking between the two strands or between a strand and a protein; breaks in a single strand (*4*) or in both strands (*5*). Any structural alteration affects DNA's function as a template for replication, but some negligible alterations are not sensed by a cell as damage to DNA.

phosphate groups linked, as by the rungs of a twisted ladder, by paired nitrogenous bases: two purines (adenine and guanine) and two pyrimidines (thymine and cytosine). Adenine always pairs with thymine and guanine with cytosine, but the sequence of the bases along a strand of DNA is variable and carries a particular coded message. Any alteration, even a slight one, in the structure of the double helix affects the functions of the DNA, one of which is to serve as a template for its own replication.

Not every DNA alteration is sensed in the cell as DNA damage. Defined precisely, DNA damage is an alteration that constitutes a stumbling block for the replication machinery and hence hampers the replication of DNA, endangering the survival of the cell. Once incurred, DNA damage calls for repair, which is accomplished by the interplay of at least a score of enzymes whose action is governed by as many genes. Repair is never totally efficient, and so many cells die. Some cells may survive, however, even though the lesions are not totally removed from their DNA, because a repair process has bypassed the lesions. Replication then reconstitutes an undamaged double helix, but one bearing a coded message different from the original one. The scars left on the DNA by such a process are mutations.

The correlation between radiation-induced cancer and radiation-induced DNA damage has long been apparent to radiation biologists, but it is only recently that molecular evidence has been found that DNA damage is a direct cause of cancer. The evidence comes from patients suffering from xeroderma pigmentosum, who are extremely sensitive to sunlight and, while they are still very young, develop skin cancers of which they may eventually die. James E. Cleaver of the University of California School of Medicine in San Francisco and Dirk Bootsma of Erasmus University in Rotterdam have demonstrated that xeroderma patients suffer from a well-characterized genetic defect: their cells cannot carry out a particular DNA-repairing process.

At noontime on a sunny day the flow of ultraviolet radiation that reaches the earth is strong enough to generate pyrimidine dimers in the DNA of exposed cells by linking two laterally adjacent thymines or a thymine and a cytosine. Most such DNA lesions in skin cells are repaired in normal people by an excision process strikingly similar to the "cut and patch" repair process in bacteria exposed to the same radiation. In xeroderma patients, however, the lesions go unrepaired, and their accumulation appears to bring on cell transformation: DNA damage breeds cancer.

Appreciation of the carcinogenic role of chemicals in the environment has come slowly, even though instances of cancer caused by occupational exposure have long been observed. As early as 1775 the British physician Sir Percival Pott correlated the incidence of cancer of the scrotum in men who had once been chimney sweeps with the accumulation of soot in their groin area many years before. Experimental findings on chemical carcinogens date back at least to 1918, when two Japanese investigators, K. Yamagiwa and K. Ichikawa, showed that skin cancers could be induced by repeated applications of coal tar to the ear skin of rabbits. One of the chemicals responsible for causing such cancers is benzo[*a*]pyrene, which is also present in soot, cigarette smoke and charred meat.

Progress toward understanding chemical carcinogenesis has been slow for at least three reasons. First of all, most chemical carcinogens are not biologically active in their original form, so that testing them in that form does not reveal their carcinogenic nature. Only about a decade ago did it become clear, as a result in particular of the investigations of James A. Miller and Elizabeth C. Miller of the University of Wisconsin, that normal metabolic processes, which convert food into substances the body can absorb and eliminate and convert harmful compounds into harmless ones, transform environmental chemicals into metabolites capable of inducing cancer. Those metabolites had to be characterized in order to show just how their parent substances are threats.

A second delaying factor was that the actively carcinogenic metabolites react with various cell components, including RNA and proteins as well as DNA. Since there are a lot of proteins, performing important functions, it was often assumed that damage to proteins might play the major role in the cancerous transformation initiated by chemical carcinogens. The key role of damage to DNA in carcinogenesis by chemicals was recognized only recently, and the results of the bacterial tests have provided strong (although indirect) evidence for such a mechanism.

Finally, the understanding of chemical carcinogenesis has been obscured by the fact that DNA damage, although it is the essential event in the initiation of carcinogenesis, does not usually in itself lead to cancerous transformation; additional factors are apparently required to promote the complex chain of cellular events culminating in the transformation. DNA-damaging agents in themselves are therefore only potential carcinogens.

As I pointed out at the beginning of this article, only a small fraction of the flood of chemicals reaching the market every year can be tested accurately by means of the standard animal assays. In order to obtain results with statistical significance a great many animals must

be (or at least should be) exposed to each tested chemical; for that reason alone a comprehensive screening of new chemicals would be impractical (other than the few, such as food additives, drugs and cosmetics, for which testing is mandated). Even when animal tests are feasible, manufacturers need low-cost, fast tests if they are to identify DNA-damaging substances while new products are still under development and alternative ones can still be sought.

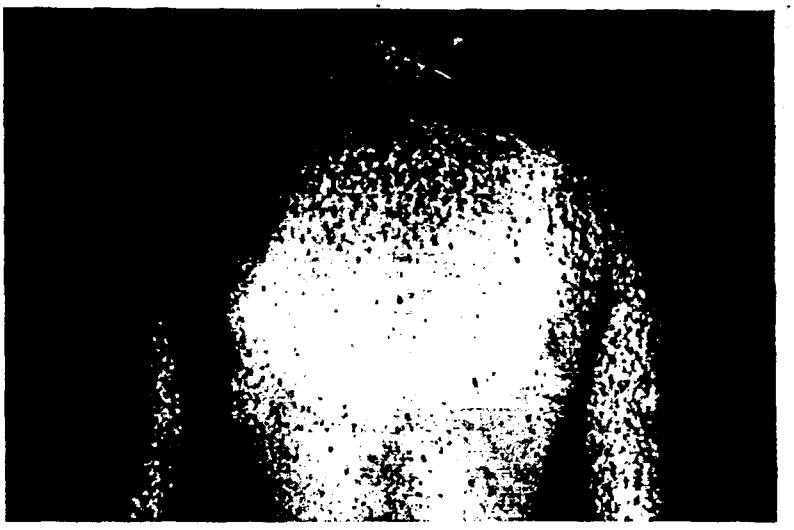
Studies of the epidemiology of human cancers have provided much information on environmental carcinogens, but such studies are of little immediate value in detecting new potential carcinogens because most human cancers appear only some 20 or 30 years after the exposure that gives rise to them. Epidemiological analysis has most frequently been successful in detecting a chemical to which an identifiable subpopulation, such as workers in a particular industry, are exposed and because of which they show a high incidence of a particular type of cancer. For some widely distributed carcinogens there are no clearly identifiable subpopulations for statistical analysis.

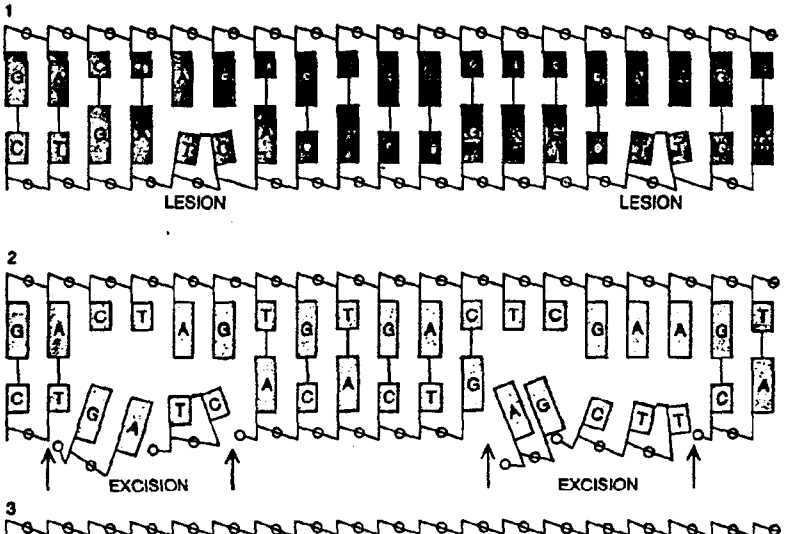
There is no doubt, then, that the present situation calls for simple, fast, inexpensive methods of detecting potential carcinogens, which is to say DNA-damaging agents. One possibility is to measure DNA damage directly in mammalian cells by determining biochemically the incidence of particular forms of DNA damage in cells exposed to a chemical. Such studies are being done by molecular biologists, and their results provide valuable standards of reference. For screening purposes, however, it is cheaper and faster to detect and measure the extent of DNA damage by scoring its manifestations in bacteria.

One of the great advantages of assays done with bacteria is the enormous biological amplification implicit in bacterial manipulations. It is easy to grow as many as a billion (10^9) bacteria per milliliter of culture medium. A mutational event such as a change in a single base pair in the bacterial DNA, which is impossible to detect by standard biochemical methods, will be revealed as a mutant bacterium. That single bacterium can be selected from among 10^9 cells because its daughter cells, and only they, will proliferate and form a colony visible to the unaided eye on an agar nutrient plate. Since a colony consists of about a million (10^6) bacteria, a rare single mutational event with a probability of, say, one in 100 million (a probability of 10^{-8}) would thus be amplified by a factor of 100 trillion (10^{14}).

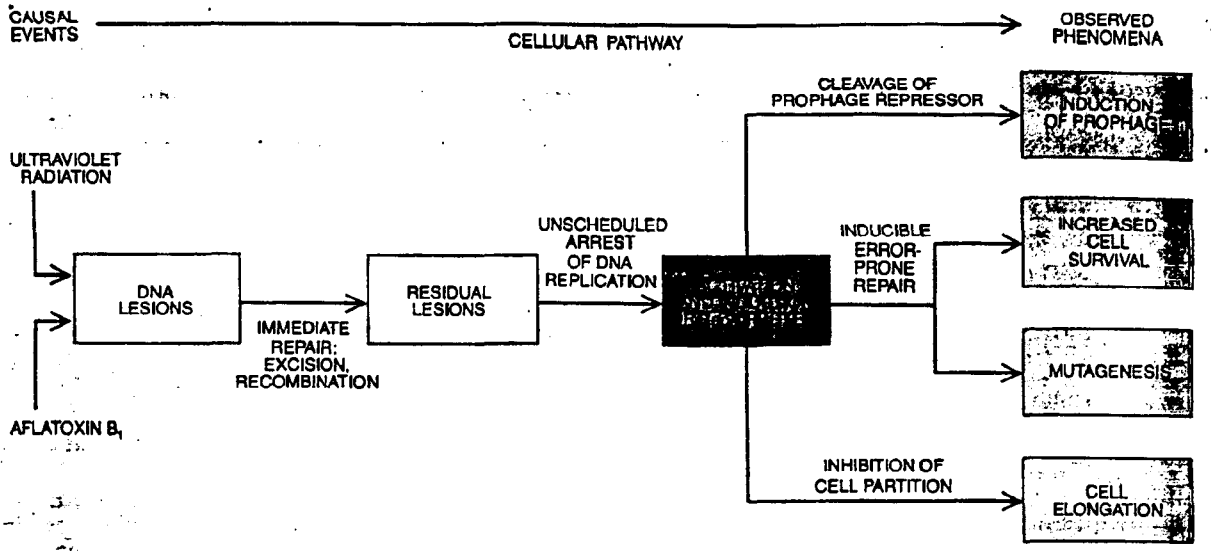
The series of cellular events that leads to mutation, and to several other manifestations of DNA damage in bacteria, has recently been somewhat clarified. The bacterium *Escherichia coli*, which inhabits the colon of a number of mammals including human beings, is genetically programmed to divide and (under optimal conditions for growth) form two daughter bacteria in 30 minutes. When the DNA of a bacterium is damaged, the bacterium may need more than two hours to resume its cycle of division (if it is not killed by the damage). During that time there is a sequence of cellular events.

Immediately after the primary DNA lesions are incurred a first round of repair is effected: an excision repair, in which the damaged segment is cut out of the DNA and replaced by an undamaged DNA sequence. Some residual lesions will remain, however. If they are bulky or clustered, the residual lesions hamper the DNA-replication machinery, and replication stops abruptly. Unless the arrest of replication is transitory

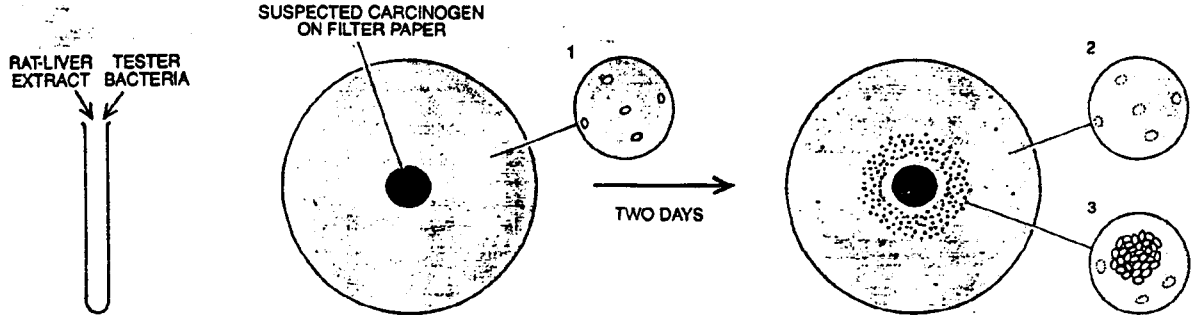




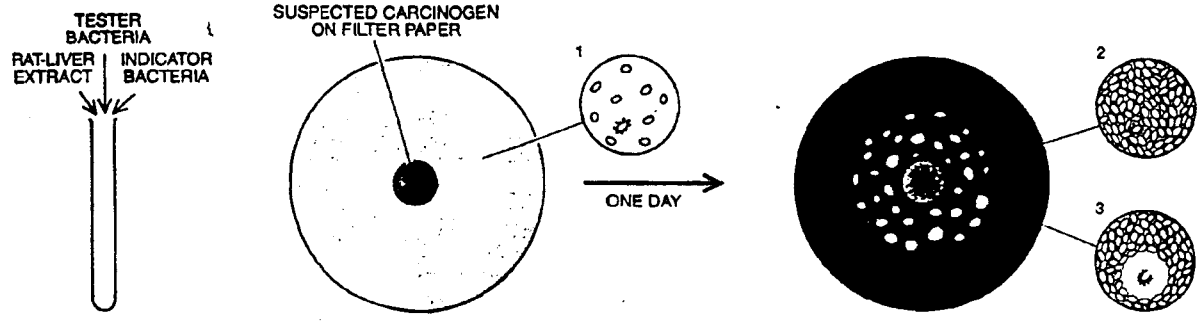
XERODERMA PIGMENTOSUM is a genetic disease whose victims develop skin cancer as a result of normal exposure to sunlight, typically on uncovered parts of the body, as is shown in the photograph at the top. These patients lack a DNA-repair process that in normal individuals removes thymine dimers, the lesions created by the ultraviolet radiation present in sunlight (1). In the normal repair process the bases in the lesion areas are first excised (2). Then the excised stretches of DNA are resynthesized and the new segments are ligated to undamaged stretches (3).



SEQUENCE OF CELLULAR EVENTS follows DNA damage imposed on *E. coli* by ultraviolet radiation or by the carcinogen aflatoxin B_1. Immediate repair processes mend most of the lesions but leave residual damage, causing an unscheduled arrest of DNA replication. This threat to cell survival activates the RecA protein to become a protein-cleaving enzyme and also induces the synthesis of more RecA. Various forms of the protein are involved in three processes that give rise to the four observed phenomena shown at right.



MUTAGENESIS is detected in the Ames test by mixing an extract of rat liver (which supplies mammalian metabolic functions) with tester bacteria (which cannot grow because a mutation makes them unable to manufacture histidine, a necessary nutrient) and plating the mixture on an agar medium so that a thin layer of bacteria covers the medium evenly, as is shown on a microscopic scale (*1*). In this "spot assay" a dose of the chemical to be tested is placed on a disk of filter paper on the tester bacteria. After two days most of the *his*$^-$ bacteria have died for lack of histidine (*2*), but DNA damage caused by the chemical diffusing out from the disk has given rise to mutations, some of which result in reversion of the *his*$^-$ mutation. The histidine-making revertant bacteria proliferate, forming visible colonies (*3*).



INDUCTION of a dormant bacterial virus, prophage lambda, is detected in the inductest. Lysogenic tester bacteria are mixed with rat-liver extract and then with indicator bacteria. The mixture is plated; the medium is covered with a thin layer of indicator bacteria interspersed with a few lysogenic bacteria (*1*). After a day most of the plate is covered by a thick lawn of indicator bacteria (*2*). Where the chemical that is being tested has diffused from the filter-paper disk the DNA damage it causes leads to the induction of mature lambda phage. The phage particles burst out of the lysogenic cells and kill indicator bacteria in the vicinity, making visible plaques on the lawn (*3*).

the cell's survival is at risk. As Miroslav Radman of the University of Brussels and Evelyn M. Witkin of Rutgers University first suggested, the following cellular adaptive mechanisms come into play to cope with the emergency:

1. A second round of repair is induced. This inducible and error-prone repair (nicknamed "SOS repair") tends to restore the structure of the DNA even though there are errors in the coded message; indeed, this adaptive response may be successful partly because it does not "bother" to follow the base-pairing rules of normal DNA replication. At any rate, the price paid for cell survival appears often to be mutagenesis.

2. Cell partition ceases. The elongation of the cell that ordinarily precedes division is protracted, and the cells may form filaments. The elongation may be adaptive in that it facilitates recombination between the two sets of damaged chromosomes in the cell, the intact segments of each combining to yield an intact chromosome.

3. If a prophage, or dormant bacterial virus, is present in the cell, it is induced to develop into a large number of mature progeny phage that burst out of the cell. This adaptive response evolved by the prophage ensures its survival when a host cell appears to be doomed to die: the rats leave the sinking ship.

Each of these adaptive responses is in part promoted by a multifunctional protein called the RecA protein (for "recombination," since defects in the protein impair recombination in general). The RecA protein appears to be required in *E. coli* not only for recombination but also for the responses listed above. The RecA protein is activated and its synthesis dramatically increased when DNA replication is blocked.

Of the various bacterial manifestations of DNA damage, Ames chose mutagenesis as the basis of his pioneering work to develop a test for potential carcinogens. His *Salmonella*–mammalian-liver assay, known generally as the Ames test, is currently the standard test and by far the most widely used. The tester organism is a strain of *Salmonella typhimurium*, another colon bacterium, bearing a mutation (*his*$^-$) that renders it unable to manufacture one of the enzymes required for the synthesis of the amino acid histidine, a necessary component of proteins. As a result of the mutation the bacterium is unable to grow in a mineral nutrient medium unless the medium is supplemented with an external supply of histidine.

On very rare occasions a *his*$^-$ mutation undergoes reversion: a back mutation restores the DNA's normal coding sequence for the needed enzyme and thereby restores the internal supply of histidine. The reversion can be scored because only the revertant bacteria form colonies on a medium that lacks histidine. Obviously the spontaneous rate of reversion, which is ordinarily very low, will be considerably enhanced if the *his*$^-$ bacteria are exposed to a chemical that induces mutations. This is the theoretical basis of the Ames test.

Actually Ames and his colleagues had to introduce three important modifications into the original *his*$^-$ strain to make it a sensitive and versatile tester bacterium. Bacteria such as *E. coli* and *S. typhimurium* have a rather impermeable envelope that reduces or even prevents the penetration of many chemicals into the cell. (This bacterial armor has evolved because the bacteria must usually survive in a hostile environment such as an intestine or a sewer.) Ames and his colleagues overcame the envelope barrier by introducing a mutation that gives rise to defects in the envelope. They went on to make the strain more sensitive to DNA-damaging agents by eliminating its capacity for excision repair, so that most of the primary lesions remain unhealed. And they introduced into the bacterium a plasmid, a foreign genetic element that makes DNA replication more error-prone. By means of these three modifications a strain was constructed in which just a few molecules of a carcinogen are able to create DNA lesions, each of which is likely to engender a mutation; of those mutations, some will be such that the internal supply of histidine is restored.

The real breakthrough, and the one that made the *Salmonella* test truly effective, was Ames's idea of mixing the tester bacteria with an extract of rat liver and thereby subjecting the tested chemical to mammalian metabolic processes. As I pointed out above, it is usually not the original form of a chemical carcinogen that is active but rather one of its metabolites. Because the liver is the body's major metabolic factory the enzymes of a rat-liver extract should convert the chemical being tested into metabolites that react with DNA—if there are any.

In practice the Ames test is usually done by adding the chemical to be examined to *his*$^-$ tester bacteria immersed in a rat-liver extract and plating the mixture on a solid nutrient medium devoid of histidine. (For demonstration purposes the chemical can instead be spotted on a disk of filter paper, which is then placed on a medium on which the bacteria, mixed with the liver preparation, have previously been plated.) After two days of incubation any cells that have undergone the reversion mutation will give rise to revertant colonies. The number of such colonies per mole of the tested chemical provides a quantitative estimate of the mutagenic potency of the chemical.

The simplicity, sensitivity and accuracy of the *Salmonella* test for screening large numbers of environmental sources of potential carcinogens has resulted in its current application in more than 2,000 governmental, industrial and academic laboratories throughout the world; it is estimated that 2,600 chemicals have been subjected to the test. Ames and his collaborator Joyce McCann have themselves validated the procedure by testing more than 300 chemicals that were previously reported, on the basis of animal experiments, to be either carcinogens or noncarcinogens. About 90 percent of the reputed carcinogens turned out to be mutagenic and about the same proportion of the noncarcinogens were negative for mutagenicity. Other mutagenicity tests have since been devised with *E. coli* as the tester bacteria, and their efficiency is about as high as that of the original *Salmonella* test.

Two impressive accomplishments of mutagenicity tests can be mentioned to give an idea of their value. In Japan the chemical furyl furamide, known as AF-2, was added to a broad range of common food products for some years as an antibacterial agent. It had not shown any carcinogenic activity in standard tests on rats in 1962 or on mice in 1971. Then in 1973 T. Sugimura and his colleagues at the National Cancer Centre in Tokyo found that AF-2 was highly mutagenic in bacteria; they could easily demonstrate the mutagenic activity of the additive contained in just one slice of fish sausage! The discovery prompted a new round of more thorough animal tests for carcinogenicity, which showed that AF-2 was indeed a carcinogen. It was withdrawn from the market. If it were not for the bacterial test, AF-2—which had passed two approved animal tests and been declared negative for carcinogenicity—would presumably still be a component of fish sausage and other Japanese food products.

In 1975 Ames and his colleagues reported that 89 percent of the major class of hair dyes sold on the U.S. market contained mutagenic compounds. Since then the cosmetic industry has modified the composition of most hair dyes. It is estimated that several tens of millions of people dye their hair in the U.S. alone, which suggests that the discontinued components had presented a considerable risk.

In spite of all their advantages the mutagenicity tests have some technical and even theoretical limitations. Since mutations are revealed in the Ames assay by a restoration of enzyme activity, any mutation that does not happen to reconstruct the precise DNA sequence that codes for the histidine-making enzyme is not observed. To take one example, the antitumor drug bleomycin, which does its therapeutic work by damaging the DNA of tumor cells (as do about half of all antitumor agents), fails to induce the mutation that is scored in the *Salmonella* test. A false-negative re-

sponse of this kind is a technical problem that can be remedied by substituting other tester bacteria or a complementary short-term test.

False-positive responses are more significant from a theoretical point of view because they may raise some doubts about the validity of mutagenicity tests for identifying potential carcinogens. The point is that some chemical reactions with DNA are highly mutagenic in bacterial and mammalian cells without, as far as one can tell, being carcinogenic. Among these are the incorporation of an analogue of one of the nitrogenous bases and the methylation of certain sites on the bases. Such reactions cause negligible alterations in DNA structure that are not sensed in the cell as DNA damage; DNA replication proceeds on schedule and the new DNA carries a readable—although wrong—coded message. This process is termed direct mutagenesis, and in scoring for mutations it should be clearly distinguished from the more frequent indirect mutagenesis. In the latter process, described above, DNA damage brings about a transient arrest in replication; replication is resumed with the help of the RecA protein on a template that carries lesions, causing mutagenesis of the newly formed strand.

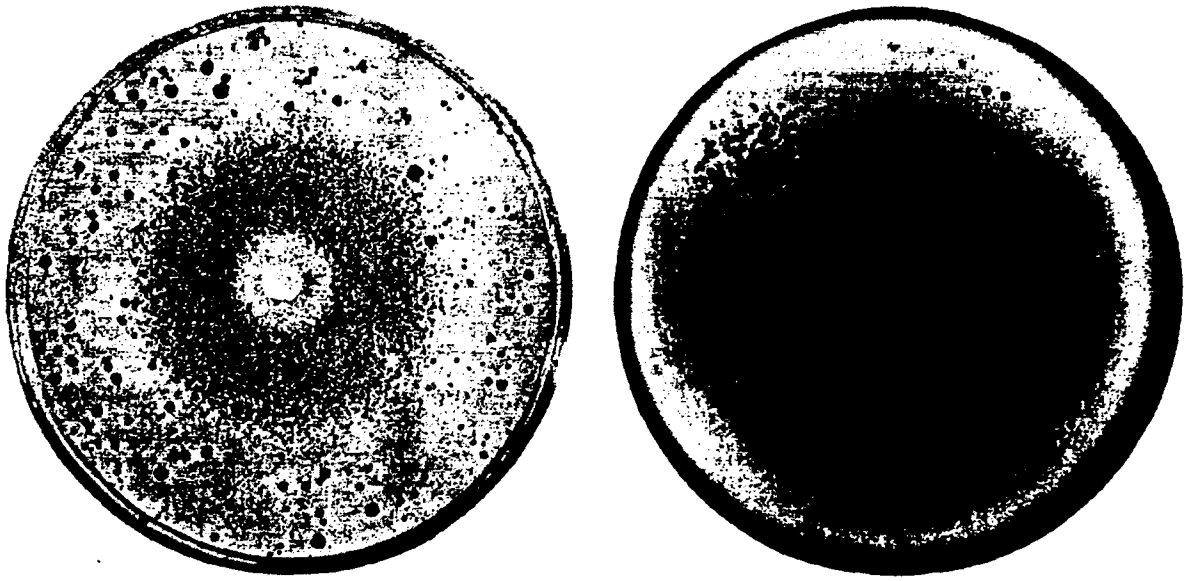
Since it is DNA damage that appears to initiate the cancerous transformation of a mammalian cell, a chemical is a potential carcinogen if it is a DNA-damaging agent, not simply because it causes mutations. A mutagen can have strong genetic effects on a biological population by altering the information encoded in the DNA of individuals' germ cells but nonetheless fail to cause cancer because it does no real DNA damage to the somatic cells: the cells of the rest of the body. Not all genetic toxic substances (chemicals that affect DNA) are potential carcinogens, whereas chemical carcinogens—because they damage DNA—are all indirect mutagens. In other words, mutagenic activity in bacteria is correlated with carcinogenesis in mammals primarily through indirect mutagenesis, which results from DNA damage.

In 1953, before the structure of DNA had even been established, André Lwoff of the Pasteur Institute in Paris anticipated that "inducible lysogenic bacteria might become a good test for carcinogenic and perhaps anticarcinogenic activity." A bacterium is said to be lysogenic when it carries, in a dormant state, the DNA of a "temperate" bacterial virus, which in this dormant state is called a prophage. One such temperate virus is phage lambda, which becomes a prophage when it is integrated into the DNA of certain lysogenic strains of *E. coli*.

When lysogenic *E. coli* bacteria are subjected to any treatment that halts DNA replication, the prophage is induced: its DNA loops out of the bacterial DNA and also directs the synthesis of proteins forming the virus particle, and a progeny of mature phage develops, bursting out of the host cell. The process is called lysogenic induction. Under normal conditions the dormant state of prophage lambda is maintained by a repressor, a protein that lies on the DNA's "operator" regions and blocks the operation of the lambda genes (except the gene that directs the synthesis of repressor to keep the prophage dormant). Jeffrey W. Roberts and Christine W. Roberts of Cornell University discovered that the induction of prophage lambda results from the cleavage of the lambda repressor; together with Nancy Craig they have recently shown that relatively pure repressor can be cleaved when it is mixed with an activated form of the RecA protein.

By triggering the activation of the RecA protein into the form that can cleave a viral repressor, DNA damage results in the induction of a dormant virus. The induction of prophage lambda can therefore serve as a test for DNA damage. Even before the mechanism of prophage development was understood at a molecular level, lysogenic induction was applied in the pharmaceutical industry to identify prospective antibiotics and antitumor drugs. When induction tests were done with ordinary strains of lysogenic *E. coli*, however, they did not give a positive response for such known carcinogens as benzo[*a*]pyrene.

Patrice Moreau, Adriana Bailone and



SPOT ASSAYS visualize the efficacy of bacterial tests qualitatively, although quantitative assays are preferable **(*see illustration on page 41*).** **In the Ames test (*left*) the tested chemical was ethyl methane sulfonate, an alkylating agent and potent mutagen. A dense halo of revertant *S. typhimurium* colonies is seen around the disk from which the mutagen diffused. (Close to the disk there is a zone in which a toxic concentration of the chemical killed all bacteria.) The larger colonies are mutant bacteria that arose spontaneously without exposure to the chemical. In the inductest (*right*) the tested chemical was aflatoxin B_1, a potent carcinogen of the liver. The aflatoxin doses placed on the four test disks were (*clockwise from top right*) 0, 20, 200 and 2,000 nanograms. In the background a few of the lysogenic *E. coli* tester bacteria have spontaneously produced mature phage that have killed the nearby indicator bacteria, producing plaques at random locations.**

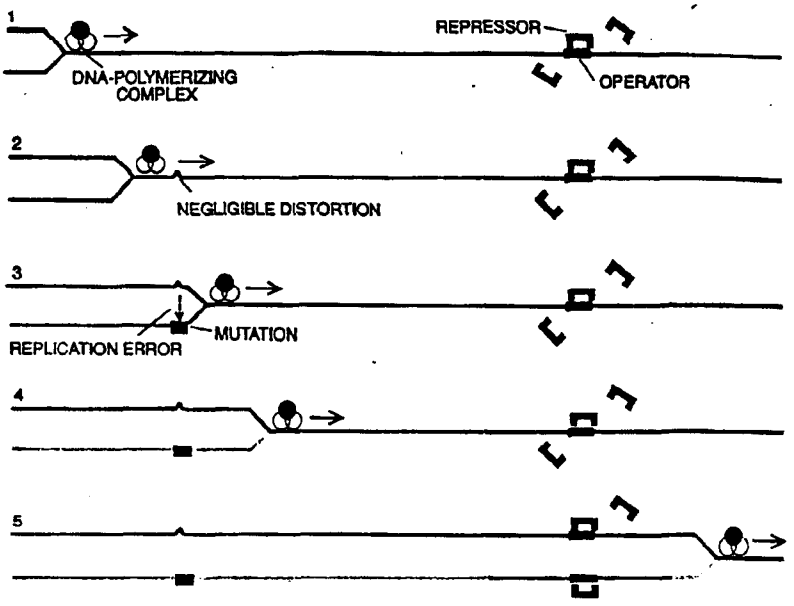
I therefore set out five years ago to renovate the lambda-induction test to make it capable of identifying all kinds of DNA-damaging agents. We reasoned that because the molecular mechanism of lysogenic induction was understood better than that of mutagenesis, a prophage-induction test should provide more insights than mutagenicity tests could into the precise effect of chemical carcinogens on DNA and should provide a supplementary tool for screening as well.

Following Ames's lead, we constructed new tester bacteria that are permeable to chemicals and deficient in excision-repair enzymes, and we assayed potential carcinogens in the presence of a rat-liver metabolizing mixture. The prophage-induction test, or inductest, has turned out to discriminate effectively between carcinogens and noncarcinogens in our laboratory and in several others.

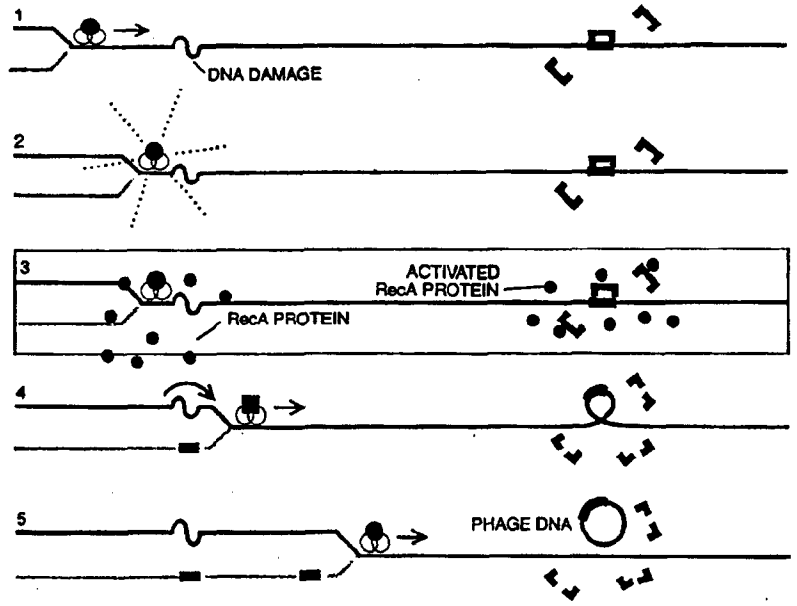
The inductest has certain advantages over mutagenicity tests. Mutations can be scored only in bacteria that survive exposure to a chemical treatment that is often toxic as well as mutagenic, and no more than one bacterium out of 1,000 is likely to be detected as a histidine revertant. In contrast, lysogenic induction can be observed, when it takes place, in the bulk of a cell population. Prophage induction is a mass effect, largely independent of whether or not cells would survive the chemical treatment; a cell that is induced to produce a progeny of phage would die in any case. The inductest can therefore continue to give a positive response for a highly toxic potential carcinogen at dose levels that would kill the tester bacteria in a mutagenicity test.

The fact that in the inductest most cells undergo a dramatic change led us to design a biochemical assay of lysogenic induction. Sankar Adhya, Maxwell E. Gottesman and Asis Das of the National Cancer Institute in the U.S. constructed a bacterial strain in which the gene for the enzyme galactokinase is hooked up to the lambda DNA in such a way that the lambda repressor blocks the synthesis of the enzyme. Alain Levine, Moreau, Steven Sedgwick and I demonstrated that when a DNA-damaging chemical activates the RecA protein in this strain, the repressor is cleaved and galactokinase is synthesized, and the amount of enzyme activity that can be detected reveals the extent of DNA damage. This biochemical assay may be the shortest of the short-term tests; it takes only half a day to test for a potential carcinogen.

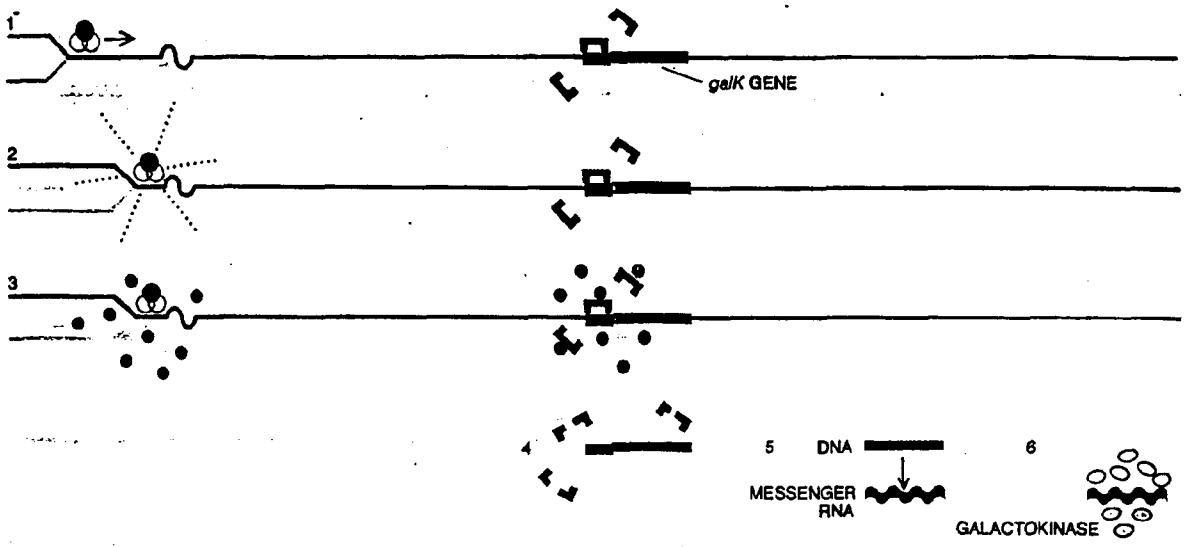
Moreau and I have also constructed strains, containing a new form of prophage lambda, in which one can identify chemicals that induce mutagenesis without damaging DNA. This lambda mu-



DIRECT MUTATION is a result of DNA replication on a minor distortion of a DNA strand. This diagram and the one below show the replication of only one strand of bacterial DNA (*blue*) carrying a piece of viral DNA, prophage lambda (*red*), which is kept in a dormant state by a repressor protein that blocks an operator region. The DNA strand is replicated by a polymerizing complex (*1*). A negligible distortion of the bacterial DNA (*2*) does not obstruct replication, but it does give rise to an error in replication, so that the newly formed DNA strand (*light blue*) carries a mutation (*3*). The negligible distortion is not sensed as DNA damage, RecA protein is not induced, repressor stays in place and prophage is replicated in dormant state (*4, 5*).



INDIRECT MUTATION results from the events that follow DNA damage (*1*), which blocks DNA replication and produces an "SOS" signal (*2*). A large amount of RecA protein (*orange*) is synthesized (*3*). The RecA somehow facilitates DNA replication on a damaged template, apparently by temporarily modifying the polymerizing complex (*4*). This replication is highly mutagenic not only at the lesion site but also elsewhere (*5*). Activated RecA cleaves the repressor (*4, right*), freeing the operator region of the prophage DNA. The prophage is thereby induced to form phage DNA (*5*), which in turn forms virus particles that burst out of bacterium.



ENZYME-INDUCTION TEST is a biochemical counterpart of the inductest. The gene, *galK*, for synthesis of galactokinase is hooked up to prophage-lambda DNA in such a way that enzyme synthesis is blocked by lambda repressor. When DNA is damaged, activated RecA protein cleaves repressor. Liberated *galK* DNA (*4*) is transcribed into messenger RNA (*5*), which is translated into galactokinase (*6*).

tatest, as we call it, reveals whether a chemical gives rise to mutations directly (that is, without causing DNA damage and therefore without inducing prophage) or indirectly (by damaging the DNA and producing active virus).

Although neither the inductest nor the lambda mutatest has yet been validated as extensively as the Ames mutagenicity test, the two appear to be valuable complementary assays, in particular for determining the nature of DNA damage and for establishing a correlation between potency in producing DNA damage in bacteria and potency in causing cancer in mammals. Perhaps more important in the long run, a biological system comprising both a cell and a dormant virus should be a valuable tool for analyzing the pathway of biochemical changes, genetic or nongenetic (cleavage of repressor, for example), that are triggered when cells are exposed to carcinogens.

Given that man is exposed to a flood of chemicals, some of which surely increase the risk of cancer, there is an urgent need to identify potential carcinogens and lower human exposure to them to a level of negligible risk and keep it there. In such an effort bacterial tests can play a major role. In order to ensure broad acceptance of uniform standards, some investigators have strongly favored the adoption of a single, standardized mutagenicity test. Often, however, it is advisable to adapt a screening effort to the particular physical and chemical properties of the substance that is under study. It would therefore seem to be sensible to subject each chemical to a battery of tests. Any test has its foibles, which give rise to false-negative or false-positive responses. Several independent and complementary determinations of a chemical's DNA-damaging capacity can provide a balanced and hence more compelling assessment of carcinogenic risk.

The pronouncement that a particular chemical is a potential carcinogen has obvious social, economic and even political implications. The chemical may present a risk to a very large population, as was the case with the food additive AF-2 in Japan. That situation was dealt with rather easily: AF-2 could be replaced by an innocuous substitute. It was dispensable, and so it was simply banned from the market. Some chemical carcinogens cannot easily be dispensed with and cannot be banned; particular problems are raised by substances as different as cigarette smoke, motor-vehicle exhaust fumes and antitumor drugs. For most indispensable carcinogens it is clearly necessary to reduce human exposure to the safest levels possible. That calls for the adoption of a broadly accepted set of safety standards to be implemented by stringent regulations.

We stand today, with regard to chemical carcinogens, about where we were with regard to ionizing radiations three decades ago. The advent of nuclear power reactors made it necessary to protect workers in the industry from direct sources of radiation and to protect everyone from exposure to radioactive effluents and solid wastes. An international agency sponsored by the United Nations worked out a set of standards and regulations designed to minimize human exposure to various sources of radiation. The regulations were broadly accepted and implemented, and they have stood the test of time. They should provide a valuable reference for evolving standards for chemical carcinogens.

The experts who set the standards for radiation safety have proceeded from two basic facts. One is that mankind is exposed to a natural background of radiations whose biological effect is not perceptible (even though in principle there is no threshold for the effects of ionizing radiations). The other is that biological damage is proportional to the energy released within the living system being considered, and that this linear relation is more or less independent of the particular source of radiation. The experts assumed that for the known sources an absorbed dose only a few times higher than the dose delivered by background radiation, and less than the level that would cause a doubling of the mutation rate in a mammalian population, would constitute a negligible risk; in general such doses were adopted as being permissible for human exposure. In the case of newly devised or newly discovered radioactive materials for which there were no accumulated experimental data, permissible values were calculated on the basis of the linear relation between biological damage and the energy released within the organism.

Since chemical pollution is worldwide, an international committee of experts should propose protective regulations for adoption by individual governments. Some principles should gain broad acceptance rather easily: for example, all unnecessary potential carcinogens should be banned. A list of carcinogens that cannot be dispensed with but to which exposure should be strictly limited will also have to be drawn up. It may be difficult, however, to reach a consensus on such a list and on the permissible environmental concentrations

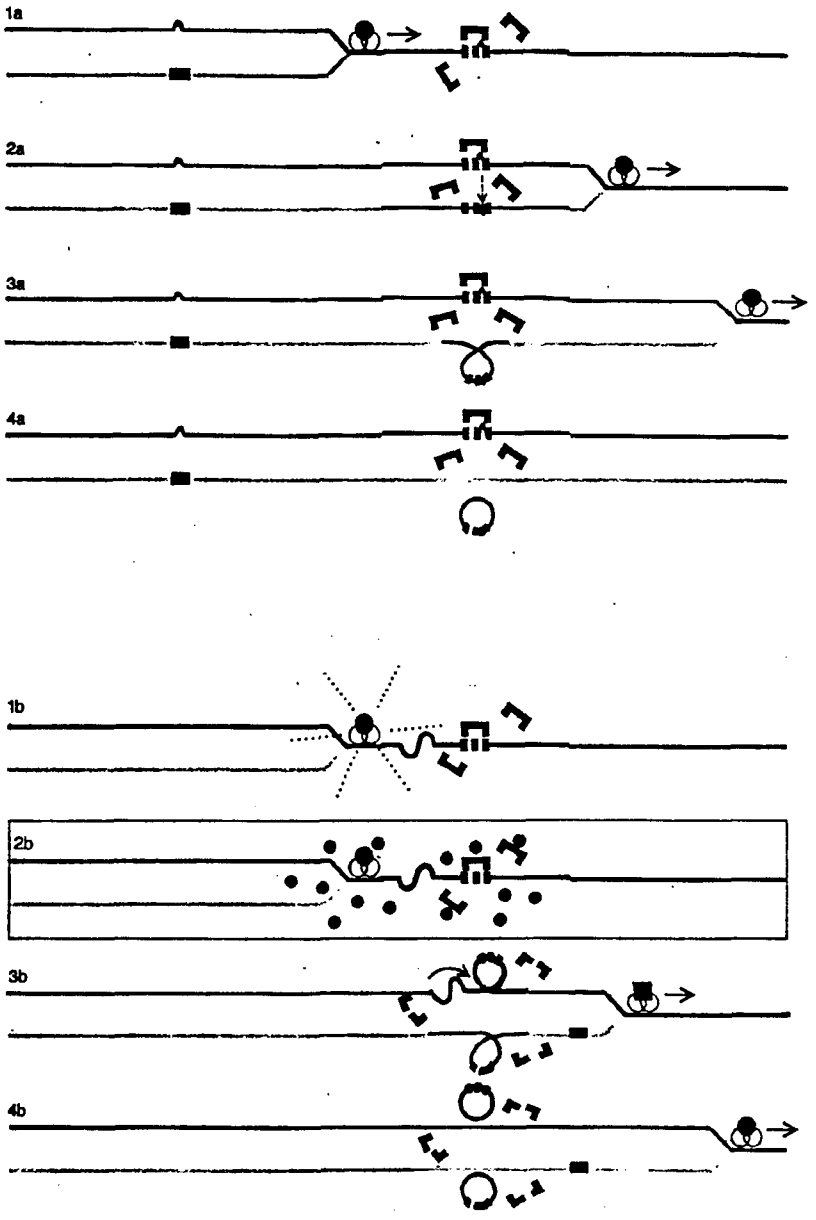
of each chemical. One would like to begin by defining permissible concentrations of indispensable carcinogens in relation to a "natural" background. The trouble is that cultural differences, social habits and other factors have considerably distorted notions of what is a natural background level of negligible risk. (For geographical, economic and religious reasons the carcinogenic background is surely lower in Salt Lake City than it is in New York.)

If a negligible-risk level can be agreed on, the next step would be to estimate, for each indispensable carcinogen, the concentrations that should be allowed for workers in the industry and (at a much lower level) for the general population. Can bacterial tests help in setting such levels? Yes, if there is proportionality between potency in causing DNA damage in bacteria and potency in causing cancer in mammals. Matthew S. Meselson and Kenneth Russell of Harvard University have attempted to demonstrate a direct relation between mutagenic potency in the *Salmonella* test and carcinogenic potency in laboratory animals. For 10 of the 14 known chemical carcinogens they considered there was a correlation.

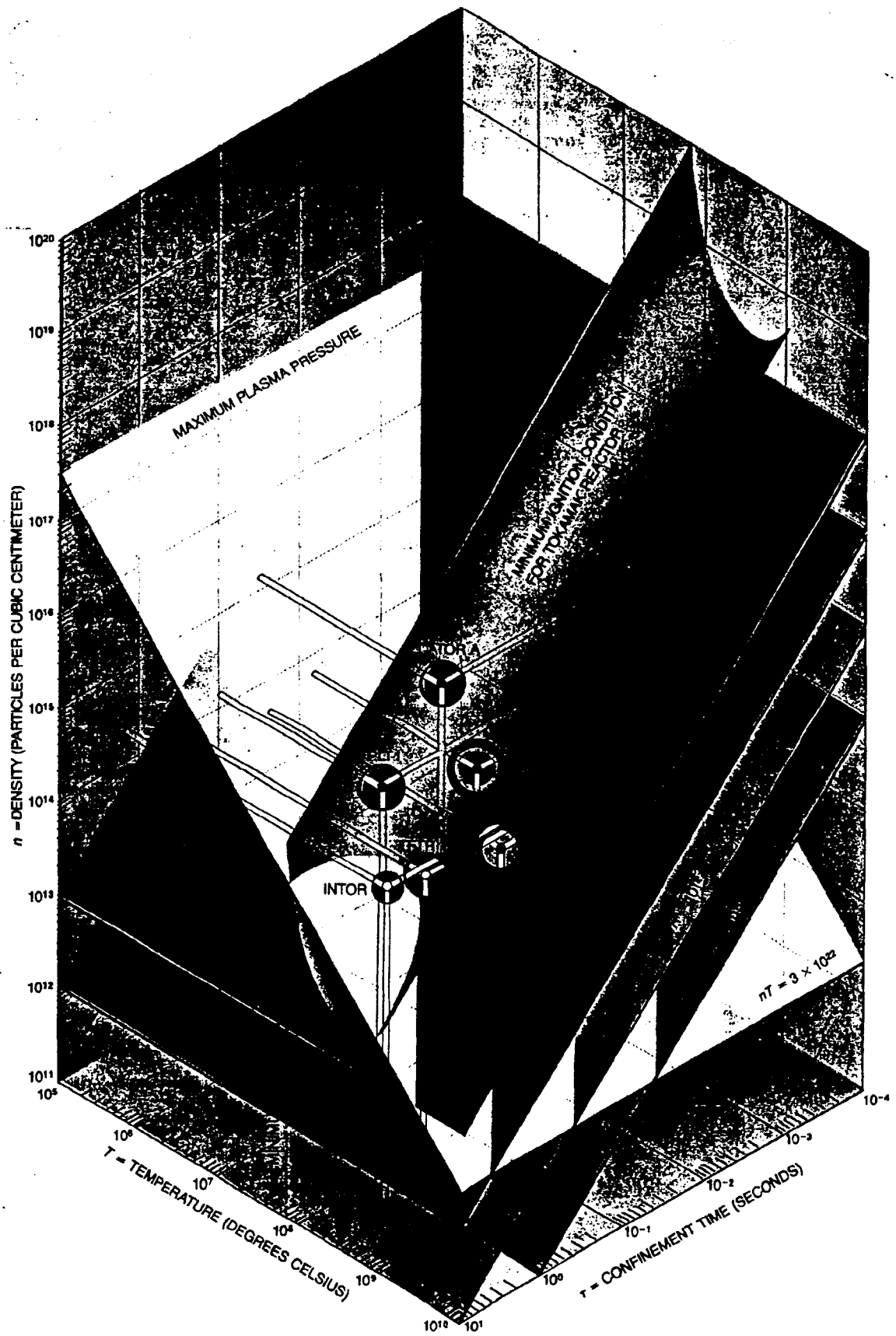
The many exceptions to the rule of correlation were to be expected because in a mammal the extent of DNA damage in a target cell depends on factors (which may be different in bacteria) such as the specific metabolism of carcinogens in the organ involved, the cells' permeability to particular metabolites and tendency to accumulate them and the extent of residual DNA damage after the cellular repair mechanisms have had their effect. Each of these variables must be determined for each individual carcinogen if one is to set valid levels for human exposure. Each chemical—in contrast to individual sources of radiation—requires a thorough specific study to determine its carcinogenic potency. Bacterial tests have good indicative value.

Even though establishing regulations will be much more difficult for chemical carcinogens than it was for radiations, the regulations should be established soon. It is wiser and safer to have safety regulations than not to have them. A large part of the human population contracts cancer; one person in five dies of it. It is important to determine the precise mechanism by which each DNA-damaging agent acts on DNA, not only to prevent cancer but also to develop better ways of curing it. About half of all the drugs administered in an effort to arrest cancer are DNA-damaging agents, and so a significant number of people are being regularly exposed, for therapeutic purposes, to such agents. By establishing, through studies in bacteria, the mechanism whereby antitumor agents affect DNA, investigators may be able to help design more drugs that are effective in curing cancer without also causing cancer.

Bacterial tests for potential carcinogens should help to answer two questions now being asked by industry, government and people at large. What chemicals are DNA-damaging agents? How much of each—in our air, our water and our food, in the products we manufacture and consume—constitutes a biological risk? These two questions will be answered satisfactorily, however, only if a third question is asked: What is the mechanism whereby each chemical affects DNA? Only basic knowledge can breed safety.



DIRECT AND INDIRECT MUTAGENESIS can be distinguished from each other by the mutatest, based on mechanisms diagrammed here. The repressor binds to a slightly modified operator (*1a, 1b*). A negligible alteration of operator DNA (*1a*) results, on replication, in a mutation (*2a*) that prevents further binding of the repressor (*3a*). The prophage develops into active phage particles (*4a*) that are selectively revealed by strain-*B* indicator bacteria, which are not sensitive to nonmutated phage; phage development independent of RecA-protein activation reveals direct mutagenesis. DNA damage (*1b*), on the other hand, leads on replication (*2b, 3b*) to RecA-dependent indirect mutagenesis, which is detected with strain-*B* indicator bacteria. The concomitant event, prophage induction (*3b, 4b*) resulting from activation of RecA, is detected with indicator bacteria of strain *A*, not with strain-*B* bacteria. Mutagenesis can be diagnosed as indirect when it occurs along with prophage induction: both are dependent on RecA.


MAXIMUM PLASMA PRESSURE
MINIMUM IGNITION CONDITION FOR TOKAMAK REACTOR
TOKAMAK
INTOR
$nT = 3 \times 10^{22}$
n = DENSITY (PARTICLES PER CUBIC CENTIMETER)
T = TEMPERATURE (DEGREES CELSIUS)
τ = CONFINEMENT TIME (SECONDS)

THE LANCET

Does this Chemical Cause Cancer in Man?

EVERY year some thousands of new chemical compounds are synthesised and brought into use in industry, and some of these inevitably escape into the environment as contaminants of food, air, water, and consumer products. In addition, in 1972 the Department of Health received 132 applications to permit clinical trials of new drugs. We need to know whether any of the new chemicals to which we are exposed will cause cancer, for we cannot afford to release another product which in 30 years' time will cause, in England alone, 30,000 deaths a year—the lung-cancer death-rate at the moment. The expectation of life for a man aged 40 has been static since 1948. All advances in medical care and social conditions have been wiped out by the combined effects of increasing lung cancer and coronary heart-disease. Ignorance, normal commercial promotion, and the willing consumer led our society 50 years ago into a pathway of development that was damaging to health, and now we do not know what to do about cigarettes or coronary heart-disease. Are we any better these days at excluding carcinogenic factors from our environment?

The conventional test of carcinogenesis involves feeding or skin application or injection of the chemical in doses starting from the maximum tolerable amount for about two years in several species (preferably including one non-rodent as well as rats and mice). The procedure is so expensive and takes so long that we do not have the resources and skilled manpower to test every new chemical. For every test, not only do hundreds of animals have to be fed and looked after, but clinical, chemical, and post-mortem examinations must also be carried out, together with histological examination of many tissues from each animal and its controls. In addition, the conventional procedure is under criticism, because the diet, strain of animal, and housing conditions affect the result. Should one use animals that are very susceptible and get spontaneous tumours, with the risk of false positives? Should one "pair-feed" the animals in order to prevent the high tumour incidence in animals that over-eat? ROE and TUCKER [1] suggest that we cannot ignore the fact that animals under test live under sex-free no-exercise conditions, with unknown virus contamination.

The refinement of animal test procedures is one suggested way out of these dilemmas. Another suggestion is that we now know enough about chemical carcinogenesis to do rapid tests employing tissue-culture and bacterial systems.[2] An attack on cellular D.N.A. (which is associated with carcinogenesis) is recognised by effects on cell transformation [3] or by the production of mutations in bacteria.[2] The advantage of such systems is that they are quick and simple enough to be applied to all new chemicals as they enter the environment. The disadvantage is that they commonly give false-positive results with chemicals which have been safely used for many years, and in some instances they give false-negative results as well. The same criticisms apply to animal tests. For instance, THORPE [4] and others have reported an increased incidence of liver tumours in mice fed on phenobarbitone. But an excellent study in human beings who had taken phenobarbitone (and also other anticonvulsants) for many years shows, not only that there is no increase of liver cancer, but that there is an overall deficit of cancer of about a third in the population taking anticonvulsants (brain tumours excepted).[5] CLEMMESEN et al., who did this work, attribute the deficit to life in the epilepsy colony, but the metabolic effects of phenobarbitone are at least as likely an explanation. It is clear enough that inducers of microsomal-enzyme synthesis, such as phenobarbitone and D.D.T., do cause liver tumours in certain strains of mice on certain diets. But phenobarbitone does not cause tumours in rats or in man. Could these inducers cause increased metabolism of endogenous or dietary chemicals, perhaps steroids, which lead to tumours in mice while in man and rats the same enzymes lead to an overall detoxication effect? The predominant effect may well depend on the exact chemical environment.

The common-sense answer seems to be to use a selection of the quick methods to screen all new compounds entering the human environment. Those that give a "positive" in the screen and those that are suspect by reason of chemical structure, those that are to be widely used, and those where industrial exposure is likely to be heavy, should also be tested in animals before use. In this way we can expect to detect major carcinogens (i.e., those that cause a high proportion of cancers in many species, like the nitrosamines, aflatoxins, and benzo-*a*-pyrene). But

1. Roe, F. J. C., Tucker, M. J. *Proc. Europ. Soc. Study Drug Tox.* 1973, 15, 171. (*Excerpta med. int. Congr. Ser.* 311.)
2. Magee, P. N. *Nature*, 1974, 249, 759.
3. Freeman, A. E., Weisburger, E. K., Weisburger, J. H., Wolford, R. G., Maryjak, J. M., Huebner, R. J. *J. natn. Cancer Inst.* 1973, 51, 799.
4. Thorpe, F. *Fd Cosmet. Tox.* 1973, 11, 433.
5. Clemmesen, J., Fredericksen, V. F., Plum, C. M. *Lancet*, 1974, i, 705.

after that we have to depend on long-term follow-up of human beings exposed to the hazard. We can expect to detect a carcinogen that produces cancers in 1 in 20 heavily exposed animals. But what if it prdduces a cancer in 1 in 50,000 persons who are exposed to a low concentration of the chemical (chloramphenicol agranulocytosis seems to be a risk of this order of magnitude)? The genetic variability of response in the human population is likely to be far wider than can possibly be tested in animals. We badly need to use the records of men exposed to chemicals at work. We need to follow the cancer history of patients in relation to drug intake. If cigarettes were today being introduced for the first time, it seems doubtful that we would detect the major carcinogenic hazard. For that we should have to depend on follow-up of smokers and non-smokers. We cannot have new products without risk, but it is irresponsible to permit new products without assessment of their effects. In devising a monitoring system, we should work back to trying to find out what existing factors in our environment cause the 50% of cancers that are thought to be due to environmental chemicals.[6] To avoid the sort of mistakes that permitted widespread use of cigarettes 50 years ago, we need record linkage. How can we have record linkage without sacrifice of privacy and its attendant political dangers? Perhaps we should start thinking of voluntary record linkage. Could industrial exposure, family food and shopping habits, medication, and patterns of illness be followed at least in a volunteer population? DOLL persuaded doctors to give information about smoking habits which enabled him to sort out the effect on lung cancer of smoking and stopping smoking. The Medical Research Council Epidemiology Unit has studies on these lines—for instance, to test the dietary-fibre hypothesis of intestinal disease. We need more epidemiology linked with laboratory facilities. More mice will not solve the problem.

Something Rotten in Scotland, and Elsewhere

SIX years ago a socio-dental study suggested that, in knowledge of oral health, the intelligent professional Englishman scored rather lower than the nine-year-old Californian schoolgirl.[7] A survey in England and Wales showed widespread neglect of teeth [8]; and now follows a report from Scotland [9] showing an even worse state of affairs. 44% of Scots over the age of sixteen have lost all their natural teeth; over half go to the dentist only when their teeth hurt; and half again, if they had an aching tooth, would opt for extraction rather than filling. Almost 1 in 5 of dentate adults said they did not usually clean their teeth as often as once a day.

Findings like these ought to tell us something about dental priorities for the National Health Service, and there are three obvious ones. Firstly, prevention of the massive amount of untreated dental decay can be brought about only by water fluoridation: continuation of the existing system is unlikely to produce substantial changes in community dental health; people will go on losing teeth, and they will go on having untreated decayed teeth. Fluoridation in the major population centres would reduce the incidence of new decay in all sections of the community. Secondly, "we need more dental-health education". This is a cliché: what is needed is education based on sound principles rather than endless unevaluated repetition of old (and now disproved) saws about apples and carrots. Such education should be incorporated in school curricula. Thirdly, at a time when dental science is advancing rapidly, the dental profession must be kept up to date by postgraduate education. The main dental diseases, decay and periodontal disease, are now well enough understood to be completely prevented (in cooperative individuals), yet practically no-one in the Scottish survey was free of disease: perhaps most telling of all was the observation that little difference existed in mean tooth loss and in periodontal disease between regular and irregular attenders. This indicates that people who go regularly to the dentist are still losing teeth. What sort of care are they getting?

The above are measures which can be taken within the existing system; but they do not go to the heart of the problem. Perhaps more than any other branch of the National Health Service, dentistry is in a critical state, and its predicament stems partly from the system of payment: dentists are paid by item of service rather than by capitation fee, as general medical practitioners are. This method causes grave problems in inflationary times. For example, the fee for a full upper and lower denture, at £16, has not changed substantially for several years, whereas in some cases the laboratory fee now exceeds £16, so that the dentist actually makes a loss. Small wonder that there is now hardly a dentist in the West End of London who does any N.H.S. work at all. This discrepancy could doubtless be remedied; but the chief defect in the item-of-service system is that it emphasises episodic rather than continuous care. The National Health Service gives no encouragement at all to the dentist and patient who, between them, wish to prevent disease and preserve teeth.

6. Doll, R., Vodopija, I. (editors). Host Environment Interactions in the Etiology of Cancer in Man. W.H.O./International Agency for Research on Cancer. Lyon, 1973.
7. Bulman, J. S., Richards, N. D., Slack, G. L., Willcocks, A. J. Demand and Need for Dental Care: a Socio-dental Study. London, 1968.
8. Gray, P. G., Todd, J. E., Slack, G. L., Bulman, J. S. Adult Dental Health in England and Wales in 1968. H.M. Stationery Office, 1969.
9. Adult Dental Health in Scotland. By J. E. TODD and A. WHITWORTH. H.M. Stationery Office, 1974. £2.95.

THE LANCET

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Viruses, Polymerases, and Cancer

David Baltimore

The study of biology is partly an exercise in natural esthetics. We derive much of our pleasure as biologists from the continuing realization of how economical, elegant, and intelligent are the accidents of evolution that have been maintained by selection. A virologist is among the luckiest of biologists because he can see into his chosen pet down to the details of all of its molecules. The virologist sees how an extreme parasite functions using just the most fundamental aspects of biological behavior.

A virus is a form of life with very simple requirements (*1*). The basic needs of a virus are a nucleic acid to be transmitted from generation to generation (the genome) and a messenger RNA to direct the synthesis of viral proteins. The critical viral proteins that the messenger RNA must encode are those that coat the genome and those that help replicate the genome. One of the great surprises of modern virology has been the discovery of the variety of genetic systems that viruses have evolved to satisfy their needs. Among the animal viruses, at least six totally different solutions to the basic requirements of a virus have been found (*2*).

If we look back to virology books of 15 years ago, we find no appreciation yet for the variety of viral genetic systems used by RNA viruses (*3*). Since then, the various systems have come into focus, the last to be recognized being that of the retroviruses (RNA tumor viruses). As each new genetic system was discovered, it was often the identification of an RNA or DNA polymerase that could be responsible for the synthesis of virus-specific nucleic acids that gave the most convincing evidence for the existence of the new system.

Now that the life-styles of different types of viruses have been delineated we can ask what relation there is between a virus' multiplication cycle and the disease it causes. In general, this question has no simple answer because disease symptoms do not correlate with the biochemical class of the virus. For instance, both poliovirus and rhinovirus are picornaviruses, but one causes an intestinal infection with paralysis while the other causes the common cold. One class of RNA viruses, however, does have a unique symptom associated with it: the biochemically defined group of viruses called the retroviruses are the only RNA viruses known to cause cancer. For a virologist interested in cancer, the problem is first to understand the molecular biology of retroviruses and then to understand how they cause the disease.

In what follows, I will review my personal involvement in uncovering the different genetic systems of RNA viruses, a story which leads to the recognition of the unique style of retroviruses. I will then consider what is known about the relationship between the biochemistry of retroviruses and their ability to be oncogenic.

As I tell my story I will mention a few of the many co-workers, teachers, and students who have influenced my thinking or contributed their labors and ideas to the products of my laboratory. To mention all of the people to whom I am indebted would make too long a list; I can only say that the honors I receive are in large measure testaments to their accomplishments.

Picornaviruses

My work on the genetic systems of RNA viruses dates back to my graduate school days. As part of my introduction to animal virology during a Cold Spring Harbor course, I heard Richard Franklin describe his then-recent experiments using autoradiography to show that Mengovirus, a picornavirus and a close relative of poliovirus, could shut off the nuclear synthesis of cellular RNA early after infection and could later induce new RNA synthesis in the cytoplasm which appeared to represent synthesis of viral RNA (*4*). I decided to go to the Rockefeller University as a graduate student with Richard Franklin in order to work on the system he had developed.

Before I began to study how picornavirus RNA was made, it was already known from the work of Simon that picornavirus RNA synthesis was independent of DNA synthesis (*5*). Furthermore, studies with actinomycin D had shown that neither synthesis nor expression of cellular DNA was involved in viral RNA synthesis (*6*). These results suggested that Mengovirus might make a cytoplasmic RNA-dependent RNA synthesis system. The concept that viruses induce synthesis of their own enzymes had strong precedents in bacteriophage systems—Seymour Cohen's work had shown decisively that new virus-specified enzymes were found in infected bacteria (*7*).

We approached the problem of the virus' effect on intracellular RNA synthesis as a question in enzymology. We first showed that the nuclei from Mengovirus-infected cells were greatly reduced in their ability to carry out cell-free DNA-dependent RNA synthesis compared to nuclei of uninfected cells (*8*). Later, we showed that cytoplasmic extracts of Mengovirus- or poliovirus-infected cells contained an RNA synthesis activity not evident in uninfected cells and not inhibited by actinomycin D (*9*). When we learned that the system made RNA of the size and structure of virion RNA (*10*), it became clear that it represented the postulated viral RNA-dependent RNA synthesis system.

While there has been extensive further analysis of crude cytoplasmic systems (*11*, *12*) and impressive enrichment of the RNA synthesis system has been achieved (*13*), no pure enzyme able to make picornavirus RNA has ever been isolated, so the detailed mechanism of viral RNA synthesis still remains obscure. Most of our knowledge of how picornavirus RNA is made has come from studies on the virus-specific RNA molecules in infected cells and their kinetics of labeling by radioactive precursors. Such research has been carried out in many laboratories (*11*, *12*); my work in this area was done in association with James Darnell and Marc Girard. Together we found and studied the relations between the poliovirus double-stranded RNA, the poliovirus replicative intermediate, and the poliovirus replication complex (*14*). Girard's precise in

The author is professor of microbiology at the Massachusetts Institute of Technology, Cambridge 02139. This article is the lecture he delivered in Stockholm, Sweden, on 12 December 1975 when he received the Nobel Prize for Physiology or Medicine, a prize he shared with Renato Dulbecco and Howard M. Temin. The article is published here with the permission of the Nobel Foundation and will also be included in the complete volume of *Les Prix Nobel en 1975* as well as in the series *Nobel Lectures* (in English) published by the Elsevier Publishing Company, Amsterdam and New York. Dulbecco's lecture appeared in the 30 April issue, and Temin's lecture will appear in a later issue.

more controls to abate pollution, or whether they lessen the impetus to change in order to accommodate the status quo—will substantially affect us all.

In discussing the pervasive effect of the Clean Air Act, I have limited myself to one section of the act and to three requirements only in that section—that emission limitations, land use controls, and transportation controls be effectuated within a state to implement ambient air quality standards established by the statute. I have not discussed the provisions of the statute relating to the siting of new stationary sources of air pollution—this alone a significant land use control—nor have I discussed the provisions relating to the control of automobile pollution through the development of improved internal combustion engines. The discussion of these provisions would only reinforce the point I have attempted to make, that the Clean Air Act necessarily does more than clean the air: it relocates industry, it changes centers of population, it alters life-styles and living patterns, and it touches immediately a broad range of interests, from the esthetic to the economic.

Cleaner than Clean Air

But there is one other requirement of the Clean Air Act that should be mentioned. Although this requirement is difficult to find explicitly set forth in the statute, the courts have found it to be in the statute implicitly, and it may have as profound an effect as any I have mentioned. The District Court for the District of Columbia, in a suit entitled Sierra Club *v.* Ruckelshaus (*9*), held that in those portions of the nation where the air is already of a quality higher than that required by the national ambient air quality standards, the air may not be permitted to deteriorate to the level of the national ambient air quality standards. This is called the principle of nondeterioration or nondegradation. The holding of the District Court for the District of Columbia was affirmed per curiam, that is, unanimously, and without an opinion, by the Court of Appeals for the District of Columbia. The matter was then taken to the Supreme Court, where the justices were divided equally, four to four (*10*), on the question (Justice Powell having taken no part in the consideration of the case). A consequence of an even division by the Supreme Court is the affirmation of the decision which it is reviewing; the nondeterioration principle, therefore, is today the law.

In what form the nondegradation decision will ultimately manifest itself is difficult to say. In compliance with the court decision, the EPA has issued regulations establishing the manner in which areas of the country having "cleaner than clean" air will be preserved (*11*), but these regulations are presently being challenged in the court by the Sierra Club and other environmental groups on the one hand and by the American Petroleum Institute, various oil companies, and the state of New Mexico on the other hand (*12*). In their most extreme form, nondegradation principles could operate so as to prevent industrial development of any consequence in those areas of the country (mostly the western states) where the air is now superior to that required by the national ambient air quality standards; new towns and cities in such areas might also be difficult to establish, as might be recreational areas, roads, and other activities that would draw automobiles. A consequence of thus freezing at their present level of development these "cleaner than clean" areas might be the confining of industry and population to those areas of the country which are now "dirty air" areas. Thus, we have another example of an environmental statute's impact going far beyond mere land and water.

Other Land Use Control Authority

In addition to administering the Clean Air Act, the EPA administers the Federal Water Pollution Control Act and a substantial number of other statutes. The Federal Water Pollution Control Act affects matters other than clean water just as fully and pervasively as the Clean Air Act affects matters other than clean air; one author commenting on one provision only of the act, section 208, observed that "it is difficult to imagine how a successful water pollution control strategy of this magnitude could be effective without extensive reforms in the state land use guidance system, or apart from other conservation, social, and economic objectives" (*13*). Clearly, the growth of almost any metropolitan area could be determined through the control of the size and location of sewage treatment plants and restrictions on hookups to these plants. It follows then that the agency that administers statutes of such comprehensive scope is itself an agency whose policies, like the statutes it administers, have an impact going far beyond the area suggested by the name of the agency.

Summary

It is clear that natural resource development and industrial expansion must be done so as to produce the least impact on our physical environment. Our energy requirements, along with the exigencies of life in modern society, demand adjustment and balance between pollution control and development. That adjustment will be made based largely on the political interests reflected in our communities, and where the pendulum will stop has yet to be determined.

References and Notes

1. *United States Code*, vol. 42, sect. 1857c-1 (1970), amending *ibid.*, sect. 1857c (Suppl. 1975).
2. ———, *ibid.*, sect. 1857b *et seq.* (1970), amending *ibid.*, sect. 1857 *et seq.* (Suppl. 1975).
3. *Fed. Reporter 2nd* 508, 743 (3rd circuit, 1975).
4. *Ibid.* 481, 162 (6th circuit, 1974).
5. Indiana and Michigan Electric Co. *v.* EPA, *ibid.* 509, 839 (7th circuit, 1975); Union Electric Company v. EPA, *ibid.* 515, 206 (8th circuit, 1975).
6. State plans are published in the *Federal Register*; each plan also appears in the *Code of Federal Regulations*. See *Code of Federal Regulations*, vol. 40, sect. 52 *et seq.* (1975). Following are two examples of provisions having the effect noted in the text:
   "No later than February 15, 1974, the City of Fairbanks shall prohibit the idling of all motor vehicles within the City unless attended by a licensed driver . . ." (Alaska's plan, *ibid.*, sect. 52.87).
   "Beginning June 1, 1975, the State of Maryland shall . . . establish a computer aided carpool matching system which is conveniently available at least to all employees of employers within the region who employ 100 or more employees" (Maryland's plan, *ibid.*, sect. 52.1104).
7. *United States Code*, vol. 42, sect. 1857c-10 and 1857f-6f (1974), amending *ibid.*, sect. 1857 *et seq.* (1970) (Suppl. 1975).
8. ———, *ibid.*, sect. 1857(b)(f) (1970) (Suppl. 1975).
9. *Fed. Suppl.* 344, 253 (District of Columbia District Court, 1972).
10. *U.S. Rep.* 412, 541 (1973).
11. *Code of Federal Regulations*, vol. 40, sect. 52.21 (1975).
12. Sierra Club *v.* EPA, civil No. 74-2063 (District of Columbia Court of Appeals, 1974); Sierra Club *v.* EPA, civil No. 74-2079 (District of Columbia Court of Appeals, 1974); Public Service Company of Colorado *v.* EPA, civil No. 75-1368 (District of Columbia Court of Appeals, 1975); Utah Power and Light Company *v.* EPA, civil No. 75-1369 (District of Columbia Court of Appeals, 1975); State of New Mexico *ex rel.* New Mexico Environmental Improvement Agency *v.* EPA, civil No. 75-1370 (District of Columbia Court of Appeals, 1975); Pacific Coal Gasification Company *v.* EPA, civil No. 75-1371 (District of Columbia Court of Appeals, 1975); Utah International Inc. *v.* EPA, civil No. 75-1372 (District of Columbia Court of Appeals, 1975); Indiana-Kentucky Electric Corporation *v.* EPA, civil No. 75-1575 (District of Columbia Court of Appeals, 1975); Dayton Power and Light Company *v.* EPA, civil No. 75-1663 (District of Columbia Court of Appeals, 1975); Buckeye Power Inc. *v.* EPA, civil No. 75-1664 (District of Columbia Court of Appeals, 1975); American Petroleum Institute *v.* EPA, civil No. 75-1665 (District of Columbia Court of Appeals, 1975); Alabama Power Company *v.* EPA, civil No. 75-1666 (District of Columbia Court of Appeals, 1975); Montana Power Company *v.* EPA, civil No. 75-1763 (District of Columbia Court of Appeals, 1975); Salt River Project Agricultural Improvement & Power District *v.* EPA, civil No. 75-1764 (District of Columbia Court of Appeals, 1975).
13. W. K. Reilly, in *Federal Environmental Law*, E. L. Dolgin and T. G. T. Guilbert, Eds. (West, St. Paul, Minn., 1974), p. 1431.
14. I thank Martin Green, special assistant to the Assistant Attorney General, for his help in preparing the original text of these remarks.

vitro analysis of RNA synthesis capped this whole series of experiments (*15*).

A crucial part of the viral genetic system is the manner of translation of the viral messenger RNA. While working on viral maturation, my first graduate student, Michael Jacobson, and I began to realize that proteolytic cleavage was an important part of the process (*16*). Our work led us to suggest that the whole 7500-nucleotide viral genome might be translated into a single continuous polypeptide that we have called a polyprotein (*17, 18*). Recently, this work culminated in the demonstration that poliovirus RNA can be translated into this continuous polyprotein in a cell-free system and that some of the cleavages that make the polyprotein into the functional proteins appear to occur in extracts of uninfected cells (*19*).

The demonstration that the poliovirus genome RNA is the messenger RNA for the synthesis of viral proteins, coupled with the demonstration of the infectivity of viral RNA (*20*), implies that the poliovirus genetic system is very simple. The existing evidence confirms this simplicity. As seen diagrammatically in Fig. 1, it appears that the incoming "plus" strand of RNA synthesizes a "minus" strand which in turn synthesizes a series of plus strands. This diagrammatic simplicity of poliovirus replication hides a fair amount of as yet undeciphered complexity, as shown by the work of Eckard Wimmer and his colleagues as well as by work in my laboratory. For instance, the 3'-ends of the virion RNA and messenger RNA are both polyadenylate [poly-(A)] and the 5'-end of the minus strand is polyuridylate [poly(U)], so we assume that they are templates for each other (*21*). But these homopolymer stretches have very variable lengths even in the progeny of a cloned virus; what then determines their length? The poly(A) serves some obscure but necessary function in the life cycle of the virus (*22*); what is this function? There is no triphosphate 5'-terminus, either free or capped, on the virion RNA or messenger RNA (*23, 24*); how then is the synthesis of these molecules initiated? The 5'-end of the virion RNA and messenger RNA are different (*24*); what does this mean?

Vesicular Stomatitis Virus

Most of the work in my laboratory until 1969 centered on poliovirus. We had assumed that all RNA viruses would be similar in their basic molecular biology, but during the 1960's results emerging from various laboratories implied

The Poliovirus Genetic System

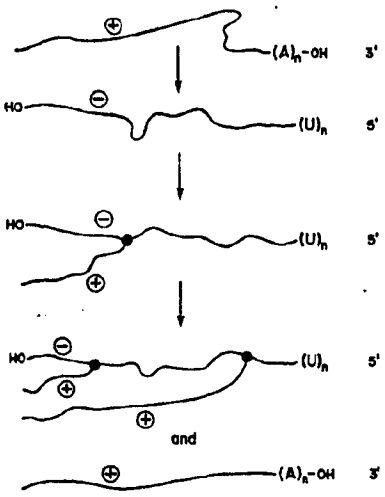

Fig. 1. Schematic representation of poliovirus-specific RNA synthesis in the cytoplasm of infected cells.

that poliovirus, rather than being a model for all RNA viruses, used one out of a collection of different viral genetic systems. Probably the first dramatic demonstration of the variety in RNA viruses came from next door to Richard Franklin's laboratory at the Rockefeller Institute, where Peter Gomatos and Igor Tamm found that reovirus has double-stranded RNA as its genome (*25*). The peculiarity of reovirus was underscored by the demonstration later that an RNA polymerase in the virion of reovirus is able to assymetrically transcribe the double-stranded RNA (*26*). This was the first virion-bound RNA-dependent RNA polymerase ever found and followed after the finding of the first nucleic acid polymerase in a virion—the DNA-dependent RNA polymerase found by Kates and McAuslan (*27*) and Munyon *et al.* (*28*) in virions of vaccinia virus.

Another observation that suggested there were profound differences among the RNA viruses was the finding that in cells infected by the paramyxoviruses, Newcastle disease virus, or Sendai virus, much of the virus-specific RNA was complementary to the virion RNA (*29*). This result was in sharp contrast to what was found in poliovirus-infected cells, where most of the virus-specific RNA was of the same polarity as virion RNA (*11*).

We branched away from concentration only on poliovirus to include the study of vesicular stomatitis virus (VSV) because of the lucky circumstances that brought Alice Huang to my laboratory. She joined me first at the Salk Institute and then we both came to MIT in 1968. Alice had studied VSV as her graduate work with Robert R. Wagner at Johns Hopkins. We decided that the techniques developed for studying poliovirus should be applied to VSV, hoping that the peculiar ability of VSV to spawn and then be inhibited by short, defective particles could be understood at the molecular level. A graduate student, Martha Stampfer, joined in this work and together we found that we had bitten off an enormous problem because VSV induced synthesis of so many species of RNA. Only three species of RNA are seen in poliovirus-infected cells, but we found at least nine RNA's in VSV-infected cells, and one of these RNA's was clearly heterogeneous (*30*)—later work showed it had four components (*31*). In our description of this work we said that nine RNA species "seems exorbitant" (*30*) but we soon realized that each RNA had its place in the cycle of growth and growth inhibition of VSV.

As we were beginning to unravel the multiple RNA's of VSV, Schaffer *et al.* (*32*) published a paper showing that the major VSV-induced RNA's in infected cells, like those induced by Sendai and Newcastle disease viruses, were complementary in base sequence to the virion RNA. We confirmed and extended that observation, showing that the virus-specific RNA recovered from the polyribosomes of infected cells (the viral messenger RNA) was all complementary to virion RNA (*33*). This finding presented a pregnant paradox: if these viruses were like poliovirus and induced a new polymerase in the infected cell, how could a virus that carried as its genome the strand of RNA complementary to messenger RNA ever start an infection? There seemed two possible solutions: the RNA came into the cells and was copied by a cellular enzyme to make the messenger RNA to initiate the infection cycle, or the RNA came into the cell carrying an RNA polymerase with it.

Because no convincing evidence for RNA-to-RNA transcription existed (or exists) for any uninfected cell, the possibility of a polymerase with the incoming RNA seemed attractive. This possibility implied that there might be polymerase activity demonstrable in disrupted virions of VSV. Almost as soon as the power of this reasoning was clear to us, we had shown the existence of the virion RNA polymerase (*34*). The demonstration of this enzyme was the piece of evidence that led to the realization that there is a huge class of viruses, now called negative strand viruses (*35*), that

all carry the strand of RNA complementary to the messenger RNA as their genome and that carry an RNA polymerase able to copy the genome RNA to form multiple messenger RNA's.

Retroviruses

The discovery of a virion polymerase in VSV led us to search for such enzymes in other viruses. Because Newcastle disease virus made a lot of complementary RNA after infection, it seemed a logical candidate; after an initial failure (*34*), we found activity in virions of that virus (*36*). But a more exciting possibility occurred to me; maybe by looking for a virion polymerase, light could be shed on the puzzle of how RNA tumor viruses multiply.

In his Nobel lecture, Howard Temin has outlined how he was led to postulate a DNA intermediate in the growth of RNA tumor viruses (*37*). Although his logic was persuasive, and seems in retrospect to have been flawless, in 1970 there were few advocates and many skeptics. Luckily, I had no experience in the field and so no axe to grind—I also had enormous respect for Howard dating back to my high school days when he had been the guru of the summer school I attended at the Jackson Laboratory in Maine. So I decided to hedge my bets—I would look for either an RNA or a DNA polymerase in virions of RNA tumor viruses.

To make the foray into tumor virology, I needed some virus. Peter Vogt first sent me some Rous sarcoma virus and, although I later used it to good advantage, I initially assayed for an RNA polymerase in this viral preparation and failed to find anything. Then George Todaro helped me utilize the resources of the Special Virus Cancer Program of the National Cancer Institute to get some Rauscher mouse leukemia virus. Using that virus preparation I set out to look for a DNA polymerase activity. With little difficulty, I was able to demonstrate that virions of Rauscher virus contained a ribonuclease-sensitive DNA polymerase activity, and, after confirming the results with Rous sarcoma virus, I knew that the machinery for making a DNA copy of the RNA genome was wrapped up inside the virions of RNA tumorviruses (*38*). Simultaneously with my work, Temin and Mizutani discovered the DNA synthesis activity in Rous sarcoma virus (*39*).

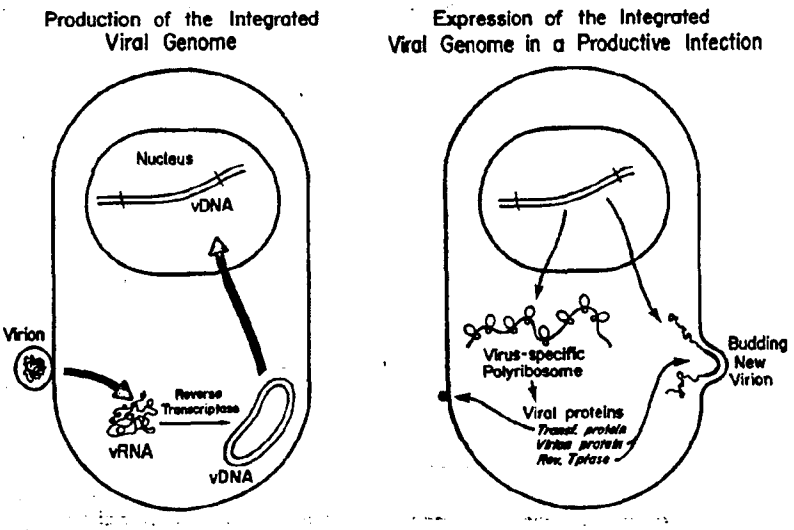

Fig. 2. The life cycle of an RNA tumor virus. On the basis of present knowledge (*42*), the life cycle of an RNA tumor virus can be separated into two parts. In the first part the virion attaches to the cell and somehow allows its RNA along with reverse transcriptase to get into the cell's cytoplasm. There the reverse transcriptase causes the synthesis of a DNA copy of the viral RNA. A fraction of the DNA can be recovered as closed, circular DNA (*43*), and it is presumably that form which integrates into the cellular DNA. Once the proviral DNA is integrated into cellular DNA it can then be expressed by the normal process of transcription. The two types of product which have been characterized are new virion RNA and messenger RNA. Much of the messenger RNA which specifies the sequence of viral protein is of the same length as the virion RNA, but there may also be shorter messenger RNA's (*48*). The virus-specific proteins have two known functions: one is to transform cells, which occurs when, for instance, a sarcoma virus infects a fibroblast; the second is to provide the protein for new virion production. The transforming protein is shown here as acting at the cell surface, but that is only a hypothesis; *v*, viral; *Transf.*, transforming; *Rev. Tptase*, reverse transcriptase.

Biochemistry of Reverse Transcriptase

Once the DNA polymerase activity had been demonstrated in the virions of what we now call retroviruses, many laboratories began to study the enzyme. A correspondent of *Nature* dubbed the enzyme "reverse transcriptase" (*40*) and this romantic name has become common parlance. About 2 years after its discovery, Howard Temin and I reviewed the literature on the enzyme (*41*). That review and later compendia (*42*) make a detailed rehash of the biochemistry of retroviruses superfluous. So, I will only present a brief sketch of the picture we have today of how a retrovirus multiplies and how the reverse transcriptase functions. I will not attempt to credit all of the people who have helped to clarify this picture.

There are two separate time periods that can be distinguished after infection of a cell by a retrovirus (Fig. 2). The first period, which lasts a few hours, involves reverse transcription and establishment of the DNA provirus as an integrated part of the cellular DNA; the second period involves the expression of the integrated genome and synthesis of progeny virions.

Analysis of the first period of retrovirus growth has focused on the types of virus-specific DNA molecules that are produced. One important type of DNA that has been found is a closed circular duplex DNA of about 5.7×10^6 daltons (*43*). This DNA can transfect cells with one-hit kinetics (*44*) and therefore contains the total viral genetic information. Other DNA's that may be on the way to becoming the closed circular form are also evident (*45*). It has been hard to get definitive evidence as to what DNA form integrates but presumably it is the circular duplex DNA. Whatever the form that integrates, the evidence is quite good for acquisition of proviral DNA by the chromosomes of infected cells (*46, 47*).

The second period in a productive retrovirus growth cycle starts when the integrated genome begins making viral RNA (*48*). Synthesis of viral proteins and progeny virions ensues, and the cell ever after continues to make viral products except for variations imposed by the cell cycle (*49*). Among the viral proteins made in the infected cell may be a product that changes the growth properties of the cell (*50*); in such a case the retrovirus becomes a tumor virus.

The second period of the infection cycle can be dissociated from the first in a number of experimental systems. For instance, mammalian cells infected by avian viruses can gain viral DNA but not

express it (*46*). Also, cells can have viral genomes that they inherited from their ancestors, and such genomes are generally not being transcribed. Nonexpressed genomes can be activated: bromodeoxyuridine and iododeoxyuridine, for instance, stimulate the expression of inherited, silent viral genomes (*51*). The exact mechanism of activation of the genome for transcription, initiation of the transcript, and termination of transcription are obscure, as are any processing events of the initial transcript which may occur.

It is evident that the key piece of machinery provided by the virus for this unique life cycle is the reverse transcriptase. Purified reverse transcriptase has the properties of most DNA polymerases: it is a primer-dependent enzyme that makes DNA in a 5′ → 3′ direction using deoxyribonucleoside triphosphates as substrates and taking the direction of a template for determining the base sequence of the product. The enzyme differs from normal cellular DNA polymerases by having a unique polypeptide structure, having an associated ribonuclease H activity, and being able to make copies of RNA templates as readily as DNA templates (*41*). Genetics has shown us that the avian leukosis viral enzyme, at least, is encoded by viral RNA and needed only in the first period of the infection cycle (*52*).

The primer dependence of the reverse transcriptase means that the enzyme can only elongate nucleic acid molecules, it cannot initiate DNA synthesis de novo. How then does the enzyme initiate the copying of viral RNA? The answer is that the genome RNA has attached to it a primer RNA molecule which is, in the case of avian leukosis viruses, cellular tryptophan transfer RNA (*53*). The avian leukosis virus reverse transcriptase has a high-affinity binding site for that transfer RNA which the enzyme presumably uses for precise initiation of reverse transcription (*54*).

Retroviruses and Cancer

The last 15 years of research in animal virology has allowed us to see the diversity of genetic systems used by the various types of RNA viruses and has most recently shown us how distinct the retroviruses are from the others. Rather than using an entirely RNA-dependent replication and transcription machinery, the retroviruses have included the DNA provirus in their life cycle. Having a DNA intermediate does not make their mode of growth especially complicated—the DNA formally takes the place of the "minus" strand in the picornavirus genetic system—but the DNA is probably the clue to why retroviruses are the only ones able to cause cancer. The DNA provides the necessary stability to the virus-cell interaction so that a viral gene product can permanently change the growth properties of an infected cell. Equally significant, the DNA stage is probably important to the ease with which retroviruses carry out genetic recombination (*55*); it is quite possible that the recombination system can bring together host cell genetic information with viral information and that in this way nononcogenic retroviruses become oncogenic (*56*).

So the inclusion of a proviral stage in the retrovirus life cycle may provide critical capabilities toward the development of an oncogenic potential. But the actual transformation of cells by retroviruses is a highly selective process; each type of oncogenic virus transforms a very limited range of cell types (*57*). If we assume that all transforming genes of viruses are like those of Rous sarcoma virus, genes that are not necessary to the growth of the virus (*50, 58*), then we can postulate that each type of transforming virus makes a specific type of transforming protein. Such a protein, by this model, would not be critically involved in the multiplication cycle of the virus. Isolation of such transforming proteins and elucidation of their mechanism of action is the present challenge of cancer virology. Not only will such work help us to understand carcinogenesis, it may also be important to the study of developmental biology because of the intimate relationship between the differentiated state of cells and the type of virus able to transform them.

Another implication of the occurrence of a proviral stage in the life cycle of retroviruses is that cells can harbor such viruses as genetically silent DNA molecules. In fact, in most, if not all, animal species, the normal cells of the body have DNA related to one or more types of retroviruses (*59*). They receive that DNA by inheritance, not infection, and in favorable cases it can be as precisely located in the chromosomes as any gene (*60*). What is the significance of these genes that look like viruses?

There have been three types of explanations for virus-related genes that are inherited in the germ line of so many animal species:

1) They are an aspect of the normal genetic complement of the animal and they are virus-related because they are the progenitors of retroviruses. These genes play some important role in the life of the animal and so are not dispensable. This explanation is basically the protovirus hypothesis put forward by Howard Temin (*37*).

2) They are genes inserted into the chromosome of some ancestral animal by a retrovirus infection of the germ cells of that animal. Because once the provirus is integrated it remains stably associated with the chromosome, the viral genes are inherited by progeny of the original infected animal. There is one force that can eliminate such genes from a species, the slow but inexorable process of mutation. As part of this explanation of inherited viral genomes, therefore, it has been suggested that the viral genes have some positive influence on the life of the animal and so are maintained by positive selection. This explanation is closely related to the virogene-oncogene hypothesis (*61*).

3) The previous explanation can be modified by the exclusion of any positive role for viral genes in the life of the infected animal. There are a number of reasons why positive selection may be an unnecessary attribute to postulate. For one thing, mutations are rare events and totally inactivating mutations are much rarer. Also, the virus can be genetically invigorated by becoming a replicating virus in the body of the animal and then reinfecting the germ line. When the virus starts multiplying as an independent entity, the burden of debilitating mutations it might have accumulated could be purged if a sufficient number of generations intervened between the activation of the latent provirus and its reintegration into the germ line. The reintegration might even replace the original provirus (*62*). If the viral genes were not transcribed in most cells that have the viral genome, as appears to be the case, the negative effect of having one or a few integrated genomes would be slight and probably insufficient to cause a serious negative selection against animals with inherited proviruses.

I would argue that the third explanation above is the one most likely to be correct. It is an explanation that maintains the separation of viral activities and cellular activities and does not require the ad hoc postulation of beneficial properties of viral products. It treats retroviruses like any other virus, as an entity with its own life-style and its own accommodation with evolution.

In summary, I have tried here to develop the view of retroviruses as one of a number of solutions to the problem of creating a virus. Each virus directs synthesis of two critical classes of proteins:

proteins for replication and proteins for constructing the virus particle. By encoding the reverse transcriptase, retroviruses have evolved the ability to integrate themselves into the cell chromosome as a provirus. This is a very sheltered environment in which to live; only mutation interferes with the continual transmission of the virus to the progeny of an animal that is infected in its germ cells. In this context, the ability of some retroviruses to cause cancer is a gratuitous one. But it is today the most challenging and important attribute of these retroviruses and the one that will dominate future research efforts in this area.

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NEWS AND COMMENT

Anatomy of a Decision: How the Nation Declared War on Swine Flu

Late on the afternoon of 24 March, President Gerald R. Ford appeared before White House reporters to discuss "a subject of vast importance to all Americans"—the appearance of a new strain of flu. A month earlier, scientists had discovered that Army recruits at Fort Dix, New Jersey, were infected by "swine flu virus." One of them had died. The last time an outbreak of swine flu appeared in the United States was in 1918–19 when, the President told reporters, "a widespread and very deadly flu epidemic" killed 548,000 Americans of all ages. The 1918 epidemic was really a pandemic; in successive waves, swine flu swept around the world, leaving 20 million persons dead. It is said that the pandemic killed more individuals in a short period of time than any other catastrophe in history.

After consulting with his top health advisers and virologists from throughout the country, Ford was concerned about the "very real possibility" of a dangerous epidemic in the United States next fall and winter. To head off this threat, the President announced that he would ask Congress to appropriate $135 million right away to buy enough vaccine to inoculate "every man, woman, and child in the United States."

This would be the largest immunization campaign ever launched in this country, far more ambitious even than the polio immunization drives of the 1960's. Then, about 100 million Americans received polio vaccine during a period of a year and a half. Ford was launching a campaign to vaccinate twice as many citizens in a matter of months. Officials said that all his health advisers thought

From the Molecular Biology of Oncogenic DNA Viruses to Cancer

Renato Dulbecco

Oncogenic viruses, able to elicit tumor formation in animals, have been on the scientific scene for many years. After the early discovery of Ellerman and Bang at the beginning of this century, Peyton Rous opened up the field in the next decade and in prophetic words gave a good hint of things to come. However, these discoveries were soon forgotten, and only after a long eclipse was interest in oncogenic viruses revived in the 1950's. My involvement in this field began at that time, when Rubin and Temin worked in my laboratory with the Rous sarcoma virus. When polyoma virus, a new oncogenic virus with different properties, was isolated in 1958, I jumped at the new opportunity and started working with it. Within a short time polyoma virus became the main interest of my laboratory, to be joined, a few years later, by SV40, another papovavirus. It became clear fairly soon that the molecular biology of these viruses could be worked out, and I set out to find the molecular basis of cancer induction. The results that I and a number of brilliant young collaborators have obtained during the following 15 years have brought us close to that goal. I will review the most interesting steps of our work and will then ask some questions concerning the nature of cancer and about perspectives for prevention and treatment. I stress the relevance of my work for cancer research because I believe that science must be useful to man.

 The author is deputy director for research at the Imperial Cancer Research Fund Laboratories, Lincoln's Inn Fields, London, W.C.2, and a fellow of the Salk Institute, San Diego, California. This article is the lecture he delivered in Stockholm, Sweden on 12 December 1975, when he received the Nobel Prize in Physiology or Medicine, a prize he shared with David Baltimore and Howard M. Temin. The article is published here with the permission of the Nobel Foundation and will also be included in the complete volume of *Les Prix Nobel en 1975* as well as in the series *Nobel Lectures* (in English) published by the Elsevier Publishing Company, Amsterdam and New York. The lectures by Baltimore and Temin will appear in later issues.

Integration: The Provirus

Let me start with a brief review of our work on the molecular events in transformation. The first results, crucial for future developments, showed that polyoma virus could be assayed in certain cell cultures (*1*), which we call permissive, and could induce a cancer-like state in other cultures (*2*) in which the virus does not grow, which we call nonpermissive. The induction of the cancer-like state in vitro was called transformation. We were able to show that the virus contains DNA (*3*), and within a few years we gave the first evidence of its cyclic, or circular, shape (*4*), which is important for two critical biological events: DNA replication and integration. In integration, which we discovered a few years later with the virus SV40 (*5*), the viral DNA becomes a provirus, that is, it establishes permanent, covalent bonds with the cellular DNA. The cyclic configuration explains how a complete molecule of the SV40 DNA can be integrated without losses.

Integration is one of the key events in virus-induced cell transformation. It explained the persistence of the transformed state in the cell clone deriving from a transformed cell, since the provirus replicates with the cellular DNA. It also permitted us to resolve one of the main questions about the role of viruses in transformation. It was known at the time that papovaviruses leave their footprints in the cells of the cancers they induce and those they transform in vitro, in the form of characteristic antigens. However, it was not known whether the antigens were expressed by viral genes or by derepressed cellular genes. Hence, it was uncertain whether cells were transformed by the expression of viral genes persisting in the cells or, alternatively, whether the virus altered the cells by a hit-and-run mechanism, changing the expression of cellular genes and then leaving. The demonstration that viral DNA is integrated in the cells, in conjunction with the finding that the provirus is transcribed into messenger RNA (*6*) hundreds of generations after the establishment of a transformed clone, made the hit-and-run hypothesis unlikely and supported a continuing role of viral gene functions in determining transformation. This possibility was later supported by observations with abortively transformed cells, which behave as transformed only for several generations after infection, but then return to normal (*7*). When they are back to normal these cells no longer contain the viral DNA (*8*).

The viral genes that remain unexpressed in the transformed cells, such as those for capsid protein in SV40-transformed cells, were also interesting, although in a different way. In fact their expression could be renewed in heterokaryons formed by fusing transformed cells with permissive cells (*9*), a result that gave the first evidence that the viral functions are under the control of cellular functions. The provirus thus became a tool for studying regulation of DNA transcription in animal cells. Subsequently, the presence of giant RNA's containing viral sequences in the nucleus of transformed or lytically infected cells (*10*) raised the question of the initiation and termination signals for transcription in animal cells, as well as the question of processing of nuclear RNA precursors of messenger RNA, questions that are still largely unresolved.

Viral Functions in Transformation

In the meantime efforts were directed at identifying the viral genes transcribed in the transformed cells. It was established that in lytic infection with SV40 the whole viral DNA is transcribed in two nearly equal parts—one early, before the inception of replication of the viral DNA, the other late, after DNA replication has begun—and that the early RNA is also present in transformed cells (*6*). Subsequently, the early and the late messengers were found to be transcribed from different DNA strands (*11*), an observation that facilitated further characterization of the viral transcripts. Later work in other laboratories with specific fragments produced by restriction endonucleases confirmed and refined these findings, and the results were extended to adenoviruses by showing that a segment of the early part of that DNA is always present and transcribed in transformed cells (*12*).

These facts suggested that some early viral function is essential for maintaining the transformed state but they could also be interpreted differently: for instance, transformation might be caused by the mere presence of the viral DNA in the cellular DNA, the persistent viral functions being perhaps required for establishing and maintaining integration.

Attempts were made to solve the dilemma by isolating temperature-sensitive mutants affecting either initiation or maintenance of transformation. Many transformation mutants were found, with mutations all clustered in a segment of the early region of the viral DNA, designated as the A gene, but they were all initiation mutants (*13*). These mutations prevent the onset of transformation at high but not at low temperature, and cells transformed at low temperature remain transformed at high temperature. It was not possible to find clear-cut maintenance mutants, that is, mutants capable of causing a complete reversion of the phenotype when cells transformed at low temperature were shifted to high temperature. However, careful observation later showed that the initiation mutants were also partial maintenance mutants, since the cells they transform undergo a partial reversion of phenotype at high temperature (*14*). This result shows that the viral genes play a continuing role in transformation; however, the failure to obtain complete maintenance mutants suggests that the relation between viral gene expression and cell phenotype is complex.

Search for the Viral Transforming Protein

Further progress in this subject has been achieved by studying the proteins specified by the early region of the viral DNA. This work has centered around the so-called T antigen (*15*) present in the nucleus of cells infected or transformed by SV40; the synthesis and properties of this antigen are affected by mutations of the A gene (*16*). In nonpermissive transformed cells the antigen is a protein with molecular weight of about 94,000 daltons (*17*), which binds firmly to double-stranded DNA but without much specificity (*18*). That the T antigen is specified by the viral DNA is strongly suggested by its in vitro synthesis by a wheat germ extract primed with various messengers (*19*), especially since the size of the product depends on the nature of the messenger. Thus, when the messenger was viral RNA made in vitro by transcribing SV40 DNA with *Escherichia coli* RNA polymerase, an antigenic protein of about 62,000 daltons was synthesized; but when messenger RNA extracted from infected cells was used, the protein synthesized was, like the T antigen of transformed cells, of about 94,000 daltons. The discrepancy of the two molecular weights makes it very unlikely that the T antigen is a cellular protein modified by a viral function, because two different proteins would have to be modified in the same extract depending on the messenger used. In contrast, the synthesis of a shorter polypeptide chain with the artificial messenger may be justified by the absence of accessory signals, such as the special nucleotide sequence present at the 5'-end, known as "cap," polyadenylate at the 3'-end, and possibly other modifications. Further definition of these findings awaits peptide maps of the various products.

Since the early, transforming, part of the SV40 genome can specify proteins of a molecular weight of about 100,000 daltons altogether, the T antigen is likely to be its sole product and, therefore, to be the transforming protein. However, the same protein must also initiate viral DNA replication, which cannot begin at high temperature in cells infected by mutants of the A gene. The different functions in transformation and lytic infection could be performed by different domains of the same protein, or could result from modifications (such as phosphorylation and glycosylation) or from processing. Processing of SV40 T antigen seems to occur in lytically infected cells which contain a smaller T antigen of about 84,000 daltons; this smaller size contrasts with the regular size (94,000 daltons) of the antigen specified in vitro by messenger RNA extracted from the same cells (*17*). Whether the two forms of the antigen have different roles in transformation and DNA replication remains to be established.

Since the transforming protein should control both initiation and maintenance of transformation, the partial reversion of the phenotype of cells transformed by A mutants when shifted to high temperature may be explained by a decreased requirement for the transforming protein once transformation has taken place, which in turn could result from a positive feedback stabilizing the transformed state. For instance, unstable protein monomers specified by the mutated gene might form self-stabilizing oligomers (*20*), or the transforming protein might generate changes that tend to favor the transformed state. An example of the latter model is the β-galactosidase induction in *E. coli* which is maintained by inducer concentrations much smaller than that required for initiating induction, because inducer is pumped into the cells by the induced permease (*21*). I wonder whether a certain degree of self-stabilization of the state of gene expression is a general property of animal cells which has developed for maintaining differentiation.

Cellular Events in Transformation

I now turn to cellular events participating in transformation, which will be the main problem after the remaining questions on the role of the virus have been answered. Among the cellular events are functional changes and mutations. Some functional changes, which affect many cellular properties, are associated with the shift of resting cells to a growing state after infection with polyoma virus or SV40 (*22*); other changes observed in transformed cells and in cancer cells in general consist of the reexpression of cellular genes normally expressed in a preceding state of differentiation, in fetal life (*23*). These functional changes might be caused by the binding of transforming proteins to DNA; if so, they may be mediated by an alteration of transcription of the cellular DNA. However, we do not know whether the transcription pattern changes, because experiments based on competition hybridization have given ambiguous results. Perhaps the methodology is not good enough. Cloning of cellular DNA fragments in phages or plasmids may afford the necessary probes for carrying out significant experiments.

In order to understand further how the virus deregulates cellular growth we would need detailed knowledge of the mechanisms of growth regulation in animal cells, which is now lacking. However, certain useful ideas about growth regulation are now available, and can be used to draw inferences about the action of the virus. Thus it seems clear that with a given cell type, growth regulation involves a complex chain of events, beginning with extracellular regulators of many kinds, probably interacting with the cell plasma membrane. Cytoplasmic mediators then appear to transmit regulatory signals from the plasma membrane to the nucleus, where they perhaps control DNA-binding proteins similar to the transforming protein of papovaviruses. The complexity of growth regulation increases markedly when different cell

types are considered, since they seem to recognize different sets of extracellular regulators and may have different mediators and DNA-binding proteins.

Proceeding from this general picture it would be tempting to propose that the viral transforming protein replaces one of the normal nuclear regulatory proteins of the cell and, being unaffected by the mediators that control the normal protein, keeps growth-related transcription going, bypassing the signals of the plasma membrane. If so, however, the transformed state should be dominant over the normal state in cell hybrids, whereas the contrary is usually true (*24*). On the other hand, the dominance of the normal state could be explained if the transformed cells had a changed surface, unable to respond to regulatory signals. Such a change could result from the reexpression of fetal functions to make the transformed cells anachronistic, that is, belonging to a stage of differentiation inappropriate to that of the organism which contains them. The cells with an anachronistic surface, being insensitive to the growth regulators which operate on adult cells in the adult organisms, would grow without control. A striking support of the role of cell anachronism in cancer has been obtained with teratoma, a tumor originating when cells from an early embryo are transplanted to an adult environment. When, after many transplants, cells of this tumor are introduced back into a blastocyst (an early embryo), they return to normal (*25*), presumably because the internal growth control of the cells becomes again matched by the environmental regulators of the recipient embryo. In this model a hybrid cell formed by fusing a transformed and a normal cell may be untransformed if the normal partner contributes normal surface components which respond to the normal extracellular regulators. For this result to be possible, anachronistic transcription after cell fusion should not be initiated on the DNA deriving from the normal parent. The virological studies suggest that this may well be the case, since the initiation of transformation seems to require much more transforming protein than its maintenance.

It would be important to recognize the developmental period in which the anachronistic genes of transformed or cancer cells are normally expressed, not only for understanding but possibly also for controlling cancer. In fact, if the growth regulators specific for the periods expressed in cancer cells could be identified, they could be used for halting the growth of the cancer cells.

Role of Cellular Mutations

I will now consider the other cellular events important in viral transformation: cellular mutations. Several results suggest that cellular mutations may be needed for obtaining the full state of transformation with papovaviruses. Thus, after infection primary cultures generate clones with various degrees of transformation, some of which appear to undergo full transformation in steps (*26*) that may correspond to the occurrence of cellular mutations. Cells that achieve full transformation immediately, as is common with permanent lines, may have already undergone similar mutations before infection. Some cellular mutations occurring in transformed cells may even be virus-induced, because in the early stages of transformation by papovaviruses cells of primary cultures have frequent chromatid breaks (*26*). Conversely, cells fully transformed by SV40 can revert to a relatively normal phenotype although they still contain normal viral DNA and T antigen (*27*). It is conceivable that these mutations are reversions of mutations of the former kind, which enhance the transformed state of the cells. Stepwise transformation may occur not only with viruses. Thus I have observed it in primary cultures exposed to a chemical carcinogen. In this experiment fully transformed cells evolved from the normal cells, which have limited life. The normal cells first generate cells with unlimited life but unable to form colonies in agar, then cells with progressively increasing colony-forming efficiency in agar, and finally cells that reach 100 percent efficiency.

All these observations show the important role of cellular mutations in cell transformation induced by different agents. This conclusion is reinforced and generalized by additional findings, such as (i) the experimental enhancement of the transforming activity of viruses by mutagenic agents (*28*); (ii) the elevated cancer frequency in some genetic diseases; and (iii) the evidence that most carcinogens are promutagens, that is, generate mutagenic substances when acted upon by normal metabolism (*29*). Most of the carcinogens themselves must be activated by metabolism in a similar way in order to induce cancer.

Prospects for Cancer Prevention

I now turn to some general deductions concerning the etiology and possible prevention of human cancer which derive from the various points I have discussed so far. One deduction, deriving from the persistence of the viral DNA in the cells, is that we can test whether a given DNA virus is a possible agent of human cancer by looking for its DNA in the cancer cells. I think that much more extensive surveys than those carried out so far are warranted, but they should have a sensitivity sufficient to detect fragments of viral DNA of about 1 million daltons, which is within the reach of modern technology, even with the most difficult viruses. A positive finding would be significant because DNA viruses do not appear to exist in widespread endogenous forms.

Another deduction is that somatic mutations are one of the fundamental ingredients of cancer, although they appear to require the occurrence of several other events not yet understood. The role of mutations in turn suggests that the incidence of cancer in man could be reduced by identifying as many promutagens as possible, and by eliminating them from the environment. One important feature of this approach to cancer prevention is that it can be started now, since these substances can be identified with simple bacterial tests suitable for mass screening (*30*). The feasibility of prevention is shown by the fact that the promutagens already identified in a preliminary screening, such as tobacco or some hair dyes, are inessential for human life (*31*).

However, it is practically difficult to achieve a substantial reduction of the use of these substances, as shown by the example of tobacco. According to epidemiological evidence, tobacco smoke is the agent of human lung cancer, which in Britain is responsible for one in eight of all male deaths (*32*). Yet only mild sanctions have been imposed on tobacco products, such as a vague health warning on cigarette packets which sounds rather like an official endorsement. Any limitation on the use of tobacco is left to the individual, although it is clear that the individual cannot easily exercise voluntary restraint in the face of very effective advertisements, especially as one does not usually appreciate the danger of a cumulative action over a long period of time.

The lax attitude of governments toward tobacco probably also derives from the difficulty of appreciating epidemiological evidence, especially since this evidence is contradicted from time to time by single-minded individuals who use incomplete or even erroneous analyses of the data and whose views are

magnified out of all proportion by the media. However, the recent recognition that tobacco smoke contains promutagens contributes direct experimental evidence on the dangers of tobacco smoke, evidence on which there cannot be any equivocation. I, therefore, call on governments to act toward severely discouraging tobacco consumption, and to act now because it will be at least 30 years before their action has its full effect.

Although tobacco smoke is a striking example of an environmental carcinogen, many others are known and probably many more remain to be identified. Identification by conventional tests is difficult because they are costly and laborious, but they can now be replaced by the bacterial tests for promutagens. Since the tests are easy and inexpensive it should be possible to investigate many normal constituents of the environment, and every new compound before it is offered to the public. The feasibility of such a program is borne out by the finding that most of the commonly available substances are not promutagens (*31*). Given the strong correlation between mutagenicity and carcinogenicity (29), any promutagen is suspect and, if at all possible, should be withdrawn.

In fact, this is precisely the attitude that scientists have taken for themselves concerning the experiments in genetic engineering, which carry the theoretical possibility of creating new viruslike molecules endowed with carcinogenic activity. Although the danger is only hypothetical, experiments that might be very useful for science and society have been postponed until they can be carried out under the strictest safeguards (*33*). Governments have accepted this position and are eager to impose severe restrictions on the performance of these experiments. While I fully approve of their concern, I cannot help noticing that they follow a double standard: if there is any doubt you must discourage experiments, but if there is any doubt you cannot discourage cigarettes.

Biologists and Society

This discussion about cancer prevention is a development of the experimental results obtained in the field of oncogenic viruses, but it is also strongly influenced by the new social conscience of many scientists. Historically, science and society have gone separate ways, although society has provided the funds for science to grow and in return science has given society all the material things it enjoys. In recent years, however, the separation between science and society has become excessive, and the consequences are felt especially by biologists. Thus, while we spend our life asking questions about the nature of cancer and ways to prevent or cure it, society merrily produces oncogenic substances and permeates the environment with them. Society does not seem prepared to accept the sacrifices required for effective prevention of cancer. The situation is clearly unacceptable, and we biologists would like to see it corrected. We have ourselves begun to put our house in order, by banning some experiments that may contain a risk for mankind. We would like to see society take a similar attitude, abandoning selfish practices that are dangerous for society itself. We would also like to see a new cooperation of science and society for the benefit of all mankind and hope that the dominant forces in society will recognize that this is a necessity.

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How the Liver Metabolizes Foreign Substances

Among the most significant of the liver's chemical transformations are the inactivation of drugs, the detoxification of environmental pollutants and the activation of chemicals that can cause cancer

by Attallah Kappas and Alvito P. Alvares

The intensity and duration of the action of most drugs is determined in large part by their rate of metabolism. If nothing else happened to a drug after it entered the body and reached its target organ, for example, it might continue to act indefinitely. Something does happen, however: most drugs are transformed into inactive substances and then excreted. The biotransformation can occur in any of several tissues and organs. Some drugs are transformed chemically in the intestine, some in the lung, the kidney or the skin. By far the greatest number of these chemical reactions are carried out in the liver, which metabolizes not only drugs but also most of the other foreign chemicals to which the body is exposed. Biotransformation in the liver is therefore a critical factor not only in drug therapy but also in defending the body against the toxic effects of a wide variety of environmental chemicals such as insecticides, herbicides, dyes, food preservatives and a number of substances that are suspected of inducing cancer. The central step in the metabolism of most of these agents involves an oxidation reaction mediated by a complex of enzymes that has come under intensive study in recent years in our laboratory at the Rockefeller University Hospital and in other laboratories.

The liver is the largest organ in the body (it weighs about three pounds in an adult) and has diverse functions. It serves, first of all, as the primary receiving depot, chemical-processing plant and distribution center for almost everything that enters the body through the walls of the alimentary canal. All the blood that has absorbed digested food and other substances from the intestines enters the liver through the large portal vein, which ramifies into fine channels through which the blood perfuses slowly among the liver cells. Here nutrients and other foreign substances are removed, metabolized, in some cases stored and then released into the general circulation. Amino acids, for example, are made into proteins and other nitrogenous compounds; glucose is converted into glycogen and stored, to be converted back into glucose and released as required. And drugs and other toxic substances are detoxified. Not everything is metabolized on the first passage of blood through the liver, of course; drugs, for example, are given in doses such that a sufficient amount of the drug moves through the liver to its site of action and is transformed later, on return visits to the liver [*see illustration on page 25*]. The liver also produces bile, which is a secretion that aids in the digestion of fats when it is released into the small intestine and is also a vehicle for the excretion of transformed substances and other waste products of metabolism.

The biotransformation of drugs and other foreign compounds in the liver is accomplished by several remarkable enzyme systems that can metabolize a wide variety of structurally unrelated drugs, toxic agents and environmental pollutants, which enter the body primarily through ingestion but also through the lungs and the skin. The enzyme systems are built into the membranes of the endoplasmic reticulum of the liver cells, a network of interconnected channels that is present in the cytoplasm of most animal cells. There are two kinds of endoplasmic reticulum, rough and smooth, and they differ in both form and function. The surfaces of the rough membranes are studded with ribosomes, small granules that translate the genetic code into the sequences of amino acids that constitute proteins. The smooth membranes have no ribosomes. In the liver a major function of both kinds of membrane is to assemble the enzymatic complexes that transform foreign substances and then to serve as the site of those transformations. Unlike some other cellular subsystems, the endoplasmic reticulum cannot be separated from cells as an intact structure. If liver cells are homogenized and then centrifuged, the tubular reticulum breaks up and bits of the membranes are sealed off to form the tiny vesicles, or sacs, called microsomes. The microsomal fraction thus obtained from liver cells is a convenient natural source of enzymes for laboratory studies of liver-cell metabolism.

Drugs and other foreign compounds are metabolized in the liver by a rather small number of reactions: oxidations, reductions, hydrolyses and conjugations. Their essential effect is to convert lipophilic, or fat-soluble, compounds into hydrophilic, or water-soluble, ones. The hydrophilic compounds are the more readily removed from the blood by the kidneys and excreted.

Oxidation accounts for most of the transformations, largely because there are so many different ways in which a compound can be oxidized [*see illustration on pages 28 and 29*]. The alkyl side chains of barbiturates and some other drugs, for example, are oxidized to form alcohols. In the case of compounds incorporating aromatic rings, including polycyclic hydrocarbons (such as those in cigarette smoke) and many drugs, a hydroxyl group is inserted into the ring.

accomplished as a result of the establishment of certain new rural practices.

The first of these is related to household economics at the production-team level. Individual household members receive a share (in money and produce) of the annual earnings of their team; the share is proportional to the number of work points each has earned during the year. For example, a 70-year-old grandmother in a suburban Shanghai commune told me that she and nine of the other 10 members of the household worked regularly in the fields. The only nonproducer was a five-year.

The family received a monthly advance of the equivalent of $80 against joint annual earnings. In 1973 share of the team's income after operating expenses and taxes were deducted was $2,100, or about $200 per person.

Withholding for taxes and expenses and to provide funds for reinvestment absorbs about half of the gross income of a commune. For example, the manager of the suburban Rainbow Bridge Commune in Shanghai told us that 30 percent of the commune's gross income was required to meet the cost of its agricultural endeavors. Another 11 percent went into a fund for capital reinvestment, 4 percent was paid to the government as tax and 3 percent went into a welfare fund for the support of new mothers and the elderly. That left 52 percent of the gross income for distribution to individuals in proportion to the work points earned.

Such a work-point system simultaneously accomplishes two objectives. It provides a strong incentive for increased overall output in order to maximize the amount that is divided among the individuals in a household. At the same time it encourages householders and production teams alike to keep their numbers from increasing, so that the maximum earnings are divided into the fewest possible shares. The latter constraint, of course, places the disadvantages of population growth squarely before each individual. This economic consideration must generate substantial social pressure in favor of small families.

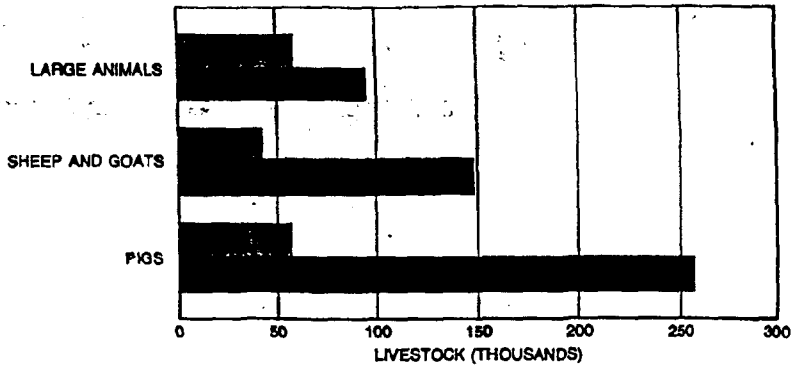
Other growth-limiting factors are at work. As one example, it is said that improved health care has greatly reduced infant mortality, thus undercutting the validity of the traditional view that many births are needed to ensure the survival of even one offspring to maturity. Another example is the current program of providing care for the aged, one of the objectives of the 3 percent welfare withholding reported by the Shanghai commune. We visited one such residence for the elderly, maintained by a commune near Peking. Normally, we were told, each family cared for its own elders. Nonetheless, some individuals inevitably lost their families or became separated from them; that was the case with the 75 residents we saw. They were encouraged to engage in handicrafts; meanwhile they were housed, clothed and fed at commune expense, and a clinic attached to the residence looked after their health needs. Custodial care of this kind, if it were widely practiced, would diminish the validity of another traditional view that favors multiple births: the view that one needs to have many children in order to ensure one's welfare in old age.

A third growth-limiting factor that may even now be undergoing a crucial test is the continued existence of private landholdings throughout rural China. We were told that when the commune system was established in the late 1950's, between 5 and 7 percent of the arable land in each commune was set aside for private use. The land was parceled out at a fixed rate: each adult in a household was assigned one 150th of a hectare. The household children received private land too, but only the two eldest children in each household were eligible. If a household had more than two children at the time of the land assignment, or if more children arrived later, those children received no share. In such a family there would simply be less private land per person. In its early days the allocation system probably had relatively little effect on rural family planning. Today, nearly a generation later, the system must act as a powerful social force favoring small families.

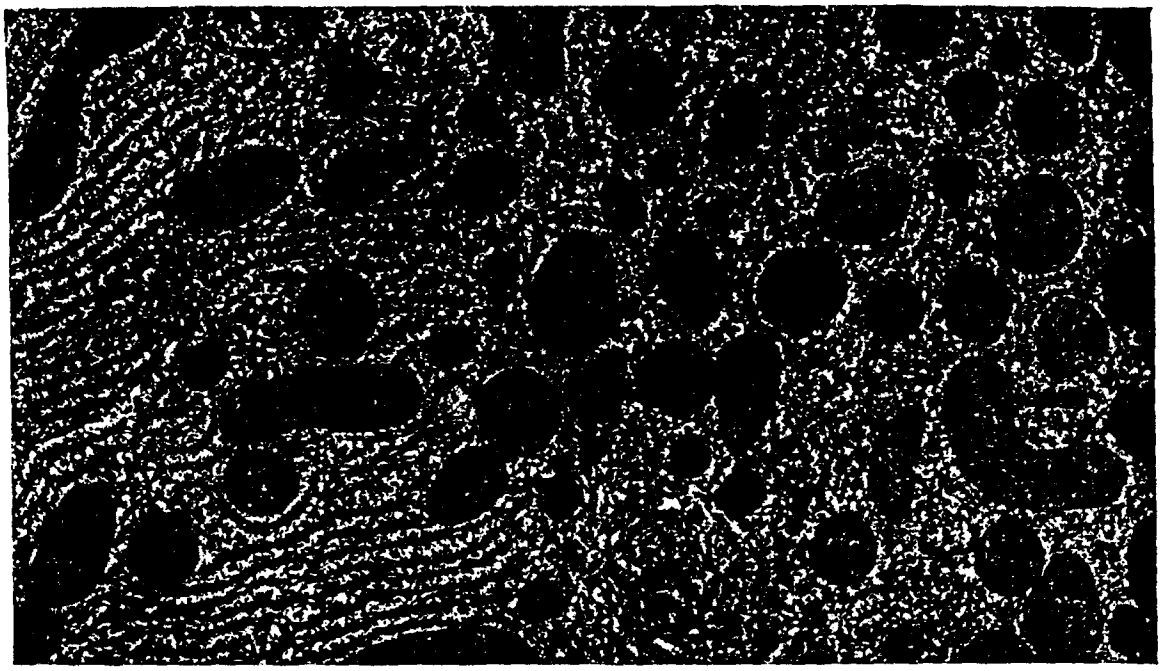
In summary, with respect to agricultural production in China now and in the near future our group was inclined to accept the government's assertion that self-sufficiency was achieved in 1971, when some 250 million metric tons of rice, wheat and other major foodstuffs were harvested. Furthermore, given a continuation of the present aggressive and coordinated effort it seems that China will be able to achieve substantial increases in agricultural production over the next decade. Whether the increase for any particular crop will be as little as 20 percent or as much as 50 percent will probably prove to be a function of present yields. For example, rice yields are already high, so that an increase of 50 percent in the annual harvest will be much harder to achieve than a 50 percent increase in the maize or sorghum harvest.

China nonetheless faces serious long-term problems. If the country's remarkable agricultural advance is to continue, two of these problems must soon be resolved. The first problem lies in the current Chinese policy that emphasizes applied and decentralized agricultural research for the sake of immediate increases in food production. Although the short-term benefits of this policy are obviously important, the policy must be complemented in the near future by similar emphasis on more basic scientific investigation.

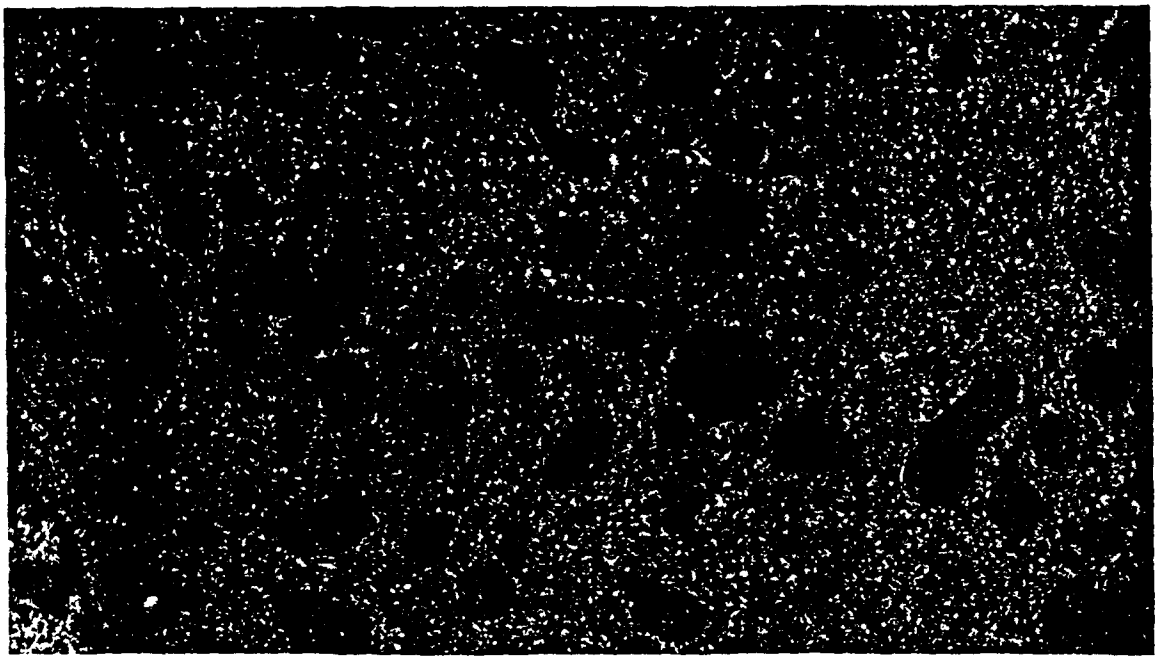
The second problem has to do with the government's remarkably comprehensive efforts to retard the growth of China's population. Only if this policy meets with success will the Chinese people continue to receive the benefits of increased agricultural production and the accompanying advances in living standards that so many of China's people now seem to expect.



LIVESTOCK IN CHINA are compared according to categories; estimates, made by the U.S. Department of Agriculture, are for 1949 (*gray*) and 1972 (*color*). Sheep and goats are largely confined to grasslands and mountains in the west and northwest. That is also true of range cattle, lumped together here with large draft animals: horses, mules, donkeys and water buffaloes. Pigs are by far the most numerous of all the domestic mammals in China.



ENDOPLASMIC RETICULUM of a rat liver cell is enlarged 18,000 diameters in this electron micrograph made by Edward S. Reynolds of the Harvard Medical School. Most of the biotransformations of foreign substances take place in this system of membranous tubules. The "rough" endoplasmic reticulum is covered with ribosomes, the structures in which proteins are synthesized; it appears here as fairly linear double membranes studded with black dots. The "smooth" endoplasmic reticulum lacks ribosomes and forms a more branching, tubular network; there are patches of it between the mitochondria, the fine-grained gray objects, at right.

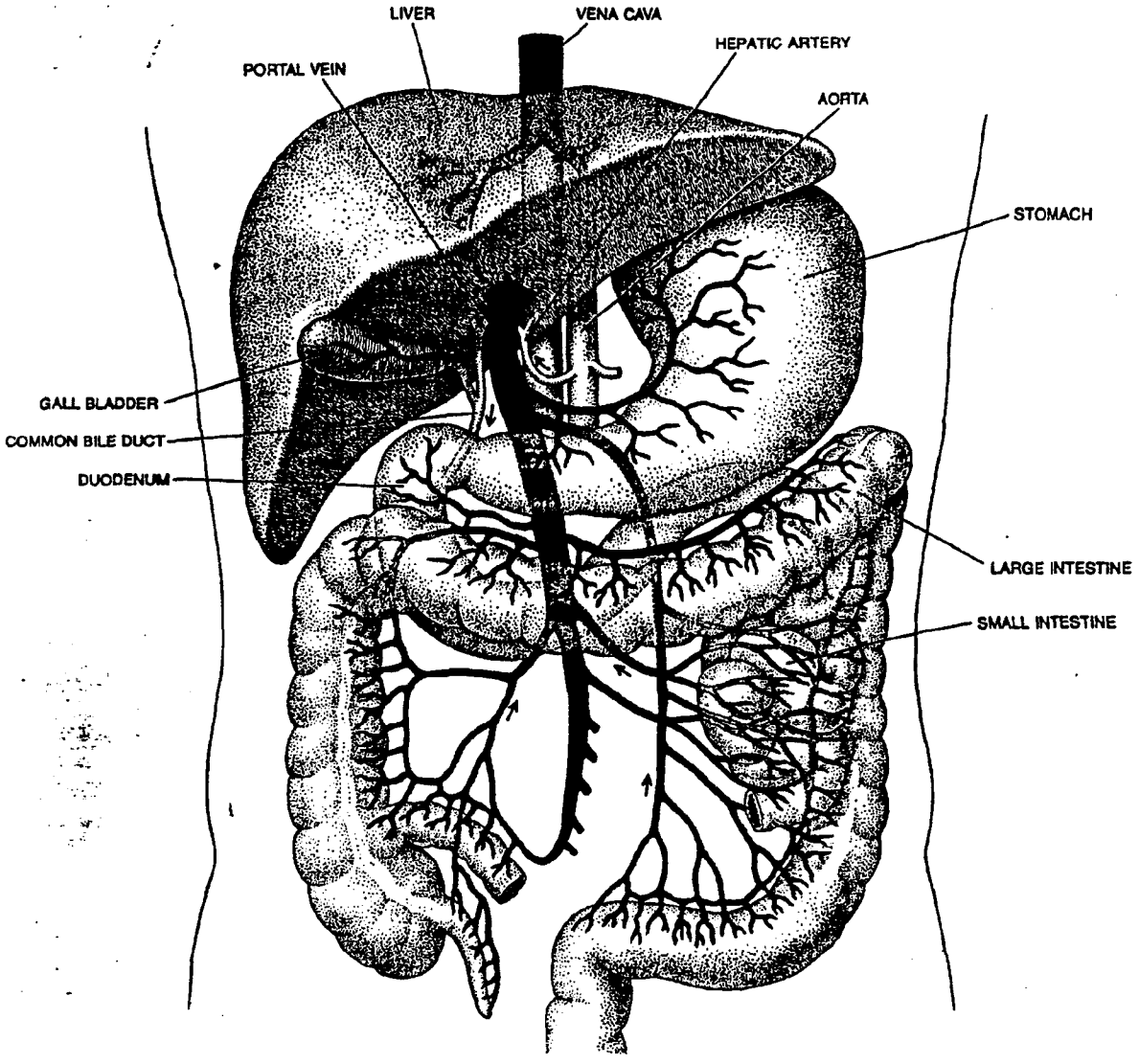


SMOOTH ENDOPLASMIC RETICULUM proliferates when the synthesis of the enzyme systems it contains is stimulated by drugs. This micrograph, also made by Reynolds and reproduced at the same scale as the one at the top of the page, is of a cell from the liver of a rat that was treated with phenobarbital. The drug stimulates the synthesis of cytochrome *P*-450, an enzyme located in the endoplasmic reticulum membranes. The cytoplasm of the cell has expanded because of a striking increase in the smooth endoplasmic reticulum membranes, which now fill most of the space between the more dispersed mitochondria. Enzyme induction by one drug can significantly affect the metabolism, and thus the activity, of other drugs that are metabolized by the same enzymes.

In other cases alkyl groups are removed from either nitrogen or oxygen atoms, amino groups are removed or sulfoxides are formed. Reduction and hydrolysis are also catalyzed by liver enzymes, but these reactions are less common than oxidation.

Conjugation of a chemical is combination with some natural constituent of the body such as the glucose derivative glucuronic acid, the amino acid glycine or the tripeptide glutathione. In the presence of the appropriate enzyme these natural agents can combine readily with compounds that have carboxyl (COOH), sulfhydryl (SH), amino (NH_2) or hydroxyl (OH) groups. Some drugs have these groups when they are in their active form and are handled in the liver by conjugation; for most drugs, however, conjugation is a second step that comes after metabolism by oxidation, reduction or hydrolysis. Almost without exception the conjugated compound is devoid of pharmacological (or any biological) activity.

Clearly the enzyme systems that catalyze these reactions were not invented by the mammalian organism in order to cope specifically with drugs or novel pollutants. Their basic physiological role has presumably been to metabolize endogenous substrates: substances normally present in the body. For example, liver microsomal enzymes oxidize steroid hormones, cholesterol and fatty acids. The products of these oxidations may then be conjugated (with glucuronic acid, for instance) and excreted. Bili-



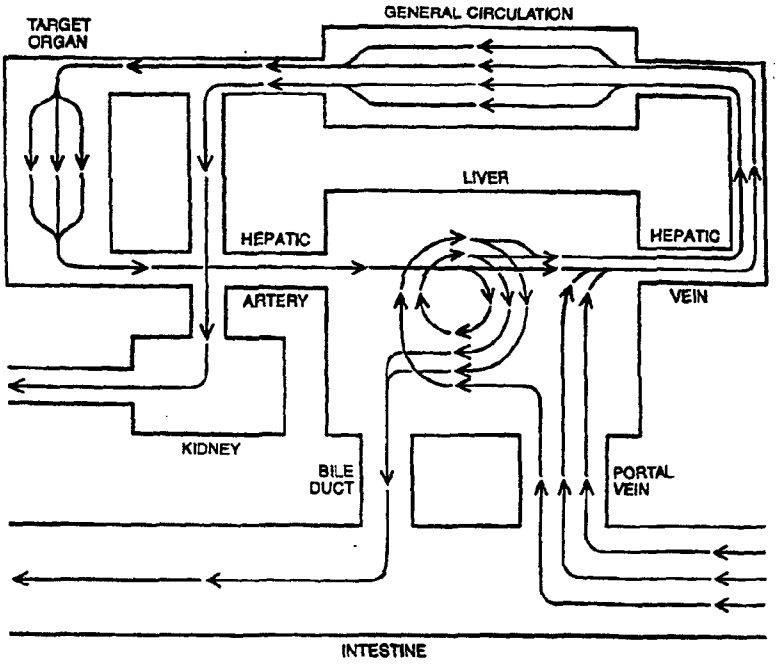
LIVER is a primary site of metabolism of substances entering the body through the alimentary tract. All the fine blood vessels that absorb nutrients and other substances through the wall of the intestines come together and enter the liver through the portal vein. The liver's supply of oxygenated blood from the heart enters through the hepatic artery. After passing through the sinusoids of the liver (*see illustration on page 26*) the blood is collected by the hepatic veins, which feed into the vena cava. The liver secretes bile, which is collected by the bile duct, stored in the gall bladder and emptied into the duodenum, the first segment of the small intestine.

rubin, a product of the oxidation of heme, the red pigment of hemoglobin, is an example. It is normally prepared for excretion by glucuronide conjugation. The rate of formation of glucuronides is generally low in newborn infants, however, because of a deficiency of the enzyme glucuronyl transferase. As a result bilirubin may not be conjugated and excreted at an adequate rate. Excessive amounts of it can then accumulate and cause grave damage to the brain, a condition called kernicterus.

It has been apparent for some time that the human fetus and the newborn infant are far more sensitive than adults to many drugs. A number of drugs can pass across the placenta, so that obstetricians need to exert care in administering them to an expectant mother. Barbiturates or morphine given to a woman during childbirth can be stored in the infant's tissues and cause respiratory depression and occasionally death. The explanation for the sensitivity of infants to drugs has emerged from a number of reports in recent years on the maturation of the capacity to metabolize and conjugate drugs. These studies make it clear that the capacity to oxidize and conjugate is negligible in the mammalian fetus and newborn animal and increases after birth at a rate that varies with the species, the type of reaction and the drug. Impaired drug metabolism increases the intensity and duration of drug action. For example, newborn mice treated with a dose of the hypnotic drug hexobarbital equivalent to 10 milligrams per kilogram of body weight sleep more than six hours, whereas adult mice given 10 times as large a dose sleep less than an hour.

The adverse effect of drugs on newborn infants as a result of inefficient metabolic conversion is illustrated by the striking "gray-baby syndrome" in infants that may come a few days after treatment with the antibiotic chloramphenicol: abdominal distention, respiratory difficulty, cyanosis (blue skin color as a result of insufficient oxygenation of the blood) and shock. The condition is apparently the result of deficient metabolism of the antibiotic and deficient conjugation with glucuronic acid. In adults about 90 percent of the antibiotic is excreted in the urine in the form of conjugated metabolites within 24 hours after it has been orally administered; only a small amount is excreted unchanged. In comparison a 10-day-old infant in one study excreted less than 50 percent of the drug in 24 hours.

Rates of drug metabolism are very different in different species, and the effective dosage varies accordingly. The anti-inflammatory agent phenylbutazone is metabolized slowly in man; its half-life in the plasma averages about three days. In the horse, the dog, the rabbit, the rat and the guinea pig, however, the drug is metabolized much more quickly and the half-life ranges from three to six hours. (Knowledge of the rapid rate of metabolism of phenylbutazone in the horse is of practical importance because the drug is administered to treat arthritic conditions in racehorses.) A dose of hexobarbital (adjusted for the body weight of the animal) that makes mice sleep for an average of 12 minutes puts rabbits to sleep for 49 minutes, rats for 90 minutes and dogs for 315 minutes. When the enzymatic oxidation of hexobarbital by microsomes from the liver of these animals was measured, the fastest oxidation was carried out, as expected, by microsomes from the mouse; the rates of oxidation by microsomes from the rabbit, the rat and the dog were proportionately lower. Differences in the rates and patterns of drug metabolism in the various animals seem to explain most of the species differences in effect, but there may also be differences in the distribution of the drug, the response of the target tissues and excretion.



PATHWAY of a drug that is transformed in the liver is shown schematically. The drug (*colored arrows*) enters the liver through the large portal vein, passes into the general circulation, has its effect on the target organ and eventually returns to the liver. On each passage through the liver a fraction of the drug is converted, usually into inactive metabolites (*black arrows*). The metabolites may be carried by the bile into the intestines for excretion or may pass through the circulation to the kidneys, to be excreted into the urine.

Even among human patients there are marked individual variations in the metabolism of drugs that are handled primarily by microsomal enzymes. The variability causes some patients to metabolize a drug so quickly that therapeutically effective blood and tissue levels are difficult to achieve and others to metabolize the drug so slowly that they suffer toxic effects. It can therefore be difficult for the physician to predict just what dosage of some drugs will provide a safe and therapeutic effect in an individual patient. Very large individual differences have been noted in the metabolism of the coumarin-derivative anticoagulant drug Dicumarol; the half-life can vary from seven to 74 hours. That makes it hard to predict how much of the drug will provide the desired anticoagulant effect in a patient. Marked variations have also been observed in the metabolism in man of phenylbutazone and of diphenylhydantoin, a drug that is administered to control epileptic seizures.

Genetic factors may play an impor-

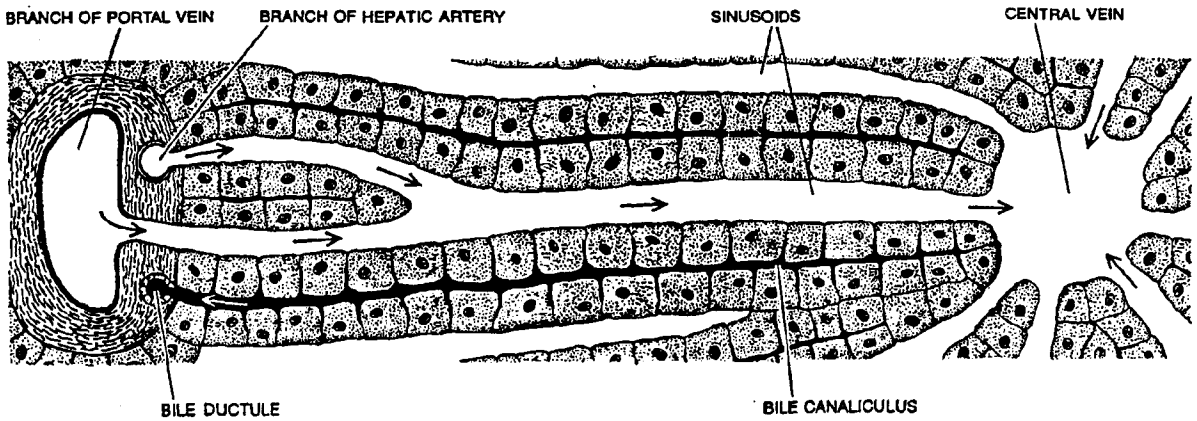
tant role in the metabolism of drugs, as Elliot S. Vesell of the Pennsylvania State University College of Medicine has shown. The large individual differences in Dicumarol half-life persist to some extent when fraternal twins are compared but almost disappear in identical twins, and similar results have been reported for other drugs studied in twins. The antituberculosis drug isoniazid has been the subject of a number of investigations covering different populations. Isoniazid is metabolized in man primarily by an acetylation reaction catalyzed by the enzyme *N*-acetyl transferase. Soon after the drug was introduced it became apparent that individuals vary in their ability to acetylate it. The distribution of the acetylation rates for a group of subjects tends to be bimodal, that is, plotting the rate against the number of patients exhibiting each rate yields a curve with two peaks. Apparently there are two classes of individuals: those who acetylate isoniazid rapidly and excrete the drug primarily as acetylisoniazid and those who acetylate and excrete the drug more slowly and excrete more of it in an unchanged form. The frequency of rapid inactivators is about 90 percent in a population of Eskimos or of Japanese, whereas among both whites and blacks in North America there are about as many slow inactivators as there are rapid ones.

A clear relation has now been established between this variation in acetylation rate and the incidence of isoniazid toxicity. Several studies have shown that the slow inactivators are more susceptible to a toxic effect of isoniazid: a disorder of the peripheral nerves caused by a specific vitamin B_6 deficiency resulting from an interaction of the drug and the vitamin.

The first indication that microsomal enzymes were special kinds of complexes was developed some 20 years ago by Gerald C. Mueller and James A. Miller at the University of Wisconsin. They discovered that compounds known as aminoazo dyes could be oxidized (specifically, *N*-demethylated) by liver microsomes and that the oxidation required molecular oxygen (O_2) and the coenzyme NADPH (reduced nicotinamide-adenine dinucleotide phosphate). Soon afterward Bernard B. Brodie's group at the National Heart and Lung Institute showed that there was a similar requirement for the oxidative metabolism of a number of drugs. In 1957 Howard S. Mason of the University of Oregon Medical School proposed that such oxidations are catalyzed by a class of "mixed-function oxidases": enzyme complexes that require oxygen and NADPH, are nonspecific (they catalyze the oxidation of different kinds of compounds) and catalyze the consumption of a molecule of oxygen for each molecule of the drug or other substrate, with one atom of oxygen appearing in the metabolized substrate and the other atom usually combining with two hydrogen atoms to form water.

The key enzyme of these oxidases is cytochrome *P*-450. A cytochrome is a complex of protein and heme, the iron-containing ring structure that is the oxygen-binding component of hemoglobin. Like hemoglobin, the various cytochromes serve to bind oxygen, which they deliver to their substrates in such processes as cell respiration. Cytochrome *P*-450 gets its designation from the fact that in the reduced form it binds carbon monoxide and then absorbs light most intensely at a wavelength of 450 nanometers. The amplitude of the absorbance peak is the basis of quantitative studies of the enzyme. In the mixed-function oxidases cytochrome *P*-450 serves as the terminal oxidase: it accepts electrons passed along by several intermediates, binds oxygen and then delivers the oxygen to oxidize its substrate and (usually) produce water [*see illustration on page 30*]. In addition to NADPH the system includes the enzyme cytochrome *P*-450 reductase. Another heme protein, cytochrome b_5, is present in liver microsomes and may participate in drug oxidations, but its precise function is not clear.

Nor is it known just how the various components of the mixed-function oxidase complex are arrayed in the tubular membranes of the endoplasmic reticulum. It appears that the reductase and cytochrome b_5 are on the outside of the membranes, with cytochrome *P*-450 in the deeper layers. The enzyme glucuronyl transferase, which catalyzes the most important conjugation reaction, is also present within the membranes. The drug or other fat-soluble compound to be transformed is presumably bound and



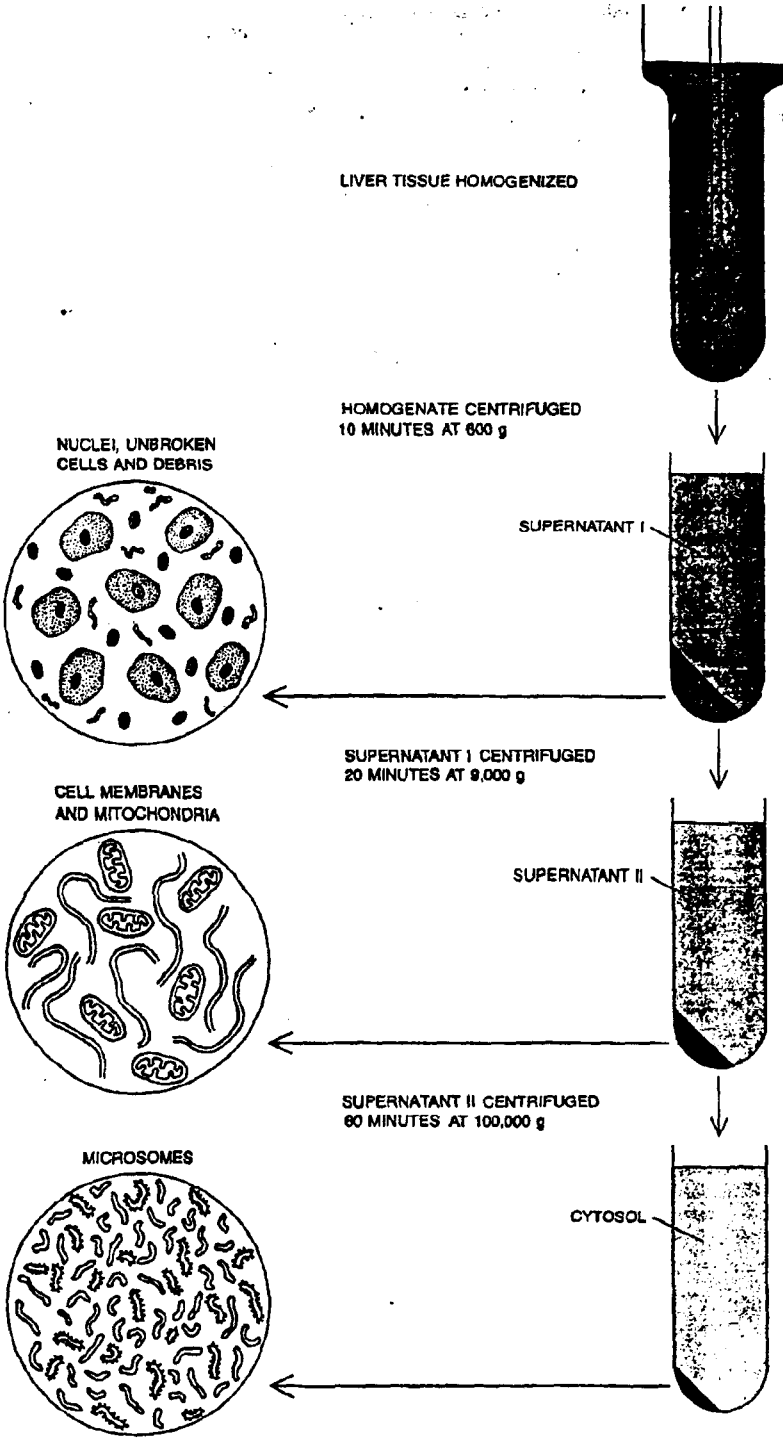
LIVER LOBULE, the functional unit of liver tissue, is defined by branches of the portal vein, the hepatic artery, the bile duct and lymphatic vessels, which run together through the tissue, outlining the lobules. (The lymphatics are not shown here.) The venous blood, with its nutrients and other ingested substances, and the oxygenated arterial blood enter the fine sinusoids and perfuse the liver cells, which carry out the metabolic functions discussed in the text. The blood drains into a central vein in each lobule and thence to sublobular and hepatic veins. The liver cells also secrete bile, which is collected by bile canaliculi that feed into bile ductules and eventually into the main bile duct. This two-dimensional view of part of a lobule is highly simplified and diagrammatic.

metabolized by the mixed-function oxidase system that contains cytochrome *P*-450 and then is often converted into a highly water-soluble compound by conjugation with glucuronic acid; the transformed product passes into the lumen, or central channel, of the tubular membranes and is excreted from the cell into the bile or the bloodstream.

The cytochrome *P*-450 mixed-function oxidase system has come to be recognized as having a central role in the body's defense against chemical agents, whether they are normal body constituents or are introduced from the environment. It is now clear that the system is responsible for the detoxification of many of the potentially harmful environmental pollutants. The system is highly inducible, that is, its activity can be greatly increased by exposure to a wide variety of environmental agents and drugs that act as substrates for the system. Such chemicals stimulate the synthesis of cytochrome *P*-450 and other components of the complex. More than 200 steroid hormones, drugs, insecticides, carcinogens and other foreign chemicals are now known to stimulate drug metabolism in experimental animals, and many of them have been shown to do the same thing in man. On the other hand, some substances (such as lead and other heavy metals) inhibit the mixed-function oxidase system, although they are fewer in number and less diverse than the inducers. The *P*-450 system is also the site of much competitive interaction among drugs and other chemicals that are undergoing transformation.

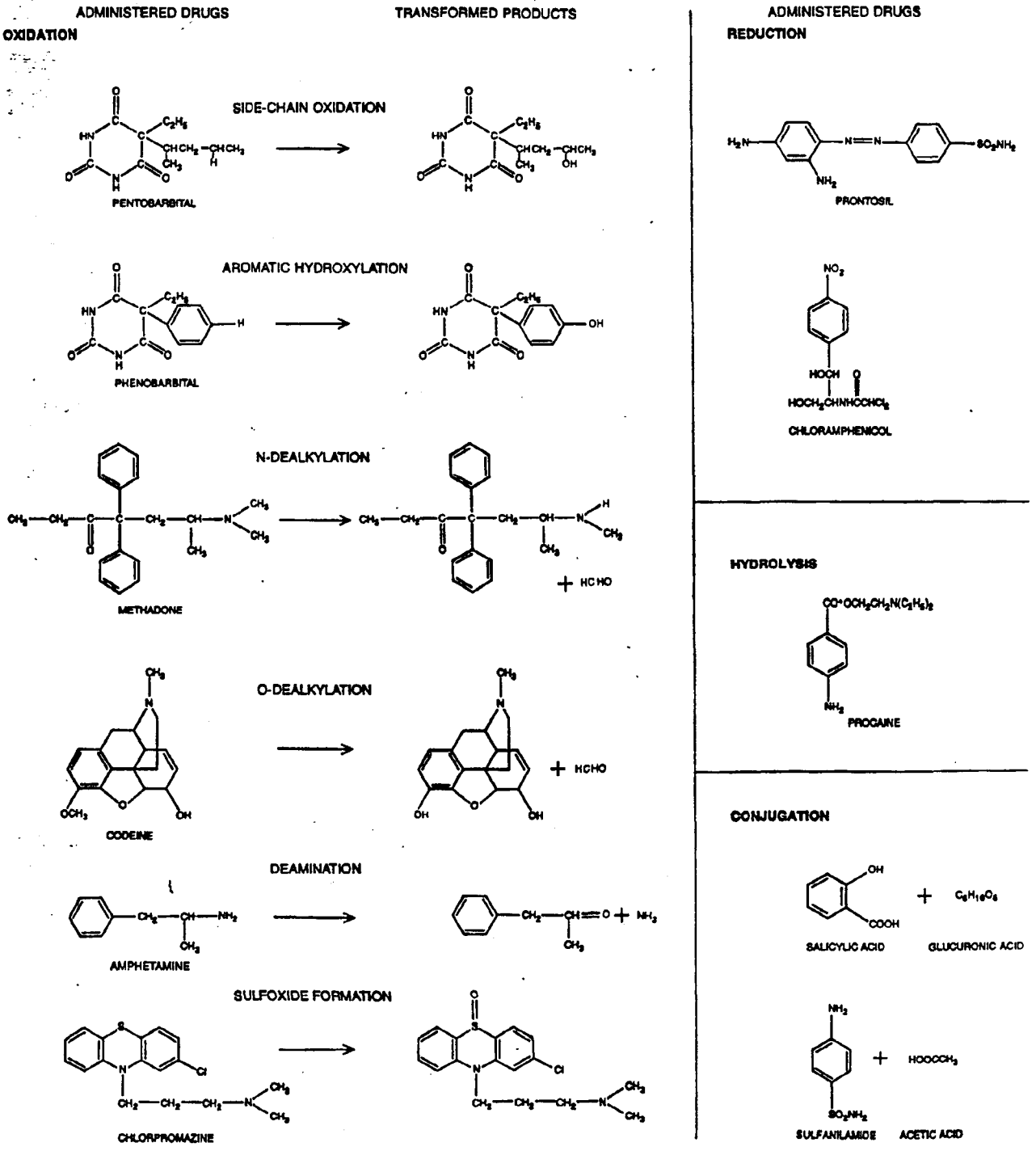
The consequences of this inducibility, inhibition and competition have important implications in drug therapy. Patients are often given several drugs at the same time. Certain combinations can have unpredictable and often undesirable effects if one drug inhibits or stimulates the metabolism of another or competes with it. For example, phenylbutazone, the coumarin anticoagulants and chloramphenicol compete with the metabolic inactivation of tolbutamide, a drug given to reduce the blood-sugar level in diabetics; the competition can lead to excessive tolbutamide activity and thus to serious hypoglycemia, or low blood sugar.

Phenobarbital is a prime example of a drug that has a different effect: enzyme induction. When rats are treated with phenobarbital, there can be a three- to fourfold increase in the microsomal content of cytochrome *P*-450 and a two-



LIVER MICROSOMES, the membrane structures that contain most of the liver enzymes engaged in detoxification, are isolated by spinning homogenized liver tissue at successively higher speeds, which produce successively higher gravity (*g*) forces, in a centrifuge. Homogenized liver tissue is centrifuged for 10 minutes at 600 *g*; a pellet of dense material, primarily whole cells, cell debris and cell nuclei, collects at the bottom of the tube. The supernatant, or liquid portion, is centrifuged more strongly, isolating less dense structures such as mitochondria and pieces of membrane. When supernatant II is spun at very high *g* forces in an ultracentrifuge, the microsomes are separated from the cytosol, or cell fluid.

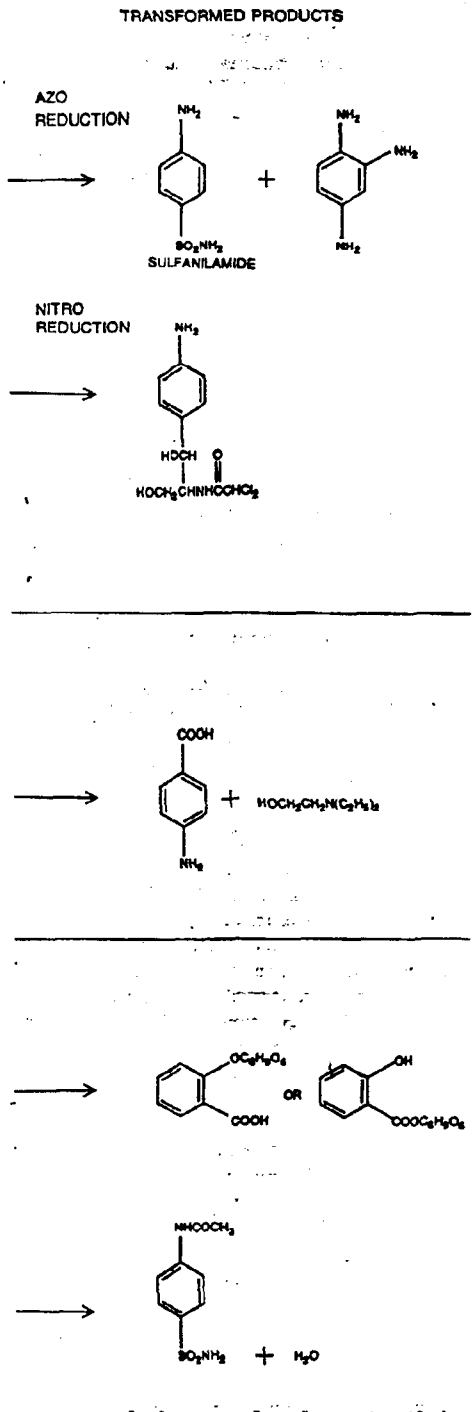
fold increase in the reductase, and drugs such as methadone and ethylmorphine are metabolized three or four times as fast. In accordance with these experimental observations chronic administration of phenobarbital to patients decreases the effects of many drugs by hastening their inactivation. Sedative doses of the drug reduce the concentration in the blood plasma of phenylbutazone, the analgesic antipyrine and the coumarin anticoagulants; it also decreases their pharmacological actions. Anticoagulant therapy is particularly sensitive to dosage. A patient who is satisfactorily maintained on a given coumarin-drug dosage while he is being given phenobarbital as a sedative may hemorrhage when the phenobarbital is withdrawn because the P-450 system is no longer being induced and the anticoagu-



BIOTRANSFORMATIONS carried out in the liver include oxidation, reduction, hydrolysis and conjugation, examples of which are given here. The molecular sites of each reaction are indicated in color. In a few cases transformation converts an inactive form of a drug (such as Prontosil) into an active form (sulfanilamide), but most of the reactions lead to inactivation. Conjugation with a nat-

lant is therefore being inactivated more slowly.

Alcohol is converted into acetaldehyde largely in the liver, perhaps to some extent by the mixed-function oxidase system: heavy drinkers are found to have an increased concentration of



ural substance such as glucuronic acid often follows metabolism of drugs and facilitates excretion. Not all the by-products are shown.

cytochrome *P*-450. The habitual consumption of alcohol therefore stimulates the metabolism of a wide variety of drugs. This helps to explain why heavy drinkers are less affected than other people by barbiturates and other sedatives—when they are sober. A single very large dose of alcohol taken together with another drug, on the other hand, inhibits the drug's metabolism, presumably by competing with the drug for the appropriate enzymes. This effect, in addition to the depressant effect of alcohol on the central nervous system, helps to explain the enhanced sensitivity to barbiturates and other sedatives of a person who has been drinking heavily. The synergistic actions of alcohol and sedatives in the brain can cause death.

The inducibility of microsomal enzymes by drugs suggests a form of therapy for certain conditions in which normal body constituents ordinarily metabolized by such enzymes are present in excessive amounts. Long-term administration of phenobarbital, for example, can lower the concentration of bilirubin, the pigment that produces jaundice, in the blood of patients with chronic obstruction of bile flow in the liver. The excessive bilirubin levels that are normally observed in infants after birth can also be markedly reduced if the mother is given a small dose of phenobarbital for a number of days before delivery. Presumably the drug crosses the placenta and stimulates the conjugating enzyme system that is ordinarily slow to develop in the fetus and the newborn infant.

Halogenated hydrocarbon insecticides such as DDT are potent stimulators of drug and steroid sex-hormone metabolism in mammals and in birds; the breakdown of sex hormones explains in part the devastating effects of DDT on reproduction in some bird populations. The minimum exposure to DDT that will stimulate the metabolism of pentobarbital and decrease its hypnotic action in experimental animals is one that results in concentrations of from 10 to 15 micrograms of DDT per gram of fat; that is a level commonly found in human fat tissues. Among the other insecticides that induce microsomal enzymes in experimental animals are chlordane, aldrin and dieldrin. It is interesting that piperonyl butoxide, a synergist that was added to insecticides to inhibit the enzymatic defenses insects had developed against DDT and its chemical relatives, also inhibits the activity of the microsomal enzymes in the mammalian liver.

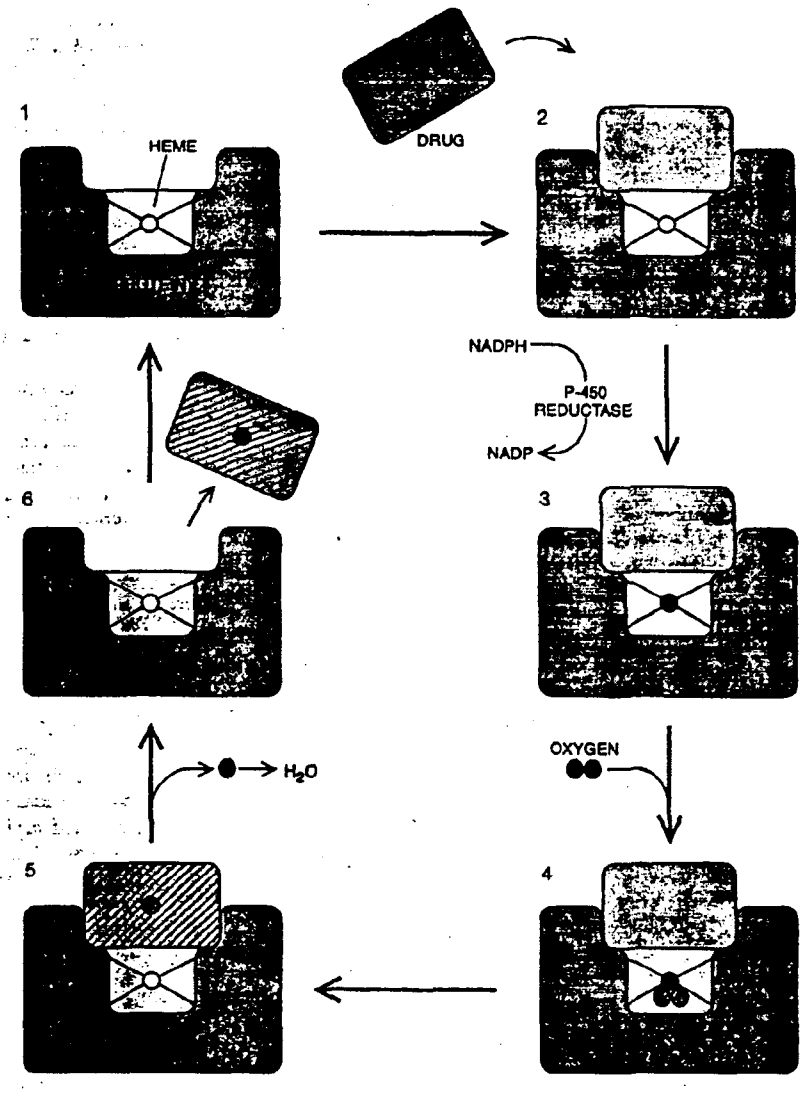
The polychlorinated biphenyls (PCB's) constitute another class of environmental pollutants that have been shown to induce microsomal enzymes. The PCB's are lubricants, heat-exchange fluids, insulators, plasticizers for paints and plastic compounds and a major component of the lens-immersion oil used in microscopy. Whereas some of the consumer-product applications have recently been curtailed, the immersion oils are still handled daily by many laboratory workers. PCB's have been found in the tissues of numerous bird and fish species and in human fat and milk, although the route of entry into the human body has not been accurately determined. In recent experiments we have been able to show that the application of pure PCB's or of microscope immersion oil to the skin of experimental animals in very small amounts (one microliter) causes a marked increase in mixed-function oxidase activity and reduces the pharmacological effect of zoxazolamine, a muscle relaxant, and of hexobarbital in the live animal. These findings suggest that trivial skin exposure to chemicals can have significant and perhaps harmful biological effects in man.

A number of chemicals to which human beings are regularly exposed have been identified as chemical carcinogens, that is, they cause cancers when they are applied to the skin of experimental animals or otherwise administered to them. Clearly a factor that inhibits or stimulates the metabolism of such compounds may affect the development of human cancers. Benzpyrene, benzanthracene and similar polycyclic aromatic hydrocarbons are among the most ubiquitous carcinogens: they are present in tobacco smoke, in polluted city air and in charcoal-broiled and smoked foods. The polycyclic hydrocarbons are metabolized by a mixed-function oxidase enzyme that in this case is called aryl hydrocarbon hydroxylase because of the particular oxidation it catalyzes. Like other microsomal oxidations, this one requires NADPH and molecular oxygen, but the terminal oxidase of the system induced by the polycyclic hydrocarbons is somewhat different from the oxidase induced by drugs. The catalytic properties of the cytochrome are changed, as are its spectral properties: the absorbance maximum of the complex of carbon monoxide with the reduced cytochrome is at 448 nanometers rather than at 450, and so the enzyme is designated cytochrome *P*-448. Apart from the polycyclic hydrocarbons, only one other class of compounds has so far been noted to induce the formation of cytochrome *P*-448: we have found that the PCB's induce

some of the newly identified cytochrome along with cytochrome *P*-450. This suggests the possibility, for which there is developing evidence in animals, that the PCB's too may have carcinogenic properties.

The aryl hydrocarbon hydroxylase system has been the subject of intensive investigation as a possible link in the causation of some cancers. The reason is that rather than detoxifying its polycyclic-hydrocarbon substrates it seems to make some of them more toxic: intermediates of polycyclic-hydrocarbon metabolism such as epoxides are more active in the malignant transformation of tissue-cultured cells than the parent products are. Moreover, the enzyme system for carcinogen metabolism is available at many sites in the body that are exposed to polycyclic hydrocarbons and is induced not only in the liver but also in the gastrointestinal tract, the kidneys, the skin and the lungs. Cigarette smoking markedly induces aryl hydrocarbon hydroxylase activity even in the human placenta, as Allan H. Conney, Richard M. Welch and their colleagues showed at the Wellcome Research Laboratories. Little or no such enzyme activity was found in placentas from nonsmokers.

We have investigated the cytochrome P-448 system in human skin and have found that there is marked variability in the hydroxylase activity of different skin samples and that incubation of skin in tissue culture with the polycyclic hydrocarbon benzanthracene induces more enzyme activity. A skin biopsy is easy to do, so that assaying skin aryl hydroxylase in the presence or absence of polycyclic hydrocarbons may provide a convenient test of individual differences in the capacity to metabolize certain environmental carcinogens. The possibility that induction of aryl hydrocarbon hydroxylase is of considerable significance in chemical carcinogenesis is suggested by the results of some recent experiments performed by Gottfried Kellermann and his associates at the University of Texas. They found that the inducibility of such hydroxylase activity in lymphocytes is significantly greater in cigarette smokers who have lung cancer than it is in healthy nonsmokers.



OXIDATION OF A DRUG by the enzyme cytochrome *P*-450 is visualized here as a sequential process. The enzyme (1) is a complex of a protein and the oxygen-binding compound heme, which contains an iron atom that is initially in the ferric (Fe^{+++}) form (*open circle*). The cytochrome binds the drug (2). Then (3) the enzyme cytochrome *P*-450 reductase, utilizing the coenzyme NADPH, reduces the iron of the heme to the ferrous (Fe^{++}) form (*black dot*), in which it can bind a molecule of oxygen (4). It supplies one atom of oxygen to oxidize the drug and one generally to form water, in the process reverting to its oxidized form (5). The drug, oxidized and in most cases inactive, is thereupon released (6).

It is clear that the liver's mixed-function oxidase system is implicated in a number of processes affecting human health. The ability of a drug or other foreign substance to stimulate the metabolism of another drug by the system may explain some of the adverse drug reactions observed in clinical practice. Drug interactions have been well documented in anticoagulant therapy; more research is required to explore other drug interactions, since most patients are given several drugs at the same time. The ability of environmental pollutants to modify drug action is now under active investigation. It is clear that insecticides, for example, stimulate drug-metabolizing enzymes and that heavy-metal substances such as lead and methyl mercury inhibit the enzymes; the clinical significance of these effects may be appreciable in populations that are exposed occupationally to such agents. Finally, recently acquired evidence seems to indicate that the induction of aryl hydrocarbon hydroxylase in human tissues by polycyclic hydrocarbons may play a significant role in chemical carcinogenesis. Research on this biological action of carcinogens may provide important leads toward predicting the susceptibility of individuals to certain kinds of chemically induced cancer.

SLAVERY IN ANTS

Certain species of ants raid the nests of other species for ants to work in their own nest. Some raiding species have become so specialized that they are no longer capable of feeding themselves

by Edward O. Wilson

The institution of slavery is not unique to human societies. No fewer than 35 species of ants, constituting six independently evolved groups, depend at least to some extent on slave labor for their existence. The techniques by which they raid other ant colonies to strengthen their labor force rank among the most sophisticated behavior patterns found anywhere in the insect world. Most of the slave-making ant species are so specialized as raiders that they starve to death if they are deprived of their slaves. Together they display an evolutionary descent that begins with casual raiding by otherwise free-living colonies, passes through the development of full-blown warrior societies and ends with a degeneration so advanced that the workers can no longer even conduct raids.

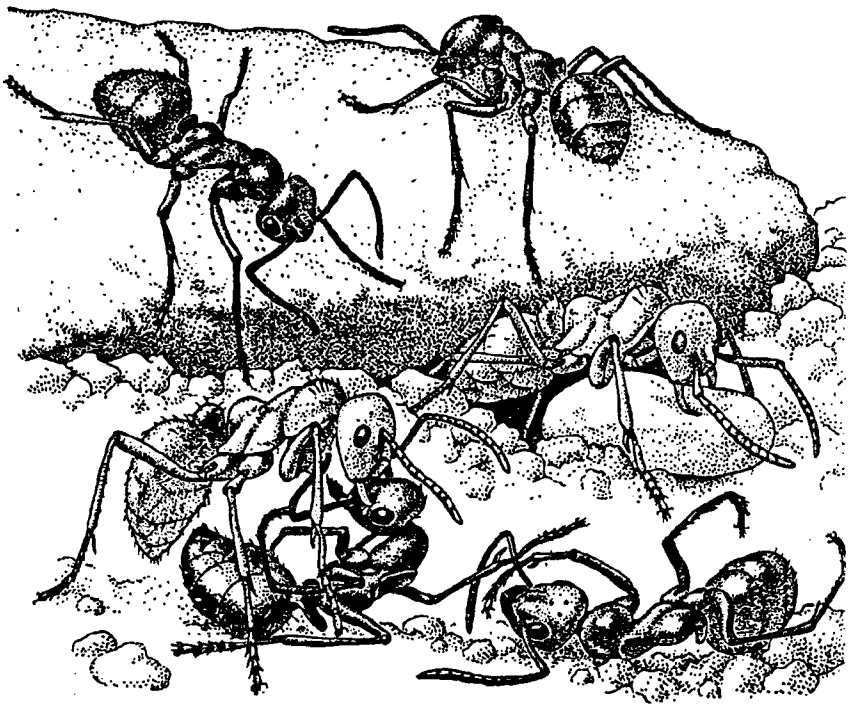
Slavery in ants differs from slavery in human societies in one key respect: the ant slaves are always members of other completely free-living species that themselves do not take slaves. In this regard the ant slaves perhaps more closely resemble domestic animals—except that the slaves are not allowed to reproduce and they are equal or superior to their captors in social organization.

The famous Amazon ants of the genus *Polyergus* are excellent examples of advanced slave makers. The workers are strongly specialized for fighting. Their mandibles, which are shaped like miniature sabers, are ideally suited for puncturing the bodies of other ants but are poorly suited for any of the routine tasks that occupy ordinary ant workers. Indeed, when *Polyergus* ants are in their home nest their only activities are begging food from their slaves and cleaning themselves ("burnishing their ruddy armor," as the entomologist William Morton Wheeler once put it).

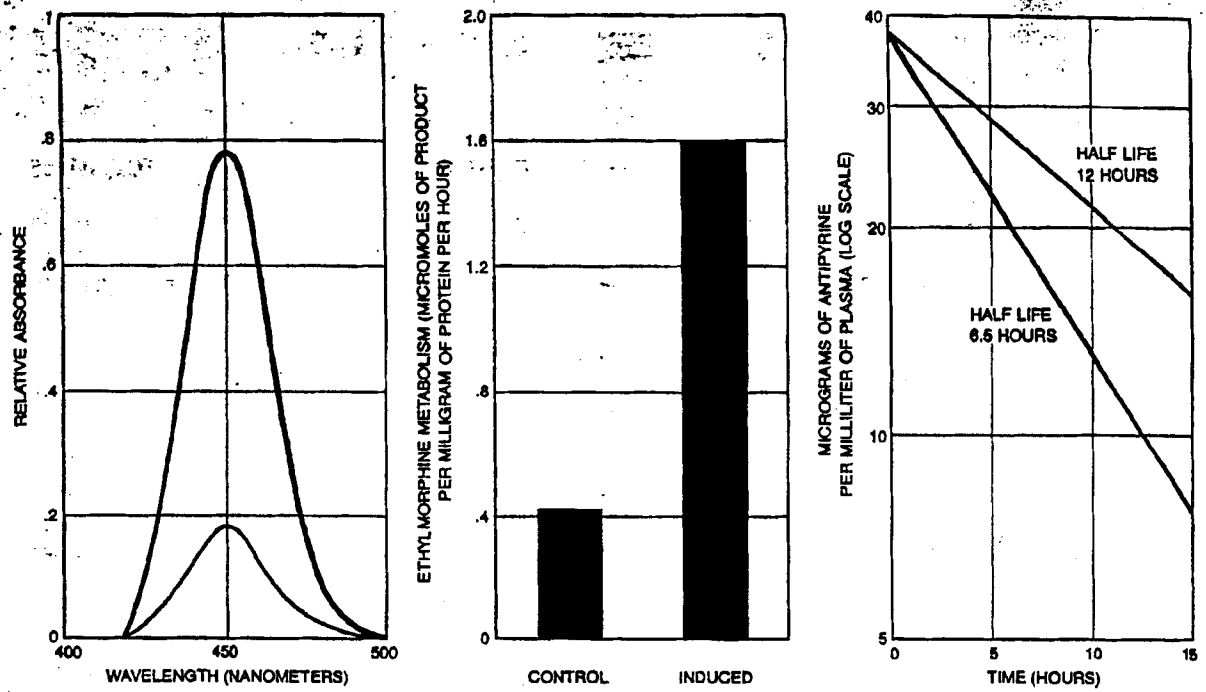
When *Polyergus* ants launch a raid, however, they are completely transformed. They swarm out of the nest in a solid phalanx and march swiftly and directly to a nest of the slave species. They destroy the resisting defenders by puncturing their bodies and then seize and carry off the cocoons containing the pupae of worker ants.

When the captured pupae hatch, the workers that emerge accept their captors as sisters; they make no distinction between their genetic siblings and the *Polyergus* ants. The workers launch into the round of tasks for which they have been genetically programmed, with the slave makers being the incidental beneficiaries. Since the slaves are members of the worker caste, they cannot reproduce. In order to maintain an adequate labor force, the slave-making ants must periodically conduct additional raids.

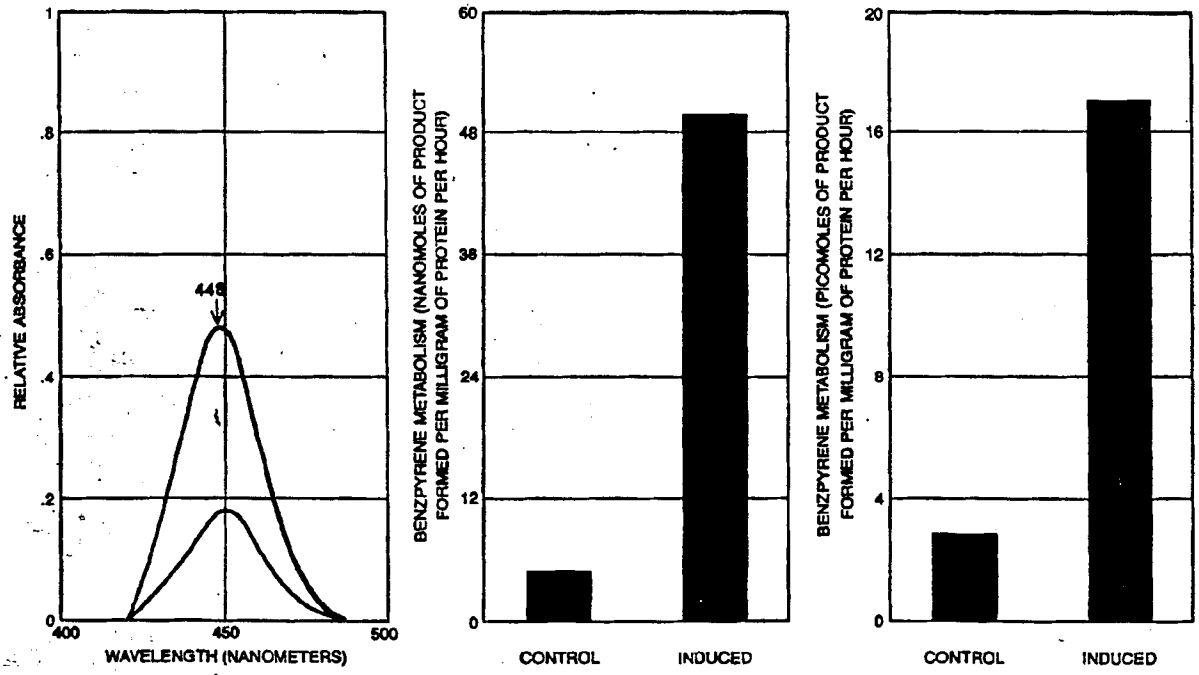
It is a remarkable fact that ants of slave-making species are found only in cold climates. Although the vast majority of ants live in the Tropics and the



RAID BY SLAVE-MAKING AMAZON ANTS of the species *Polyergus rufescens* (*light color*) against a colony of the slave species *Formica fusca* (*dark color*) is depicted. The *fusca* ants make their nest in dry soil under a stone. The raiding Amazon ants kill resisting



PHENOBARBITAL induces cytochrome *P*-450 and thus stimulates the metabolism of drugs oxidized by that enzyme. The curves (*left*) show the light-absorbance spectrum, with a peak at 450 nanometers, characteristic of reduced cytochrome *P*-450 complexed with carbon monoxide, the usual means of identifying and quantifying the enzyme. In a rat treated with phenobarbital the cytochrome *P*-450 content of microsomes (*colored curve*) is substantially higher than that of microsomes from an untreated rat (*black curve*). More of the test drug ethylmorphine is therefore metabolized (*center*) by liver tissue from a phenobarbital-treated rat (*colored bar*) than by the same amount of tissue from an untreated rat (*gray*). Similarly, phenobarbital speeds up metabolism of the drug antipyrine in man (*right*): antipyrine level in plasma falls faster after phenobarbital treatment (*colored curve*) than ordinarily (*black*).



CARCINOGENS induce a different species of cytochrome, cytochrome *P*-448. The curves (*left*) compare the absorbance of microsomes from the liver of a rat treated with the carcinogen methylcholanthrene (*color*) with the absorbance of control microsomes (*black*); the induced cytochrome has a peak at 448 nanometers. Methylcholanthrene enhances metabolism of another carcinogen, benzpyrene (*center*): the microsomes of treated rats produce more metabolite (*color*) than control microsomes (*gray*) do. Similarly, treating human skin in tissue culture with the carcinogen benzanthracene stimulates skin-cell metabolism of benzpyrene (*right*).

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NEWS AND COMMENT

Cancer Institute: Expert Charges Neglect of Carcinogenesis Studies

A prominent scientist has resigned from directorship of a key program in the National Cancer Institute for reasons which, if well founded, could provoke a serious perturbation in the agency's affairs. The scientist, Umberto Saffiotti, heads the NCI's program in chemical carcinogenesis, a subject whose importance has been increasingly acknowledged by cancer epidemiologists, by regulatory agencies, and in Congress. A threatened fragmentation of the program which could prolong the public's exposure to carcinogens is one of the reasons behind his decision to quit.

NCI director Frank Rauscher pays tribute to Saffiotti's scientific expertise but regards the issue of his resignation as the result of a difference in approach to the management of certain programs under his control, which Rauscher believes could have been pushed ahead faster.

Saffiotti believes that the carcinogenesis program has long been denied the manpower necessary to keep pace with its growing responsibilities. It has only 20 percent more staff than in 1968, but almost 8 times the amount of money to administer. This year the program received only 3 of the 79 new staff positions assigned to the NCI, although Rauscher told the House Appropriations Committee that he was giving Saffiotti the highest priority possible. With a current budget of $47 million, the program conducts basic research on chemical carcinogenesis as well as developing bioassay tests for carcinogens.

Saffiotti also feels that he and other colleagues with relevant expertise have been excluded from a series of decisions on chemical carcinogenesis, the most recent being the announcement of a National Clearinghouse on Environmental Carcinogenesis, on which he says he was not consulted until a late stage. The final straw for Saffiotti was a recent decision to split away from his program the responsibility for developing bioassay tests for chemical carcinogens. The move will, in his view, compromise the scientific credibility of the tests, delay their being put into action, and increase the time that people will be exposed to the chemicals the tests may show to be carcinogenic.

Although he has been asked to remain as director for the research part of the carcinogenesis program, Saffiotti has chosen to resign altogether from the program management, lest he seem by staying to concur with the decision on the bioassay tests. He plans to take up full time research in his laboratory at the NCI. "I am glad to call it quits rather than endorse a mode of operation I disagree with," he told *Science* in an interview last week before announcing his resignation.

Saffiotti adds that a fundamental reason for resigning is his belief that active scientists have very little voice in setting policy or priorities in his division of the NCI, and that the division is being run by managers with the help of scientists rather than the other way around. Because of the growth of successive layers of bureaucracy, whose actions are not accountable to detailed peer review by scientists, Saffiotti says, "There seems to be a growing gap between the top policy-making decisions of the institute and the

expertise which is needed to make these complicated value judgments."

Saffiotti's resignation is likely to be taken seriously because he is well known outside the NCI. He has been an active researcher in chemical carcinogenesis for 20 years and has headed the NCI program since 1968. Evaluations of data by his program staff have played an important part in regulatory decisions banning various chemicals, such as the pesticides aldrin and dieldrin. As the NCI's leading expert on chemical carcinogenesis, he has given frequent testimony before congressional committees and is a key figure in the field.

NCI director Rauscher calls Saffiotti a "superb scientist" but ascribes the reasons for his resignation to a difference in managerial approach. In a brief conversation before going out of town, Rauscher told *Science* he had been unable to assign as many staff positions as he would have liked to the carcinogenesis program but that Saffiotti could have reassigned his own staff to the bioassay area. The NCI position has been discussed in greater detail by deputy director Guy Newell and by James Peters, director of the division of cancer cause and prevention, to which the carcinogenesis program belongs.

Part of Saffiotti's unhappiness about the role of scientists in NCI policy-making relates to the fact that Peters, as division director, has to make decisions affecting chemical carcinogenesis research even though he has no scientific research experience. Peters, a veterinarian by background, notes that he has a master of public health degree in epidemiology and worked for 5 years as Saffiotti's deputy in the carcinogenesis program. Asked if he has the expertise to make decisions about carcinogenesis research, Peters says that he feels perfectly qualified to do so: "While I don't have the bench-side experience that Saffiotti has, I am in a better position to make decisions because from my position as division director I have an overall view of the program's needs within the division and of its relationship to other agencies."

The specific issues which prompted Saffiotti to resign are hard to assess because he and the NCI top management are to some extent talking past each other. Saffiotti discusses the issues in terms of their impact on research, Rauscher and Peters in terms of managerial and public relations aspects. A principal point of disagreement concerns the management of bioassay tests for carcinogens, both in vivo tests in which a chemical is fed to animals for an extended period, and in vitro tests in which cell cultures are the test medium. The in vitro tests, still in the process of development, promise to be very much quicker and cheaper than the $100,000-per-chemical tests in animals.

Umberto Saffiotti

What triggered Saffiotti's resignation was a decision to split the validation of the in vitro tests from the basic research, leaving the research in Saffiotti's program and moving the validation to Peters's office. The project will not receive any more staff in Peters's office, but, according to Rauscher, it will be "in an organizational position of higher visibility and emphasis." Rauscher says he has promised Congress that the NCI would press ahead with the in vitro bioassays. "Umberto feels this has been done. I feel that more could be done. Some of the advice I have been getting from some people is that he has not been moving fast enough," Rauscher says.

Saffiotti's position is that he has been moving ahead as fast as he could, but that the tests are at an extremely delicate stage where the state of the art varies from one laboratory to another and expertise at the bench counts for almost everything. To divorce research from development at this stage will, in his opinion, crucially delay the validation of the tests. "All the experts in the field are saying, 'Go full steam ahead in developing the tests, but don't stop to test chemicals because you will get a lot of test data you can't interpret properly.' " Saffiotti observes.

Peters and Newell say that there is no question at all about Saffiotti's managerial ability, but that they believed the tests could be developed more quickly. Asked the basis for disagreeing with Saffiotti's scientific judgment, Peters says he regards the decision as managerial, not scientific, but that outside scientists were consulted. The discussions took place on an informal basis over a long period of time until the decision just emerged, Peters says. A member of the NCI advisory board says that the decision to split off validation of the in vitro tests "ought to be made with a good deal of advice from scientific people who are not members of the NCI. That is what advisory committees are for."

Rauscher, Newell, and Peters also seem to hold Saffiotti to blame, though without directly saying so, for the bottleneck that has arisen in assessing and disseminating the results of in vivo tests. Some 200 chemicals have been put through animal feeding tests, but the results have not yet been published. Saffiotti says that he warned of the bottleneck 3 years ago, at the time the tests were started, but never received the staff he needed to get the results out. He was unable to transfer staff from elsewhere in his program without seriously damaging other program areas, which themselves are of high priority in interpreting the results of carcinogenicity tests and their relevance to man.

Newell and Peters indirectly criticize Saffiotti for a reluctance to delegate tasks to contractors. "I feel these things can be done without direct control by active scientists," remarks Newell of Saffiotti's general approach to management. "I believe that you can go out and buy expertise and you can get good expertise. With the in vivo tests the protocols are so well developed that it is sort of cookbook," Peters says.

Saffiotti rejects this attitude, believing that unless the contractors are rigorously supervised, the tests will not be done properly, and a sloppy test is a total waste of investment. "There is no such thing as cookbook stuff at any stage of the way," says Saffiotti. As the Kennedy hearings on commercial laboratory operations have shown (page 531, this issue), it is crucial to set high standards for contractor performance, Saffiotti contends. "In fact, it is almost axiomatic that if bioassays are used to claim a negative result for regulatory purposes, the sloppier the test the 'safer' will the product appear—unless one investigates the adequacy of the procedures used."

In a letter of resignation sent on 23 April, Saffiotti ascribes the backlog in publishing the test results to a "tragic policy" by the NCI of failing to provide his bioassay team with sufficient staff. Reassignment and disruptions in his present staff appear likely "to lead to further delays in the publication of well-docu-

mented evidence of cancer hazards," he says in his letter. "I cannot accept any longer a situation which in fact deprives the regulatory agencies, industry, labor, consumers and the scientific community of data of urgent public health value: it is *people* who are now exposed to toxic agents and who are not protected because the necessary support was not provided in time."

Sloppy Tests

Saffiotti considers that he has had "at best only a few opportunities to discuss and participate in major policy decisions at the Institute level." Rauscher, he says, has received advice from his program "at second or third hand." The problem, as he sees it, is layers of bureaucracy separating the NCI director from his scientific experts. "There is a huge bureaucracy in the front office—some 200 people, few of whom are active scientists—and our own division director has built up a small layer of people in his front office," Saffiotti says. "Essentially the direct involvement in research stops at our level and all the rest is bureaucratic overhead. This may be the inevitable result of the very rapid growth of the NCI's budget, but the fact is that it is so."

The observation is serious, if true, not least because one of the principal arguments for giving the cancer institute greater autonomy within the National Institutes of Health was to free it from encumbering layers of bureaucracy. Rauscher, however, says he has never refused to see Saffiotti. Peters observes that he is the only person between Saffiotti and the NCI director, and that he has a larger program but smaller staff than any other division in the NCI.

One apparent example of the exclusion of experts from their proper role is that of a proposed interagency committee on the assessment of carcinogens. Peters has nominated himself as the NCI member. Asked if Saffiotti wouldn't be better qualified, Peters says no, because the committee is a broad-based policy group. But the chairman of the committee, Roy Albert of the Environmental Protection Agency, says that the committee will be a technical group making scientific judgments. Asked why Peters was a member, Albert said, "It does look peculiar that he is on it without being an expert in carcinogenesis or having that background, but I view it as only on the basis of setting up a channel to the NCI's experts such as [H.F.] Kraybill and Saffiotti." Peters has designated Kraybill as an alternate on the committee.

The bureaucracy's actions are not always accountable to peer review by outside scientists, Saffiotti believes. For example, the division of cancer cause and prevention has disbanded the peer review group it used to have. Thus although there are peer review groups at the program level, the decisions taken at divisional level, such as on allocation between the three programs (carcinogenesis, virology, and epidemiology) are not subject to direct peer review. Peters says the divisional level peer review group was disbanded because it did not make sense to have experts in one program area reviewing work done in another. According to Rauscher, however, "Active researchers have probably never had a greater input to the NCI—we have 62 advisory committees and I don't know of a single recommendation from them over which I have control which I did not implement."

An attempt to test the opinion of scientists in the chemical carcinogenesis field and on the NCI advisory board produced general praise of Saffiotti's program—"He has done as well as anybody could possibly do," says one of the outside advisers to his program. There seems to be a general reluctance to comment on the specifics of the issue, about which most people expressed ignorance. Members of the NCI advisory board were unaware even of the way in which the NCI's 79 staff positions had been distributed. "Internal NCI affairs are very complex, and outsiders comment on them at their peril because there are so many political issues involved," observes Nobel laureate Howard Temin of the University of Wisconsin.

Saffiotti's complaints may or may not be fully justified, but his resignation has at the least drawn attention to what he regards as a critical shortage of support for the carcinogenesis program.

—NICHOLAS WADE

Clinical Labs: Bills Aimed at Correcting "Massive" Problems

In medical practice today, physicians and patients rely increasingly on the results of laboratory tests to tell them what is wrong. And what is wrong, it seems, in an appalling number of cases, is the test itself. It is almost impossible to get accurate data on the quality of work performed in the 64,000 to 94,000 clinical laboratories in the United States (no one is sure exactly how many there are), but the Senate has evidence that "from 7 to 26 percent of all lab tests may be in error."* Considering the number of laboratory tests performed in any year, that is a lot of error.

According to figures from the Senate subcommittee on health, chaired by Edward M. Kennedy (D–Mass.), 4½ billion lab tests were conducted in this country in 1975; that comes out to 12 million a day. By the end of the year, the nation's total bill for laboratory tests hit $12 billion, which is roughly equal to 10 percent of the entire cost of health care for that year. Reports by the Senate's Special Committee on Aging, which conducted an investigation of Medicare fraud in the lab business, the Senate's subcommittee on health, and others reveal that problems with clinical laboratories are "massive and widespread." As Kennedy has said, "Clinical laboratories perform inadequately and constitute a threat to the public health."

In the Senate, Kennedy and Jacob K. Javits (R–N.Y.) have sought a remedy through the Clinical Laboratories Improvement Act of 1975, which is likely to go to the floor for action soon. Representative Paul G. Rogers (D–Fla.) has introduced similar, though not identical, legislation in the House. And the Administration, which is perfectly willing to concede there is a problem, is trying to block legislative action on the grounds that there already exist adequate powers to regulate clinical laboratories and that the government is now going to use them. But Congress is not going to hold its breath until that happens.

Nine years ago, Senator Javits in-

*"Fraud and abuse among clinical laboratories," a staff report prepared for the U.S. Senate's Special Committee on Aging, 1976.

NAE Elects 104 New Members

The National Academy of Engineering, established to share the responsibility given the National Academy of Sciences under its congressional charter to examine questions of science and technology at the request of the federal government, has elected 104 new members. This addition brings the total membership to 685. The new members are as follows:

H. Norman Abramson, Southwest Research Institute; **Harold M. Agnew**, Los Alamos Scientific Laboratory; **Clarence R. Allen**, California Institute of Technology; **Alfredo H.-S. Ang**, University of Illinois, Urbana-Champaign; **Horace S. Beattie**, IBM Corporation; **Daniel Berg**, Westinghouse Electric Corporation; **Donald J. Blickwede**, Bethlehem Steel Corporation; **John E. Breen**, University of Texas, Austin; **Charles M. Brinckerhoff**, Consulting Engineer, New York; **Frederick P. Brooks, Jr.**, University of North Carolina; **Donald B. Broughton**, UOP Process Division; **Bernard Budiansky**, Harvard University.

Joseph E. Burke, General Electric Research and Development Center; **Marvin Camras**, IIT Research Institute; **Dayton H. Clewell**, Mobil Oil Corporation; **Julian D. Cole**, University of California, Los Angeles; **John W. Coltman**, Westinghouse Research Laboratories; **Franklin S. Cooper**, Haskins Laboratories; **F. J. Corbató**, Massachusetts Institute of Technology; **Ruth M. Davis**, Department of Commerce; **Anthony J. DeMaria**, United Technologies Research Center; **Ira Dyer**, Massachusetts Institute of Technology; **Milton C. Edlund**, Virginia Polytechnic Institute and State University; **Lloyd E. Elkins**, Amoco Production Company; **Martin A. Elliott**, Energy Consultant, Texas; **Richard S. Engelbrecht**, University of Illinois, Urbana-Champaign; **Elliott M. Estes**, General Motors Corporation; **Joseph Feinstein**, Varian Associates; **Steven J. Fenves**, Carnegie-Mellon University; **Michael Field**, Metcut Research Associates Inc.

Merton C. Flemings, Massachusetts Institute of Technology; **Charles F. Fogarty**, Texasgulf Inc.; **Gerard F. Fox**, Howard Needles Tammen & Bergendoff; **Alfred M. Freudenthal**, George Washington University; **King-Sun Fu**, Purdue University; **Douglas W. Fuerstenau**, University of California, Berkeley; **Robert A. Fuhrman**, Lockheed Missiles and Space Company, Inc.; **Solomon W. Golomb**, University of Southern California; **John B. Goodenough**, Lincoln Laboratory; **Robert C. Gooding**, Naval Sea Systems Command; **Arthur G. Hansen**, Purdue University; **Edwin L. Harder**, Pittsburgh, Pennsylvania; **Milton Harris**, Washington, D.C.; **Herman A. Haus**, Massachusetts Institute of Technology; **Arthur Hauspurg**, Consolidated Edison Company of New York Inc.; **Heinz Heinemann**, Mobil Research & Development Corporation; **Joseph M. Hendrie**, Brookhaven National Laboratory; **Abraham Hertzberg**, University of Washington.

Wilmot N. Hess, National Oceanic and Atmospheric Administration; **William C. Hittinger**, RCA Corporation; **Charles H. Holley**, General Electric Company; **Joe W. Johnson**, University of California, Berkeley; **Robert L. Johnson**, McDonnell Douglas Astronautics Company; **Donald J. Jordan**, Glastonbury, Connecticut; **Joseph H. Keenan**, Massachusetts Institute of Technology; **Robert W. Keyes**, IBM T. J. Watson Research Center; **Lee A. Kilgore**, Consulting Engineer, Pennsylvania; **Gordon S. Kino**, Stanford University; **Leon Lapidus**, Princeton University; **Milton Levenson**, Electric Power Research Institute; **Joseph T. Ling**, 3M Company; **Ray K. Linsley**, Hydrocomp, Inc.; **John P. Longwell**, Exxon Research and Engineering Company; **Bruce T. Lundin**, NASA Lewis Research Center; **John D. Mackenzie**, University of California, Los Angeles; **Enrique A. J. Marcatili**, Bell Laboratories; **Hans M. Mark**, NASA Ames Research Center; **Sidney Metzger**, Communications Satellite Corporation.

Herbert L. Misch, Ford Motor Company; **James K. Mitchell**, University of California, Berkeley; **Gordon E. Moore**, Intel Corporation; **Ben Morecll**, Pittsburgh, Pennsylvania; **Richard S. Morse**, Massachusetts Institute of Technology Development Foundation, Inc.; **Albert G. Mumma**, Short Hills, New Jersey; **Peter Murray**, Westinghouse Advanced Reactors Division; **Eugene F. O'Neill**, Bell Laboratories; **Henry J. Ongerth**, California State Department of Health; **Jack S. Parker**, General Electric Company; **Norman F. Parker**, Varian Associates; **Thomas H. Pigford**, University of California, Berkeley; **Egor P. Popov**, University of California, Berkeley; **Jacob Rabinow**, Department of Commerce; **Eric Reissner**, University of California, San Diego; **James B. Reswick**, Rancho Los Amigos Hospital; **Allen S. Russell**, Aluminum Company of America; **Robert S. Schechter**, University of Texas, Austin; **Reinhardt Schuhmann, Jr.**, Purdue University.

David Slepian, Bell Laboratories; **William P. Slichter**, Bell Laboratories; **Arthur C. Stern**, University of North Carolina; **Archie W. Straiton**, University of Texas, Austin; **Morgan C. Sze**, Lummus Company; **Harold A. Thomas, Jr.**, Harvard University; **Chang-Lin Tien**, University of California, Berkeley; **Milton Van Dyke**, Stanford University; **Henning E. Von Gierke**, Aerospace Medical Research Laboratory; **John B. Wachtman, Jr.**, Department of Commerce; **William M. Webster**, RCA Laboratories; **Johannes Weertman**, Northwestern University; **Roy F. Weston**, Roy F. Weston, Inc.; **Richard T. Whitcomb**, NASA Langley Research Center; **J. Ernest Wilkins, Jr.**, Howard University; **Amnon Yariv**, California Institute of Technology; **Alfred A. Yee**, Alfred A. Yee & Associates, Inc.

troduced the first piece of legislation covering the operation of clinical labs—the Clinical Laboratories Improvement Act of 1967. That law, intended to guarantee high quality lab work, authorized the Center for Disease Control (CDC) in Atlanta, Georgia, to test the proficiency of clinical labs; but its authority was restricted to those laboratories that engage in interstate commerce, estimated to be a mere 6 percent of the total. CDC officials believe that those 900-odd labs that come under their purview represent the best in the country, but the Center's own analyses of the labs' performance indicates that all is not well, even among the best.

As part of its quality monitoring program, CDC sends sample specimens of various sorts to laboratories to see if they are identified correctly. Sometimes the laboratories know they are being tested, sometimes not. Among recent discouraging findings are these:

- Thirty-one percent of labs, which knew they were being tested, failed to identify sickled red blood cells.
- Leukemia was mistakenly diagnosed by more than 10 percent of labs in one test.
- Mononucleosis is incorrectly diagnosed as much as one-third of the time.
- Seventeen of 22 laboratories correctly identified drugs in urine samples when they knew they were being tested, but 16 of those 22 labs missed the drug identification 60 percent of the time in a repeat quiz when they received urine labeled as patient specimens rather than as CDC test material.
- Five to 12 percent of the time, laboratories "find something" when CDC sends them slides that contain nothing of consequence.

Sometimes the errors are trivial, but sometimes they are of more than passing consequence. Even people in the laboratory business acknowledge problems of error and some capitalize on them, as is shown in an advertisement in the November–December 1975 issue of *Cadence* (the journal of the American Society for Medical Technology), which is devoted to the issues of achieving quality assurance by legislation. A two-page ad shows a dramatic sketch of a young woman lying in a hospital bed, her husband, head bowed, staring out of the window. The ad, in bold letters, reads, "She searched her conscience, conferred with her husband and went through with the abortion. But there was no fetus there." The ad is for a particular pregnancy test that is claimed to meet a "high standard of accuracy."

The Senate has reached the conclusion that the reason for all these sorts of problems is that clinical laboratories and labo-

"That letter disqualified them," he said. "If we'd tried to appoint them we'd have had resignations from half the committee."

The episode has led some participants to question the adequacy of the Academy's procedures for uncovering bias among prospective committee members. The list of names from which the committee was chosen was generated primarily by the Academy staff with help from relevant consultants, and Hastings added some names of his own. Then Hastings, after analyzing a list of the fields of expertise needed and the potential candidates from those fields, indicated whom he wanted as committee members. Hastings told *Science*, "I personally wasn't sensitive to any stands taken by these people."

Hastings' recommendations then had to gain the approval of other key figures in the Academy. But it was only *after* the committee members were appointed that they were asked to fill out bias statements indicating, among other things, any views they might have expressed publicly on the issues to be considered by the committee. By that time, it would have been embarrassing to ask anyone to withdraw, though Academy officials say they have done so on occasion in the past.

Some Academy representatives are suggesting that appointments should be made conditional upon review of the bias statement, but others consider it presumptious to ask scientists to reveal their stockholdings, commercial affiliations, grant support, and other such matters if they are not sure they will actually be appointed. And for every scientist who wants to tighten up the bias procedure, there is another who wants to weaken it. Schwan, for example, considers it "an awful thing" for the Academy to ask what stands he has taken on an issue. "It intimidates my freedom of expression," he said. "Where's the borderline between such things and what happens in Russia?"—PHILIP M. BOFFEY

Chemical Carcinogens: Industry Adopts Controversial "Quick" Tests

For generations, industry has been introducing new chemicals into the environment in staggering numbers without really knowing whether they might be hazardous. And the public, assuming there was nothing to be done, or not thinking about it, has passively tolerated the situation. But then the environmental movement came along, as did the calculations by epidemiologists that a large proportion of all cancers are environmentally caused. As a result, there has been growing pressure to force industry to evaluate new chemicals before they are released, on the theory that safety should be tested in the laboratory and not in the environment. And there is a good chance that Congress this year will pass the Toxic Substances Control Act that would mandate premarket testing (*Science*, 13 February).

The major impediment to premarket testing has been the lack of a test system that is reliable, fast, and cheap. However, during the past few years some progress has been made in that area, largely because of the leadership of biochemist Bruce Ames of the University of California, Berkeley. Ames developed a simple system for taking a quick look at the mutagenic, and by implication, carcinogenic, properties of chemicals.

No one yet is sure just how reliable a predictor the Ames test—a bacterial system—is, but it is generally thought to be the best available of its type. In view of the probable passage of the Toxic Substances Control Act, the chemical industry has begun, during the past year or so, to use the Ames, and other quick tests, on its own in order to get some idea of the safety of new products. In the process, industry itself may help to answer questions about the value of various types of screening systems by generating sufficient volume of data on which to base scientific judgments.

At present, the only officially recognized way to test a chemical for carcinogenicity is to see whether it causes cancer in laboratory animals, which takes 2 to 3 years and costs about $100,000 per chemical. Citing the time and money involved, industries have been notoriously reluctant to routinely screen new products in animals. On the other hand, they hesitate to invest huge sums of money in the development of new products without knowing whether those products will later be banned as carcinogens. Therefore, industries have seized on a variety of quick and inexpensive tests that, they hope, will tell them whether substances are carcinogens. This has led to a curious situation in which industries are implicitly endorsing the tests at the same time that scientists and legislators deliberate over whether companies should be forced to use them.

The extensive use by industries of these quick tests is hailed by many scientists as a change in the tradition of wanton release of chemicals into the environment even while debate continues on how to evaluate potentially harmful substances in accord with the pending Toxic Substances Control Act. Ames reports that 60 or 70 major companies, including such giants as American Cyanamid, Inc., Merck Sharp & Dohme, and Dupont have asked him to supply them with the strains of salmonella bacteria he uses in his system. Numerous other firms do not test their products themselves but send them to commercial laboratories for testing.

Companies are reluctant to discuss their uses of the quick tests, but Ames relates one story told to him by investigators at American Cyanamid's agricultural division. It seems that American Cyanamid found what looked like a promising new pesticide that turned out to be highly mutagenic when tested in Ames' bacterial strains. Not willing to just drop their new product the investigators of American Cyanamid took a second look and found that this mutagenic effect was due, not to the primary chemical but to an impurity in the pesticide. Now, Ames reports, the company has removed the mutagen from the pesticide and has decided that it is worthwhile to go the full route with the purified product by testing it in animals.

The Ames test is based on the presumption that many cancers are related to mutations or some sort of damage to the DNA of a cell and, therefore, that agents that are mutagenic are likely to be carcinogenic as well. After searching through innumerable bacterial strains, Ames hit upon some mutants of *Salmonella typhimurium* that have lost the ability to make the amino acid histidine. Consequently, in a histidine-free culture medium, these bacteria cannot grow. What Ames has shown is that, when these bacteria are exposed to mutagenic chemicals, they undergo additional muta-

tions that can have the effect of repairing the original defect. What it amounts to is that in the presence of a mutagenic chemical, the bacteria begin to grow again, forming colonies that show up as white spots. A particularly handy feature of the test is that powerful mutagens will cause a larger number of bacteria to revert than will less potent ones, thereby providing at least some indication of how potentially hazardous a suspect chemical may be. The test is cheap—it costs only $200 per chemical—and fast—it can be completed in 3 days.

Other quick assays for mutagenic chemicals are based on yeast, fruit flies, and mammalian cells grown in culture. At present, no other assay is as widely used as the Ames test, but each is being studied extensively.

Commercial laboratories report that the recent interest by industries in quick tests has provided them with a substantial increase in business. David Brusick of Litton Bionetics in Kensington, Maryland, says that his firm has had some contracts to screen chemicals for the past 2 years but that the vast majority of its clients have been signed up in the past few months. Now Litton Bionetics does the tests for about 50 companies. Clients include pharmaceutical companies, manufacturers of agricultural chemicals, producers of pigments and dyes, and other companies that may be marketing toxic substances.

Litton Bionetics, like most other commercial laboratories, offers its clients a range of quick tests, including the Ames test, which is almost always performed first. In many instances, a firm is told that its chemical is mutagenic in the Ames test and will then request other quick tests of the chemical before deciding what course of action to take. The cost of a whole battery of tests is less than one-tenth of the cost of a cancer test in which laboratory animals are used.

The widespread use of the Ames test is a tribute to the decade of work put in by Ames and his associates. They have

Medical Devices Law Is on the Books at Last

Congress has finally given the Food and Drug Administration explicit authority to regulate medical devices in a law signed by the President at the end of May. The event concluded 15 years of intermittent congressional efforts to fill a regulatory gap that was becoming ever more evident with leaping advances in medical technology.

Efforts to pass a devices law began in earnest following a 1970 report by a study group at the Department of Health, Education, and Welfare, which revealed that medical devices had been implicated in 10,000 injuries and 731 deaths between 1963 and 1969. Most of the deaths resulted from malfunctioning heart valves and pacemakers.

The new amendments to the Food, Drug and Cosmetic Act for the first time empower FDA to review and approve high-risk devices before they go on the market. The law puts all medical devices from tongue depressors to artificial organs into three categories. Classification, to be done by outside panels appointed by the secretary of HEW, will be made according to the potential danger of each device and the availability of information sufficient to formulate safe standards governing its design, manufacture, and use.

Devices put in class III, the most stringent category, will require FDA clearance before they are marketed. This applies to devices that are deemed to be life supporting or life sustaining or are implanted in the body. Class II devices must conform to standards to be promulgated either by groups from outside the government or by the government. Class I devices are subject to "general controls," which means they basically won't be regulated any more than they are now. This is equivalent to the "generally recognized as safe" designation for food additives.

Until now, the only explicit statutory authority the FDA has had to regulate devices has come from the 1938 drug law which permits the agency to take action against any device found to be "misbranded" or "adulterated." In 1969 the concept that devices could be regulated as drugs within the law was elaborated by a Supreme Court decision which ruled that Bacto-Unidisk, a paper disk used for testing bacterial sensitivity to drugs, should be classified as a drug. But since then, only a handful of devices, such as copper IUD's and soft contact lenses, have been regulated as drugs.

According to a lawyer on the staff of Senator Gaylord Nelson (D–Wis.), who is largely responsible for strengthening the bill from its earlier versions, what the new law does is shift the burden of proof that a device is safe and effective from the FDA (which only had the power to intervene after a device was on the market) to the manufacturer. The law requires that every "new" device—that is, every one that is introduced after passage of the law and is not "substantially equivalent" to something already in use—must be automatically put in class III. From there, panels have 6 months to decide whether to approve it and whether to reclassify it in class I or II. All "old" devices—those already on the market—that are implantable or life sustaining also go into class III. Their manufacturers are given 3 years from the date of the law's enactment to get marketing approval. Devices now covered by new drug applications would also probably go into class III.

Passage of the law has taken a remarkably long time considering the fact that some sort of legislation has been widely thought to be not only desirable but inevitable. Even device manufacturers have supported it as being far preferable to alternative and even more stringent regulatory procedures. Their main complaint about the new law, according to a spokesman from the Pharmaceutical Manufacturers' Association, is that class III is unnecessarily broad and that the restrictions in this class will impede the flow of new devices onto the market.

As for consumer advocates, the chief problem, according to attorney Anita Johnson of Ralph Nader's Health Research Group, is that the major classification decisions are to be made by committees of nongovernment personnel. Johnson believes outsiders are more lax and subject to conflicts of interest, and that the only way to ensure accountability is to keep all responsibility on the backs of public servants. "We must decide whether we want outsiders to be making basic public health decisions," she says. Johnson also calls the standards-setting procedures "a Rube Goldberg machine" that offers numerous opportunities for industry interests to create obstructions and delays.

The new law covers about 12,000 devices, products of a more than $3-billion-a-year industry. According to an FDA official, about 10 percent of all devices would go into the premarketing approval category and half would be allowed to stay under general controls.—C.H.

shown that 90 percent, or 156, of 174 known carcinogens cause mutations in their bacterial strains. By contrast, few of the 109 "noncarcinogens" that they have tested are mutagens. Because a wide variety of classes of chemical carcinogens are mutagens in his system, Ames argues that this bacterial test system provides a reliable screen for potentially harmful chemicals.

As an example of the usefulness of quick tests for carcinogenicity, Ames tells the story of a preservative (furofuramide) that was extensively used in Japan. It was tested in animals and found not to cause cancer. The chemical, however, did cause mutations in bacteria. It was subsequently retested in animals, found to be carcinogenic, and banned. Now, Ames reports, bacterial mutagenicity tests are extensively used in Japan. In particular, the Japanese require that all pesticides be shown not to cause mutations in bacteria. The Japanese are also taking very seriously the finding that hair dyes are potent bacterial mutagens and are currently trying to develop hair dyes that do not have this drawback, according to Ames.

Despite these arguments in favor of the Ames and similar tests, it is by no means clear how results of these tests are to be interpreted. One problem is that, with the data obtained so far, the logic behind the test evaluations goes the wrong way. Ames can say that 90 percent of all tested carcinogens are mutagens in his bacterial strains but he cannot say what the probability is that a chemical that is a mutagen will turn out to be a carcinogen.

Some investigators believe that this difficulty can be partially remedied by the use of more than one test system. A positive result in several quick tests might carry more weight than a positive result in the Ames test alone. Mammalian cell systems are of particular interest to some investigators who believe it would be intellectually more satisfying to detect DNA damage to these cells or transformation of them into tumor cells than to detect mutations in bacteria. Mammalian cell test systems are not yet extensively used, however, because these tests cost 5 to 10 times more than the Ames test and neither they nor the other quick tests have the data base or the sensitivity of the Ames test.

Legislators and officials at the National Cancer Institute have expressed interest in the quick tests but have hesitated to endorse any of them because of the difficulties in interpreting results. Yet rapid and inexpensive screens for harmful chemicals are needed if the Toxic Substances Control Act is to be economically feasible. The ultimate solution to the problem with the quick tests lies in obtaining more data. Then it will be possible to correlate results from quick tests with results from animal tests for carcinogens in a statistically convincing manner. By their recent extensive use of the quick tests, industries seem to be making a substantial contribution to the data base that must exist for these tests to be evaluated.—GINA BARI KOLATA

Nuclear Testing: U.S.–Soviet Treaties Viewed with Doubts and Misgivings

Control of nuclear arms is a matter about which the public, aware of the generally frustrating history of arms negotiations, has learned to lower its expectations. Accordingly, the treaties negotiated by U.S. and Soviet officials to limit underground weapons tests and peaceful nuclear explosions have not been awaited with much excitement or anticipation. But, now that the Ford Administration is finally ready to submit them to the Senate for ratification, these treaties give rise to so many doubts and objections that many senators will find it a close question whether they are marginally better than nothing or whether they are actually worse than nothing.

The treaties now subject to ratification go back to the Moscow summit of July 1974, Richard Nixon's last hurrah before he was forced to resign over Watergate. With the strategic arms limitations talks still at an impasse, the President seized the opportunity to sign a treaty limiting underground tests to a certain maximum yield or "threshold."

Had the treaty banned all underground tests or even all tests susceptible to unambiguous "verification" by seismic monitoring, it would have been applauded by the private arms control community that is made up in good part of groups such as the Federation of American Scientists (FAS) and the Arms Control Association (ACA). (A number of former government officials with responsibilities for arms control are active in both the FAS and the ACA.) But the treaty Nixon brought back from Moscow was instantly put down as a mockery by many of these arms controllers, who wanted a treaty that would effectively discourage further weapons development and inhibit nuclear proliferation.

By prohibiting testing in the atmosphere, in outer space, and under water, the Limited Test Ban Treaty (LTBT) of 1963 had stopped the dangerous radioactive contamination of the world environment. But the United States and the Soviet Union had simply moved their ambitious programs of testing underground, and, consequently, the LTBT had constrained the arms race little if at all.

The Threshold Test Ban Treaty (TTBT) signed by President Nixon and General Secretary L. I. Brezhnev in 1974 clearly would not do much to constrain the arms race, either. The threshold, set at 150 kilotons, would allow the testing of weapons 10 times more powerful than the one that destroyed Hiroshima. Also, this threshold would bear no relation to verification capabilities, which have been improved to the point that even explosions at yields of 10 kilotons or less may not escape detection.

Furthermore, many arms controllers objected strongly to the fact that, permissive as it was, the treaty would not take effect until 31 March 1976, thus leaving time for each of the two superpowers to carry out a series of tests at high yields. They objected, too, to a glaringly evident loophole in the TTBT—"peaceful" explosions, of whatever magnitude, were not covered even though such explosions can be indistinguishable from weapons tests.

Administration officials acknowledged that plugging this loophole was priority business. Assurances were given that the TTBT would not be submitted for ratification unless it was accompanied by an agreement on peaceful nuclear explosions, or "PNE's." And, sure enough, after a year and a half of hard negotiations, a PNE treaty (PNET) was signed on 28 May, in ceremonies conducted simultaneously in the White House and the Kremlin.

After its fashion, the PNET does address some of the concerns of the arms

controllers. No individual PNE would be allowed to exceed 150 kilotons, which is to say that the threshold for weapons tests would also apply to PNE's. And, in the event either nation should plan a simultaneous detonation of two or more peaceful devices with an aggregate yield greater than 150 kilotons, the other could have observers present to monitor the event on-site.

With the yield limited to 150 kilotons, no PNE would produce militarily useful information not otherwise obtainable through weapons tests allowable under the TTBT. Also, properly equipped observers present for simultaneous multiple explosions could verify that no individual explosion has exceeded the threshold, something which could not be accomplished by seismic monitoring at a distance.

The negotiations for the PNET began in late 1974 and were not successfully concluded until this past April. The United States would have readily agreed to ban PNE's altogether. After spending more than $160 million on earth-moving (cratering) and gas-stimulation experiments with PNE's, the U.S. government had pretty well decided that their promise was illusory. But the Soviets, who had experimented with a number of different PNE applications, professed an especially keen interest in pursuing grandiose plans to use PNE's for canal building. There might be some simultaneous multiple explosions having an aggregate yield of several megatons, with some individual explosions of up to 400 kilotons or greater. In the hope that they would be able to continue conducting PNE's without restrictions, the Soviets had agreed in principle at the Moscow summit to allow some PNE's to be witnessed by on-site observers, subject to such terms and conditions as the negotiators might later arrive at. And this was, in fact, a significant concession in light of the traditional Russian aversion to the idea of on-site inspections.

But, by the time negotiations for the PNET got under way it had become apparent that the Senate would probably reject the TTBT unless PNE's as well as weapons tests were made subject to the 150-kiloton threshold, with adequate provisions for verification.

The on-site observer provisions ultimately agreed to testify to the delicate balance that had to be struck to satisfy both the U.S. negotiators' insistence on effective verification and the Soviets' insistence on minimizing any American intrusion. For instance, American observers witnessing a Soviet PNE would bring two identical sets of yield monitoring equipment and allow the Soviets to choose the set actually to be used; the other set would be turned over temporarily to the Soviets. This, together with other conditions meticulously spelled out in the treaty, would make any unauthorized snooping hard to get away with.

The nation carrying out PNE's would be required to provide the other party with a substantial amount of geologic, hydrologic, and other data bearing on the interpretation of seismic signals and the measurement of explosive yields. In general, the larger the PNE, the more data required. Such data would be essential to verification because, unlike weapons tests conducted solely at designated test sites (about which there would be information exchanges), PNE's might be carried out under widely varying conditions.

The question whether the TTBT and the PNET represent a gain or a loss for the cause of arms control must be considered from the standpoint of weapons development, nuclear proliferation, and the chances for a comprehensive test ban.

The TTBT would impose some constraints on weapons development, but they would be clearly marginal, especially in light of the numerous kinds of high-yield weapons already in the U.S. and Soviet inventories and of all the testing done between July 1974 and the end of March 1976. During this period, the United States conducted 12 announced tests at yields over 200 kilotons; a number of these tests were at yields of up to a megaton. The Soviets also detonated a number of high-yield devices, including several in the multimegaton range.

From the standpoint of nuclear proliferation, the 150-kiloton threshold does indeed seem to make light of the superpowers' solemn obligation under the Nonproliferation Treaty of 1970 to seek arms reductions and a comprehensive test ban. Furthermore, the PNET in a sense legitimizes PNE's, although it does underscore the fact that a PNE and a weapons test can be indistinguishable.

Arms controllers can only hope that, as some people in the Ford Administration are now suggesting, the PNET's on-site observer and data requirements may discourage the Soviets from undertaking a major program of PNE's. But, if such a PNE program is carried out by the Soviets, this could encourage some nonnuclear nations to develop nuclear devices, either out of a genuine interest in PNE technology or from a recognition that PNE's offer a convenient mask for a fledgling nuclear weapons program.

As for whether the TTBT and the PNET would improve chances for a comprehensive test ban or harm them, one person's speculation may be as good as another's. The treaties would establish a threshold from which negotiators could work downward. But many arms controllers believe that, if the treaties are formally ratified, the U.S. and Soviet governments will not resume serious test ban negotiations during the TTBT's initial 5-year term (renewal is automatic unless either party withdraws).

The treaty does call for negotiations looking toward a comprehensive test ban, but, in light of its other provisions, this has the ring of an empty promise. There is even the very real likelihood that, if the Soviets try to employ PNE's in canal building, they will violate the 1963 test ban treaty. Expert opinion holds that some "venting" of radioactive debris would inevitably occur and that part of this material probably would drift across international boundaries.

Yet, despite all doubts and misgivings, if the TTBT and the PNET are sent to the Senate floor this year and brought to a vote, they are likely to be ratified. This is so because even such senators as Edward Kennedy, sponsor of a resolution calling for a comprehensive test ban, are afraid that Senate rejection of the treaties might undermine hopes for further arms control agreements.

On the other hand, there may be a better than even chance that in this election year time will run out before the Senate brings itself to act. Indeed, senators who regard the treaties dubiously may contrive to make this happen. If time does run out this year, the question of ratification will go over until 1977 when a newly elected President will have to review it. If the president should be someone like Jimmy Carter, who favors an immediate moratorium on testing, the treaties' fate will be in serious doubt.

—LUTHER J. CARTER

Clarification: The article, "Pesticides: Three EPA attorneys quit and hoist a warning flag" (19 March) referred to aldrin and dieldrin and heptachlor and chlordane as "two pairs of compounds found to be potent carcinogens." Most uses of both aldrin/dieldrin and heptachlor/chlordane were in fact ordered suspended by the administrator of the Environmental Protection Agency as an "imminent hazard" to human health and the environment. But some readers may take the words "potent carcinogen" to mean that a compound has been determined by federal authorities to be carcinogenic in laboratory animals at relatively low dose levels. No such finding has been made with respect to heptachlor/chlordane.

In the case of these compounds, the administrator overruled the decision of the administrative law judge, who held that to suspend their use as an imminent hazard was not justified. He felt "hesitantly unwilling at this time to find that heptachlor/chlordane are conclusively carcinogens in laboratory animals." The administrator concluded, however, that heptachlor is a carcinogen in both the rat and the mouse. Weighing the risks of continued use of heptachlor/chlordane against the benefits, he decided that most uses should be suspended. His ruling is now under review by the U.S. Circuit Court of Appeals for the District of Columbia.—L.J.C.

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Zeppel

The Metabolism of Alcohol

Overconsumption of alcohol can cause cirrhosis and death not only because alcoholism promotes malnutrition but also because alcohol and its products disturb liver metabolism and damage the liver cells

by Charles S. Lieber

For all the attention being directed toward heroin, cocaine and marijuana, the favorite mood-altering drug in the U.S., as it is in almost every human society, is alcohol. Its psychic effects, both pleasant and unpleasant, are well enough known. What is less well known is that alcohol, in different quantities for different people, is a toxic drug: its overconsumption taxes the body's economy, produces pathological changes in liver tissue and function and can cause disability and death. As the incidence of alcoholism has risen in the U.S. population, so has the incidence of cirrhosis of the liver, which in 1974 climbed past both arteriosclerosis and influenza and pneumonia to rank seventh among the leading causes of death; in some urban areas (including New York City) it is actually the third most frequent cause of death between the ages of 25 and 65. Such a disease deserves intensive study. It is only in recent years that the changes in liver tissue and liver function have been directly associated with specific steps in the metabolism of alcohol, for the first time giving some hope that rational methods can be developed for preventing and treating alcoholic liver disease.

What we call alcohol is of course ethyl alcohol, or ethanol (CH_3CH_2OH). On the earth ethanol is probably almost as old as life itself. Whereas for man it is something to be ingested, it is a waste product for yeasts, the microorganisms that manufacture it. Ethanol is what is left over after fermentation, the process by which yeasts, through the agency of their particular enzymes, derive energy from various vegetable sugars. Man probably encountered ethanol during prehistoric times in the form of naturally fermented fruit juice (wine), honey (mead) and malted grain (beer).

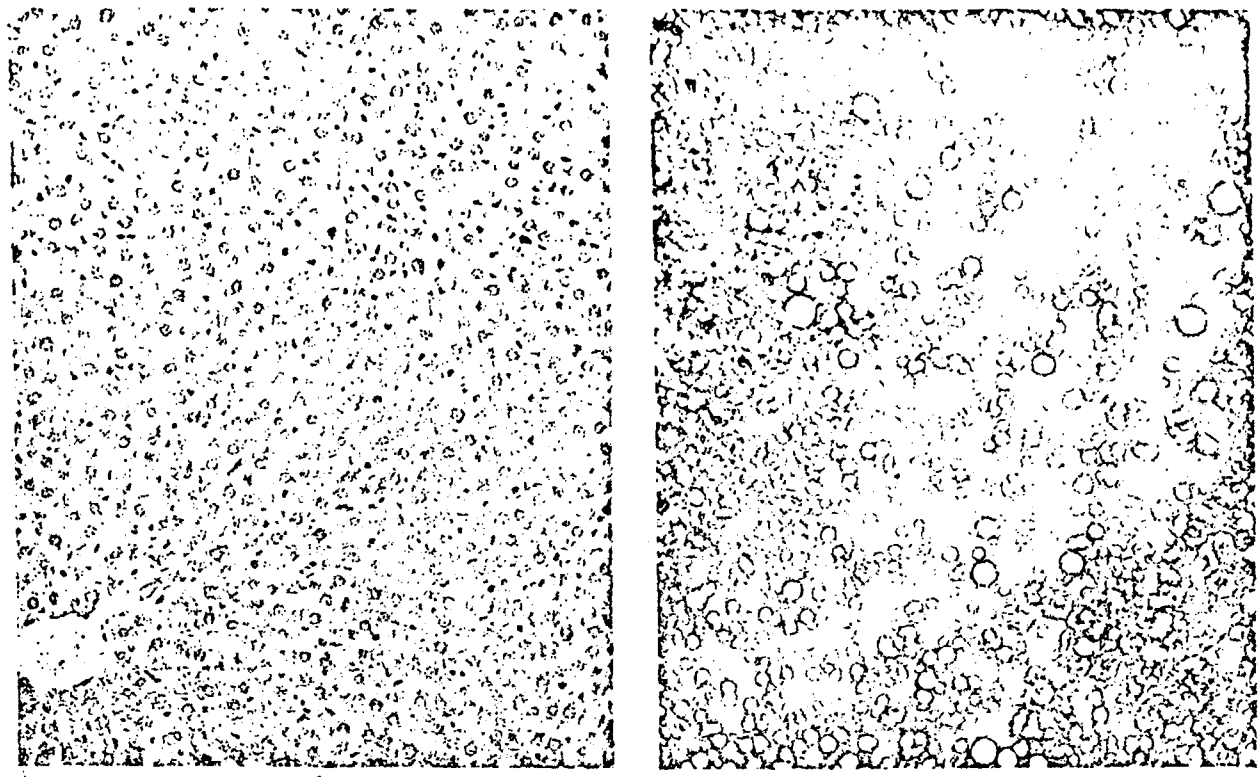
At least since the anatomical studies of Vesalius in the 16th century it has been recognized that heavy ingestion of alcohol is associated with diseases of several tissues, notably those of the liver. Until recently, however, such disease was attributed not to alcohol itself but to the malnutrition that is so often an accompaniment of excessive drinking. Alcohol was considered not a drug but a special kind of food with interesting psychotropic effects, and one that was metabolized by the body like any other energy-rich food. As recently as 1949 the distinguished physiologist Charles H. Best and his colleagues wrote that alcohol's metabolic contribution was simply to supply calories and that "there is no more evidence of a specific toxic effect of pure ethyl alcohol upon liver cells than there is for one due to sugar." Perhaps it was wishful thinking on the part of people in general and physicians in particular that installed as accepted fact the concept that alcohol lacked intrinsic toxicity.

It was a rather naïve belief. Ethanol has little in common with other energy-rich compounds. Carbohydrates and fats can be synthesized in the body as well as ingested in the diet, but alcohol is essentially foreign to the body. As do carbohydrates and fats, alcohol has a high caloric value and is readily absorbed from the gastrointestinal tract; unlike them, however, it is not effectively stored in the tissues. Moreover, very little alcohol can be disposed of through the lungs or the kidneys, so that the body can get rid of alcohol only by oxidizing it. Again unlike fat and most carbohydrates, which can be oxidized by almost all tissues, alcohol must be oxidized in the liver, the organ that contains the bulk of the enzymes necessary for initiating the process. The organ-specificity of alcohol explains to a large extent the concentration of so many of alcohol's deleterious effects in the liver, which is the body's main chemical plant, the primary site of metabolic processes ranging from the synthesis of proteins to the detoxification of drugs and other foreign substances.

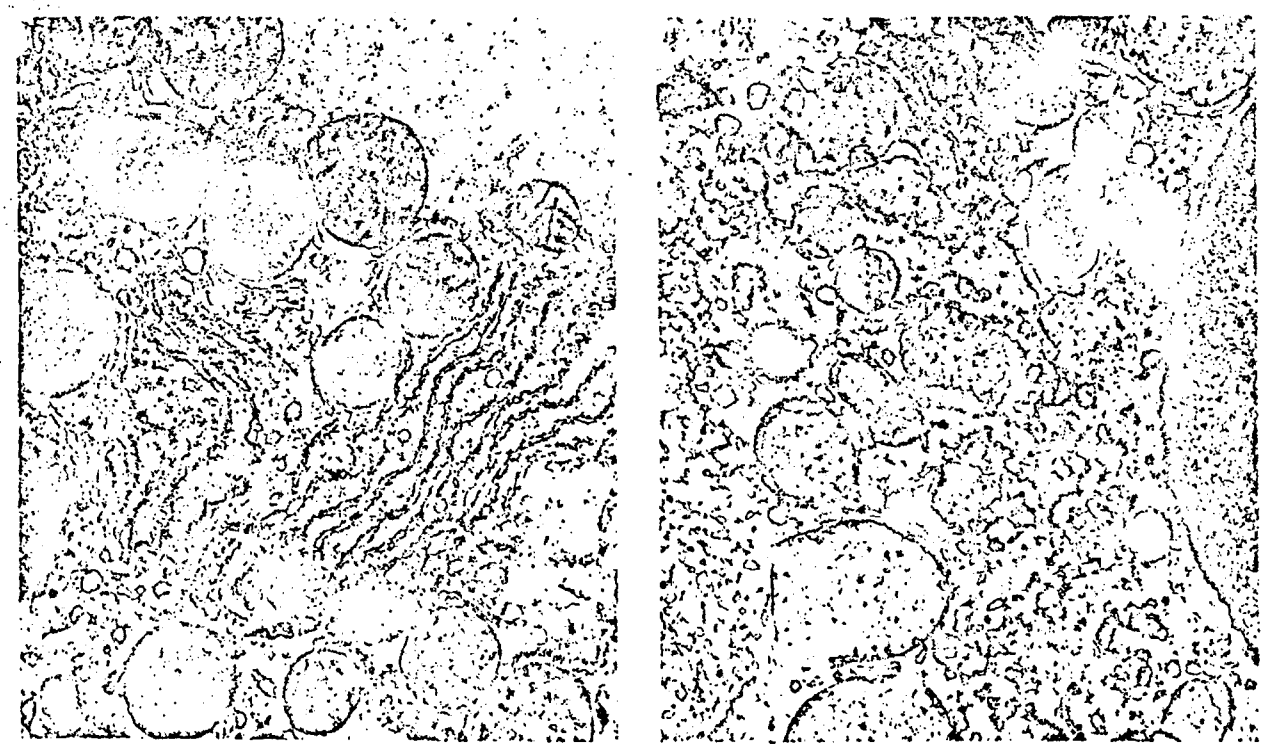
The purpose of this article is to relate how our studies at the Bronx Veterans Administration Hospital and the Mount Sinai School of Medicine of the City University of New York demonstrated that alcohol's toxicity for the liver is independent of poor diet and have shown how specific events in the metabolism of alcohol are linked to specific biochemical and structural changes in the liver and other tissues.

There are reasons enough, both socioeconomic and physiological, for an alcoholic to suffer from malnutrition. The alcoholic spends his time, money and energy drinking and may tend to disregard such routine tasks as the preparation of meals and even eating. Unlike other drugs, alcohol has a high caloric value: 7.1 calories per gram, so that a pint of 86-proof spirits (not an unusual daily intake for an alcoholic) represents about half of the daily caloric requirement. As a result the appetite for food may be diminished. Ethanol calories, however, are "empty calories," quite unassociated with nutritive proteins, minerals and vitamins. Moreover, alcohol directly enhances malnutrition. By causing inflammation of the stomach, pancreas and intestine it can impair the digestion of food and absorption of nutrients into the blood; malnutrition can in turn cause the intestine to function still less effectively. Finally, ethanol and its primary conversion product, acetaldehyde, can interfere with the activation of vitamins by liver cells. As a result of all these effects malnutrition is common in alcoholics, and malnutrition alone does unquestionably impair liver function, as is observed in experimental animals that are fed severely deficient diets.

It was therefore quite natural to speculate



FATTY LIVER was produced in laboratory rats by alcohol in spite of an adequate diet. Liver-tissue sections from two rats are enlarged 240 diameters in these photomicrographs. One rat (*left*) was fed a liquid diet without alcohol. The other (*right*) had a liquid diet containing ethanol as 36 percent of total calories. After 24 days the micrograph of the ethanol-fed rat's liver shows numerous globular fat droplets.



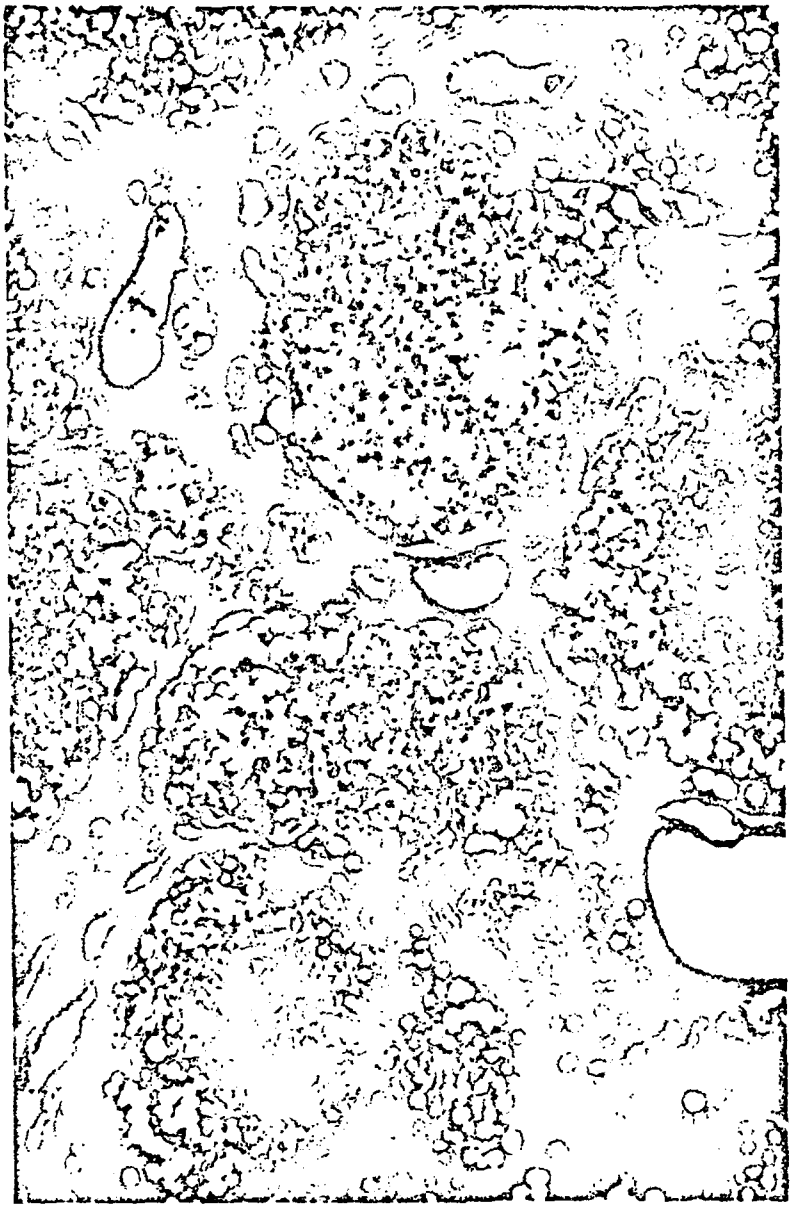
MICROSTRUCTURAL CHANGES are revealed in electron micrographs made by Oscar A. Iseri and the author, in which part of the liver cell is enlarged 16,000 diameters. In the control liver (*left*) the ray organelles with infolding membranes (cristae) are normal mitochondria; between them the parallel arrays of ribosome-studded rough endoplasmic reticulum are seen, along with some vesicular (sac-shaped) smooth endoplasmic reticulum. In the liver of the ethanol-fed rat (*right*) the mitochondria are swollen and distorted; in some mitochondria the external membrane and cristae are disrupted. There is marked proliferation of the smooth endoplasmic reticulum.

that malnutrition might be the cause of alcoholic liver disease even if one recognized that alcohol was not a food. The fact remains that in medical practice one encounters alcoholics who develop liver disease in spite of what is apparently an adequate diet. In the late 1950's I therefore began to wonder whether alcohol might exert direct toxic effects on the liver. Such a finding would clearly be of therapeutic as well as theoretical interest. Belief in the exclusively nutritional theory was leading many physicians to advise alcoholic patients that normal liver function could be maintained in spite of heavy alcohol consumption as long as the diet was adequate. If that was not true, it would be good to know it.

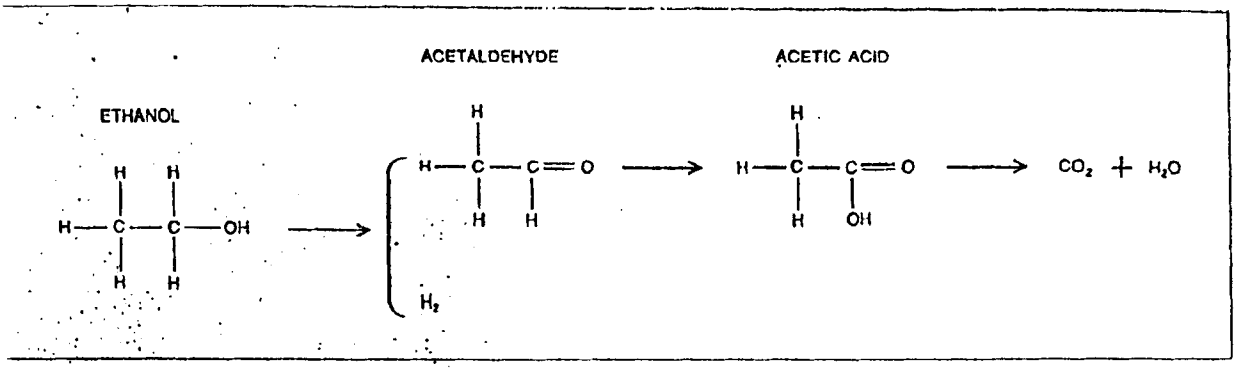
The importance of the issue, along with our confidence that even heavy consumption of alcohol carries a negligible risk if it is only for a brief period and under supervised conditions, led to a decision to study the effect of ethanol directly in volunteers. We fed the volunteers a nutritionally optimal low-fat diet, in which protein accounted for 25 percent of the calories, or two and a half times the recommended percentage. That meant beef for breakfast, cheese and fish for lunch and meat or fowl for dinner, with supplementary vitamin and mineral capsules. The volunteers also had six drinks a day, a total of about 10 ounces of 86-proof alcohol. On that regimen there was a progressive rise in liver fat; routine thin-needle biopsy revealed significant fat accumulation after a few days, and there was an average eightfold increase over the 18-day course of the experiment. Here was the characteristic "fatty liver," the first (and typically reversible) stage of liver disease. There were striking changes in the ultrastructure of the liver cell: the mitochondria, the energy-converting organelles, were enlarged and distorted, and the smooth membranes of the endoplasmic reticulum, the site of enzymes associated with the metabolism of alcohol and other substances, proliferated. These liver changes were brought about by a rather moderate ingestion of ethanol, which did not result in any clinical signs of intoxication; the blood levels peaked at from 80 to 90 milligrams of alcohol per 100 milliliters of blood, which is below the levels (100 or 150 milligrams) that constitute evidence of excessive drinking in most states. Drunkenness is no prerequisite for the development of liver damage.

In order to verify these effects and study their mechanisms in detail we needed to reproduce them in an experimental animal. The laboratory rat is a common subject for such experiments. Ethanol is usually fed to rats in their drinking water, however, and under these circumstances the animals generally refuse to take enough ethanol to develop liver injury. Leonore M. DeCarli and I countered the rat's aversion to alcohol by developing a new technique: we incorporated the ethanol in a nutritionally adequate but totally liquid diet, so that in order to eat or drink the animals also had to take the alcohol. With this method we produced fatty liver in rats in spite of an adequate and fully controlled diet. The changes in the liver were comparable to those produced in man, both on gross examination and when tissue was viewed with the light microscope [*see top illustration on opposite page*]. The ultrastructural changes seen with the electron microscope were also similar, including enlargement and disruption of the mitochondria and proliferation of the smooth endoplasmic reticulum, part of the system of involuted membranes within the cell [*see bottom illustration on opposite page*].

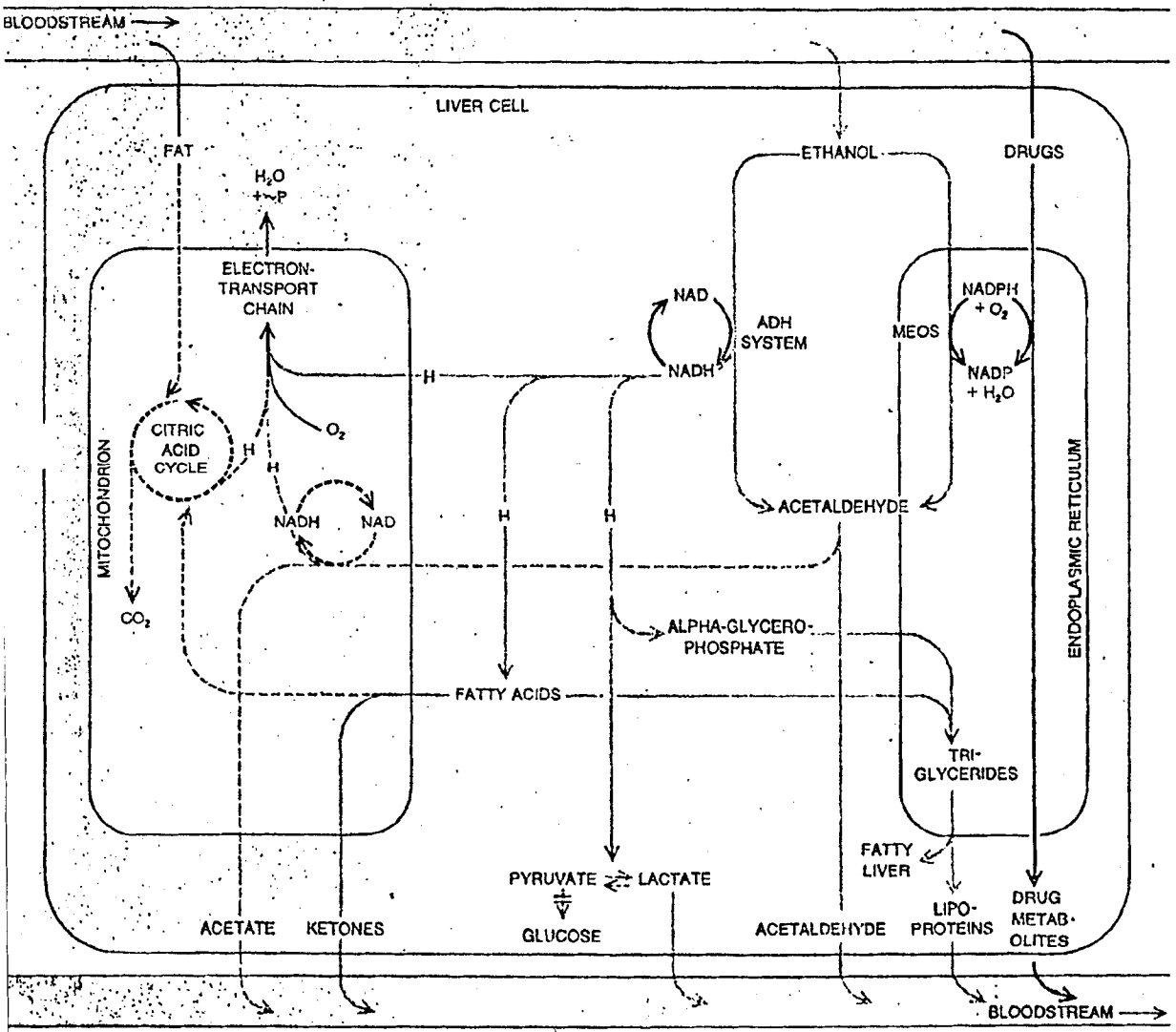
Although our alcohol-fed rats developed a fatty liver, they did not go on to incur the more severe forms of alcoholic liver injury seen in human beings. The first of these stages is alcoholic hepatitis, in which decreased liver-cell function leads to the death of cells, inflammation and a mortality rate of from 10 to 30 percent. The final stage is cirrhosis, when fibrous scars disrupt the normal architecture of the liver and give rise to a number of potentially fatal compli-



CIRRHOSIS OF LIVER was produced in a baboon after four years of heavy alcohol consumption. This section of the baboon's liver, enlarged 70 diameters, shows the broad strands of fibrous connective tissue, like scar tissue, that have disrupted the once orderly arrays of liver cells, segregating nodules of irregularly arranged cells and heavily concentrated globules of fat.



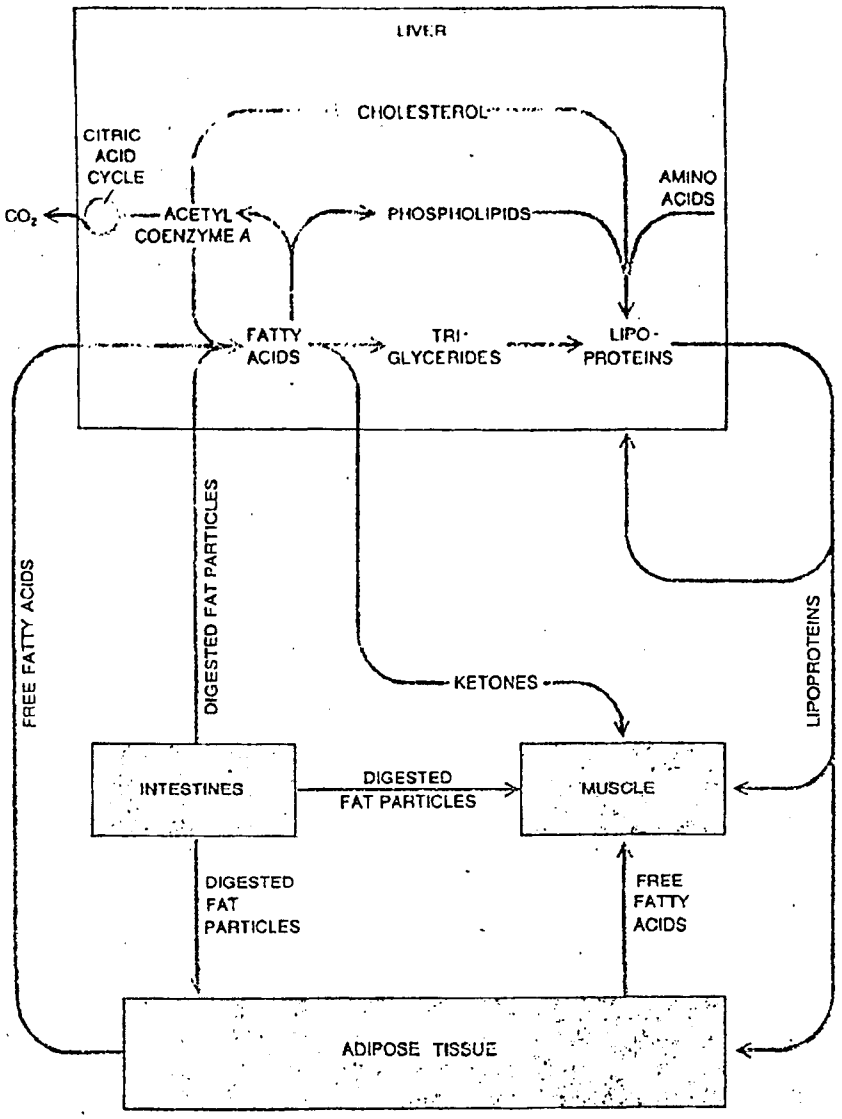
OXIDATION OF ETHYL ALCOHOL (ethanol) is accomplished in several stages. In the liver cells two hydrogen atoms are removed from each molecule of ethanol to form acetaldehyde. The acetaldehyde is normally oxidized, primarily in the liver, to form acetic acid (as acetates), which is eventually broken down into carbon dioxide and water. The illustration omits the various enzymes and cofactors.



METABOLISM OF ETHANOL in the liver cell by the alcohol-dehydrogenase (*ADH*) system and the microsomal ethanol-oxidizing system (*MEOS*) is represented schematically, with the two ethanol pathways and the movements of their products shown in color. Pathways that operate in the absence of ethanol are shown in black and those whose activity is decreased by ethanol are shown by broken lines. In the primary ethanol pathway alcohol dehydrogenase catalyzes the removal of hydrogen atoms (*H*) through reduction of a cofactor, nicotinamide adenine dinucleotide (*NAD*). The excess hydrogen is shunted into various processes. For example, it supplies the energy-producing electron-transport system, supplanting fats, the normal fuel. Hydrogen also contributes to the synthesis of excess triglycerides, or fats. A secondary pathway that comes into play at high alcohol levels utilizes the microsomal system, which also metabolizes certain drugs and other foreign compounds. Here the cofactor is reduced nicotinamide adenine dinucleotide phosphate (*NADPH*).

cations. We could imagine two reasons for the rats' failure to develop hepatitis and cirrhosis. Even with our liquid diet their alcohol intake did not exceed 36 percent of the al calories, which corresponds to moderconsumption in man. Moreover, it takes from five to 25 years of steady drinking for a human being to develop cirrhosis, and a rat lives for only about two years.

We turned to the baboon, which is long-lived and more closely related to man, and which can tolerate liquid diets containing alcohol as 50 percent of the total calories, about the same as an alcoholic human being. At that level the baboons were visibly intoxicated, and when alcohol was temporarily withdrawn, they gave signs of physical dependence, such as tremors and seizures. At the Laboratory for Experimental Medicine and Surgery in Primates we put 16 baboons on the ethanol-rich diet and 16 others on an alcohol-free control diet with the same caloric content. Whereas the liver remained normal in the control animals, all 16 animals given ethanol soon showed excessive fat accumulation in the organ; five of them showed typical alcoholic hepatitis, and six of the animals kept on the ethanol diet developed cirrhosis after between two and four years. The rat and baboon experiments demonstrated clearly that severe liver injury can be produced by prolonged heavy ingestion of alcohol even when the diet is good. They also gave us for the first time experimental animal models that reproduce all the liver lesions seen in the alcoholic human being. The models have enabled us to find biochemical explanations for the early stages of alcoholic liver disease (fatty liver and the metabolic disturbances associated with it) and for some of the persistent lesions that seem to lead to the more severe stages: hepatitis and cirrhosis.



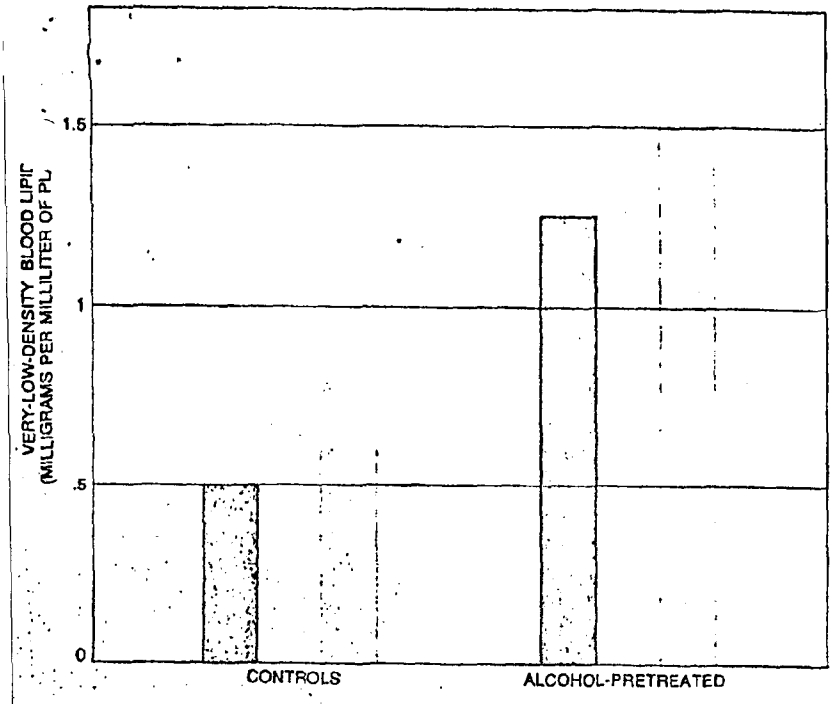
LIVER FAT comes from three sources: from the diet, by way of the intestines; in the form of free fatty acids mobilized from storage in adipose tissue, and through synthesis within the liver itself. A fatty liver can develop if there is an excessive supply of fat or if disposal of fat from the liver (by oxidation to carbon dioxide or by secretion into the blood as lipoproteins) is impaired.

The first step in alcohol's primary metabolic pathway is catalyzed by the enzyme alcohol dehydrogenase. (In spite of its name, alcohol dehydrogenase serves as a generalized remover of hydrogen atoms from various compounds, including some steroids; it was therefore probably present in the liver of the prehistoric men who first sampled alcohol, available to take on what has since become a major function.) Alcohol dehydrogenase catalyzes the transfer of hydrogen atoms from ethanol to a cofactor, nicotinamide adenine dinucleotide (NAD), converting the ethanol into acetaldehyde. The acetaldehyde is then oxidized, primarily in the liver, to form acetate, which is eventually converted into carbon dioxide and water. A number of the metabolic effects of alcohol are directly linked to the two first products of its oxidation: hydrogen and acetaldehyde.

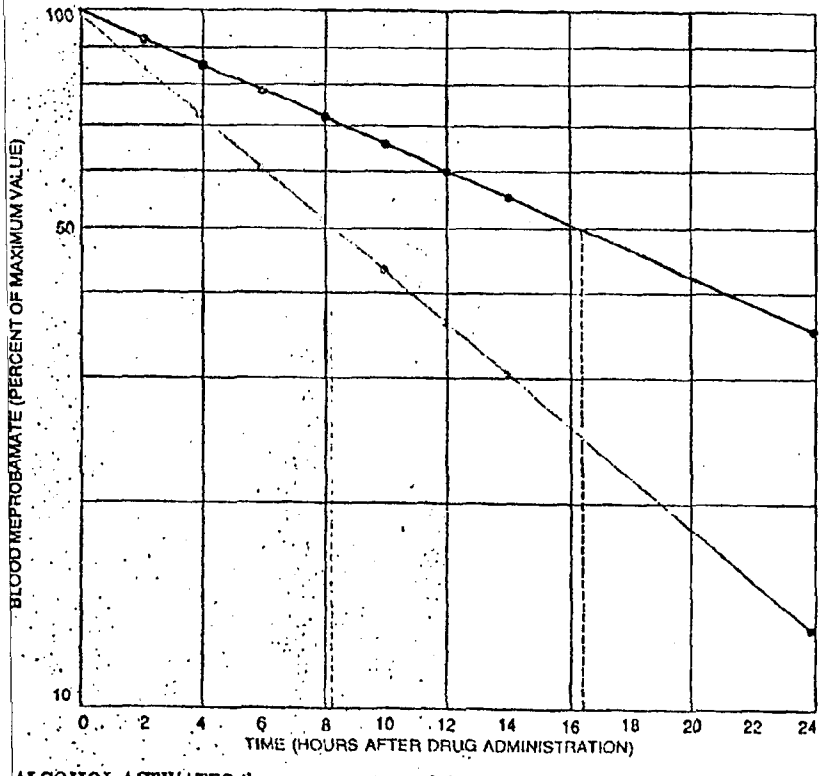
The excess hydrogen from alcohol unbalances the liver cell's chemistry. In order to live the cell must get rid of the hydrogen, and it does so by shunting hydrogen ions into one or more of several pathways dependent on them, sometimes with deleterious effects. One such pathway is the process whereby amino acids (derived from the breakdown in the liver of proteins) are converted, with pyruvate as an intermediate, into glucose. In the presence of excess hydrogen ions the process is turned in a different direction: the pyruvate is reduced to lactate instead of being converted into glucose. Blood sugar is derived primarily from three sources: gluconeogenesis, or synthesis from amino acids in the liver, breakdown of glycogen stored in the tissues and conversion of carbohydrates in the diet. If the alcoholic person has been drinking and not eating, there are no dietary carbohydrates and glycogen may be used up; if gluconeogenesis is then blocked by the diversion of pyruvate to lactate, the level of sugar in the blood will be lowered. Low blood sugar, or hypoglycemia, is known to be a complication of acute alcoholism, but it is often overlooked. When an intoxicated person is brought into a hospital emergency room, it is important to test for hypoglycemia, since crucial organs, including the brain, can be critically affected by a lack of sugar; some deaths of "unknown origin" in alcoholics may be attributable to the condition.

The lactate buildup from excess hydrogen has other effects. The lactate moves into the blood, bringing on lactic acidosis. In the kidney it interferes with the excretion of uric acid. A high uric acid level in the blood (hyperuricemia) exacerbates gout, so that the process I have described may explain the ancient clinical observation that excessive drinking can trigger or aggravate gout attacks.

There are other ways in which the liver cell can rid itself of alcohol's excess hydro-



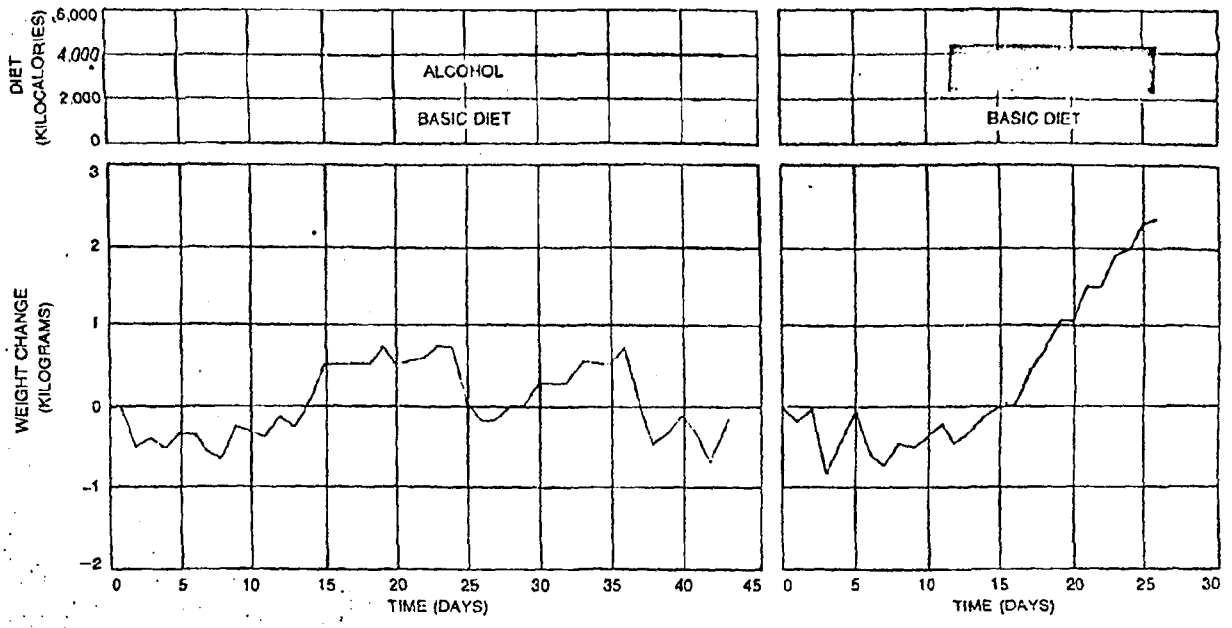
ALCOHOL-FED RATS develop a tendency toward hyperlipemia, or high blood fat. Animals were fed either the control diet or the alcohol-containing diet of the same caloric value for about a month. Then, after fasting, the animals were given a "load" of one or the other diet. The rats' fasting blood-lipid levels had been about the same. In response to the load the animals that had been chronic alcohol consumers (*right*) developed markedly higher blood-lipid levels, whether the load contained alcohol (*colored bars*) or did not contain alcohol (*gray bars*).



ALCOHOL ACTIVATES the enzyme systems of the smooth endoplasmic reticulum, stepping up the metabolism of certain drugs. The curves show the disappearance from the blood of a dose of the tranquilizer meprobamate given to volunteers before (*black*) and after (*color*) a [illegible] of alcohol ingestion. Alcohol activation cuts the drug's half-life (*broken lines*) in half.

gen. Several of them involve the formation of lipids, or fat. The hydrogen can be shunted directly into the synthesis of alpha-glycerophosphate and fatty acids. Those are the two precursors of the triglycerides, and triglycerides are the lipids that accumulate in the alcoholic fatty liver. The main mechanism for disposing of hydrogen is more indirect, but it has a similar result. The hydrogen is transferred to the mitochondria, the cell organelles that produce the energy for liver functions. Normally it is fat that is oxidized—in effect burned—in the mitochondrial citric acid cycle to produce usable energy in the form of phosphate ions in an energized state. The plentiful hydrogen from alcohol, however, provides an alternate fuel that is oxidized instead of the hydrogen from fat. In promoting the oxidation of alcohol the substitution exacts a price: the lipids accumulate, again leading to fatty liver. If alcohol is ingested along with a diet containing fat, the fat of dietary origin accumulates in the liver; even when alcohol is taken with a low-fat diet, fat made in the liver itself is deposited there. In addition, when alcohol is ingested in very large quantities, it can trigger hormonal discharges that mobilize fat from stores of adipose tissue and move it toward the liver [*see illustration on preceding page*].

What can the liver do with the accumulating fat? One possibility is for it to be secreted into the bloodstream, which delivers blood lipids to provide fuel for peripheral tissues such as muscle and deposits extra supplies for storage in adipose tissue. The secretion is complicated by the fact that lipids are not soluble in water; they have to be made soluble by being wrapped in a thin coat of protein to form lipoproteins. The assembly of lipoproteins is carried out in the liver in the smooth membranes of the endoplasmic reticulum. I have alluded to the proliferation of the smooth endoplasmic reticulum that comes with heavy alcohol consumption in both rats and human beings, which I observed with Oscar A. Iseri, Bernard P. Lane and Emanuel Rubin. The proliferation is reflected, Jean-Gil Joly, Lawrence Feinman and I found, in the increased activity of certain enzymes in the smooth reticulum, which enlarges the liver's capacity for secreting lipoproteins. A liver that has thus adapted to alcohol after being conditioned by a period of heavy alcohol intake will respond with exaggerated secretion of lipoproteins even after a normal meal is eaten, producing hyperlipemia, or an abnormally high level of fat in the blood. Enrique Baraona and I observed that effect in rats, and William H. Perlow, Stephen A. Borowsky and I have now verified it in man. The effect is of particular significance in people who have underlying abnormalities of either lipid or carbohydrate metabolism and therefore a propensity for elevated blood-lipid levels. Hyperlipemia is a major predisposing factor for heart attacks. What is usually overlooked is that in individuals with preexisting hyperlipemia alcohol is proba-



ALCOHOL CALORIES are apparently not fully equivalent to other calories. When a basic 2,200-calorie diet was supplemented by 2,000 calories of alcohol (*left*) and of chocolate (*right*), the gain in weight caused by the alcohol was much less marked and more erratic.

bly the single most important aggravating factor that is amenable to correction.

Still another way for the liver to dispose of excess fat is to convert some of it into the water-soluble breakdown products called ketone bodies and secrete them into the bloodstream. In some susceptible people this response may be exaggerated, resulting in an elevation of ketone bodies in the blood that mimics the condition known as ketoacidosis in diabetic patients.

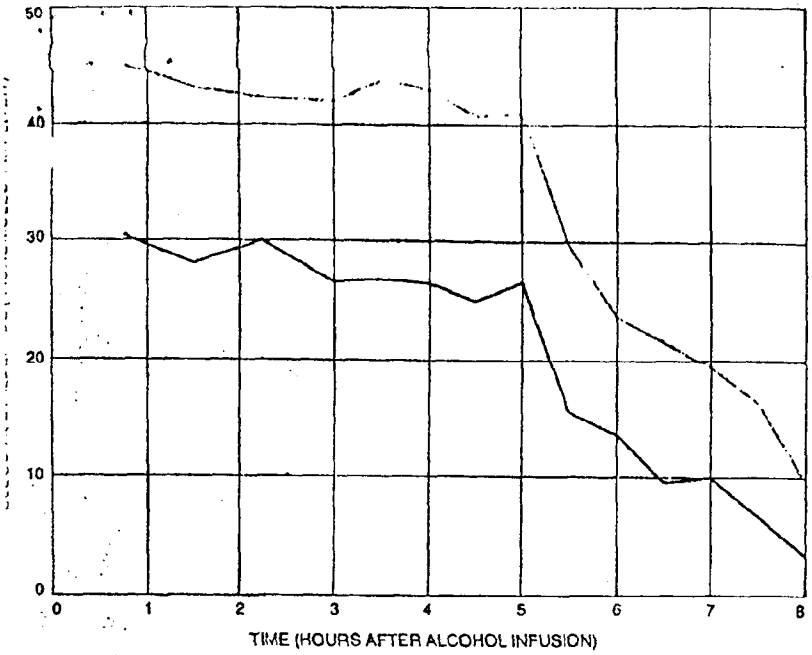
The conversion of lipids into lipoproteins is just one of a number of functions of the endoplasmic reticulum of liver cells, which also inactivates a wide variety of drugs and other foreign substances and converts them into water-soluble products that can be excreted [see "How the Liver Metabolizes Foreign Substances," by Attallah Kappas and Alvito P. Alvares; SCIENTIFIC AMERICAN, June, 1975]. I therefore wondered whether the proliferation of smooth reticulum after chronic alcohol consumption would be reflected in an increased capacity of the alcoholic liver to metabolize various drugs. That turned out to be the case. After repeated administration of alcohol (but before the evolution of severe liver injury) the enzymes of the smooth reticulum that inactivate tranquilizers, anticoagulants and other drugs and that detoxify certain food additives, cancer-causing substances and insecticides do increase their activity, enhancing the body's capacity to rid itself of these compounds. For example, Prem S. Misra and I gave the tranquilizer meprobamate to volunteers and measured its rate of disappearance from the bloodstream. The time for the blood level of the drug to fall to half its original value decreased from 16 hours during a control period to eight hours after a month of alcohol consumption.

Anesthesiologists had known for many years that larger doses of sedatives are required to achieve a given effect in alcoholics than in other people. That drug tolerance was attributed to adaptation in the central nervous system: increased resistance to sedatives by the brain of the alcoholic. Our finding pointed to a metabolic adaptation as well: increased capacity of the alcoholic liver to inactivate and excrete sedatives and other compounds detoxified by the endoplasmic reticulum. At the adaptive stage of their disease (which, as we shall see, does not continue indefinitely) alcoholics therefore require larger doses of many drugs. That is true, however, only when the individual is sober. When the alcoholic has been drinking, the effect is quite the opposite. The reason, we found, is that one of the drugs the smooth reticulum metabolizes is alcohol itself, by way of an accessory pathway that supplements the basic alcohol-dehydrogenase system.

DeCarli and I demonstrated the accessory pathway by spinning liver tissue in the ultracentrifuge and isolating the endoplasmic reticulum as what is called the microsomal fraction. We then found that a preparation of the microsomal fraction would oxidize ethanol. We called the accessory pathway the microsomal ethanol-oxidizing system, and Rolf Teschke, Kunihiko Ohnishi and I were able to obtain it in a semipurified form. We have found that this microsomal ethanol pathway comes into operation after the blood alcohol reaches a certain level. The alcohol thus enters into competition with other drugs whose metabolism shares some elements of the microsomal system, thereby slowing down the metabolism of those drugs and enhancing their effect. That is why simultaneous drinking and taking of tranquilizers is particularly dangerous: the alcohol can accentuate the action of the drug not only because the effect of the two drugs on the brain may be additive but also because the presence of alcohol can interfere with the liver's capacity to inactivate the drug, so that at a given dosage more of the drug remains active for a longer time.

Along with the rest of the microsomal enzyme systems, the ethanol-oxidizing system adapts to heavy alcohol consumption by increasing its activity, thus contributing to alcohol tolerance. The alcoholic's ability to drink more than most nonalcoholics is primarily due to brain tolerance, a progressive decrease in the effectiveness of alcohol's action on the brain. The alcoholic also develops an increased capacity to metabolize alcohol, not only through the microsomal system but also through the alcohol-dehydrogenase pathway and perhaps a third pathway that depends on the enzyme catalase. After heavy or prolonged drinking, however, this adaptation can be offset by progressive liver injury, so that the liver's overall ability to handle alcohol remains about the same or even decreases.

The microsomal changes I have described are beneficial in that they help to rid the liver of fat and speed the detoxification of many drugs, food additives and other foreign compounds. Hyperlipemia is one undesirable concomitant of the adaptive process, and there are others. Some foreign substances are activated rather than inactivated by the conversions they undergo in



ACETALDEHYDE LEVEL IN BLOOD is higher in alcoholics than in nonalcoholic individuals. Identical quantities of pure ethanol were infused into the blood of alcoholic and nonalcoholic volunteers, achieving similar blood-alcohol levels. The acetaldehyde plateau was higher in the alcoholics (*colored curve*) than in the nonalcoholics (*black*). Apparently the alcoholics metabolized acetaldehyde less effectively, perhaps because of ethanol-induced liver damage.

the endoplasmic reticulum. Certain potentially cancer-causing substances become carcinogenic only after activation by the microsomes, and other substances become ... to the liver itself only after such acti-

For example, exposure to carbon etrachloride is known to cause liver damage, but the familiar dry-cleaning agent is harmless to the liver until it is activated by the liver-cell endoplasmic reticulum. Yasushi Hasumura and I found that the increase in microsomal activity induced by alcohol enhances the toxicity of carbon tetrachloride: rats chronically treated with alcohol were much more susceptible to the toxic effects of carbon tetrachloride than matched control animals. This effect presumably explains the clinical observation that alcoholics are particularly susceptible to carbon tetrachloride poisoning in dry-cleaning plants that still use the compound. The enhanced susceptibility of alcoholics very probably extends to a number of other foreign compounds that are harmless to most people but that may become toxic in the alcoholic as an undesirable side effect of increased microsomal activity.

Another side effect is energy waste. Microsomal activity requires energy. Moreover, it is a peculiarity of the various microsomal oxidations that they produce heat without conserving chemical energy. This could conceivably contribute to the impaired growth we observe in animals that are fed ethanol, since the production of heat beyond what is required to maintain body temperature represents a waste of energy. Such waste may explain at least in part my observation with Romano C. Pirola that the addition of ethanol to the diet results in less weight gain than the addition of the same number of calories from other sources [*see illustration on preceding page*]. That is, with respect to body weight ethanol calories apparently do not fully "count," at least in alcoholics and when the alcohol intake is large.

Apart from the metabolic complications of excess hydrogen and of the adaptive changes in microsomal activity, heavy alcohol consumption has direct toxic effects on liver tissue. It appears that a central role in the toxicity of alcohol may be played by acetaldehyde, a product of alcohol's several metabolic pathways that is extremely reactive and that affects most tissues in the body. Most of the acetaldehyde is converted into acetate by the liver mitochondria, but some of it escapes into the bloodstream. Both the alcohol dehydrogenase and the microsomal ethanol-oxidizing pathways become saturated when the liver is loaded with a large amount of alcohol, so that the acetaldehyde level in the blood reaches a plateau and stays there until the alcohol level drops, at which point the microsomal pathway apparently cuts out. Mark A. Korsten, Shohei Matsuzaki, Feinman and I found that the acetaldehyde plateau is significantly higher in alcoholics than it is in nonalcoholics, even when the same amount of alcohol has been given to both groups and the same blood level of alcohol has been attained [*see illustration at left*]. This abnormally high acetaldehyde level in alcoholics could result from faster metabolism of alcohol to form acetaldehyde (because of the adaptive increase in microsomal activity) or from slower disposition of the acetaldehyde (because of impaired acetaldehyde metabolism).

Probably both factors are involved, at least in the beginning. I have mentioned the striking alterations in the mitochondria revealed by the electron microscope even in the early stages of heavy alcohol consumption. Hasamura and I isolated the damaged mitochondria and found that their capacity for metabolizing acetaldehyde to acetate is reduced. Acetaldehyde itself may be responsible for part of the decrease in mitochondrial function: Arthur I. Cederbaum, Rubin and I showed that it has a toxic effect on the organelles. The alcoholic may therefore be the victim of a vicious circle: a high acetaldehyde level impairs mitochondrial function in the liver, acetaldehyde metabolism is decreased, more acetaldehyde accumulates and causes further liver damage. The acetaldehyde affects not only the liver but also other tissues. For example, Marcus A. Rothschild and his colleagues at the Manhattan Veterans Administration Hospital have reported that acetaldehyde levels no higher than we have measured in the blood of alcoholics can inhibit the synthesis of proteins in heart muscle. Such an effect could in part explain the impaired cardiac function that is common in alcoholics. Acetaldehyde may affect the functioning of other muscles too.

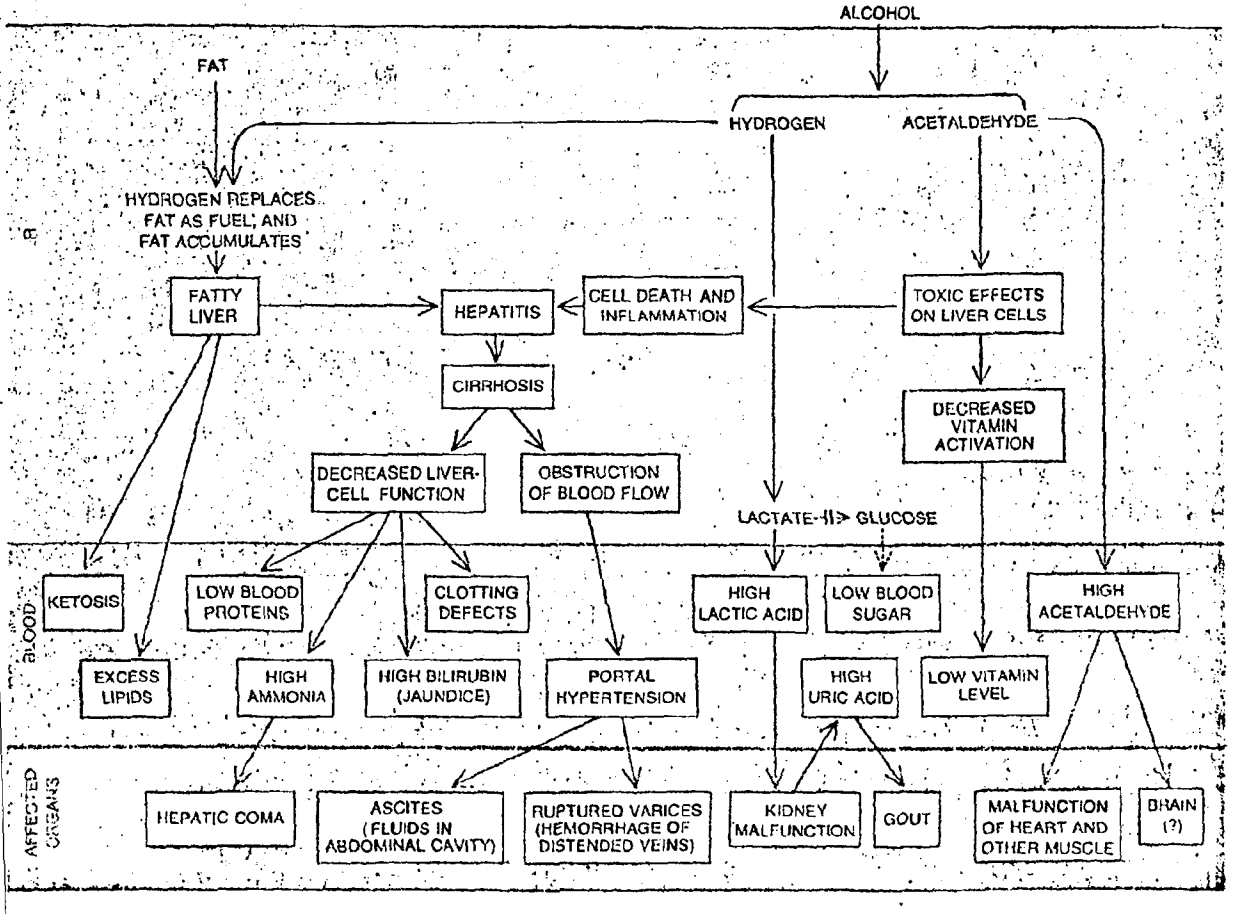
Acetaldehyde has striking effects on the brain. Several investigators have suggested that acetaldehyde rather than alcohol itself may be responsible for the development of the dependence that, along with tolerance, characterizes alcohol addiction. Dependence manifests itself by a state of extreme discomfort, often accompanied by physiological disturbances such as tremors and seizures, produced by withdrawal of a drug. Several mechanisms mediated by acetaldehyde have been proposed to explain dependence but none has yet been confirmed. One proposal begins with the fact that certain amine neurotransmitters, which transmit nerve impulses from one cell to another in the brain, are inactivated by the enzyme monoamine oxidase to form an aldehyde that is then converted into an acid. The conversion to acid requires an enzyme that is also active in the metabolism of acetaldehyde. Virginia E. Davis and Michael J. Walsh of the Houston Veterans Administration Hospital have suggested that if acetaldehyde is present in the brain, it may compete for the enzyme; unmetabolized neurotransmitter aldehydes may therefore accumulate; the aldehydes may then combine with the neurotransmitter, forming compounds that are startlingly similar to certain morphine derivatives known for their ability to promote dependence.

Another possibility, pointed out by Ger-

ald Cohen of Mount Sinai, is that the acetaldehyde combines directly with amines to form isoquinoline derivatives, potent psychoactive compounds that could play a role in developing dependence. Alcohol dependence is probably determined by a number of factors acting in concert. It is at least possible, however, that acetaldehyde is involved in the predisposition to alcoholism: a predisposed person may have deficiencies of acetaldehyde metabolism that make for a higher blood acetaldehyde level and a propensity to dependence because of the effect of the higher acetaldehyde level on the brain.

If heavy drinking continues, reversible fatty liver evolves, in most people, toward more severe and irreversible liver disease: hepatitis and then cirrhosis. Just why and how the liver may lose its ability to adapt to the alcohol load is not clearly established. Even in the fatty-liver stage there are some indications that more severe lesions are developing. There may be a ballooning of the liver cells, which is usually attributed to fat accumulation. Baraona, Maria-Anna Leo, Borowsky and I have noted that proteins, of which the liver is the major synthesizer, also pile up in the cells, whose ability to export proteins is somehow depressed by large amounts of alcohol. The engorgement of liver cells with fat and protein interferes with their normal functioning. So does the reduced energy production to which I have alluded. For all these reasons some liver cells may die, and the necrosis can trigger an inflammatory process that is characteristic of the stage called alcoholic hepatitis. The acute reduction in liver-cell function at this stage is enough to cause death in some patients.

Cell necrosis and inflammation in turn promote the next stage: fibrosis, or the development of scar tissue, the hallmark of cirrhosis. The fibrous connective-tissue barriers between groups of liver cells interfere with the flow of blood to and from the cells, further decreasing liver function. Partially obstructed in the liver, blood backs up, increasing the pressure in the portal system, which brings blood to the liver from the intestines. Abnormal channels may therefore develop in the venous system so that some of the blood can bypass the circulatory block established by the cirrhotic liver. Some newly overloaded veins (notably in the esophagus) may become distended, and such veins, called varices, can rupture and hemorrhage. Bleeding varices are a major cause of death in cirrhosis. Moreover, under high pressure plasma leaks out of the portal-system blood vessels. Extra lymph is also formed, and it leaks out of the lymphatic vessels. Both sources contribute to ascites, the accumulation of fluid in the abdominal cavity. A final complication stems from the liver's inability to clear from the blood ammonia and other nitrogenous compounds produced by bacteria in the intestines. As such compounds accumulate they act on the brain and may cause functional disturbances, hepatic coma and death.



COMPLICATIONS of excessive alcohol consumption stem largely from excess hydrogen and from acetaldehyde. Hydrogen produces fatty liver and hyperlipemia, high blood lactic acid and low blood sugar. The accumulation of fat, the effect of acetaldehyde on liver cells and other factors as yet unknown lead to alcoholic hepatitis. The r step is cirrhosis. The consequent impairment of liver function disturbs blood chemistry, notably causing a high ammonia level, which can lead to coma and death. Cirrhosis also distorts liver structure, inhibiting blood flow. High pressure in vessels supplying the liver may cause ruptured varices and accumulation of fluid in abdominal cavity. There are individual differences in response to alcohol; in particular, not all heavy drinkers develop hepatitis and cirrhosis.

Looking at Genes in the Workplace

Screening for individual susceptibilities and monitoring of the workplace promise to stir up a host of legal, ethical, and scientific questions

The Office of Technology Assessment (OTA) raised some eyebrows at a congressional hearing last month when it said that at least 59 major corporations may be planning to inaugurate some kind of genetic testing of employees in the foreseeable future. This apparent surge of interest is occurring despite the fact that very few tests have been developed to screen employees for susceptibilities to certain chemicals, and their usefulness so far is questionable. Moreover, the whole idea of genetic screening is looked on with great suspicion by labor unions.

The OTA's testimony was part of a report of preliminary findings from a study of the problems and promises posed by two types of genetic testing. One is biochemical genetic testing of individual workers for certain genetic traits; the other is cytogenetic monitoring, which entails testing groups of workers for aberrations in chromosomes that might occur from exposure to chemicals. The OTA said only six companies are currently engaged in such testing, down from 17 who have done it in the past 10 years. The dip mainly reflects a decline in testing for the sickle cell trait among blacks. The OTA presented its figures at hearings held by a subcommittee of the House Science and Technology Committee chaired by Representative Albert Gore, Jr. (D–Tenn.), who predicted that genetic testing "is likely to become the source of legal concerns very soon."

Indeed, although the field is in its infancy it raises a host of concerns relating to confidentiality, the use of human subjects in research, discrimination in the workplace, and the proper use of findings—particularly when the implications of such findings are not known. The subject is especially sensitive because of its rancorous setting in the world of labor-management relations. Increasing interest in the subject is evidenced by the OTA study as well as a government-funded project at the Hastings Institute, which is examining the ethical implications.

The two types of testing at issue are sometimes confused in the rhetoric of the debate, but they pose somewhat different problems for society. Biochemical genetic screening for "susceptible" workers is the more politically controversial of the two since it is aimed at pinpointing individuals who may be unsuitable for certain types of jobs, but it falls into a time-honored tradition. In the past, makers of tar and creosote denied employment to fair-skinned and freckled people because of their higher susceptibility to skin cancer. The railroads for years have been screening out applicants whose x-rays show a potential for back problems. More recently, companies have been grappling with the difficult problem of how to prevent pregnant women from being exposed to chemicals that might cause birth defects. Genetic screening is at least as problematic because it could lead to discrimination against certain racial and ethnic groups, and because in most cases it is impossible to predict when a person with a given trait will suffer from exposure to a given substance.

There are probably thousands of genetic deficiencies that render individuals unusually vulnerable to certain chemicals, but only a few are known presently and several, such as the sickle cell trait, occur primarily to members of minority groups. The sickle cell trait, which some believe could interfere with the blood's oxygen-carrying capacity in a person exposed to oxidizing chemicals, is probably not a very useful one to know about because there is no solid evidence that it makes any difference in the workplace. Indeed, the Air Force Academy recently reversed a 10-year-old policy of barring blacks with the trait from pilot training because no one could demonstrate that a low-oxygen environment would precipitate a medical crisis.

There are several other traits, though, that give cause for concern. One—which also predominates among minorities—is called G6PD or glucose-6-phosphate dehydrogenase deficiency. This occurs in 10 percent of American black males and a higher percentage of Mediterranean Jews. It can predispose carriers to a hemolytic crisis causing anemia from lack of oxygen resulting from exposure to certain chemicals such as napthalene. Another much-talked-about deficiency is that for alpha-1 antitrypsin (AAT) which can predispose individuals to lung disorders and emphysema from exposure to lung irritants. The severe form of the deficiency, which only affects one person in 2000 or 3000, is clearly linked to emphysema. But among those who carry the heterozygous trait—2 to 4 percent of the population—no ill effects can be reliably predicted.

For a while during the 1970's, Dow Chemical Company and Du Pont Corporation did limited testing for AAT and G6PD deficiencies in employees assigned to work with cyanogenic compounds. However, according to Du Pont's medical director Bruce Karrh, tests were terminated because they did not supply any additional useful information. The only company *Science* could learn of (the OTA poll is anonymous) that currently does any genetic screening is Du Pont, which screens blacks for the sickle cell trait. This program was begun in 1972 at the request of black employees at a time when sickle cell anemia was getting a lot of publicity and screening for the trait was regarded as a public service. Although the information is not used for employment decisions but only, said Karrh, for the "information and edification" of black employees, the company came in for a good bit of criticism following a series of articles in the *New York Times* in early 1980.

There appears to be very little support at present for biochemical genetic screening within the scientific community. The tests are regarded as arbitrary and, although valid, not very predictive. Says Gilbert Omenn of the University of Washington, "The extent of debate is way out of proportion to the scientific knowledge and any test applications at this time." This being so, the political objections to such screening begin to appear compelling. Union officials such as Anthony Mazzochi of the Oil, Chemical and Atomic Workers Union have decried such testing as having devastating potential for discriminating against certain groups of workers and creating, in effect, leper colonies of susceptible workers who may be assigned to low-paying jobs because they are regarded as genetically unfit.

Although companies are by no means rushing into the business of genetic screening, many critics believe they are attracted to it as an alternative to cleaning up the workplace. If genetically hy-

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scientists who have already been subject to the audit say zealous and inexperienced auditors may not be painting a fair portrait. At the University of Wisconsin at Madison, one indignant scientist said the audit team seemed preoccupied with equipment such as minicomputers and kept asking naïve questions about why they were needed. There were no scientists on the team, and answers provided seemed to go over the heads of the auditors. The scientist also complained that audits seemed to be held when the principal investigator was absent, a situation in which the person responsible for the equipment is not in a position to defend or explain apparent discrepancies.—WILLIAM J. BROAD

FDA to Reexamine Bendectin Data

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The FDA last looked at Bendectin studies in September of 1980 at which time its panel of experts examined data from animal studies and 13 epidemiological studies and concluded that there is no demonstrated relation between the drug and birth defects. However, it is impossible to prove that any drug is harmless. The panel recognized a "residual uncertainty" about Bendectin's safety during pregnancy (*Science*, 31 October 1980, p. 518).

Despite the FDA's conclusions, a growing number of parents are convinced that Bendectin caused birth defects in their children. More than 100 lawsuits have been filed against Merrell-Dow, Bendectin's manufacturer, although the plaintiffs lost in the one case that did go to trial. Recently, public attention has been focused again on Bendectin as a result of newspaper stories indicating that there is new evidence which shows a strong link between Bendectin and birth defects.

The FDA's decision to reexamine all the Bendectin data was prompted by a meeting on 8 April between FDA commissioner Arthur Hull Hayes, Surgeon General C. Everett Koop, Representative Doug Walgren (D–Pa.), and Harry Meyer, director of the National Center for Drugs and Biologics.

Susan McFalls, a member of Walgren's staff, says the congressman is concerned by some new data that he believes may implicate Bendectin in birth defects. There is a rat study showing that Bendectin may cause diaphragmatic hernias, a potentially fatal defect in which the stomach and other organs get into the lung cavity through a hole in the diaphragm. There is a monkey study showing that Bendectin may cause ventricular septal defect, a hole between the chambers of the heart. And there is evidence from a new in vitro test for teratogens that Bendectin may cause birth defects. "We have been concerned for some time because we have seen both these defects [diaphragmatic hernias and ventricular septal defects] in reports from physicians and patients," McFalls says.

Ann Wilk, a reviewing pharmacologist at the FDA, notes the very preliminary nature of the new Bendectin studies. The rat study, done by Reimer Roll of Bundesgesundheitsamt in West Berlin, is still unpublished and the FDA has only a summary statement and reams of raw data which the agency is now having translated into English. Roll apparently sees a slight incidence of diaphragmatic hernias at very high doses of Bendectin with no dose-response effect. But, says Wilk, the FDA cannot yet say anything about his methods. In the meantime, Roll is repeating his study and the FDA is repeating it "with some modifications," according to Wilk.

The monkey study, conducted by Andrew Hendrickx of the Primate Research Center at the University of California at Davis, also is unpublished except in abstract form. Hendrickx, however, notified the FDA of his results in May of 1981. He gave 12 cynomolgus monkeys 10 to 20 times the normal human dose of Bendectin throughout the major period when organs are developing. Two of the monkeys aborted their fetuses. He then examined seven of the remaining fetuses about 2 months prior to term. In four of the seven, he saw an intraventricular septal defect, but it is not clear what this finding means because fetal monkeys normally have a hole in the septum earlier during development. When Hendrickx examined the three monkey babies that were carried to full-term, he found that they were completely normal. Wilk asks of the septal holes that Hendrickx found in the four fetuses, "Was this a delay in development or would it persist until birth?"

Merrell-Dow is now funding Hendrickx in a much larger study, which will take 2½ years and will be double-blind. There will be four groups of monkeys, 20 pregnancies in each group, and three doses of Bendectin.

In the meantime, another small-scale monkey study, involving nine animals and conducted by Harold McClure of Yerkes Primate Research Center, showed no effect of Bendectin on rhesus monkey fetuses.

After hearing of these animal studies, the FDA requested that researchers conducting epidemiological studies, including Boston University's Drug Epidemiology Unit and the Centers for Disease Control, look for an association between Bendectin and heart defects or diaphragmatic hernias. In these studies none has been found as yet.

The in vitro study that McFalls referred to was conducted by John Hassell of the National Institute of Dental Research. He added Bendectin to cultured cells that normally would develop into cells resembling cartilage. The added Bendectin prevented this development. Hassell and Wilk concur that it is difficult to evaluate this study because no in vitro test has yet been validated—it has not been shown that these tests can reliably distinguish known teratogens from substances known not to harm fetuses.

At the present time, the FDA is considering a labeling change for Bendectin to reflect the possibility that the recent animal studies may turn out to demonstrate teratogenicity. Bendectin's label now says there is no evidence of teratogenicity in animal tests. But the agency scientists feel that, in the final analysis, their best data will be from continued epidemiological monitoring of Bendectin. And, as yet, there is no persuasive evidence from human studies that the drug causes birth defects.—GINA KOLATA

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persensitive workers can be identified and removed from some plants, the argument goes, companies may be able to press successfully for less stringent occupational exposure standards. Some observers, including toxicologist Samuel Epstein of the University of Illinois, contend that industry is putting a big emphasis on "weeding out the susceptibles" rather than taking the steps necessary to clean up the environment entirely. Others argue that the latter is not always technically feasible. Companies nowadays are getting very interested in preventive medicine and are trying to educate workers on the dangers of alcohol, smoking, overeating, and lack of exercise. But some see even this as evading their real responsibilities and trying to "blame the victim," as Epstein puts it. If such plainly positive activities on the part of management are looked on with suspicion, then a very extensive set of legal and ethical safeguards indeed will have to be designed to make genetic screening acceptable.

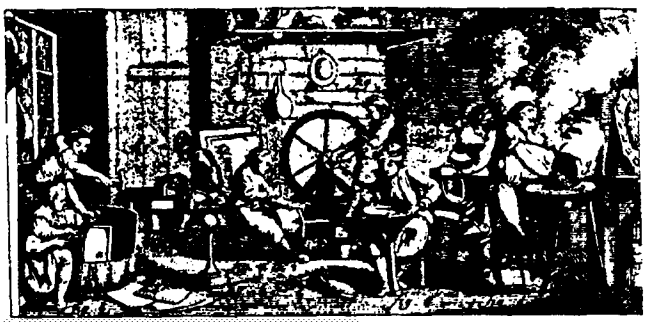
Cytogenetic monitoring, on the other hand, is not as controversial politically but probably more so scientifically. This involves monitoring a group of workers over time to test for increases in chromosome aberrations. What may be the earliest such program was carried on for about 10 years at Dow under the supervision of then medical director Jack Kilian who is now at the University of Texas. The program proceeded smoothly until Kilian's group found what it regarded as significantly raised levels of chromosomal aberrations among workers exposed to benzene and epichlorohydrin. The results of the studies are very controversial and Dow, which had doubts about their validity, went back to a "research mode," according to its present medical director. Now it is following groups of workers who have not been exposed to chemicals to see what effect other factors, such as age, smoking, and time of year, have on the rate of chromosome aberrations.

Another instance of industrial involvement in cytogenetic monitoring is a research program at Johnson & Johnson. Researchers have been monitoring the effects of ethylene oxide, a sterilant gas, on workers at three plants with three different levels of exposure, matched with three control groups. After 6 months they found that employees in the plant with the highest exposure had significantly higher incidence of a chromosomal abnormality known as SCE than the control group, according to a preliminary report by the company. They thereupon discontinued the use of ethylene oxide at that plant. The company is also doing chromosome studies on individuals before they are placed in jobs involving potential exposure to ethylene oxide.

Cytogenetic monitoring is of dubious value in determining an individual's susceptibility to cancer. All that can be said is that some substances that cause cancer cause aberrations, and, therefore, a group with high numbers of aberrations may be more susceptible. But an individual from that group who develops cancer may not be one with significant chromosome damage. The only good epidemiological data linking chromosome aberrations to cancer comes from Hiroshima and Nagasaki survivors. They were exposed to massive radiation doses, which may have caused chromosome damage and other changes on a scale far greater than that caused by exposure to chemicals, so the Japanese survivors may not tell us much about the effect of lower levels of cell damage.

Nonetheless, some scientists, including Kilian, believe there is no reason to delay in introducing cytogenetic monitoring into the workplace since the association between chromosome damage and cancer has been established beyond question. Margery Shaw of the University of Texas concurs. She believes enough close correlations already have been found—between mutagenicity and carcinogenicity; between carcinogens and substances that disrupt DNA; between chromosome breakage and SCE's, for example—that chromosome monitoring can be used as an indicator of potential health hazards much as badges are used to monitor workers' exposures to radiation. Marvin Legator, also of the University of Texas, contends that cytogenetic monitoring, in combination with other tests for abnormalities in semen and mutagens in urine, "can serve as an advance warning system" of hazardous chemicals in an environment. But, he says, progress has been stymied because industry does not want to use the tests until they are validated and industry is the only party in a position to validate them. Kilian asserts that industries do not want to get involved because cytogenetic monitoring will open up new possibilities for getting sued by workers.

Other scientists believe there is still too much uncertainty surrounding the technology. Richard Albertini of the University of Vermont says "the big problem with a monitoring system is that we don't have a disease outcome" and thus it is impossible to meaningfully interpret the results of any tests. J. Grant Brewen, cytogeneticist at Allied Chemical Corporation, puts it more succinctly: there is "not a shred of evidence," he says, that directly links chromosome damage to any disease. Brewen has another problem with cytogenetic monitoring: he says that counting structural aberrations in chromosomes is not particularly useful because to achieve substantial positive results would require very high levels of exposure, high enough to manifest themselves in systemic toxicity. He believes there is more promise in looking at SCE's, which are numerically more sensitive. However, he warns that while chromosome changes can indicate effects of chemicals, the absence of such changes do not necessarily mean there has been no effect on the organism. Therefore, he says, cytogenetic monitoring could lead to a "false sense of security."



Tinkers

Most observers seem to agree that neither biochemical genetic screening nor cytogenetic monitoring is going to be widely adopted any time soon. Although the OTA reports 59 companies are thinking about it, the uncertainty is too great and the potential usefulness too questionable for many to actually begin programs in the near future. Unions have a lot more things on their minds which seem to them more important than genetic screening. And to industries, opening up new programs of genetic testing may just seem like asking for trouble.

—CONSTANCE HOLDEN

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British Out of IIASA, Americans May Stay In

Proponents of continued American participation in the International Institute for Applied Systems Analysis (IIASA) are plugging away at finding an alternative to the U.S. government sponsorship that runs out at the end of the year. They are encouraged by the possibility that the American Academy of Arts and Sciences will agree to replace the National Academy of Sciences (NAS) as the U.S. "national member organization" and are seeking to raise money from industry and foundations to pay the U.S. way.

The Reagan Administration last year decided to discontinue federal funding for IIASA (*Science*, 2 April 1982, p. 35), an East-West think tank established near Vienna in the early 1970's in the early glow of détente. When the withdrawal of government support became likely, an IIASA-U.S. Planning Group was formed in this country by those favoring a continued American presence at the institute. The group set out to secure alternative sources of funding and a replacement for the quasi-public NAS, which is bowing out as national member organization.

Approaches to the American Academy were made. Although those involved in the efforts decline to discuss developments until the academy's council has acted, hopes appear to be high that the academy will consent to become the U.S. member.

Founded in 1790, the American Academy is an honorary society with about 2300 members elected from among scholars and national leaders in the sciences, arts, and humanities. The organization, which is headquartered in Boston, publishes the quarterly *Daedalus* and operates an interdisciplinary study center.

Results of the planning group's efforts to raise funds are also currently under wraps. Here too, however, there seems to be optimism that the campaign, headed by former NASA administrator T. Keith Glennan, will be productive in enlisting support from industry and foundations. Chairman of the planning group is Charles Maechling, Jr., a Washington attorney who negotiated the original U.S. membership in IIASA.

IIASA recently received another blow when Britain's Royal Society confirmed that Britain would withdraw from IIASA at the end of the year. The British departure will not impart as serious a financial jolt as that of the United States. Britain is one of 15 "Class B" IIASA members who contribute about $350,000 a year each, while the United States and the Soviet Union have each been contributing some $2.3 million a year to the institute's operating budget of about $10 million.

The official reason given for the British action was a change in research policy at the Department of Environment which has been the source of funds for IIASA membership. There also were reports that influential members and staff of the Royal Society were critical of IIASA's research plan in particular, and less than enthusiastic about applied systems analysis in general.

IIASA is said to be carrying on with assurances of continued backing from the Soviet Union and other member countries, is cutting administrative overhead and research plans to conform to the reduced budget and, figuratively, leaving the gate open to make it easy for the strays to return to the fold.—***John Walsh***

Using Experience to Calculate Nuclear Risks

A new and provocative estimate* of the risks of nuclear plant accidents came to light in early July, the first such analysis to base its findings on the actual record of a decade of industry performance. Earlier work has relied more on probabilistic calculations than on experience.

The new report, called the Accident Sequence Precursor (ASP) study, was written for the Nuclear Regulatory Commission (NRC) by the consulting firm, Science Applications, Inc. An antinuclear lobby, Critical Mass, released a draft of the report over the 4th of July weekend, citing it as evidence that officials have drastically increased their estimates of the risk of an accident.

The report's chief finding is that during the 1970's, the chances for having a Three Mile Island accident were one per 1000 years of reactor operation. At Three Mile Island, the fuel core was partially melted. This contrasts with the official risk estimate published by the NRC in 1974, which said that a fuel core meltdown would occur only once in 20,000 years of operation. The second figure comes from the *Reactor Safety Study* (WASH-1400), prepared for the NRC by Norman Rasmussen of MIT.

The comparison suggests that Rasmussen's work underestimated the real risk by a factor of 20. However, one industry spokesman, David Rossin of the Nuclear Safety Analysis Center, points out that WASH-1400 did not try to estimate the risk of the kind of accident that occurred at Three Mile Island. WASH-1400 looked instead at the chances for a total meltdown, a more severe and presumably rarer event. Rossin says it is wrong to compare WASH-1400 with ASP.

One of the reasons ASP was commissioned was that the NRC was criticized in 1978 for giving so much attention to WASH-1400's probabilistic theory when a better source of information—actual operating experience—was available. One review committee said the NRC ought to make a broad survey of nuclear plant operating records and use these to draw up risk estimates based on general experience.

The ASP report attempts to do this. The authors looked through 19,400 "licensee event reports" sent to the NRC between 1969 and 1979. After sifting through them, the authors identified 169 as "precursors of accident sequences," and 52 as "significant events." Drawing on these cases, they calculated the frequency with which certain accident sequences might occur and the frequency with which safety systems would fail. They concluded that severe core damage of the kind seen at Three Mile Island would occur between 1.7 and 4.5 times per 1000 years of reactor operation. Thus, if 1000 reactors were in operation, there would be at least one severe accident a year. At present there are only 74 commercial reactors in the United States.

*"Precursors to Potential Severe Core Damage Accidents: 1969–1979, a Status Report," by J. W. Minarick and C. A. Kukielka, published by the Nuclear Regulatory Commission (NUREG-CR 2497), June 1982.

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efficient, and the morphology of large-scale jets argues strongly for nonrelativistic motion of the jets. If these jets are only mildly supersonic and dominated by nonrelativistic plasma, then the turbulent boundary layer that surrounds the jet may provide the required particle acceleration, magnetic-field amplification, and transport of metal-enriched gas far from the interstellar medium. Slow jets increase the total energy requirements for the source, but not beyond the limits of credibility.

Compact jets are also inefficient, and arguments can be made for their relativistic or nonrelativistic motion. At present there is no clear-cut resolution of this issue, but it is fair to say that a consistent picture could be constructed wherein all jets are nonrelativistic. Observations of jet phenomena in extragalactic radio sources seem to require modifications of earlier models for the central energy source, and, although these have yet to be done in detail, models of the interaction of jets with their environment supply encouraging agreement with observations.

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Criteria for Evidence of Chemical Carcinogenicity

Interdisciplinary Panel on Carcinogenicity

Criteria for assessing the evidence for the carcinogenicity of chemicals have been described in several documents including a 1977 report of the Subcommittee on Environmental Carcinogenesis of the National Cancer Advisory Board (NCAB) (*1*) and the preambles to the monograph series of the International Agency for Research on Cancer on the evaluation of the carcinogenic risk of chemicals to humans (*2*). The report of the subcommittee of the NCAB recommended that "The general criteria should be reviewed on a continuing basis and revised in the light of new knowledge."

The Interdisciplinary Panel on Chemical Carcinogenicity was convened by Philippe Shubik at the request of the American Industrial Health Council to reevaluate the criteria for assessing the evidence for the carcinogenicity of chemicals as a result of recent increases in the quantity of data and research in carcinogenesis (*3*). The panel met on 31 October and 1 November 1983 and 24 and 25 February 1984 in Washington, D.C. The report that follows is particularly concerned with those areas that have been most affected by advances in knowledge.

The panel expresses its concurrence with the philosophy that was well expressed by the NCAB subcommittee (*1*):

> The criteria that are described are general guidelines and not rigid, universal criteria. The complexity of the problem dictates that the evaluation of potential human hazards of a given agent must be individualized in terms of the chemical and metabolic aspects of that agent, its intended use(s), the data available at the time the decision must be made, and other factors pertinent to the case under consideration. Each case must be considered on its own and the criteria appropriate for one agent may not necessarily apply to another.

The panel also agreed that the generalized definitions and significance of benign and malignant neoplasm presented in the NCAB subcommittee's report were adequate for present purposes without modification (*4*).

Since 1977 numerous bioassays of chemicals have been reported from in vivo studies; many in vitro tests have been undertaken and new in vitro tests have been devised. As a result much new research on the mechanisms of action of chemical carcinogens has been reported and suggestions put forward for classifying carcinogens; the influence of biometrics has been prominent in the development of a variety of mathematical models for risk assessment; and there have been controversies about the overall quantification of risk from chemical carcinogens to the human population. These factors have been influential in determining the priorities for the present deliberations.

Evidence Derived from Human Studies

Clusters of cases of a specific type of cancer associated with a particular exposure suggest that certain chemicals or combinations of chemicals may be carcinogenic to man. This has been the basis for much of our current knowledge of occupational cancer occurrence. The problems that confront the epidemiologist in such situations include the reality of the excess cancer incidence, the difficulty of estimating exposure, and

The members of the panel were: Philippe Shubik, Green College, Oxford University, chairman; Arnold Brown, University of Wisconsin–Madison; Charles Brown, National Cancer Institute, Bethesda, Maryland; J. R. P. Cabral, International Agency for Research on Cancer; David Clayson, Bureau of Chemical Safety, Health Protection Branch, Health and Welfare, Canada; Ian Higgins, University of Michigan; Wayne Levin, Hoffmann-La Roche, Inc.; Peter Magee, Fels Research Institute, Temple University, School of Medicine; Mortimer L. Mendelsohn, Lawrence Livermore National Laboratory; Robert A. Squire, Johns Hopkins University, School of Medicine; Bruce K. Bernard, Scientific Research Associates, Inc., rapporteur. Requests for reprints should be addressed to Dr. Philippe Shubik, Green College, Radcliffe Observatory, Oxford OX2 6HG, England.

the exclusion of confounding factors (5).

Potential carcinogenicity may be suggested by descriptive (hypothesis generating) epidemiological studies that explore the relationship between exposure to some factor or factors and the development of cancer in general or cancer at specific sites.

Descriptive studies are usually classified as exploring relationships with respect to time, place, or personal characteristics. Observations relate incidence of, or mortality from, cancer in general or cancer at specific sites to the introduction, increasing use, and removal of potentially carcinogenic exposures; to differences between geographical locations; and to personal exposures at work, from hobbies, leisure activities, food, water, and behavior. A primary problem is posed by difficulty in excluding confounding factors.

Analytical studies (hypothesis testing), in contrast, are carefully designed to explore hypothesized relationships and again two types are recognized: (i) case comparison studies in which the past history of exposure and personal characteristics of persons with cancer are contrasted with the same characteristics of those who do not have cancer and (ii) cohort studies in which the personal histories and exposures of a sample of the population are documented and the sample is followed over the years to determine the rate of occurrence of cancer. This rate is then related to the categories of exposure determined at the outset. The latency of cancer introduces difficulties into both types of study.

A helpful type of study is often possible in industries where good occupational hygiene records have been kept. Here it may be possible to define a group of employees whose exposures can be estimated at some time in the past. By relating categories of past exposure to subsequent mortality the waiting period needed in current cohort studies can be eliminated. Such studies are usually referred to as historical mortality studies.

An important factor in assessing carcinogenicity in man is the demonstration of dose response. Whenever possible, the dose estimated from level and duration of exposure should be related to cancer incidence or mortality.

One of the main problems of epidemiologic studies has been the difficulty of measuring exposure and estimating dose. Often studies of occupational exposure have been based on qualitative assessments such as high, medium, or low. Major improvements could be realized through better documentation of human exposure to potential carcinogens. Personal sampling has been used increasingly. For substances where portable sampling instruments are available, this provides a more relevant approach to individual exposure. We look forward to the time when such personal sampling can be coupled effectively to health outcomes.

Human data provide the only direct evidence that a chemical produces cancer in man. Negative epidemiologic results cannot ensure complete absence of carcinogenic hazard from a chemical or a process. Negative epidemiological results may indicate that the duration from the first exposure was too short, the sample size was too small, the dose was too low, or the substance to which people were exposed was not a carcinogen. In epidemiology, as in other disciplines, it is impossible to prove a negative. The lowest degree of risk that is likely to be directly detected by epidemiological means can be estimated, but risks below this can only be inferred by extrapolation from data obtained at higher exposures. Negative results thus indicate the limits within which a specific type of exposure will not affect the incidence of cancer in man.

Because of their central role in the identification of human risk, epidemiological studies are indispensable and require substantial expansion. Strengths and weaknesses of such studies, as is the case with experimental data, should be evaluated individually for robustness and weight.

Summary. The Interdisciplinary Panel on Carcinogenicity reviewed and reevaluated criteria for assessing evidence of carcinogenicity of chemical substances. The panel reviewed criteria applicable to data derived from human epidemiological studies and from both in vivo and in vitro laboratory studies. A critical appraisal of all these sources of information led to the conclusion that the characterization of human risk always requires interdisciplinary evaluation of the entire array of data on a case-by-case basis. Animal studies, whenever possible, should be augmented by studies of mechanisms, metabolism, and pharmacodynamics. Such studies may assist in assessing risk to man. Recognizing the utility of such data should point the way for better assessment in the future.

Evidence from Long-Term Bioassays

The carcinogenicity of a substance in animals is established when administration in adequately designed and conducted experiments results in an increase in the incidence of one or more types of malignant (or, where appropriate, a combination of benign and malignant) neoplasms in treated animals as compared to untreated animals maintained under identical conditions except for exposure to the test compound. Determination that the incidence of neoplasms increases as the result of exposure to the test compound requires a full biological, pathological, and statistical evaluation. Statistics assist in evaluating the biological conclusion, but a biological conclusion is not determined by the statistical results (6).

Certain data derived from animal studies, although suggestive of carcinogenic risk, may require additional study before extrapolation to conditions of human exposure can be made. For example, there are different tumor end points in bioassays conducted in vivo, some of which are enhancements of the background incidence of neoplasms seen in the untreated control animals. It is not uncommon for some of the control rodents in such studies to develop incidences of even 100 percent of neoplasms of a particular kind. If control animals develop, say, 50 percent of a certain kind of neoplasm, and this incidence is significantly increased in treated animals, or if there is a decreased latency period for the occurrence of such tumors, this is usually classified as an example of carcinogenesis. In other studies the end point may be represented by the occurrence of neoplasms that are not seen at all or only rarely seen in the controls. Instances of decreased latency or increased incidence of neoplasms in animals with a high incidence in controls requires full evaluation using a high level of statistical significance and, if possible, an analysis of the incidence in historical controls. The potentially large variation in the incidence of these neoplasms adds to the uncertainty in the evaluation of their significance.

The interpretation of these different patterns of tumor development in test animals should recognize the likelihood that they represent different mechanisms and may have different significance to human risk. Each experiment must be assessed according to the nature of the end points in the test animals, and different weights of evidence may be accorded

for purposes of extrapolation to humans.

Instances in which the only manifestation of carcinogenesis is a decreased latent period or an increased number of neoplasms with a high incidence in controls may require validation in further experiments before being extrapolated to humans. Some of these enhancements may be a result of relatively nonspecific effects such as changed caloric intake, the use of high doses leading to excessive cytotoxicity and cell proliferation, or other experimental conditions, which may not be relevant to human exposure.

Special bioassays of chemicals are sometimes undertaken to demonstrate effects such as cocarcinogenesis and promotion (7). These terms were originally meant to describe specific experimental conditions but are now widely used to describe modifications of the effects of a variety of carcinogens. There is no doubt that when the mechanisms of carcinogenesis are fully understood the role of certain modifying factors will prove to be of considerable practical importance. Presently, the relevance of these factors in human carcinogenesis cannot be determined, and for the moment such bioassays can only be used as ancillary evidence in assessing data for potential human hazard.

The statistical evaluation of carcinogenesis results makes use of experimental variables. These may include the time for observation of neoplasm (latent period), incidences of neoplasms, and time to death from neoplasms and other causes. The determination of time to neoplasm often requires an experimental design in which subgroups of animals are killed and examined at frequent intervals during the experiment in order to identify the onset of neoplasia. The exceptions to this added requirement are the instances in which neoplasms, such as those in the skin, can be diagnosed clinically from the time of their first appearance. However, most experimentally induced or enhanced animal neoplasms are not rapidly fatal, and clinical observations or terminal autopsies do not generally provide a valid basis for estimating time to onset of neoplasia.

In most cases, neoplasms of different cellular origin appear to originate independently. Consequently, it is reasonable for purposes of statistical analysis to evaluate tumors of different cellular origins separately.

In all instances the robustness of the data must be ensured. This may vary not only with the particular model chosen for the study, but also with practical conditions such as experience and training of personnel, adequacy of facilities, and avoidance of confounding factors. The strength of the data depends in part upon its robustness. Robustness in turn is a function of scientific design, execution, analyses, and interpretation of the study. An important element is the rigorous adherence to codes of good laboratory practices (8) and suitable quality assurance procedures.

Evidence from Short-Term Tests

Early markers of neoplasia. Many investigators have long hoped to find early tissue changes that would predict carcinogenicity, but this has so far eluded discovery. A recent approach is the effort to correlate enzyme-altered foci in the liver with eventual hepatoma occurrence. So far, the correlation has been too inconstant for this test alone to be a satisfactory basis for classifying compounds as carcinogens. A variety of other short-term tests for identification of preneoplastic lesions or markers of neoplastic transformations have been suggested, but they are still at the research stage; the panel expects that this area will receive increasing attention (9).

Tests for genetic alterations. Beginning over a decade ago, in vitro tests for genetic changes were developed and rapidly applied to the practical problem of carcinogen identification. This approach was spurred on by the belief that genetic alteration in somatic cells is closely linked to one or more of the stages of carcinogenesis, and by the early results which showed that the coupling of metabolic activation to relatively simple bacterial assays for mutation gave results highly correlated with the carcinogenicity of certain groups of chemicals.

Approximately 100 of these tests are now available. They involve many organisms ranging from prokaryotes to human cells and can be performed under various conditions ranging from studies of isolated DNA to observation of cells in vitro and in vivo. The tests can be grouped into three general categories based upon their end point:

1) Tests for DNA damage including adduct formation, strand breakage, prophage induction, and DNA repair.

2) Tests for mutagenicity, including forward and reverse mutation as evidenced by alterations of DNA, gene products, or cellular behavior.

3) Tests of chromosomal effects including aneuploidy, structural aberrations, micronuclei, and sister chromatid exchange.

In the main these tests are effective at measuring their intended genetic end points and, when used in batteries, are effective for identifying genetic effects of chemical toxins. It is less clear how well these tests identify chemical carcinogens or how they should be used in the absence of corroborative data on carcinogenicity.

The initially high correlation observed between genetic change and carcinogenicity has decreased with the enlargement of the set of chemicals tested and with the separation of test development from test deployment. Estimates of correlations between findings in such tests and determination of carcinogenicity in vivo varies, depending on the chemical class, test type, and laboratory. At present, the overall performance of short-term tests, as judged by the proportion of correct results for chemicals classified by carcinogenesis bioassay, is in the range of 50 to 70 percent. Although often significantly better than chance, these results are not adequate to allow reliance on short-term tests alone in the determination of carcinogenicity. However, thousands of chemicals have yielded positive results in short-term tests and require further analysis. This has created a gnawing problem for scientists, regulators, industrialists, and the public. Pending developments that may clarify these issues are: (i) continued improvements in the assays, particularly in regard to standardization and metabolic activation; (ii) expanding the chemical classes to which such assays respond; (iii) analyses of large data bases of results from short-term tests with carcinogens and noncarcinogens so that patterns of response and validation of performance can be established; (iv) extensions of assay methods to easily obtained human samples for coupling to epidemiological studies; and (v) clarification of those mechanisms of carcinogenesis that these tests are intended to simulate.

Tests for transformation in vitro. Changes in growth, colony formation, and colony morphology in culture have been related to exposure to carcinogens and to tumorgenicity when the affected cells are transplanted to animals. The use of cell transformation has become a major research tool for those studying mechanisms of carcinogens by viral, chemical, and physical agents. The methods have also been applied to the identification of chemical carcinogens but with less success and reportedly poor predictivity. However, in view of the potential inherent in these methods to elucidate some aspects of cancer causation, continued research and validation of these methods is to be encouraged.

Metabolism and Pharmacokinetics

Administration of a chemical to an animal is usually followed by its absorption and distribution throughout the animal's body. In some cases the physical properties of the chemical, such as lack of solubility or the presence of an ionized form at normal physiological *p*H, may lead either to the substance not being absorbed from the gastrointestinal tract or to its localization at the site of administration. Once in the body, the chemical will usually be metabolized by a number of enzymes that are present in different tissues. Overall, the beneficial effect of metabolism is to solubilize the chemical and thus facilitate its excretion from the body (*10*).

The pharmacokinetics of chemical carcinogens is of special importance because at elevated doses the body may be unable to clear the chemical as rapidly as it is administered so that, after a lag, the chemical may attain toxic concentrations. Pharmacokinetic studies are also important because they may determine the concentration of a carcinogen or its metabolites at particular sites. As in other enzyme systems, metabolic enzymes become saturated as the amount of substrate increases. In such a situation, enzymes with a low affinity for the substrate may become more involved in the metabolic process. Overall, such an effect will appear as a discontinuity in a plot of the percentage of an agent converted to a specific metabolite versus the administered dose of the agent. Such a discontinuity has been called a metabolic threshold and is important in assessing the effects of the high doses given in animal studies relative to the lower doses to which humans are normally exposed.

With many carcinogens, metabolism leads to the formation of reactive species as well as more readily excreted metabolic products. Reactive metabolites are formed from most major classes of chemical carcinogens, such as *N*-nitroso compounds, aromatic amines, polycyclic aromatic hydrocarbons, and aflatoxins. In each case the reactive metabolite is capable of reacting with macromolecules, including DNA, the target that is assumed to be critical for initiating carcinogenesis. The chemical structures of the DNA adducts derived from these interactions have been established in many cases and lend support to the suspected structure of the metabolite.

The activities of metabolic enzymes may be markedly affected by the physiological and pathological condition of the host and by environmental factors. Enzyme induction has been the most investigated method for modifying enzyme activity. It can either enhance the formation rate of the active metabolite or stimulate other pathways that produce inactive metabolites. The magnitude of induction varies with the inducing agent, the substrate used to assay the activity of the enzymes and the species, strain, and sex of animal. The biological consequences of enzyme induction on the carcinogenic response depend not only on rates and pathways of metabolism but also on disposition and clearance rates of active metabolites.

The situation becomes more complicated when you consider the complex nature of the mixed function oxidase system involved in the transformation of many chemical carcinogens to active metabolites. For example, multiple isozymes of cytochrome P-450, that is, forms with different amino acid sequences, exist in a given tissue, each with different, but overlapping, substrate capacities. Many are regulated independently, so various factors lead to selective increases or decreases in specific isozymes. The amount of each isozyme present in different individuals is an important factor to consider in the variability of human response to chemical carcinogens.

These parameters limit extrapolation of metabolic data from test species to the human situation. Comprehensive metabolic studies in different species can, however, provide valuable data for comparison with that data obtained from human studies. Once the metabolic profile of a particular carcinogen is established from animal studies, human tissues can be tested in vitro to determine if the same metabolites are formed.

In most in vitro tests the activation system determines the outcome. Numerous factors influence the metabolism of foreign chemicals in vivo, and results from short-term tests are often influenced by the selection and preparation of the metabolizing systems. Consequently, no single system in vitro can be totally satisfactory or can exactly mimic pharmacokinetic parameters that influence the fate and disposition of the test chemical in vivo. Nevertheless, when used appropriately, in vitro metabolic activation systems can provide valuable insight about the generation of potentially carcinogenic intermediates.

The Mechanism of Carcinogenesis

The classification of carcinogens on the basis of their mechanisms of action would greatly accelerate carcinogen identification and provide logical approaches to methods of cancer prevention. A working group from the International Agency for Research on Cancer (IARC) (*11*) concluded that, at present, no classification of carcinogens according to mechanisms could be exhaustive or definitive. However, elucidation of mechanisms has value in the identification and evaluation of specific carcinogens (*12*).

Our panel concurs with the IARC's conclusion: in certain situations, the particular mechanisms of action of some carcinogens provide guidelines for preventive measures. These situations include carcinogenic effects directly related to the hormonal changes caused by certain compounds and carcinogenic effects in the bladder caused by compounds that induce bladder calculi. These carcinogenic effects can be dealt with differently from those of compounds that induce cancer without the apparent intervention of other physiological or pathological factors.

In spite of the inability to derive a generic classification of carcinogens, case-by-case scientific evaluations may be reached for individual substances. Chemical carcinogens can, in principle, be divided into two categories. One category gives nonthreshold dose responses, is stochastic in mechanism, and may have some probability of producing carcinogenic effects at any dose. The second category gives threshold dose responses and, theoretically, has a no-effect level. A few chemicals can be placed, provisionally, in one category or the other; but for the bulk of chemical carcinogens, we are currently unable to discern in which compartment they fall. By dealing with chemicals case-by-case and by studying mechanism, we can look forward to doing better than this.

Extrapolation from Experimental Data

Animal experiments are commonly conducted with higher exposure levels than those normally encountered by humans. Therefore, the first step in a quantitative evaluation is to extrapolate the results from high doses to effects from doses corresponding to human exposure. The second step is to extrapolate across species from animal experimentation to the human situation. Both steps entail large uncertainties (*13*).

The first step, extrapolation from high to low doses, depends upon the presumed dose-response relationship. A number of mathematical models have been proposed for this extrapolation,

each being necessarily simplistic. These dose-response models are quantitatively similar to one another in the range of experimentally observable response rates, that is, 10 to 100 percent, yet they may yield substantially different estimates at lower, unobservable response rates. Estimates for low-dose responses that differ by three to four orders of magnitude are not uncommon. This uncertainty is the single most important limitation of the models. Current knowledge does not yet allow the selection of one particular model, and experimental data from animal bioassays are not sufficient to discriminate among the competing models.

Cancer can occur through means other than exposure to a specific chemical. The manner in which this "spontaneous" response is incorporated into a dose-response model is another major source of uncertainty in extrapolating from high to low doses. Two methods have been proposed: one method assumes that the process leading to spontaneous cancer is independent of the process induced by exposure to the suspect agent; the other, that the two processes are identical. As with the different mathematical models for dose response, these two methods yield dose-response curves that are statistically indistinguishable in the observable response range but yield substantially different extrapolations. Other sources of uncertainty in extrapolating from high to low doses include the possible existence of thresholds, determination of the effective dose at the site of action (compared to the administered dose), and the mechanism of carcinogenic action.

The second step in extrapolation, from the laboratory to the outside world, involves additional sources of uncertainty. The route of exposure for the laboratory animal is often different from that for exposed humans, and the responses of the two species to the carcinogenic insult may be substantially different. Experimental animals are often genetically homogeneous and share nearly identical environmental conditions; however, they do not always agree, qualitatively and quantitatively, in their carcinogenic response. In addition, they may differ in the site of their carcinogenic response. Humans are a genetically outbred species living under widely diverse environments; they are exposed to a large variety of carcinogens and noncarcinogenic modifying factors that may enhance or even inhibit the agent in question. There are unknown biases involved in extrapolating dose response obtained under homogeneous experimental conditions to heterogeneous environmental conditions.

Different age-related patterns of exposure may have a substantial effect on risk. Experimental animals are usually exposed to a near-constant level for most of their lifetime, whereas human exposure patterns may vary widely from day to day. The results of extrapolating from one exposure pattern to another are dependent upon assumptions about the mechanism of carcinogenic action, thus providing another degree of uncertainty to extrapolation.

Further development of biometric models should be encouraged in order to provide statistical analyses for evaluation along with all relevant scientific data. Pending elucidation of the mechanisms of cancer, statistical estimations of the relation between exposure and response will be most helpful when the models incorporate pharmacokinetic data and the time between exposure and tumor development, distinguish between the administered doses and target doses, and correct for the duration of exposure and competing risks. The most probable estimates should always be presented together with the confidence limits of the estimates. Enough data should be presented to show how well the estimates fit the experimental data; and assumptions incorporated in the model, together with any uncertainties, should be clearly stated.

The Overall Assessment Process

Chemical carcinogenesis is a rapidly moving field, and great quantities of data have been accumulated during the past decade. Even though an individual experiment may yield only suggestive information, this information may be of considerable importance when considered together with other data (*14*).

Clearly, when the primary source of data comes from epidemiological studies in man, it may be possible to evaluate a chemical and institute scientifically based preventive measures. However, even in the instances where data are available from humans, the data must be supplemented with information from other sources before a conclusion can be reached.

For example, toxicological evaluation of carcinogenicity has classically relied upon long-term in vivo studies as the primary source of data. Such studies have been performed in a routine manner, and evaluations have followed predetermined formulas. This rote method is rapidly giving way to evaluations that take into account findings from in vitro tests, metabolism studies, and biometric analyses as well as any other available information. One of these methods alone cannot produce a reliable estimate of a chemical's risk to man, but taken together they provide an estimate with a high level of confidence.

Carcinogens act via different mechanisms, which results in their having different magnitudes of risk to man. Even though there is no basis for the exact extrapolation of risk from experimental animal to man, current advances, if exploited to the fullest, can provide a basis for distinguishing the degrees of risk from different carcinogens. The scientific criteria should be reviewed often, and scientific advances should be fully adopted.

The scientific characterization of human risks from carcinogens involves the evaluation and integration of data from many disciplines. It requires scientific impartiality to review all appropriate data, both negative and positive, including statistical estimations of low-dose response. Quantitative characterization of human risk requires scientific experience and judgment in order to weigh the evidence. Because of the strengths and weaknesses of the data to be evaluated in the assessment of human risk and the complexity of the problem, case-by-case analysis is most appropriate (*15*).

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3. The panel acknowledges support of the workshop by the American Industrial Health Council.
4. In (*1*), p. 461, column 2: "This subcommittee has found it useful to state generalized definitions of malignant and benign neoplasms, recognizing that in practice the diagnosis of a particular neoplasm is an operational one based on convention, experience, and experimental data.

"A malignant neoplasm is composed of a population of cells displaying progressive growth and varying degrees of autonomy and cellular atypia. It displays, or it has the capacity for, invasion of normal tissues, metastasis, and causing death to the host. Benign neoplasms are a less autonomous population of cells, exhibit little or no cellular atypia or invasion of normal tissues, and do not metastasize. In particular cases, however, benign neoplasms may endanger the life of the host by a variety of mechanisms, including hemorrhage, encroachment on a vital organ, or unregulated hormone production. The cytologic and histologic criteria utilized in determining whether a lesion is benign or malignant differ depending upon the tissue in which the neoplasm arises. Evaluation of whether a specific lesion is benign or malignant should, therefore, follow standard criteria used by experimental oncologists and pathologists with emphasis on correlation of the histopathologic pattern with the biologic behavior of the lesion or type of lesion. In equivocal cases, the diagnosis of a specific lesion may require a panel of experts, recognizing that they may not always agree.

"Depending upon the particular case, benign

neoplasms may represent a stage in the evolution of a malignant neoplasm and in other cases they may be "end points" which do not readily undergo transition to malignant neoplasms."

In this report, we have used the term "neoplasm" freely to describe both benign and malignant neoplasms.

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15. In today's phraseology, this report has been concerned with scientific risk assessment and not with risk management. The process of risk management, by definition, begins after risk assessment has determined that a risk to a human population exists. Whereas "assessment" deals with biological significance, "management" deals with the possible alternative regulatory actions. Included in risk management may be evaluations of costs, feasibilities, risk-benefit ratios, availability of replacement substances or processes, and the level of risk that is acceptable to the society in question. Management of risks is a political, social, and economic issue. Scientists acting as scientists have a role in this phase, but it is limited to ensuring that the biological meaning of the risk is understood throughout the process.

RESEARCH ARTICLE

Antibodies to Human c-*myc* Oncogene Product: Evidence of an Evolutionarily Conserved Protein Induced During Cell Proliferation

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William DeGrado, Philip Leder

The viral oncogene v-*myc*, which is harbored by avian myelocytomatosis viruses, is derived from a cellular gene (c-*myc*) found in all vertebrates (*1*). The cellular *myc* gene contains three exon sequences transcribed from two promoters located either just 5′ of or just within the first exon (*2–4*). Considerable interest has been shown in the c-*myc* gene since Burkitt lymphoma cells show translocations that have brought the c-*myc* gene in close proximity to the immunoglobulin heavy chain locus, t(8;14), or the immunoglobulin light chain loci, t(2;8) and t(8;22) (*5*). The molecular mechanism by which c-*myc* oncogenicity can occur, however, remains obscure although several mechanisms regarding its activation have been proposed. These include a transcriptional activation of the c-*myc* gene resulting from the chromosomal translocation (*6*), a deregulation of the c-*myc* gene allowing constitutively high levels of expression (*7*), a removal of an untranslated 5′ exon, thus facilitating c-*myc* gene expression at the translational level (*8*), release of a transcriptional repressor (*9*), a differential usage of promoters in normal and malignant cells or somatic mutations occurring at a high level as a result of its proximity to the immunoglobulin locus (*10*).

The protein product of the c-*myc* gene is, most likely, responsible for c-*myc* oncogenicity, and some information on potential properties of the c-myc protein has been obtained from studies with the myelocytomatosis viruses. The transformation specific protein from MC29-type viruses is synthesized as part of a polyprotein with a molecular weight of 110,000 (110K), in which v-myc protein is fused to the gag protein (*11*). The p110K$^{gag\text{-}myc}$ polyprotein is found largely in the cell nucleus, binds to double-stranded DNA, and at least a fraction of the protein is associated with chromatin (*11*). Two other members of the myelo-

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15. SUPPLEMENTARY NOTES

16. ABSTRACT

This draft report is a comprehensive summary and compilation of the scientific evidence related to the hypothesis that cancer rates in human populations are associated with their exposure to pollutants present in the ambient air. Critical comments on the strength and weaknesses of the studies are presented and general methodological problems in the conduct and interpretation of the studies are discussed. No overall judgments about the weight of the entire body of scientific evidence are presented. This draft is being circulated for technical review and comment.

17. KEY WORDS AND DOCUMENT ANALYSIS

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and case reports of mesotheliomas among residents in neighborhoods surrounding asbestos mines and factories. However, these studies yielded no specific evidence for exposure other than location of residence. Environmental exposure to asbestos also results from other activities (e.g., wearing out of brake linings in automobiles). In one study of urban dwellers, nearly all (96%) had asbestos fibers in their lungs (Churg and Warnock 1977). This suggests that asbestos from diverse sources, particularly airborne asbestos, may be an important problem for additional study.

3. Vinyl Chloride

Cases of the rare cancer, angiosarcoma of the liver (ASL), have been reported among individuals living near vinyl chloride fabrication, or polymerization, plants. Brady et al. (1977) studied the cases of ASL reported to the Tumor Registry of the Cancer Control Board of the New York State Department of Health during the years 1958-1975. For each of these cases a matched control with an internal malignant tumor other than primary liver cancer was selected from the registry. Cases and controls were matched on age (same 5-year age group), race, sex, county of residence, and vital status. Relatives of both the subjects and matched controls were interviewed in order to obtain information on potential exposure to vinyl chloride (VC), arsenic (As), or thorium oxide (ThO_2), as well as medical, familial, residential, and occupational histories. Of the 26 cases of ASL diagnosed during 1958-1975, 7 had direct exposure

to VC, As, or ThO_2 ($p<0.02$). Of the remaining 19, 5 lived within one mile of a VC fabrication or polymerization plant. Although this is suggestive of an association, no statistical test of the possibility of this finding arising by chance was reported. Due to the small number of cases and the lack of monitoring data directly demonstrating exposure, no firm conclusions are possible.

Infante (1976) studied the mortality patterns of residents of four Ohio communities with polyvinyl chloride (PVC) production facilities. Using data for the Ohio white population as the standard, SMRs were calculated for central nervous system (CNS) cancer, leukemia and aleukemia, and lymphomas. He found that in these four communities the number of observed CNS cancers for both sexes combined during 1958-1973 was significantly greater than that expected (38 observed vs. 24.07 expected $p<0.001$). SMRs were also calculated for each of the counties excluding the areas surrounding the PVC facilities, but no significant excesses were found.

This study was reviewed by Air Products and Chemicals (1980), who commented that interpretation of this study is complicated by the fact that (1) the increase in CNS tumors was observed primarily in males, and (2) most of the excess occurred in one part of the study area (Painesville). They argued that these factors seriously challenge any conclusions of association of vinyl chloride with community cancer risks.

To these criticisms should be added the failure to control for occupational exposure, race, and socioeconomic status.

Infante (1976) has also been criticized by the Society of the Plastics Industry (1980) for including North Ridgeville in the study group while not including other cities located as close as North Ridgeville or closer to the PVC facilities (e.g., Mentor, Ohio). If North Ridgeville is excluded from the study group, the excess in CNS tumors remains significant ($p<0.05$, one-sided test), however.

4. Petrochemical and Other Chemical Emissions

A number of studies have indicated that workers exposed to a wide range of industrial chemicals are at increased risk of developing cancer (Althouse et al. 1980). An increased risk of bladder cancer has been reported among workers exposed to benzidine (Case et al. 1954) and paints (Cole et al. 1972). Exposure to polycyclic aromatic hydrocarbons (found in crude petroleum, catalytically cracked oils, soot, and other pyrolysis products) has been associated with increased incidence of cutaneous and pulmonary cancers in workers (Doll et al. 1972, Lloyd 1971, Hammond et al. 1976, Fraumeni 1975).

Blot et al. (1977) studied cancer mortality patterns for 1950-1969 in the U.S. counties where the petroleum and petrochemical industries are most heavily concentrated. Using methods similar to those of Blot and Fraumeni (1975) described above, it was found that male residents of these counties experienced significantly higher rates for cancers of the lung, nasal cavity

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GGT is a highly sensitive indicator of liver disease, regardless of the disorder's origin.

Gamma-Glutamyltransferase in the Laboratory Evaluation of Liver Disease

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KEY POINTS

1. The biochemistry, physiology, tissue distribution, and assay methods for gamma-glutamyltransferase (GGT) are discussed in detail.
2. GGT is a highly sensitive indicator of liver disease, regardless of whether the origin is inflammatory, space-occupying, or obstructive. It is a useful screening test for alcoholism, particularly because GGT levels are not elevated in social drinkers.
3. GGT is not elevated after infancy, in bone or muscle disorder, or during pregnancy, as are other enzyme tests.

Whereas the transaminases, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), and alkaline phosphate (AP) could be termed the "classical" liver enzymes, gamma-glutamyltransferase (GGT) is a relative newcomer, particularly in clinical practice in the United States. Much of the early clinical work on this interesting enzyme has been done in Europe.[1] In fact, in most European countries, requests for GGT as an integral parameter of the hepatic profile are at least as frequent as those for ALT and AP.[2]

In recent years, the use of GGT in the United States has been growing at a very rapid pace, in fact, much faster than for any other liver test.[3] Most likely, the reasons for this recognition of GGT are its specificity for liver diseases, its reputation as the most sensitive enzyme test of liver disease, and its growing use in the laboratory assessment of the chronic alcoholic.

This article attempts to interpret some of the recent studies that have stimulated interest in GGT.

Structure and Function of GGT

Before we discuss the use of GGT as an indicator of chronic

alcohol consumption and in the detection and differentiation of liver disease, it will be of interest to review briefly the biochemistry, physiological role, and tissue distribution of this enzyme.

GGT is widely distributed in human tissues. It is present in very high concentrations in the brush border of the proximal tubules of the kidney. In fact, the kidney is the human tissue richest in GGT. The content of GGT is second and third highest in human pancreas and liver, respectively. At the cellular level, GGT is located primarily on the outside surface of cell membranes (Figure 1).[4] The primary localization sites in the normal human liver are the canalicular portion of the hepatocyte membrane and, to a lesser extent, the plasma membrane of epithelial cells lining the bile ducts.[5]

What are the location and origin of elevated GGT in various liver diseases? If we take alcoholic fatty liver as an example, we start to see spreading of GGT to parts of the hepatocyte membrane beyond the canalicular region.[6] The enzyme is still located on the outside surface of the membrane, but in much higher concentrations and spread out over the entire membrane surface. In cholestatic liver disease, where one primarily sees proliferating bile ducts, high concentrations can be found in the membrane fraction of proliferating ducts.[7]

A more detailed look at the structure of GGT reveals that the enzyme is composed of two subunits, a smaller and a larger one.[8] One end of the large subunit has a sequence of approximately 40 hydrophobic amino acids which are probably responsible for the tight binding of the enzyme to the lipid matrix of the plasma membrane.[9] The remainder of the molecule is coated with carbohydrate.[10] So one can think of GGT as a plasma membrane-bound glycoprotein with the hydrophobic part of the molecule serving as an anchor to the lipid membrane and the hydrophilic sugar groups facilitating interaction of the enzyme with its plasma environment.

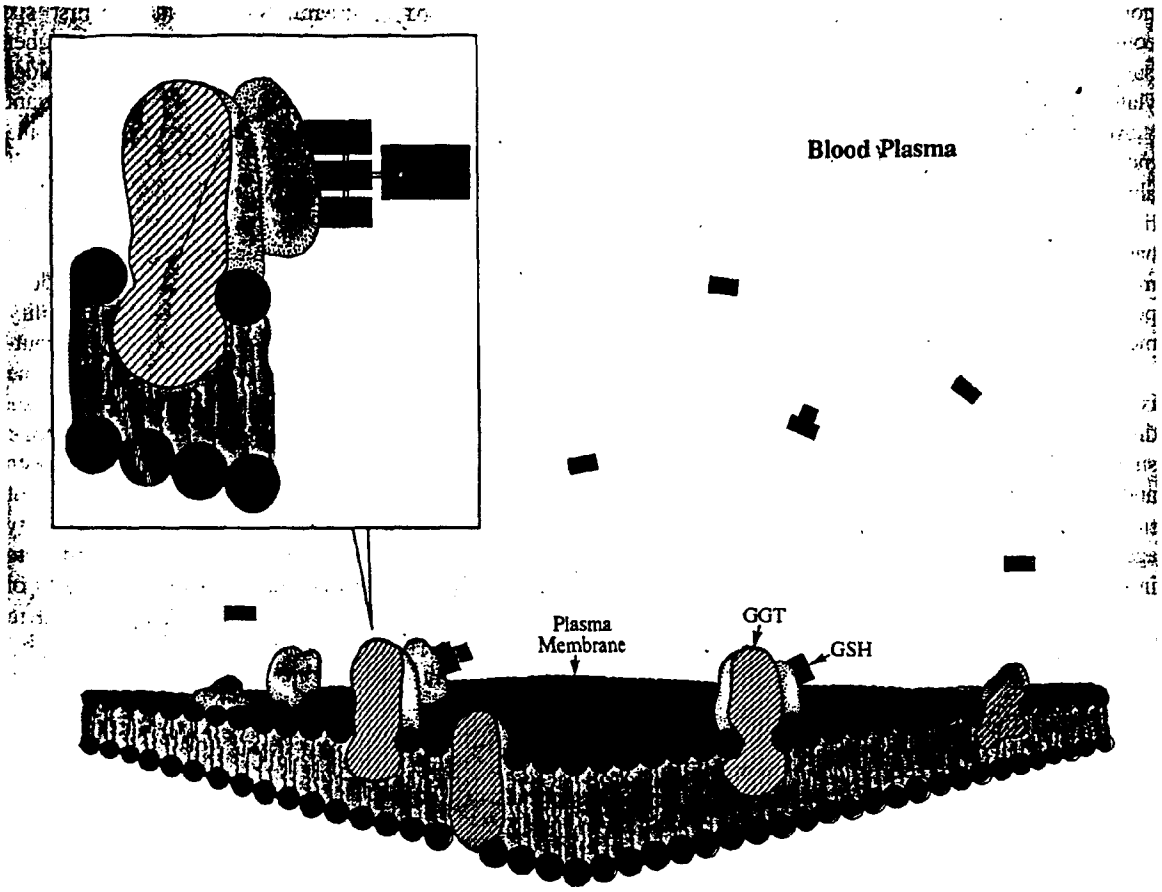
What is the role of this enzyme in human metabolism? To the best of our knowledge, the primary physiological role of GGT is the hydrolysis of glutathione.[11,12] The products of the hydrolysis reaction are L-glutamate and the dipeptide, cysteinylglycine. Cysteinylglycine is further broken down into its constituent amino acids. The three amino acids L-glutamate, cysteine, and glycine now become available for reutilization. One of the hallmark aspects of intermediary metabolism is that the chemical constituents of the human body, as well as of all living organisms, undergo continuous synthesis and breakdown. We do not always understand why that is the case, and we certainly do not understand why it is important for glutathione to undergo synthesis and breakdown continuously. But, nevertheless, it does occur, and the key enzyme in this catabolic pathway is GGT.

It should be emphasized that s-conjugates of glutathione are also natural substrates for GGT. These conjugates are produced in the body through the glutathione-s-transferase pathway after exposure to drugs or environmental toxins. By catalyzing the hydrolysis of these s-conjugated compounds, GGT plays a role in one of the pathways for the elimination of some drugs and toxins referred to as the mercapturic acid pathway.[13]

Test Performance and Prelaboratory Factors

In interpreting a laboratory result (normal or abnormal), one has to be concerned with three main factors: the technical aspects of the test and the extent that they might influence the test result, the prelaboratory or nondisease factors that might produce an abnormal result, and the possible pathological causes of an abnormal result.

Let us briefly discuss the first two factors before we consider the interpretation of abnormal results. As far as technical performance is concerned, the most important parameters to consider are temperature and pH control, the choice of substrate, the concentration and purity of the substrates, buffer selection, selection of the wavelength for monitoring the reaction, and, of course, the standardization and performance of the instrument with which the test is performed. The International Federation of Clinical Chemistry (IFCC) has recently proposed a reference method for the assay of GGT which has several advantages.[14] The reaction scheme for this assay is shown in Figure 2. This in vitro assay takes advantage of the ability of GGT to transfer the γ-glutamyl moiety of the donor substrate to a variety of acceptor substrates, including



1. Cell Membrane Location of GGT—This schematic presentation indicates the location of GGT on the surface of the cell membrane. At this membrane site, GGT catalyzes the hydrolysis of glutathione (GSH) and glutathione conjugates.

water, a variety of amino acids and small peptides, as well as the donor substrate itself. Both the donor and the acceptor substrate are nonphysiological molecules that were selected to achieve optimal test results. The L-γ-glutamyl-3-carboxy-4-nitroanilide (the donor substrate) is a colorless compound. The rate of formation of the yellow-colored product (5-amino-2-nitrobenzoate) of GGT's catalytic action on the donor substrate is determined by monitoring the production of the yellow color at 410 nm. The reaction is carried out at the optimal pH of 7.9 and at 30°C.

To my knowledge, this is the simplest and least expensive enzyme assay in the world. It consists of only two reagents, L-γ-glutamyl-3-carboxy-4-nitroanilide and glycylglycine, in addition to water. Glycylglycine, the acceptor substrate, plays a dual role in the assay since it also serves as the reaction buffer. An important feature of the donor substrate in the IFCC method is the presence in the nitroanilide ring of a carboxyl group. This facilitates the water solubility of the donor substrate.[14]

The noncarboxylated donor substrate is only poorly soluble in water and requires prior heating or the use of dilute HCl for solubiliza-

tion. The carboxylated substrate is considerably more stable in aqueous solution than the noncarboxylated substrate.

Another point of interest is that most reference methods are not always easily adaptable to the routine laboratory. In this case, we have an example of a reference method that is probably simpler to perform than most GGT routine methods currently in use.

The second area of consideration is to determine what effects nondisease or prelaboratory factors, such as serum sample storage time and temperature, alcohol consumption, medications, diet, exercise, age, and sex, have on GGT activity in patients' sera.

Serum GGT activity is quite stable. Activity remains constant for five days at 4°C and for at least seven months at −70°C. Thus, if samples require storage for more than one working day, no error will be introduced by storage in a refrigerator or freezer until the assay can be performed.

The most noted clinical feature of GGT is its sensitivity to alcohol consumption. There is now a large convincing body of evidence describing the response of GGT to various types and doses of alcohol.[15-18] The key question here is, "How much alcohol must an individual consume before one can observe an interpretable elevation of GGT?" Because of the paramount importance of this question in the face of the magnitude of the nation's alcoholism problem, we will later give this question some special consideration.

Another important and well-known nondisease parameter is the type of medication. GGT activity in serum is elevated by medications, particularly the anticonvulsants which produce elevations up to about four times normal.[19] Small increases in serum GGT activity above the reference range are seen in some people who are grossly obese. No significant change in GGT activity occurs after strenuous exercise.

A recent study on reference ranges of GGT in children makes an important point concerning the influence of age on the interpretation of hepatic enzyme levels.[20] These authors demonstrated that the immature liver, which has higher concentrations of GGT than the normal adult liver, pours out more GGT into the infant's blood stream, so that in the first six months of life we see much higher serum levels compared with older age groups. But the important point that these investigators make is the fact that after six months of age the reference interval for GGT does not vary much with age in young, growing children. In contrast, alkaline phosphatase does not exhibit that degree of stability in this age group, due to output of bone osteoblastic alkaline phosphatase.[21] As a result, we see higher levels throughout childhood than we see in adulthood. Even more compromising is the spike of alkaline phosphatase at puberty which may vary from individual to individual in intensity and time of onset. It is, therefore, difficult to develop reference ranges in this age group that allow maximum sensitivity in detecting liver disease. The conclusion of this work is that after six months of age, GGT is a much more reliable indicator of liver disease in children than alkaline phosphatase.

The serum levels of GGT in the normal human are somewhat sex-dependent, with males having higher levels than females.[22] One should, therefore, have access to male and female reference ranges.

L-γ-glutamyl-3-carboxy-4-nitroanilide (γ-glutamyl donor substrate) + glycylglycine (acceptor substrate)

↓ GGT

5-amino-2-nitrobenzoate + γ-glutamyl-glycylglycine

2. GGT Assay—The reaction scheme for GGT assay as recently proposed by the IFCC.

Interpretation of Elevated Results

There are a variety of clinical and subclinical conditions that can lead to GGT levels elevated above the individual subject's own reference value as well as above the reference range of a healthy population. As far as the IFCC reference method is concerned, well-defined

reference intervals for male and female populations will soon be forthcoming. Due to procedural optimization, these intervals will be somewhat higher than the ones currently in use.

Since one of the prime uses of GGT is in the recognition and monitoring of the chronic alcoholic, some of the most informative clinical studies on this subject will be reviewed. A very interesting epidemiologic study took place in Malmo, Sweden, and was reported in *Preventive Medicine*.[23] In conjunction with a department of preventive medicine, this group looked at two populations of male birth cohorts, one born in 1926, the other in 1927. Total participation was 3,050, which represented 76% of all males born at that time in that city. A comprehensive clinical screen, including a detailed history and a comprehensive biochemical profile, was performed on each subject.

The main focus was to determine what caused elevated levels of GGT in these individuals. The experimental finding was that about 10% of these people had abnormally high values of GGT activity. The key finding of the study was that 72% of the subjects with abnormal GGT results were heavy, regular consumers of alcohol; 9% were chronic consumers of drugs such as anticonvulsants, antiphlogistics, and mixed analgesics containing phenobarbital. Organic liver disease was present in 2.7% of the subjects with abnormal GGT results; 1.8% had a diagnosis of chronic hepatitis and 0.9% had biliary cirrhosis. There were also other laboratory abnormalities: 41% of the individuals with an elevated GGT also had elevated ALT, 40% had elevated HDL cholesterol, and 34% had elevated AST. Interestingly, only 6% had detectable levels of alcohol and 1% had abnormal bilirubin levels. Since this was the only biochemical abnormality in about half of the people with elevated GGT, the bottom line of this extensive study appears to be the sensitivity of GGT as a biochemical indicator of heavy drinking and possibly of early covert liver disease. Numerous other publications exist that tend to support the evidence provided by the Malmo group. It would go beyond the scope of this article to report on them in great detail. Instead, let us briefly discuss the conclusions drawn in three epidemiological studies.

One was published in *Clinical Chemistry* by a French group.[24] These authors tested GGT, iron, mean cell volume (MCV), mean cell hemoglobin concentration (MCHC), urea, uric acid, and ALT in an unselected population of 3,930 men. The self-reported alcohol consumption of these people was compared with their levels of the biological parameters by principal components and multiple regression analysis. The authors were able to show that the three variables that correlated most significantly with alcohol consumption were GGT levels, MCV, and the use of tobacco. These three parameters contribute as much information as the whole set of ten variables examined, according to these workers, and they came to the conclusion that particularly GGT levels "can be considered a screening test." However, correlation coefficients which served as an index of correlation with the quantity of alcohol consumed were only between 0.34 and 0.43 when the previously mentioned three variables were combined. The authors, therefore, recommended that the search for discriminative biological indices of the quantity of alcohol consumption needs to continue.

Chick et al.,[25] in Edinburgh, Scotland, came to similar conclusions as the French group. They assessed the usefulness of GGT and MCV determinations in 266 company executives and 222 manual workers of two alcohol production firms. The correlations with admitted drinking were significantly higher than the French study. A man with an MCV of over 98 and a

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GGT level above 50 U/L had a 62% chance of admitting to drinking over 450 gm of alcohol per week. In their conclusions, these workers stated: "In those patients in whom there is an alcohol-induced elevation of one of these tests, cessation or reduction of drinking leads to a fall in the next three to six weeks, which provides the clinician with a useful indication of treatment progress.[26-28] Patients whose goal is reduction of drinking rather than abstinence find serial test results an aid to monitoring their consumption."

In another study, a group of investigators analyzed the relationship between alcohol consumption, GGT levels, MCV, and tobacco consumption.[29] In this study, 455 apparently healthy outpatients were asked to report their daily alcohol consumption. Multiple regression analysis confirmed the superiority of GGT levels over MCV as a laboratory marker of alcohol intake, as well as the advantage of using them together. On this basis, the authors proposed a "rough estimation of alcohol consumption as a first step in mass screening of heavy drinkers." Using 80 gm of pure alcohol a day (equivalent to one liter of wine) as a cut-off point, the authors were able to identify correctly three out of four "heavy" drinkers. As in the previously cited studies, no allowance was made for the possibility of underreporting alcohol intake in the subjects with elevated GGT levels. Thus, of all laboratory tests studied, the level of GGT has been shown to be the most sensitive indicator of chronic alcohol consumption.

Another important factor to consider is how to determine the effect on GGT activity of "normal" social drinking in healthy subjects without any indication of liver disease. We have pursued this question in our laboratory and have conducted a study with 22 normal subjects, none of whom were regular consumers of large amounts of alcohol. In a one-evening drinking session, these individuals consumed several drinks of the alcoholic beverages of their choice over a period of approximately four hours. A baseline value was taken the day before the consumption. Postconsumption levels were determined after one, two, and four days, respectively. What we found was that there was no significant increase in GGT activity in the serum of any of these volunteers after we had subjected the data to analysis of variance. Even when compared with each individual's own baseline value, no significant increase was observable. The same was found for the other enzymes, ALT, AST, AP, and glutamate dehydrogenase.

In another study conducted by Belfrage and co-workers,[15] eight healthy young medical and dental students were subjected to a uniform alcohol regimen after a period of abstinence from alcohol beverages of several weeks. Every subject had one 16 ounce can of beer at noon, 6 p.m., 8 p.m., and 10 p.m. every day for five weeks. That amounts to about 60 to 70 gm of alcohol per day. There were slight variations in grams of alcohol per kilogram of body weight due to the varying weight of the students. It was found that after one week of drinking, there was no significant change of the GGT level when compared with the predrinking control value. The same applied to the transaminases, AP, bilirubin, and bromosulfophthalein (BSP) retention. At the end of the study, after five weeks of consuming four cans of beer a day, there was a small but statistically significant increase in the GGT value in each individual. The range of increase was between 11 and 44 U/L when compared with each person's own predrinking reference value. All of the postdrinking GGT values, however, were within the reference range of a healthy population. In no case was there an elevation above the upper limit of normal.

In another clinical study, Statland and colleagues studied the effects in nine healthy subjects consuming 75 gm of alcohol per kilogram of body weight on each of three successive evenings.[16] Again, predrinking and postdrinking values were determined. Maximal changes in GGT activity were found at 60 hours, varying between 0.1 and 3.5 U/L, with an average increase of 1.2 U/L.

These increases were certainly statistically significant because they occurred in all subjects. However, they were very small, and it may be difficult to reproduce these data in the average routine laboratory. Again, all increases were within the normal range.

What is the "hard core" of information that ties all these studies together? What conclusions can we make? We conclude from these

studies that normal social drinking is not likely to produce an abnormal GGT level in an otherwise healthy person. Only from an extended period of chronic drinking (e.g., at least five weeks) is one likely to see abnormal GGT values. We must realize the danger that is always involved if one extrapolates from studies on a few isolated populations to the whole universe. But keeping this reservation in mind, it is reasonable to draw a conclusion similar to that of the author of one article[30] on this subject: "It has been repeatedly demonstrated that in the chronic alcoholic, serum GGT levels fall upon withdrawal, and rise with reexposure to alcohol.[31-33] On the basis of these observations, the GGT test is now being used by alcohol treatment centers in documenting the success of therapy and in identifying patients who relapse following therapy. Clearly, the GGT test—by providing an advance warning of alcohol related liver damage (alteration), and by enabling newly discovered alcoholic patients to admit their problem, seek help, and thereby arrest their disease—could turn out to be among the most important contributions to the practice of preventive medicine."

Let us now turn to the question of whether the measurement of serum GGT contributes information to other diagnostic aspects of liver disease. One aspect is the high sensitivity of GGT as a general liver screening test, functioning as an indicator of intoxication in people whose livers are otherwise normal. One case that happened in our laboratory points this out quite clearly. A man in his middle thirties presented to the emergency room of our hospital. He indicated that he was forced to swallow an organic liquid and did not know what it was. Examination of the gastric contents revealed that this man had consumed a kerosene-like petroleum fraction. The functions of the kidneys, pancreas, and liver were monitored. Interestingly, in the first 24 hours following the incident, there was a sevenfold rise in amylase, indicating a pancreatitis-like reaction. After one day, amylase rapidly returned to normal, followed two or three days later by GGT elevation of ten times the upper limit of normal. There was no clinical indication of jaundice, and bilirubin and the other liver enzymes were only mildly elevated. Total bilirubin was 1.5 mg/dl, and the transaminases were less than one and a half times the upper limit of normal. During days four and five following the episode, the GGT level rapidly returned to normal, indicating that after some intermittent involvement, the liver had fully recovered.

Another possible application of GGT levels is in the differentiation of neonatal hepatitis from biliary atresia where levels are extremely high. In a recently published study, the authors followed seven children who had presented with the suspicion of biliary atresia.[34] A series of laboratory tests were performed, including a full liver panel and α_1-antitrypsin in order to rule out α_1-antitrypsin deficiency. The average value of GGT was about 760 U/L compared with a value of 183 in a group of children with neonatal hepatitis. Using 300 U/L as a cutoff point below which the diagnosis would be neonatal hepatitis and above which it would be biliary atresia, 16 out of the 17 children were correctly classified. Clearly, this is a preliminary finding, yet it may turn out to make a contribution to the differential diagnosis of these two childhood liver disorders. This is a very important differential diagnosis, since treatment of biliary atresia may include surgery, whereas surgery is contraindicated in children with neonatal hepatitis. Interestingly, in the two cases with α_1-antitrypsin deficiency, GGT activities were 869 and 2,700, both above the average value for children with biliary atresia. In these cases, the differentiation was made on the basis of the levels of α_1-antitrypsin (low in children with α_1-antitrypsin deficiency, normal in children with biliary atresia). It appears that the lack of the trypsin inhibitor has caused increased proteolytic cleavage of GGT from the external surface of cell membranes and an increase in activity in serum.

A study on the diagnostic utility of GGT in various forms of liver disease and the behavior of GGT in comparison with other liver parameters was published by Lum and Gambino in 1972.[35] The paper by E. and F.W. Schmidt is another study of clinical utility of serum GGT measurements.[36] If we extract the key information from these two publications, we can summarize the diagnostic contributions of serum GGT.

- GGT is a highly sensitive indica-

tor of liver disease, regardless of whether the disorder involves inflammation of the liver, a space-occupying lesion, or obstruction of the biliary tract.

- It is usually most significantly elevated by obstructive liver disease. It is superior to alkaline phosphatase and leucine aminopeptidase, both in terms of diagnostic sensitivity and specificity.
- In the absence of other laboratory evidence, GGT elevation is a sensitive screening test for occult alcoholism.
- GGT has good specificity for the liver compared with AP and AST, since it is not elevated in bone disorders or during pregnancy, as is AP, nor is GGT elevated in skeletal muscle disease, as is AST.
- GGT elevation is a sensitive and inexpensive assay which is useful as an indirect indicator of the tissue source of AP.
- The test also helps to differentiate mechanical and viral cholestasis from drug-induced cholestasis. In the first group, GGT and AP are elevated approximately to an equal degree. However, in drug-induced cholestasis, GGT and AP are disproportionately elevated, with the former being much more significantly elevated above normal than the latter.

GGT is also useful in anicteric patients with cancer in whom elevated GGT levels indicate liver metastases. ◀

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