CHAPTER 7

IMPORTANCE OF THE SEQUENCE OF TISSUE INJURY AND RECOVERY

Many aspects of the acute effects produced by combinations of drugs or toxic chemicals are known. The clinical signs and symptoms of such episodes are usually recognized with ease. Anatomopathological lesions are clear-cut and can be related without great difficulty to the offending agents. Acute interactions may involve and may even be quantitated by several end points, such as modification of metabolic pathways, physiologic parameters, appearance of enzymes indicating damage to particular organs in the serum, or defined tissue lesions such as inflammatory changes or acute cell death in target organs. The possible sequelae and significance of acute interactions have been well documented in experiments with both animals and humans. These are discussed more thoroughly in other parts of this report.

Much less is known about the effects of chronic interactions. However, there is no doubt that many of the most prevalent human diseases might not be caused by one agent but by two or even more. For certain diseases, such as liver cirrhosis, coronary heart disease, cardiovascular disease, chronic lung disease, and chronic kidney disease, there is evidence that the pathologic process, developing within months or years rather than within days or weeks, may be caused, aggravated, and modified by chemicals, diet, infectious agents, and genetic background (Lee and Kotin, 1972).

There is very little experimental evidence to permit unequivocal identification of the roles played by suspected etiologic agents in any of these chronic forms of toxicity. Moreover, there is an almost limitless number of agents and combinations thereof to which an individual may be exposed throughout life. The temporal relationship may vary as do levels of exposure in endless numbers of possible combinations. Changes in diet, in endogenous hormonal function, and in exposure to infectious agents may complicate the picture even further. Thus, all these events may produce an altered biological response. Chronic beryllium disease is such an example (Tepper et al., 1961). In people exposed to beryllium dust and fumes, the onset of disease often occurs a year or more after exposure to the offending agent, beryllium. In a few cases, the disease may progress asymptomatically, and in many patients it follows an undramatic course. In some patients, however, additional toxic insults or altered hormonal homeostasis such as pregnancy may cause an acute exacerbation of the disease. Some experimental evidence shows that this is the direct consequence of a translocation of the toxic agent, beryllium, to a new and different target site within the organism (Clary and Stokinger, 1973). Other evidence points to immunologic actions (Reeves, 1977). Nevertheless, it is obvious that the interaction of two offending agents may sometimes dramatically alter the development of the disease.

There are only a limited number of ways in which cells and tissue can react to insults by chemicals. When we imply that a chemical such as carbon tetrachloride, mercury, or silica dust causes specific toxicity, we often find it necessary to define the specific

lesion by its localization in a given target organ or tissue, e.g., the liver, kidney, or lung. In the final analysis, however, the reaction at each of these tissues may be essentially the same: cell death, inflammation, degenerative changes, tissue recovery, or abnormal growth. It is therefore necessary to understand the general pathogenetic mechanisms leading to responses caused by chronic interactions between two or more chemicals. Two possible examples are discussed below.

TWO-STAGE CARCINOGENESIS

Two-stage carcinogenesis may be a special example of interactions between chemicals. The concept of two-stage carcinogenesis was originally developed for mouse skin more than 30 years ago (Berenblum, 1941; Berenblum and Shubik, 1947; Friedewald and Raus, 1944). Since then it has undergone many refinements and has also been one of the thoroughly explored models of carcinogenesis, from the molecular level to the whole animal (Boutwell, 1974).

It is useful to distinguish three possible forms of two-stage carcinogenesis: cocarcinogenesis, initiation-promotion, and enhancement of carcinogenesis (Berenblum, 1978).

Cocarcinogenesis

In cocarcinogenesis, exposure to two weak carcinogens usually results in the formation of more tumors than would occur after exposure to either agent alone. The temporal relationship between the exposures to the two agents does not appear to be critical. Exposure may be simultaneous or sequential, and the order of sequence does not matter. Cocarcinogenesis has been observed in mouse skin, as well as in many other organs and species.

Initiation-Promotion

In classical initiation-promotion, the sequence of events is critical. The initiating agent produces an irreversible and presumably heritable change in skin epithelial cells. If this is followed by repeated topical applications of a second agent, the promoter, tumor development is accelerated and more tumors are formed. The most commonly used promoters are only weakly carcinogenic and, in most systems of two-stage carcinogenesis, are devoid of any carcinogenic action. Two very strict criteria (one related to dose, the other to time) are used to determine a true initiation-promotion phenomenon. True promoters are capable of eliciting tumor formation even when the original dose of the initiator is too small (subcarcinogenic) to produce tumors on its own. The other element is time. Promoters are effective even when they are first applied weeks or months after initiation. Administration of the promoting agent before the initiator never enhances tumor formation. Therefore, interaction between initiator and promoter takes place only if there is a strict temporal sequence of events between exposure to the two agents.

Enhancement

Somewhere in between those two extremes (cocarcinogenesis or true promoting activity) are effects produced by a third class of compounds--those that enhance carcinogenesis. Much evidence indicates that these compounds may act as promoters without fulfilling the strict requirements of tumor-promoting agents. However, these compounds are of particular interest since they provide experimental evidence that the concept of two-stage carcinogenesis

applies to organs other than mouse skin. Among the noncarcinogenic or weakly carcinogenic agents that do not enhance tumor formation are: the commonly used drug phenobarbital, the food additive butylated hydroxytoluene (BHT), the artificial sweeteners saccharin and cyclamates, and endogenous agents such as bile salts.

Peraino and associates (1978) have provided the most complete evidence that liver tumorigenesis most likely proceeds via a two-stage process that is analogous to that of tumor development in mouse skin. They selected the potent carcinogen N-2-fluorenylacetamide (AAF) as an initiating agent and fed it to rats at a level of 0.02% in the diet for various lengths of time. The number of hepatic tumors that were determined 260 days after beginning the exposure was proportional to the lengths of exposure to AAF. Of the animals exposed for 11 days, 2% developed tumors; of the animals exposed for 260 days, 26% developed tumors. However, tumorigenesis was greatly enhanced if the AAF diet was followed by a diet containing 0.05% phenobarbital. If exposure to the phenobarbital was begun immediately after exposure to AAF and continued until termination of the experiment, the incidence of tumors increased by 4 to 8 times. When the duration of exposure to phenobarbital was gradually reduced, the investigators observed that as few as 20 days of exposure to the drug increased the number of liver tumors per tumor-carrying rat although it failed to increase the percentage of rats carrying liver tumors. In further experiments, the animals were maintained on a control diet for various lengths of time after exposure to AAF before being fed phenobarbital. Tumor incidence appeared to be controlled by duration of exposure to

phenobarbital rather than by the length of time after AAF treatment before the exposure to phenobarbital was initiated.

Liver tumorigenesis was also enhanced when rats were exposed to another carcinogen, p-dimethylaminoazobenzene (Peraino et al., 1978) or to the weak carcinogen 2-methyldimethylaminobenzene (Kitagawa et al., 1979). In mice that were susceptible to developing spontaneous liver tumors, phenobarbital greatly enhanced the development of tumors (Peraino et al., 1973; Ponomarkor et al., 1976). Thus, phenobarbital has many characteristics of a promoting agent. However, when control animals were exposed to the initiator without subsequent exposure to phenobarbital, a certain number of animals (between 2% and 10%) always developed tumors (Peraino et al., 1978).

For phenobarbital to be labeled as a true promoting agent, it would have to enhance tumor formation in the livers of animals exposed to an apparently subcarcinogenic amount of AAF. Further studies will be necessary to identify an experimental system in which this criterion, usually applied to two-stage carcinogenesis in mouse skin, can be met.

There has been a large epidemiological study to determine whether the observed enhancement of tumorigenesis in laboratory animals by phenobarbital is potentially harmful to humans. In epileptic patients treated chronically with high doses of phenobarbital, there was no evidence of increased tumor incidence (Clemmesen et al., 1974).

BHT also enhances tumor formation in suitably initiated tissue. Exposure of rodents to dietary AAF followed by a diet containing 0.5% BHT (but not 0.05% BHT) enhanced tumor formation in the liver

(Peraino et al., 1977). Witschi et al. (1977) have shown a similar enhancement of tumor formation in the mouse lung. Certain strains of mice develop pulmonary adenomata (tumors derived from the type II alveolar epithelial cells) within 4 to 6 months when treated with a variety of carcinogens (Shimkin and Stoner, 1975). To examine whether it was possible to enhance tumor formation in the lung, the investigators gave urethan to mice at a dose producing 100% tumor incidence in the lung. In the control animals, an average of four tumors per lung was found 3.5 months later, and 12 tumors per lung, 6 months later. The experimental animals were given 13 injections of BHT beginning 1 week after injection of urethan. The dose of BHT caused acute necrosis of type I alveolar cells followed by a proliferation of type II alveolar cells (Hirai et al., 1977). In animals treated with BHT, an average of 12 tumors per lung was observed 3.5 months after exposure to urethan, and 19 tumors per lung after 6 months. The difference between the treated and control groups was statistically significant (Witschi and Lock, 1979). Thus, BHT appears to increase the number of tumors formed and to accelerate their growth to some extent. The minimum number of BHT treatments that increased the number of lung tumors was four. Tumor formation was also enhanced by BHT if the interval between exposure to urethan and the first BHT treatment was extended up to 6 weeks. When the treatment was reversed, i.e., the 13 weekly BHT injections were given before exposure to urethan, tumor incidence was the same in controls as it was in the BHT-treated animals (Witschi and Lock, 1979).

Although BHT enhances formation of tumors in both liver and lung, like phenobarbital it may not be labelled a promoting agent

in the truest sense of the word. When lower concentrations of urethan are given, the number of tumors formed after BHT is proportional to the urethan dose. Moreover, at the lowest level of urethan tested, enhanced tumor formation can no longer be found (Witschi and Lock, 1979).

Nonetheless, the experiments with BHT highlight an important aspect of the possible interaction between carcinogens and an agent that has never shown to be a carcinogen per se but one that is capable of enhancing carcinogenesis under certain conditions. Several studies have shown that BHT can provide protection against chemical carcinogens, if it is administered before the carcinogen (Wattenberg, 1978). On the other hand, some data (Peraino et al., 1977; Witschi et al., 1977) suggest that BHT, if present after administration of the carcinogen, no longer protects but rather enhances and accelerates tumor formation.

The temporal sequence of exposure appears to be the most critical factor in determining the quantitative nature of the interaction. This is even more evident from comparisons of two studies in which the same species (rats) and comparable levels of the same carcinogen (AAF) were used and the same tumor (hepatocarcinoma) was studied. In animals treated with BHT prior to exposure to the carcinogen, tumor incidence was reduced (Ulland et al., 1973). In animals exposed to BHT after the carcinogen, tumor incidence was increased (Peraino et al., 1977). In chronic interactions, such as those in two-stage carcinogenesis, timing of exposure appears to be a very critical factor and should be considered when assessing risk.

Two-stage carcinogenesis has also been demonstrated in the bladder epithelium. Hicks et al. (1978) found that saccharin and sodium cyclamate were capable of greatly increasing formation of tumors following a single administration via urinary catheter of the potent alkylating agent N-methyl-N-nitrosourea (MNU). Of particular importance was their observation that both sweeteners produced bladder tumors in animals that had received an initiating noncarcinogenic dose of MNU. This system appears to satisfy at least one criteria of two-stage carcinogenesis: that a promoter also produces tumor formation following a subcarcinogenic dose of the initiator. It has not been shown that either sweetener is capable of enhancing tumor formation if the interval between exposure to the carcinogen and exposure to the promoting agent is prolonged nor has it been shown that reversal of the procedure, i.e., exposure to saccharin or cyclamate followed by the carcinogen, is without effect. Nevertheless, the findings are not only of considerable theoretical importance but are also significant for estimating the risk of using these artificial sweeteners in food and drink for humans. This has been discussed in greater detail in report of the National Academy of Sciences Panel on Saccharin and Its Impurities (1978).

Finally, there is some evidence to suggest that the formation of colon tumors is slightly enhanced by dietary constituents and by bile salts. Many aspects of this mechanism suggest that this might be yet another example of two-stage carcinogenesis (Reddy et al., 1978).

In summary, there is now compelling experimental evidence to suggest that two-stage carcinogenesis applies to epithelial tissue other than mouse skin. Two-stage carcinogenesis is a particular form of interaction between two chemicals. Perhaps the most important aspect of this interaction is that the temporal sequence of exposure is the determining factor in the eventual outcome.

LUNG FIBROSIS

Acute interaction between two chemicals or between a chemical and a physical agent may also play a role in the development of another form of chronic tissue injury, lung fibrosis, which is quite common in humans. Etiologic agents responsible for its development are infectious agents, inhaled toxic dusts and fumes (e.g., metals such as cadmium, beryllium, and aluminum or fibrogenic dusts such as silica and asbestos), physical agents (e.g., irradiation of the thorax), and oxygen in abnormal concentrations (Morgan and Seaton, 1975). Lung fibrosis might also follow exposure to bloodborne toxicants such as paraquat (Smith et al., 1974) as well as to a number of drugs, especially several antineoplastic agents (e.g., bleomycin and methotrexate) (Sostman et al., 1977). Alterations in the cell population of the lung and the arrangement of interstitial collagen resulting from the loss of coordinated control of collagen synthesis and degradation within the wall of the pulmonary alveoli are common features of lung fibrosis. They result in impairment of gas exchange across the alveolar capillary barrier and reduced ventilation due to decreased compliance and changes of the elastic properties of the lung (Crystal et al., 1978). The biochemical events accompanying

these changes have been studied extensively in slices of lung tissue exposed to an offending agent in vitro, in whole animals, and in specimens of lung tissue obtained via biopsy (Fulmer and Crystal, 1976). Lung fibrosis progresses slowly: the disease may develop and cause death within a few years or it may run a more prolonged course, resulting in crippling pulmonary functions.

Recent work suggests that some forms of lung fibrosis could be caused by an interaction between two chemicals or between a chemical and a physical agent in the alveolar zone of the lung. The experimental evidence, which is still far from complete, may be summarized as follows: in the mouse lung (Hirai et al., 1977) and, to much lesser extent, in the lung of female rats (Larsen and Tarding, 1978) the antioxidant BHT causes acute damage and necrosis of the type I epithelial cells, which line 95% of the alveolar surface. In male rats and in other species, BHT has not yet been found to produce a similar sequence of events. However, necrosis of alveolar type I cells is a common form of acute toxic lung injury and may be induced in various species, including humans, by a large number of toxic inhalants as well as by agents carried into the lungs via the bloodstream (Witschi and Côté, 1977b).

Once the epithelial layer of the alveolus has become damaged, the necrotic cells eventually disintegrate and detach from the basement membrane. As a general rule, these defects are quite often repaired quickly and efficiently (Witschi, 1976). It is now well established that recovery of the tissue is caused by a proliferation of the type II alveolar cells, which are the stem cells of the alveolar epithelium. In a normal lung, this cell population represents

approximately 14% of the total pulmonary parenchymal cells (Weibel et al., 1976). The area that they cover is even smaller since the cells are of cuboidal shape and usually sit in the corners of the alveoli. Following injury to the type I epithelial cells, the type II cells begin to proliferate and to divide. During the next few days portions of the cytoplasm display signs of active movement under the electron microscope. Thin sheets of cytoplasm begin to extend from the body of the cells and to creep over the denuded areas of the basement membrane. Several days after the insult the cells have assumed a shape that is no longer morphologically distinct from type I alveolar cells. This process restores an essentially normal air-blood barrier.

While type II cells are dividing, they appear to be vulnerable to toxic and physical agents. In a resting lung, cell damage usually becomes apparent only after several days of exposure to oxygen concentrations of 90% or more (Adamson et al., 1970; Gould et al., 1972). On the other hand, proliferating epithelial cells may be prevented from carrying out DNA synthesis and, presumably, from dividing by exposure to between 40% and 60% oxygen for as short a time as 16 hours (Witschi and Côté, 1977a). In a normal lung, acute cell damage may be caused by X-rays, usually after administration of 3,000 to 5,000 rads (Phillips and Margolis, 1972). Dividing cells may be affected by as little as 100 rads from X-rays or 50 rads from neutrons. Higher doses of irradiation (400 to 800 rads from X-rays) might prevent recovery and proliferation of lung epithelial cells within 2 to 3 weeks (Meyer et al., 1980).

Physical and chemical agents that are present during the recovery of alveolar wall tissue following a primary insult can affect

an effective repair mechanism. One consequence of this is an increase in total lung collagen content (Haschek and Witschi, 1979). Animals given a dose of BHT that produces uniform and widespread alveolar type I cell damage and then exposed to between 70% and 90% oxygen for 4 to 6 days develop extensive and uniform interstitial fibrosis within 2 to 3 weeks. This has been verified histopathologically and quantitated by measuring total lung hydroxyproline. More than twice the amount of hydroxyproline found in a normal lung may accumulate under these conditions. Calculations of the amount of collagen added to a normal lung by exposure to BHT alone, oxygen alone, or the combination of the two agents show that the combined effect of BHT and oxygen adds substantially more collagen to the lung than does either exposure by itself. Thus, the effect of the combined treatment is not only additive but also synergistic. Similar observations have been made by killing the dividing epithelial cells with X-rays 1 day after exposure to BHT (Haschek et al., 1980).

The synergistic interaction between BHT and oxygen occurs only if there is a critical timing between administration of the two agents. For example, if animals are exposed to 70% oxygen for 6 days immediately following exposure to BHT, fibrosis is apparent 2 weeks after the exposure. If oxygen exposure is begun 6 days after administration of BHT only, no fibrosis develops. Exposure to 70% oxygen for 7 days followed by administration of BHT does not result in a synergistic interaction between the two agents (Haschek and Witschi, 1979).

Similar results are obtained with X-rays. Fibrosis is produced only if the thorax is irradiated 1 day before exposure to BHT, immediately after exposure, or 1 day after exposure. Irradiation 2 or 3 days before or 2 to 6 days after exposure to BHT will not result in fibrosis (Haschek et al., 1980). However, if fibrosis develops following the interaction between BHT and X-rays, it will persist up to 6 months and possibly longer after one single episode (Witschi et al., 1980).

The data summarized above show that exposure to oxygen results only in fibrosis if exposure to oxygen begins soon after exposure to BHT, but not if exposure is delayed until 6 days later. Similarly, X-rays may also induce fibrosis only if the lung parenchyma is irradiated either shortly before or shortly after exposure to BHT. Previous studies have shown that following BHT-induced lung injury there is initially a wave of epithelial cell proliferation followed only later by division of fibroblasts and capillary endothelial cells (Adamson et al., 1977). One possible explanation of these findings is that the second insult following injury by BHT must occur within a very limited time, presumably when there is an increased susceptibility of the type II epithelial cells preparing to divide. If this critical period is missed, an apparently normal recovery of the tissue occurs.

The implications of these observations are potentially far reaching. They seem to establish a broad general principle: in the lung, fibrosis develops if one agent damages the alveolar epithelium and if another toxic agent, which must be present during a critical phase of the recovery period, subsequently inhibits normal reconstitution of the alveolar epithelium. It remains to be established whether this

principle in the pathogenesis of fibrosis following lung damage can be produced by agents other than BHT. For example, we can speculate that lung fibrosis develops if the primary damage to the alveolar epithelium is caused by toxic inhalants such as cadmium fumes (Palmer et al., 1975) or by inhalation of other metallic compounds such as nickel (National Academy of Sciences, 1975) or vanadium (U.S. Department of Health, Education, and Welfare, 1977). Interference with the recovery phase might be brought about by such inhalants as nitrogen oxide, ozone, or even tobacco smoke. The latter two agents have already been found to inhibit cell division in the alveolar zone (Penha et al., 1972) or to give rise to abnormal developments of fibrotic tissue (Frasca et al., 1974).

The pathogenetic principle outlined above might also play a role in the development of lung fibrosis during the course of therapy combining certain antineoplastic drugs and irradiation of the thorax. Thorax irradiation alone has been known for some time to trigger the development of fibrotic changes in the lung (Gross, 1977). The process usually takes several months if not years to develop fully. In most cases, radiation pneumonitis appears months after irradiation of the thorax with high doses of X-rays (3,000 rads and more), and fibrotic changes occur after only months (Rubin and Casarett, 1968). However, excessive fibrosis develops in certain patients within weeks rather than within months during the course of a treatment with both anticancer drugs and X-rays. This has been observed during treatment with actinomycin D, adriamycin, cyclophosphamide, methotrexate, or vincristine (Aisenberg, 1978; Einhorn et al., 1976; Littman et al.,

1974; Nickson, 1978; Rosen et al., 1974; Wara et al., 1976). In laboratory animals, adriamycin, actinomycin D, and cyclophosphamide in combination with thorax X-irradiation lead to accelerated necrosis and, eventually, to fibrosis (Phillips et al., 1975). In these instances the anticancer drug may cause lung damage similar to that induced by BHT. Data on bleomycin support this (Adamson, 1976; Adamson and Bowden, 1977). Therefore, thorax irradiation, if applied shortly before or during epithelial recovery, might interfere with the recovery process and fibrosis would develop.

If this hypothesis can be substantiated in further experiments, it will be important to establish precise dose-effect relationships of the two agents in the lung. It will be even more critical to know their relationships to the type of effect that is produced. If irradiation can cause fibrosis when administered during a critical phase to an animal model exposed to both BHT and X-rays, it should become possible to avoid a similar complication in humans by carefully timing the administration of drugs and irradiation of the thorax.

A similar type of interaction may cause disease in yet another region of the lung, the small airways. Small airway disease is one of the most prevalent forms of chronic lung disease (Bates, 1972; Cosio et al., 1977; Ebert, 1978). The epithelial lining of the small airways is composed essentially of two types of epithelial cells, the ciliated cells and the unciliated (Clara) cells (Clara, 1937). Ciliated cells are readily damaged by toxic inhalants, particularly by the ubiquitous air pollutants nitrogen oxide and ozone. If ciliated cells die, repair is accomplished by division of the stem cells of the bronchiolar and the Clara cells (Evans et al., 1978).

Recent work has shown that Clara cells are vulnerable to the toxic effects of several agents. These cells appear to be rich in mixed-function oxidases and are therefore capable of activating certain agents to highly reactive and toxic metabolites, an event resulting in necrosis of the Clara cells. Among such agents are 4-ipomeanol (Boyd, 1977), 3-methylfuran (Boyd et al., 1978), carbon tetrachloride (Longo et al., 1978), 3-methylindole (Huang et al., 1977), and 4-nitroquinoline-1-oxide (Terao and Otsu, 1973). Tobacco smoke can also damage Clara cells (Kilburn et al., 1974). If Clara cells are damaged when they are supposed to take part in the recovery of the bronchiolar ciliated epithelium, fibrosis might develop as it does when type II alveolar cells are damaged at the time they are supposed to repair damaged type I alveolar cells. Whether this potential interaction applies to the development of small airway disease remains to be established.

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CHAPTER 8

CONDITIONS ALTERING TOXICOLOGICAL INTERACTIONS

Conditions often subtly or dramatically alter the "rules" that scientists like to provide in order to make the scientific universe move in an orderly fashion. This becomes obvious when dealing with chemicals and their biological effects and is even more apparent when several chemicals are superimposed on a biological system simultaneously. The "rules" may then become "guidelines" since the systems within organisms react in any number of ways to cope with the additional challenge of several new chemical intruders.

There may not be a high degree of predictability to the biological alterations that result from chemical interactions. Nonetheless, there are some general "guidelines" pertaining to the type of effects that result from multiple chemical exposures, the variations to be expected under certain environmental and stress conditions, the influences of dietary and nutritional factors, and alterations produced by the presence of preexisting disease states in the host system.

EFFECTS OF ENVIRONMENTAL AND STRESS CONDITIONS

Extreme environmental conditions, such as temperature and low oxygen or water levels, profoundly affect toxicity and the disposition of foreign chemicals in the various organs. The additional influence of physical factors, such as vibration or noise, produces comparable effects. These exogenous variables may also evoke typical stress responses. Microsomal oxidative stimulation, mediated via the

hypophyseal-adrenal axis, is usually an important mechanism for this effect (Driever and Bousquet, 1965; Fuhrman and Fuhrman, 1961; Fuller et al., 1972).

Cold Environmental Temperatures

Drastic lowering of the environmental temperature establishes a condition of stress that markedly alters the metabolism of drugs and foreign agents. Principally, it activates the drug-metabolizing capacity of the mammalian liver. Typical examples are the enhancement of acetanilide microsomal hydroxylation and ring hydroxylation of 2-naphthylamine (Inscoe and Axelrod, 1960). Exposure to cold also stimulates the metabolism of aniline, but it depresses the hydroxylation of hexobarbital (Dewhurst, 1963). Continuous exposure to low ambient temperatures accelerates $^{14}CO_2$ formation in rats that have been given several doses of ethanol (deBruin, 1976; Platonow et al., 1963)

High Environmental Temperatures

Investigators have observed that elevated environmental temperatures may exert a definite adverse influence upon the response to toxic chemicals and drugs. The potentiating effect of thermal stress (30°C-40°C) upon sublethal toxicity is demonstrable for drugs (Fuhrman, 1946), ozone (Stokinger, 1957), lead (Baetjer et al., 1960), mercury (Trakhtenberg et al., 1965), thiol poisons (Savitskii, 1967), antimony (Baetjer, 1969), and various pesticides, including anticholinesterase compounds (Grigorowa and Binnewies, 1973), DDT, warfarin (Keplinger et al., 1959), and 2,4-dinitro-o-cresol (Tesic et al., 1972).

Increased respiratory and dermal exposure may be responsible for the increased excretion rates of p-nitrophenol in subjects exposed to parathion in hot environments (Funckes et al., 1963).

Dehydration

The stress condition resulting from dehydration is similar to that caused by an elevation of temperature. Severe water deprivation is associated with lowered resistance to the acute toxic effects of lead (Baetjer et al., 1960), antimony (Baetjer, 1969), and methacholine chloride (Baetjer, 1973). Conversely, dehydrated animals are no more responsive to lipid-soluble solvents than they are under normal circumstances (Baetjer, 1973). Isolated hepatic microsomes derived from rats on a water deprivation regimen have a reduced capacity to metabolize hexobarbital (Baetjer, 1970).

Hypoxia

As expected, animals with hypoxia respond abnormally to chemical exposures. Alcohol metabolizes at a decreased rate, and experimentally induced lung tumorigenesis is increased (Hueper and Conway, 1964; Zapata-Ortiz et al., 1970).

Vibration and Noise

A mutual interaction between a vibrational stress and toxic factors has been established in animals subjected to the combined application of toxic metals and a vertical vibration load (50 Hz). Histopathological examination revealed that vibration accentuates the toxicity of manganese. It also intensifies the degenerative

action of mercuric salts upon nerve elements (Levakovskaya and Neizvestnova, 1972; Mavrinsaya and Tartakovskaya, 1972). Morphological changes in the internal organs were slight on exposure to repeated doses of the organophosphate trichlorfon, but were pronounced when the doses were combined with a prolonged noise stress (Tsapko and Rappoport, 1972).

EFFECTS OF NUTRITION AND DIETARY FACTORS

The activities of the hepatic enzyme systems that metabolize foreign compounds vary with nutritional status. Starvation results in decreased rates of hydroxylation of acetanilide, demethylation of meperidine, and metabolism of hexobarbital and other compounds. The activities of all the drug-metabolizing microsomal enzymes in the livers of female rats are enhanced by starvation. Fasting animals produce smaller amounts of glucuronide conjugates than normal (Miettinen and Leskinen, 1963). When maintained on a protein- or calcium-deficient diet, rats incur a diminished rate of drug metabolism due to decreased activity of the microsomal enzymes (Dingell et al., 1966). The toxicity of acetylsalicylic acid is increased by a protein-deficient diet and is further increased by a deficiency of magnesium (West, 1964). In guinea pigs that have been deprived of ascorbic acid, there is a reduction in the rates of metabolism of acetanilide and a variety of drugs.

Malnutrition and Starvation

Dietary deficiency arising from prolonged caloric restriction does not indiscriminately result in sensitization to toxic agents

(McLean and McLean, 1969). Nutrition is a highly structured entity. Consequently, dietary inadequacy may interact with toxic factors in a subtle and complex fashion, depending on the type of poisonous agent and the state of deficiency.

Reduced intake of calories usually inhibits, rather than promotes, the tendency of animals to develop spontaneous or chemically induced neoplasms (Tannenbaum, 1959; Tannenbaum and Silverstone, 1958). Malnutrition modifies the usual responses of organisms to noxious substances by interacting with their mechanisms of absorption, storage, and biotransformation. Some toxic agents have distinct antinutritive properties, and their action is confined to interference with a specific vitamin, coenzyme, or amino acid (Gontzea and Sutzescu, 1968).

A large intake of alcohol creates a general state of avitaminosis, thereby increasing the demand for vitamins. Simultaneous dietary deficiencies of the vitamins (especially the B-complex) predispose the organism to the adverse effects of alcohol. The effects of alcohol on the liver are minimized by intake of an adequate diet and are generally accentuated by dietary imbalance (Porta et al., 1970; Tomasulo et al., 1968).

The most prominent effects of nutritional deficiency occur at the level of microsomal biotransformation. Acting as stress stimuli, adverse nutritional factors may produce stimulatory effects by increasing the amounts of microsomal enzymes. Examples include the metabolic activation of DDT (Dale et al., 1962; Deichmann et al., 1972) and aniline (Kato and Gillette, 1965) due to severe fasting. Various types of unbalanced diets and nutritional variables

can modify chemically induced stimulation of hepatic microsomal drug-metabolizing enzymes. Fasting appears to be associated with a decline in the rate at which ethanol is metabolized (Smith and Newman, 1959; Vitale et al., 1953).

Protein Deficiency

Protein depletion materially alters the toxicity of numerous xenobiotic compounds, thereby exerting either favorable or adverse influences (McLean and McLean, 1969). Generally, protein-deficient diets greatly reduce the activity of hepatic microsomal enzymes and the level of cytochrome P_{450}, resulting in diminished ability of the organism to metabolize foreign compounds. Protein deficiency is associated with increased resistance to such hepatotoxic agents as carbon tetrachloride (McLean and McLean, 1966) and dimethylnitrosamine (McLean and Magee, 1970), which are toxic by virtue of their conversion into biologically active metabolites. Protein-free diets also suppress the microsomal hydroxylation of aflatoxin B_1 (Madhavan and Gopalan, 1965). Diets containing excess protein afford some degree of protection against the hepatocarcinogenicity of aflatoxin (Polrovsky, 1969).

Increased susceptibility to chloroform is apparent in protein-depleted rats, that have been treated with microsomal enzyme inducers (McLean and McLean, 1969). Both excessive ingestion of ethanol and protein-depleted diets tend to result in accumulation of hepatic triglycerides, and their simultaneous presence exerts additive effects. A low protein diet decreases hepatic alcohol dehydrogenase activity in conjunction with depressed clearance of ethanol from the blood

(Goebell and Bode, 1971). The amount of dietary protein given to animals relates linearly to the quantities of conjugated phenols and hippuric acid that are excreted following ingestion of benzene and toluene, respectively (Gontzea et al., 1970).

The susceptibility of animals to a wide range of pesticides is influenced markedly by the dietary level and quality of protein. In protein-deficient rats susceptibility is enhanced severalfold by dieldrin (Lee et al., 1964), DDT (Lasota et al., 1973), lindane (Shtenberg, 1972), chlordane (Boyd and Stefec, 1969), endrin (Boyd and Stefec, 1969), toxaphene (Boyd and Taylor, 1971), parathion (Webb et al., 1973), malaoxon (Webb et al., 1973), fenitrothion (Lasota et al., 1973), and carbaryl (Boyd and Boulanger, 1968). Protein inadequacy results in lower levels of the microsomal detoxifying enzymes. Diets of high quality protein promote the excretion of lindane and its metabolites, thereby reducing the degree of its storage in tissues (Chadwick et al., 1973).

Dietary Factors

Animals exposed to such hepatotoxins as carbon tetrachloride benefit from a diet that is high in carbohydrate and low in fat. The continuous feeding of hypocaloric diets intensifies the hepatogenic action of carbon tetrachloride (Shakman, 1974). In most instances, the consumption of high fat diets appears to enhance chemically induced carcinogenesis (Boutwell et al., 1957). The hepatoprotective effects of diets supplemented with certain sulfur-containing amino acids (such as methionine, cysteine, and homocysteine)

in animals challenged with liver-damaging doses of carbon tetrachloride, bromobenzene, or dichloroethane are well known (Binkley, 1949; Highman et al., 1951). Certain adverse responses to carbon tetrachloride in animals may be prevented or reversed by prior or simultaneous supplementation of aspartic acid, folic acid, cysteine, thioctic acid, or thiolactone plus cysteine (Fodor et al., 1971; Oeriu et al., 1966). Dietary methionine modifies the toxicity of halogenated hydrocarbon insecticides (Waliszewski, 1972). It also accelerates the detoxication of selenium, which combines with the methyl group of methionine. This process is additionally enhanced by the presence of vitamin E or antioxidants (Levander and Morris, 1970).

Deficiency of vitamin E (α-tocopherol) is associated with lowered resistance to the action of ozone. An excess in the diet reverses some of the usual biochemical responses to the inhalant. Vitamins have proved their antidotal value in various types of intoxication. Ascorbic acid probably plays a role in the activation of microsomal enzymes, as suggested by the diminished rate of metabolism of acetanilide and lindane in scorbutic animals (Chadwick et al., 1973). Other examples of interaction between nutrition and toxicity relate to altered susceptibility resulting from changes in dietary levels of trace elements. The dietary levels of calcium and iron strongly influence the toxicity of lead (deBruin, 1976).

EFFECTS OF PERSONAL HABITS

Individual lifestyle habits and activities, such as periodic or daily alcohol consumption, variable caffein intake through coffee or

tea drinking, and smoking of tobacco or mood-altering plant products, will alter physiological and biochemical functions. These effects, since they are commonplace and often not thought to be significant by the average person, may not be considered when potential interactions with other foreign chemicals are discussed.

Nevertheless, these personal, and often unique, chemical intakes as a result of lifestyle do present potentials for chemical interactions with other, more obvious foreign compounds from occupational and other environmental sources. Savolainen et al. (1979) reported that neurochemical effects were demonstrated by increased superoxide dismutase activity in the brains of 2-month old male rats exposed to 300 ppm concentrations of xylene vapors for 5 to 18 weeks. Concomitant ingestion of ethanol enhanced the xylene effect of proteolysis but did not affect superoxide dismutase activity. This has been further emphasized by Elovaara et al. (1980) who showed that inhalation exposure to xylene, a common solvent, when coupled with ingestion of ethanol produced severe liver damage while independent exposure to xylene or ethanol failed to do so.

Physicians, supervisors, and health officials should carefully explore the personal habits of workers or patients to determine if one or more of these "disguised" chemical intakes are occurring and to incorporate this information in any evaluation of adverse effects potentially or actually occurring through interaction with other compounds.

EFFECTS OF PREEXISTING DISEASE STATES

Human patients with liver damage have an increased sensitivity to a wide variety of drugs, a phenomenon attributed to impairment of the detoxicating function of the liver. Patients with obstructive jaundice, hepatitis, cirrhosis, and other liver diseases exhibit impaired formation of glucuronide and sulfate conjugates (Muting, 1963). This reduced ability of the diseased liver to metabolize foreign compounds has been confirmed by experiments with animals. Hepatic microsomes from rabbits with obstructive jaundice show impaired metabolism of acetanilide and a number of drugs. Infection of mice with murine hepatitis virus reduces the drug-metabolizing activity of the hepatic microsomes (Kato et al., 1963b). In rats with abdominal carcinosarcomas, microsomal metabolism of foreign compounds is impaired and the activity of the microsomal enzymes of various hepatic tumors is either impaired or absent (Kato et al., 1963a). Liver regeneration is accompanied by a decrease in glycogen content and microsomal enzyme activity, both of which are restored when regeneration is complete (Dixon, 1963-1964).

Similarly, diseases of the kidney and other avenues of excretion result in reduced elimination of drugs and environmental chemicals (deBruin, 1976; Parke, 1968). Higher than expected biological levels of these compounds result, and if threshold concentrations are reached, physiological alterations and toxicity can occur. The numerous chemicals encountered daily by humans during normal activities can readily induce toxic effects if disease interferes with or reduces the effectiveness of one or more steps in biological detoxication.

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