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Research Needs for Hepatic Injury Due to Environmental Agents

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This report presents an overview of the patterns of liver injury produced by presently recognized toxic substances in the environment. Current concepts of the metabolic disposition of these substances in the liver suggest that their toxic effects may be modulated by an interplay between enzymatic pathways for activation or inactivation of foreign compounds. A better understanding of the genetic control of the enzymes involved in these pathways, as well as the mechanisms of cell death or malignancy produced by the products of these pathways, is needed. Although a number of sensitive biochemical tests of liver function are currently available, development of specific, noninvasive screening tests for the earliest manifestations of liver injury are urgently needed.

The liver plays a central role in mediating the interaction between man and his chemical environment. The liver is not only a target organ for toxic effects of many environmental substances, but also the major site for biotransformation of foreign chemicals that enter the body. A list of the patterns of liver toxicity produced by noninfectious conditions other than therapeutic agents is presented in Table 1 (*1–3*). Some industrial chemicals or contaminants naturally found in food products regularly produced dose-dependent hepatic necrosis. Cholestasis, when appearing as the sole manifestation of liver injury, is rare (*4*). Liver injury due to environmental agents is often nonspecific and inconspicuous. This presents a challenge to the clinician or epidemiologist in establishing a cause-and-effect relationship between a contact with an environmental agent and liver toxicity. Examples include fatty metamorphosis detected only by liver biopsy, or hypertrophy of the endoplasmic reticulum (microsome induction). In the United States, veno-occlusive disease, granulomas, or hepatoportal fibrosis have been associated only with a limited number of toxic substances. Cirrhosis has been found in industrial workers exposed to chlorinated organic compounds or to plant toxins, but this, too, has been observed only infrequently.

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Hepatoma, the most common form of malignancy, worldwide, is associated with geographic regions in which both hepatitis B virus and aflatoxin are endemic (*5*). In the Western world, ethanol consumption is most commonly associated with hepatocellular carcinoma, although it is uncertain whether ethanol is a carcinogen *per se*, or whether ethanol evokes malignancy by producing cirrhosis, a frequent precursor of hepatoma. An important unresolved question is whether some or all of the many halogenated aliphatic, aromatic, or polycyclic compounds that cause hepatocellular carcinoma in experimental animals also are carcinogens for human liver. Angiosarcoma, a rare tumor arising from the epithelial cells of the liver sinusoid (*6*), was a rare tumor prior to its emergence in vinyl chloride workers (*7*) or in people exposed to arsenic or Thorotrast (*7*).

Research Needs in the Pathophysiology of Hepatocellular Toxicity

A simplified schematic representation of the pathways for biotransformation of foreign substances in the liver is presented in Figure 1. Most often, environmental agents are lipophilic and, hence, are not readily excreted in aqueous media such as urine or bile. Hepatic metabolism converts these substances to more polar, readily excretable forms.

Table 1. Summary of syndromes of hepatotoxicity due to environmental agents.

Toxic manifestation	*Examples of environmental toxins reported in man*
Acute or subacute hepatic necrosis	Chlorinated aliphatics in high doses (CCl_4, tetrachloroethane); mushroom toxins; phosphorus; mycotoxins; high doses of polychlorinated biphenyls (PCB's)
Cholestasis	Methylene diamine (Epping jaundice); dinitrophenol; chromium
Mild fatty metamorphosis	Small doses of chlorinated aliphatics; some organochlorine pesticides
Hypertrophy of endoplasmic reticulum	Polychlorinated biphenyls; some organochlorine pesticides
Veno-occlusive disease	Plant toxins (pyrrolidizine alkaloids); therapeutic liver irradiation
Granuloma	Beryllium
Hepatoportal fibrosis	Vinyl chloride; arsenic
Cirrhosis	Chlorinated aliphatics; aromatics; aflatoxin; arsenic; plant toxins
Hepatocellular carcinoma	Aflatoxin; ? ethanol
Angiosarcoma	Vinyl chloride; arsenic

This involves reactions carried out largely in the endoplasmic reticulum of the hepatocyte, catalyzed by cytochrome P-450. This group of hemoprotein isoenzymes oxidizes many foreign substances (*8,9*). In some instances, bio-oxidation of foreign compounds renders them pharmacologically inactive, although most potentially toxic compounds require similar bio-oxidation reactions to be converted into toxic forms (metabolic activation). Unless promptly disposed of, these oxygenated metabolites may interact chemically with nucleic acids or other mamromolecules in the hepatocyte, initiating a chain of events ultimately thought to lead to malignancy or cytotoxicity. Prompt removal of metabolically activated toxic substances is carried out by a series of conjugating enzymes capable of converting these metabolites to water-soluble derivatives that are inactive pharmacologically (detoxification). It may be reasoned that the metabolic disposition of a foreign compound in the liver will depend upon not only the dose, but also upon differences between the rates of enzymatic activation of the compound, counteracted by the availability of pathways of inactivation of its reactive metabolites. Hence, factors that alter the levels of activating or detoxifying enzymes in the liver may be important determinants of the presence or absence of toxicity. It is well recognized that cytochrome P-450 is inducible by many environmental compounds including lipophilic drugs, some natural agents, carcinogens and environmental pollutants (*9,10*). For example, following administration to experimental animals of many organochlorine pesticides or polycyclic aromatic hydrocarbons, there is a rise in the concentration of hepatic cytochrome P-450. This appears to be an adaptive response of the liver since the cytochrome concentration promptly falls once the amounts of these chemicals in the liver have been sufficiently reduced through metabolism or redistribution to other tissues to remove the stimulus to cytochrome induction. There is substantial evidence in animals and growing evidence in man that the amounts of cytochrome P-450 and its inducibility are under genetic control (*11*). The level of cytochrome P-450 may be decreased in many physiologic or pathophysiologic states (*12*).

Remarkable progress has been made over the last 15 years in isolating and characterizing these hemoproteins and in understanding their regulation in the liver. As with the cytochrome P-450 system, there is intense interest in elucidating these conjugating enzymes which include cytoplasmic glutathione-*S*-transferases, and microsomal glucuronyl transferases and epoxide hydratases. Future support should be given to efforts to characterize the structure and regulation of cytochrome(s) P-450 and other classes of enzymes catalyzing biotransformation of environmental agents. The recent availability of advanced techniques in recombinant DNA and molecular genetics should facilitate progress in phenotyping individuals in an effort to

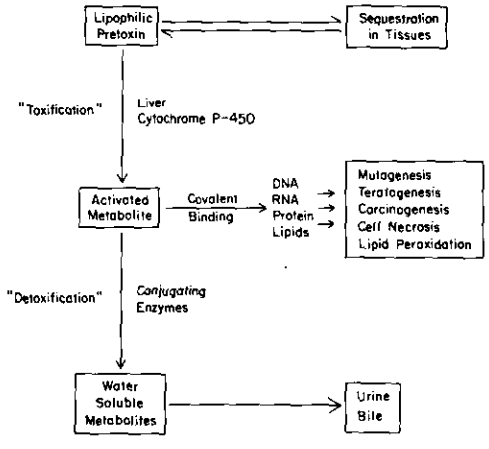

FIGURE 1. Generalized pathways for hepatic disposition of environmental chemicals. Reproduced by permission (*49*).

understand interindividual variations in sensitivity to toxic substances (*13,14*). An extremely important category of compounds commonly found in toxic dump sites are those extremely hydrophobic chemicals (e.g., organochlorine pesticides) that undergo little (if any) metabolism and elimination by the liver (Fig. 1). Chronic exposure to small amounts of these chemicals leads to their accumulation in tissues and membrane lipids. We know little regarding the biochemical and biophysical aspects of this sequestration and of the basic forces that establish distribution of these chemicals in the body. Further, since many of these compounds are toxic or carcinogenic to rodent liver, there is concern that these chemicals may also foster malignancies in human tissue. For this reason, efforts should be made to understand the mechanism of their carcinogenesis in animals. For example, do they act as "promotors" of biochemically initiated premalignant hepatocytes (see below), or do they provide a stimulus to induction of cytochrome P-450 and, hence, render the individual more susceptible to other procarcinogens?

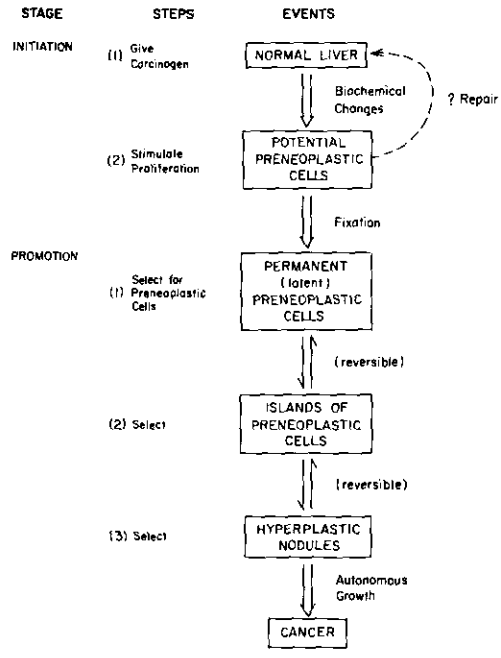

FIGURE 2. Farber's multistep model of experimental chemical carcinogenesis in the liver (*15*). Reproduced by permission (*49*).

Research Needs in the Pathophysiology of Hepatocellular Carcinoma

The problems of extrapolation from experimental animals to humans are perhaps nowhere better illustrated than in the subject of hepatocellular carcinoma. Despite the fact that many persistent chemicals in the environment cause liver tumors when administered at high doses to experimental animals, it is as yet unknown whether or not organochlorine pesticides, polyhalogenated biphenyls, etc., also produce cancer in humans exposed chronically to small amounts of these substances. Investigation of this problem in humans is difficult because the latent period for emergence of human malignancy is long, and because epidemiology is a relatively insensitive tool for establishing causation in human carcinogenesis unless the tumor is rare or unless the carcinogen is extremely potent. Solving this problem will undoubtedly require a combined approach using improved methods for clinical investigation of chemical carcinogenesis (see below, liver function tests) and also better experimental models that permit elucidation of the mechanisms of carcinogenesis of these chemicals and their dose-response relationships.

An exciting new advance in the pathophysiology of experimental chemical carcinogenesis has been the establishment of a multistage model of liver cancer, analogous to that previously established for the skin (*15-17*). For example, Farber and his associates have developed a multistep model for liver carcinogenesis which can, in general, be divided into two stages (Fig. 2). The first stage, initiation, can be produced by exposing rats to known chemical procarcinogens that, when metabolically activated, are capable of interacting with DNA and producing mutations. These short-term biochemical changes, however, will resolve immediately unless there is prompt application of a second stimulus capable of evoking hepatocyte replication (e.g., partial hepatectomy). Thus, the initiation process results in conversion of some normal hepatocytes into "preneoplastic" hepatocytes. These postulated cells are irrevocably committed to retain the potential for developing into hepatocellular carcinomas, and yet they will remain dormant for the life of the animal unless a second series of manipulations termed promotion is applied. Although there are several "promotion" protocols, they all have in common simultaneous exposure of the liver to a stimulus for hepatocyte replication, combined with an antimitogenic stimulus. Farber (*15*) has proposed that such opposing forces for replication offer a selective advantage for the preneoplastic hepatocytes. These cells proliferate, forming foci or nodules of enzyme-altered hyperplastic cells. These hyperplastic foci will revert to latent preneoplastic

hepatocytes if the promoting stimuli are stopped. However, if selection pressures are maintained, overt hepatocellular carcinomas will develop. It may be inferred from these models that carcinogenesis involves sequential exposure to a number of stimuli producing a series of rare events that infrequently lead to cancer. The individual must be exposed to both initiating and promoting influences in sufficient amounts and in the correct sequence in order for cancers to be produced. It is obvious that models such as these should be most useful for future attempts to investigate the mechanisms of carcinogenesis and also for quantitatively establishing dose dependency as a means for rational definitions of permissible amounts of initiators or promotors in the environment.

Research Needs for Screening Tests of Liver Function

Attempts to establish cause-and-effect relationships between environmental agents and their possible toxic effects would be measureably advanced by the availability of suitable screening tests. The ideal test would be noninvasive, yet sensitive enough to detect even minor forms of liver injury. It would be specific for the liver, and would be economically suitable for use in field testing of large populations of people potentially in contact with the environmental agent in question. Table 2 lists a series of biochemical tests of hepatic functions that are currently available and that could be used for screening purposes (*18*). The serum transaminases, among the most sensitive tests available, reflect damage to the hepatocyte. The alanine aminotransferase is specific for the liver, whereas the aspartate aminotransferase may also be affected by cardiac or skeletal muscle diseases. Alkaline phosphatase activity in the serum reflects functional or mechanical obstruction to bile flow including that produced by such infiltrative diseases of the liver as tumor metastases or granulomas. Unfortunately, the test is not specific for the liver since alkaline phosphatase activity is also released into the serum from bone, placenta and the intestine. Specificity for liver can be established by isolating alkaline phosphatase isoenzymes, or by measuring 5′-nucleotidase activity. The serum bilirubin also reflects biliary obstruction, but this test is less sensitive and may be affected by changes in hemoglobin metabolism irrespective of liver injury. γ-Glutamyl transpeptidase (GGTP) was initially proposed as a confirmatory test to establish a hepatobiliary source for elevated alkaline phosphatase activity. However, unlike alkaline phosphatase, GGTP is located not only in the plasma membrane of the hepatocyte, but also appears in bile ductular cells, and in the endoplasmic reticulum of the liver parenchymal cell. GGTP activity is also found in almost every other tissue of the body with the exception of bone. Hence, GGTP may be elevated by hepatocyte necrosis, by administration of drugs that produce hypertrophy of the endoplasmic reticulum of the hepatocyte (microsome induction), and by a variety of nonhepatic conditions. GGTP is frequently elevated by regular consumption of alcoholic beverages. A new commercially available test of liver function is measurement of fasting or postprandial serum bile acids (*19-23*). The liver is solely responsible for the synthesis and enterohepatic recirculation of bile acids. Therefore, biliary obstruction, hepatocellular necrosis, loss in functioning mass of the liver due to fibrosis, or extrahepatic shunts will decrease the

Table 2. Screening tests of liver function.

Test	Abnormality	Sensitive	Liver-specific
Aspartate aminotransferase AST (SGOT)	Hepatocyte necrosis	Yes	No
Alanine aminotransferase ALT (SGPT)	Hepatocyte necrosis	Yes	Yes
Alkaline phosphatase	Biliary obstruction Infiltrative diseases	Yes	No
Bilirubin	Biliary obstruction	No	No
γ-Glutamyl transpeptidase (GGTP)	Biliary obstruction Hepatocellular necrosis Ethanol Microsome induction	Yes	No
Serum bile acids	Biliary obstruction Hepatocellular necrosis Extrahepatic shunts	Yes	Yes
Aminopyrene breath test	Microsome induction Functional liver mass	No	No
Urinary glucaric acid	Microsome induction	No	No
Urinary 6β-hydroxycortisol	Microsome induction	No	No
α-Fetoprotein	Hepatoma	Yes	No

capacity of the liver to extract bile acids from the plasma. The test appears to be as sensitive as the transaminase enzymes, and except for unusual circumstances largely reflects liver diseases.

Over the last several years, a series of breath tests of liver function exemplified by the [^{14}C]-aminopyrene breath test has been developed (*24*). The administered parent drug is labeled with a radioactive or stable isotope and the labeled metabolite, CO_2, is collected with a breath-trapping device. The test appears to be somewhat less sensitive than the transaminases due to wide overlap in the range of normal values, but nevertheless is useful for reflecting microsome hypertrophy of the endoplasmic reticulum (microsome induction). These tests may reflect the amount of functioning liver mass and, therefore, are particularly useful for repeatedly examining changes in liver function over time in a given individual (*25*). Induction of the endoplasmic reticulum of the liver has also been monitored by the urinary excretion of glucaric acid, a breakdown product of microsomal glucuronic acid (*26-29*), or by urinary excretion of 6β-hydroxycortisol (*30*), a product of endogenous steroid oxidation in the liver. Many environmental agents evoke hypertrophy of the endoplasmic reticulum as their sole hepatic manifestation. It should be emphasized that it is unknown as yet whether this should be termed a toxic or an adaptive response of the liver. From the preceding discussion, it may be seen that induction of hepatic microsomal enzymes could have either a favorable or an unfavorable effect (*31*). Although tests for hepatic microsomal enzyme induction have been restricted largely to clinical pharmacologic studies of drugs, they may have wider application for problems of environmental chemicals. Therefore, it would be desirable to develop even more sensitive and specific tests for this purpose.

α-Fetoprotein is a serum test for the presence of hepatoma (*32*). Recently, the sensitivity of the test has been greatly improved by the development of a radioimmunoassay (*33,34*). This test provides the earliest practical marker of hepatoma and is useful for following the development of the tumor. Minor elevations of α-fetoprotein occur with metastatic tumors to the liver and also in nonneoplastic liver disease (*35*).

A potential problem with screening large populations with liver tests is that abnormalities may be produced by factors other than exposure to the toxic substance under investigation. Prominent among these in Western societies is ethanol. Regular consumption even of modest amounts of ethanol can produce abnormalities in one or more of the tests listed in Table 2. Histories of the amount of ethanol consumed are notoriously unreliable. Screening tests that have been used for estimating the presence and amount of ethanol consumption include AST/ALT ratio and γ-glutamyl transpeptidase (GGTP). A simple and efficient test that could be incorporated in large-scale screening protocols is estimation of the ratio of the AST to ALT. Characteristically, ethanol consumption preferentially increases the AST, while the ALT remains normal or significantly less elevated (*36-41*). A second test that has been reported to be elevated in 60-90% of populations regularly consuming ethanol is the serum GGTP (*42*). More specialized tests currently under investigation include the presence of an abnormal amount of serum transferin (*43*), plasma urate concentration (*44*), and the presence of macrocytosis (*44*).

We presently lack biochemical or morphologic markers of the carcinogenic process during the long latent period between contact with a carcinogen and the emergence of hepatocellular cancers. To help identify patients at risk, we need means to assess the body burden of the toxins, particularly their metabolically activated forms. Substantial improvements over the last ten years in analytic techniques for measuring most environmental chemicals have opened the possibility of assaying the concentrations of these substances even in the small amounts of material available from needle biopsies of the liver or tissue fat. However, these biopsies cannot be readily obtained in large population studies and must, by necessity, be restricted to carefully selected patient populations. Improved techniques for measuring these substances in blood are needed. Noninvasive tests such as neutron irradiation to detect cadmium in human liver *in vivo* (*45*) or nuclear magnetic resonance tomography (*46*) may be useful. A second important advance in assessing the body burden of potential carcinogenic material would be the opportunity to measure the amount of stable adducts formed between the activated carcinogen and genetic material. Two exciting advances in this area have recently been reported. Poirier and colleagues (*47*) have developed a radioimmunoassay capable of specifically detecting extremely small amounts of benzo(a)-pyrene–deoxyguanosine adducts. Bennett and colleagues (*48*) have developed an assay for measuring the urinary excretion of aflatoxin–guanine adducts. Moreover, the latter workers showed that the vast majority of the urinary adducts of aflatoxin–guanine adducts were derived from the liver. This important advance may provide the first liver-specific assay of chemical carcinogenesis. The availability of these and similar assays for chemical carcinogenesis in humans would offer promise of a new, exciting approach for establishing dose-response

relationships between body burdens of metabolically activated carcinogens and the subsequent emergence of toxic or carcinogenic effects.

Summary and Conclusions

Environmental agents are capable of producing many of the same patterns of liver injury observed in diseases of the liver caused by virus infection, drugs, or metabolic or genetic defects. In many instances, the changes produced are inconspicuous. The long-term consequences, especially with respect to contact with animal carcinogens, are unknown. Substantial progress has been made in identifying the hepatic enzymes involved in the metabolic activation and detoxification of environmental substances. However, additional emphasis should be placed on elucidating the mechanisms of carcinogenesis or cell death caused by metabolically activated substances. Finally, additional emphasis should be placed on the use of sensitive and specific tests of liver function now available or in the process of development to carry out systematic clinical investigations of the long-term hepatic effects of environmental chemicals.

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