TOXICOLOGY OF ORGANIC COMPOUNDS: A REVIEW OF CURRENT PROBLEMS

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In the first *Annual Review of Pharmacology*, the toxicology of organic compounds of industrial importance was discussed by Browning (25). In Volume 2, Chenoweth & Hake (37) surveyed the literature on the toxicology of the smaller halogenated aliphatic hydrocarbons in a very complete manner from about 1955 to June of 1961. In this review an attempt will be made to discuss some problems of current interest to toxicologists, choosing as materials those which are of interest principally because of the toxicologic problems which they present, and to a lesser extent because of their importance from an industrial or public health point of view. Any reviewer in the field of toxicology faces special problems not only because of the extraordinarily wide variety of journals in which data are found, but because so often the most fundamental observations are buried in an article. the title of which may not indicate any reference to the toxic compound. This is understandable because toxic compounds are often used as tools in the elucidation of some biochemical effect and are not always the primary interest of the investigator.

A second problem in the literature of toxicology has to do with the reluctance of authors to publish or of editors to accept simple descriptions of the toxicologic properties of a compound if these are essentially negative, or if they do not seem to immediately support the advance in knowledge of a toxic process. Much valuable data remain unpublished or referred to only in manufacturers' bulletins or technical literature. While such information is often not very exciting by itself, it may provide the clue to the solution of some other problem.

An attempt has been made to search for significant findings in either recent or older publications, and with due regard to the limitations mentioned above, the literature will have been at least partially examined in the following areas through June of 1962.

Halogenated Hydrocarbons

Carbon tetrachloride and chloroform.—There is an enormous amount of literature on the toxic properties of halogenated compounds which has been, however, very thoroughly analyzed in two major publications. Von Oettingen (138) covered practically all of the work in this field up until 1955, and Chenoweth & Hake (37) covered the smaller halogenated aliphatic hydrocarbons through June of 1961. The metabolic significance of the halogenated hydrocarbons was discussed by Browning (25) covering the compounds carbon tetrachloride, trichloroethylene, dichloroethane, and trichloroethane

(methyl chloroform). Lewis (88) reviewed the toxicology of carbon tetrachloride, and a brief discussion of the cytochemical effects of carbon tetrachloride was included by Bain & Mayer (4) in their summary of biochemical mechanisms of drug action.

Research in this field seems to continue to be concerned almost exclusively with the biochemical effects of carbon tetrachloride or chloroform on the liver of various laboratory animals such as the rat, although occasionally experimental clinical investigations appear (67, 126, 128). This has been a natural consequence of the great increase in knowledge of the functions of certain particles in the liver such as mitochondria, microsomes, or lysosomes. While there had been a number of previous speculations as to the possible nature of the toxic effect on the liver, such as conversion to hydrochloric acid or phosgene, Christie & Judah (38) provided evidence for associating the toxic effect with a disturbance of function in liver mitochondria. This study showed defects in the oxidation of fatty acids, some uncoupling of oxidative phosphorylation, and changes in the citric acid cycle. The theory was proposed that this was brought about by some action on the mitochondria, producing a change in permeability.

A similar theory was proposed by Beaufay *et al.* (13) in which lysosomes (cytoplasmic particles which could be partially separated from mitochondria by sedimentation methods) were found to release certain soluble hydrolytic enzymes and thus set in motion the necrotic process. Beaufay (12) describes these particles as "essentially inert little bags which upon injury by any of a variety of means, release into the medium a collection of soluble hydrolytic enzymes with an acid pH optimum." These included acid phosphatase, β-glucuronidase, cathepsin, acid ribonuclease, deoxyribonuclease, aryl sulfatases, and others. It was, however, emphasized by Beaufay that this was not a specific action of carbon tetrachloride but that these enzymes could be released from lysosomes by a variety of means, such as freezing, thawing, surfactants, etc. The point was made that if the permeability change is the primary action then substrates may migrate into the particles as well as allowing enzymes to leak out.

Still another thoery has been proposed by Recknagel and his co-workers (106). Their studies indicated that the mitochondrial functions were not altered much in the period from four to ten hours after oral administration of carbon tetrachloride; whereas, the liver glucose 6-phosphatase was markedly depressed, and the liver triglyceride content markedly increased in as little as two hours after dosing. The study of microsomal enzymes indicated that pathological changes were occurring in the endoplasmic reticulum two hours after the ingestion of carbon tetrachloride and that one of the earliest observed effects was to inhibit the ability of the liver to secrete triglycerides into the plasma. As a proof of this hypothesis, they found that in untreated rats the intravenous injection of a non-ionic detergent elevated the plasma triglycerides by as much as twelvefold. However, in rats that had been treated previously with carbon tetrachloride, the post-surfactant in-

crease in plasma triglyceride was almost completely absent and instead there was a marked increase in liver content of triglycerides. The very early effect of carbon tetrachloride on triglyceride secretion was, of course, compatible with the early histologic findings of increased liver fats.

A fourth theory has been proposed by Brody and his associates (23) which attributes the action of carbon tetrachloride primarily to a cellular anoxia of the liver resulting from a primary action on the sympathetic portion of the autonomic nervous system. The principal basis for this assumption is that the effects of carbon tetrachloride on the oxidative phosphorylation of the liver mitochondria, activation of an Mg-dependent ATPase, and deposition of lipid in the liver as well as histological changes, could be prevented to a greater or lesser extent by the use of adrenergic blocking agents, adrenalectomy, or a spinal cord section. Stern & Brody (124) recently reported that oral carbon tetrachloride administration causes a rise in urinary excretion of epinephrine and norepinephrine. Rises in catecholamine excretions were reduced after spinal cord section and the use of ganglionic blocking agents; but after adrenalectomy, carbon terachloride produced a rise in norepinephrine excretion.

A series of recent reports has provided evidence that chemical and structural changes may occur even sooner than previously supposed. Taft *et al.* (130) gave mice carbon tetrachloride in sesame oil subcutaneously and by sacrificing animals at short intervals after dosing were able to show that one of the early changes was a loss of basophilic granules in the central lobular parynchymal cell. Marked changes were seen in the endoplasmic reticulum on electro microscopy. The total RNA was the same as in untreated animals. Reynolds (111) also noted marked changes in the endoplasmic reticulum as early as one hour, and also in the structure of mitochonria, and a dispersal of basophilic bodies. There was a marked depression of glucose 6-phosphatase in the central lobular areas. An interesting finding was that in one hour the calcium content of the liver rose five times that in the control animals, being noted first in the mitochondria and then in other cells. The calcium content returned to normal in six hours, but the glucose 6-phosphatase stayed low. Ashworth & Luibel (3) gave carbon tetrachloride intraperitoneally and noted changes in the endoplasmic reticulum and in mitochondrial structure as early as one hour. There was also a reduction of oxidative and phosphorylytic enzymes. The retention of water and triglycerides in the liver cell was thought to be due to damage to energy-producing mechanisms.

Others have used perfusion techniques to demonstrate the rapid onset of similar changes. For example, Brauer *et al.* (21) perfused the rat liver with chloroform through the portal vein (the agent being added to the oxygen supply of the blood being used for perfusion). This resulted in a marked and immediate drop in oxygen consumption with only very slight evidence of any change in the resistance to flow of perfusate through the liver. There was, however, a marked drop in the pH occurring in a period of about one

hour. This pH change was reversible on removing the chloroform. The bile flow also decreased, and endoplasmic reticulum showed signs of dissolution. It was of interest that hypoxia alone to the same level of oxygen uptake did not produce the pH change. Weinstein & Murray (139) removed rat livers three and one-half hours after dosing with carbon tetrachloride and then perfused them through the portal vein. Normal control livers under such circumstances showed a continuous release of triglyceride into the perfusion medium. When C^{14}-labeled tripalmitin was given as a neutral emulsion in the perfusate, the livers from carbon tetrachloride-treated rats continued to take up tripalmitin from the perfusate while at the same time releasing less triglycerides into the outflow. These livers also incorporated more C^{14}-labeled acetate in the lipids than normal conrols. This indicated that the liver tended to retain these neutral fats.

Using a somewhat different technique, Smuckler, *et al.* (122) gave rats carbon tetrachloride orally, and to one of the groups C^{14}-labled glycine was given intravenously. These rats were later sacrificed and the perfused liver fractionated. Another group of rats was killed three hours after treatment, and the microsomes and the supernatant fluids were incubated with C^{14}-labeled glycine and various substrates. The results indicated that there was a depressed incorporation of C^{14}-glycine into the protein of mitochondria, microsomes, and into supernatant protein, the greatest difference being in the supernatant protein. They felt the major effect on protein synthesis was on the *s*-RNA step. They mention that the same findings were noted with yellow phosphorous.

In a report on the toxic effect of halogenated hydrocarbons which may have considerable significance for future investigations, Butler (28) has shown by a series of careful experiments that it is possible to demonstrate unequivocally the presence of chloroform in small quantities in the expired air of dogs which had previously been exposed to highly purified and chloroform-free carbon tetrachloride. A similar experiment was carried out with chloroform, but no methylene choride could be detected in the expired air in this case.

In vitro studies with carbon tetrachloride and chloroform using various mouse tissues and certain tissue constituents demonstrated that the mouse liver was capable of converting considerable quantities of carbon tetrachoride to chloroform and of chloroform to methylene chloride. This occurred to some extent in other tissues. It was not seen with dog blood, but some reduction of carbon tetrachloride did occur with reduced glutathione, cysteine, ascorbic acid, and cytochrome C. While no evidence has been produced for it as yet, this finding suggests the possibility of the production in cells of a "positive halogen compound" such as those discussed by Dixon (47). Such compounds are often highly toxic or lacrimators and are known to alkylate sulfhydryl compounds and possibly undergo other reactions. This hypothesis seems very attractive and might help to explain some of the puzzling problems in the toxicity of the halogens.

It is somehow difficult to conceive of carbon tetrachloride exerting its toxic effect on the cell or cell membranes or particles simply because it is nonpolar and a good solvent for fatty materials. If this were the case, then many other inert nonpolar solvents might be expected to have similar actions. It seems essential that other nonpolar solvents which do not have hepatotoxic effects should also be studied for their actions on microsomes, etc. It is not easy to account for the synergism with ethanol; however, in the case of chloroform, Kutob & Plaa (84) have suggested that pre-treatment of mice with ethanol causes an increase in liver lipids resulting in higher chloroform retention. The effects occurred when ethanol could no longer be detected in the animals.

While the theory proposed by Brody and his co-workers would put the primary location of action outside of the liver, it is again a little difficult to assume that a material which is chemically inert, nonpolar, and a fat solvent, would have such a specificity as to cause the extraordinary autonomic nervous system reaction such as he proposes simply on the grounds of its physical properties. There are also other serious difficulties with the theory of liver ischemia from release of catecholamines, etc. In the first place, in the clinical cases of pheochromocytoma there is no evidence of serious or fatal disease of the liver and kidneys. Secondly, there seems to be little evidence produced that either epinephrine or norepinephrine will produce such an intensive vasoconstriction as to produce a high degree of anoxia in cells. Epinephrine is thought to actually cause either no change or a slight increase in flow through the liver, while norepineprhine produces only a slight constricting effect (49, 77, 129). Some studies have shown an actual increase in blood flow in the liver in carbon tetrachloride poisoning, while in others a reduction in flow was considered secondary to cellular edema. Finally, in human cases of carbon tetrachloride poisoning, there may or may not be any general change in blood pressure; while there is considerable evidence of an increase in venous pressure, particularly when some tendency to cardiac failure is present, as is often the case. While the effects of cordotomy and the finding of increased catecholamine excretion in the urine seem to have been established, it seems probable that these represent some type of secondary action rather than a primary action.

While recognizing the great advances made in understanding the action of carbon tetrachloride on the liver, it would seem timely to call the attention of investigators to the fact that the major problem in human poisoning is not indeed the liver but the kidney. The majority of all human deaths from carbon tetrachloride intoxication whether by ingestion or by inhalation are the result of the well-known renal failure accompanied by cardiac failure which in most instances is secondary to the renal effects (68). Compared to humans, the common laboratory species, especially the rat, seem to be relatively resistant to the renal effects of carbon tetrachloride. When the material is taken by mouth, the liver damage is more apt to be clinically evident than it is when the exposure is by inhalation. When the exposure is at relatively

low levels and on a chronic basis, it has been supposed that liver damage will be the first to appear and that tests of liver function would be a sensitive index of over-exposure.

While there is good evidence for this in experimental animals (1), this does not seem to have been established by clinical studies. For example, Kazantis & Bomford (79) studied a group of 18 persons who had been exposed to about 45–97 ppm (290–620 mg per m^3) and found that the predominant symptoms were actually dyspepsia, nausea, occasional vomiting, depression, and irritability. Of these, nausea and anorexia were the most frequent. No abnormal signs were found on physical examination. No abnormalities were noted in the urine, and only one man was found to have a transient elevation of serum glutamic oxalate transaminase (76 units per ml) which was normal on the following test. However, only eight out of the group were tested.

Similar findings were mentioned by Stewart & Witts (125) who examined 78 persons who had been exposed under wartime conditions over a period of two or three years with probable exposures higher than those described by Kazantis & Bomford. While no actual air analysis could be obtained due to difficulties with equipment, very careful clinical studies again disclosed that gastrointestinal symptoms were predominant together with some dizziness, headaches, and mental depression. Laboratory investigations (including bilirubin, phosphatase, serum proteins, total fats, fatty acids, cholesterol, and urine examination) did not disclose any evidence of renal or hepatic disease. Gastrointestinal x-rays were thought to show some tendency to hypermotility. One of the earliest and most comprehensive animal and clinical studies of carbon tetrachloride (123) using about 96 men who had been exposed to the solvent for periods varying from 1–25 years also did not disclose obvious clinically apparent renal or liver injury.

Through the kindness of H. F. Smyth, Jr., it was possible to examine the details of the clinical studies. The mean number of years of exposure was 5.6, the mean of the individual average daily exposures (85 persons) was 40 ppm, and the mean of the individual peak exposures (85 persons) was 372 ppm. The principal symptoms were nausea (29 persons), dizziness (23 persons), and headaches (17 persons). Fifty-two persons had one or more of these symptoms. Only one palpable liver was felt, this being in a known alcoholic. There were no significant findings in the urine, although only a few of these were available as freshly voided samples. There were occasional slightly elevated icteric indices, or indirect van den Bergh tests, and occasional low serum calcium levels were noted. Restriction in carefully mapped visual fields was seen in 10 out of 93 men, the significance of this being uncertain. This report also contains much interesting animal data on guinea pigs, rats, and monkeys. Guinea pigs were especially sensitive to hepatotoxic effects while monkeys were somewhat resistant.

While clinical or laboratory evidence for liver and renal injury has not yet been established as a regular part of the human response to chronic low-level exposures, none of the above studies utilized the recently developed

enzymatic methods for detecting cellular injury. A wide choice of possible methods is now available to the investigator, and a recent critical study was made of the response of the rabbit to a six-hour inhalation of carbon tetrachloride at levels of 200 and 500 ppm (46); activities of 10 serum enzymes were correlated with liver pathology up to 72 hours after exposure. Under these conditions a rise in serum enzymes occurred before cell necrosis was seen. This was especially true with glutamic dehydrogenase. No changes were seen in alkaline phosphatase and only slight changes in leucine amino peptidase. All the others showed definite changes at 500 ppm and some change at 200 ppm. While no clear basis for preference between the actively responding enzymes was made by the authors, it would seem probable that glutamic dehydrogenase, glutamic pyruvic transaminase, and isocitric dehydrogenase might be among the better choices. No kidney function studies were made nor were any changes seen in renal cells.

Another report based on the response of rat serum glutamic pyruvic transaminase to liver injury from the solvent has appeared and seems to be correlated with the drop in glutamic pyruvic transaminase in the liver (15). Until such tests have been carefully applied to large numbers of persons under accurately known conditions of daily exposure [including biochemical tests for exposure in expired air or blood, such as those described by Stewart *et al.* (128)] their usefulness will not be known.

With regard to the extreme sensitivity of the human kidney to higher levels of exposure and changes in excretory function, there are available several important reports covering this feature of its action (52, 55, 66, 118, 120). These will not be reviewed here but should be consulted by anyone interested in understanding the nature of the changes in renal function and their correlation with cellular damage. However, there do not appear to have been many studies on the biochemical nature of the cellular injury such as have appeared for the hepatotoxic effect.

A recent report has, however, indicated a very promising method for detection of renal tubular injury (98). On the premise that the release into the serum of cellular respiratory enzymes such as isocitric and glutamic dehydrogenases is known to follow liver necrosis (109), and that lactic dehydrogenase had been noted in urine in a variety of renal disorders (114), the authors determined isocitric dehydrogenase in rat urine and serum after producing renal tubular nephrosis with uranyl nitrate. This enzyme did in fact appear in considerable quantities in the urine and reached its maximum at the same time as the tubular necrosis. Of equal interest was the fact that isocitric dehydrogenase did not appear in the serum, suggesting a preferential excretion from the tubule into the urine. If this can be confirmed in clinical cases, it may prove to be a tool of equal importance to those tests now available for hepatic damage. Since serum isocitric dehydrogenase is a sensitive indicator of liver cell necrosis, it seems possible that the application of this to blood and urine would be a useful screening device for chronically exposed persons.

It appears, therefore, as though the current threshold limit value of 25

ppm is based principally on carefully controlled animal exposures and that it is probably low enough to prevent irreversible injury in humans. It is felt, however, that this is a maximum value and that the time weighted average should not be more than 10 ppm (32, 48).

With regard to therapy of poisoning by carbon tetrachloride, there is no specific antidote, although a number of efforts have been made to develop specific treatments. One interesting report is that by Fiume & Favilli (54) who found that treatment with fairly large doses of aminoacetonitrile (a lathryogenic compound) would have a delaying action on the development of collagen formation and the cirrhotic process. The authors suggest that the inhibitory effect of aminoacetonitrile on the hepatic fibrosis from carbon tetrachloride is due to a specific inhibition of collagen formation and also an inhibition of fibroblast multiplication.

Gallagher (58) has suggested that the injection of nicotinic acid or dl-tryptophane may reduce the death rate after acute oral dosage of carbon tetrachloride in rats by stimulating synthesis of pyridine nucleotides. The influence of testosterone has been studied by Colalongo & Murari (40). The evidence of any benefit is slight and limited to the early stages of poisoning.

A report by Reddy *et al.* (107) indicates that female rats are more resistant to carbon tetrachloride than males and that oophorectomy abolished this difference. On the other hand orchidectomy in male rats did not alter the influence of cirrhosis. This report is also of interest in that pregnant female rats exposed to carbon tetrachloride developed cirrhosis to the same extent as non-pregnant rats. Surprisingly, the litters of these females were found to be normal as regards size and histology of the liver. On the other hand, weanling rats of either sex exposed to carbon tetrachloride seemed to develop cirrhosis more readily than adults.

Trichloroethylene and tetrachloroethylene.—While it is generally agreed that these solvents have little hepatotoxic effect (24), some animal studies indicate certain types of changes in liver metabolism. Kylin *et al.* (85) exposed mice to these solvents as well as chloroform for four-hour periods at various concentrations. After three days, liver fat and histology were determined and also blood serum ornithine carbamyl transferase activity [see Richard (110) for this method]. Trichloroethylene caused little fatty change or increase in fat content and no change in transferase action. Tetrachloroethylene caused some increase in fats but no change in transferase, while chloroform caused necrosis and increase in fats and transferase activity.

There have been three interesting reports on the metabolism of trichloroethylene in man. Bartonicek (10) exposed nine subjects to concentrations of about 1000 μg per liter (about 186 ppm) for periods of five hours. After exposure, measurements were made of expired air, the urinary metabolites [trichloroethanol (TCE) and trichloroacetic acid (TCA)] and concentrations in feces, sweat, and saliva. In this exper
agent was 1,066 mg (air intake at rest 6.3 liters per min) of which about 58 per cent was retained in the body. Of that retained, 77 per cent was excreted in the urine (45.4 per cent as TCE and 31.9 per cent as TCA). The feces

contained 8.4 per cent and the sweat and saliva small amounts. There were marked differences in the rate of excretion of metabolites in the urine, TCE appearing rapidly at 24 hours and declining rapidly, while TCA rose slowly to a peak at the third or fourth day. Traces could be found as long as 20 days. The ratio of TCE/TCA on the third day was 1.23. At this time, the plasma contained 2.4 mg per 100 ml of plasma, while the red blood cells contained only 0.5 mg per 100 ml.

It is of interest that the values obtained at one hour are of the same order of magnitude as those of Stewart *et al.* (127) at a shorter exposure of three hours at 211 ppm and determined by a different method. Alcohol ingestion was said to increase the TCE output at 8 to 20 days.

In another study, Bartonicek & Teisinger (11) exposed four subjects to 1 mg per liter for five hours and then gave tetraethyl thiuram disulfide in divided doses. This caused a marked decrease in output of the urinary metabolites and an increase in loss of trichloroethylene in expired air. This seems to provide proof that trichloroethylene oxidation is inhibited by this drug and adds weight to the metabolic scheme proposed by Butler (27). Since TCE is thought to be possibly a toxic or active metabolite, the suggestion was made that the disulfide might have therapeutic properties, especially in oral poisonings. More work would be necessary to establish this, however.

Attempts continue to be made to find a more sensitive index of the central nervous system action of such solvents. Zahner *et al.* (142) studied the effects of trichloroethylene vapor on rats trained to pass from the bottom of a T-shaped chamber to either the right or left branches in search of food. Normal rats show a spontaneous right-left alternation, but after exposure the frequency of regular alternations decreases. The running speed was increased by concentrations of 400 to 600 ppm but lowered at high concentrations as might be expected. A similar type of study, but using rats trained to climb a rope at a signal in search of food, was reported by Grandjean (64). Appropriate studies of central nervous system functions in humans under carefully controlled conditions of exposure would seem of interest in this connection.

While sudden delayed deaths after relatively trivial trichloroethylene exposures have been reported (26, 131), they appear to be very rare and difficult to substantiate. However, five additional cases have been reported by Hoschek (78) of which two were personally observed by him. In one of these cases, a 36-year-old man had a short but probably fairly heavy exposure during a degreasing process and walked home after work with no complaints. The next morning (a holiday) he started to do some fairly hard work in his vineyard and suddenly expired. Autopsy showed no cause for this but strongly positive tests for organic halogens were found in urine, kidney, lung, and brain. The second case in a 27-year-old man was similar in nature but the prior exposure was probably less. The attack came suddenly while walking home from work in very cold weather. The diagnosis in these cases seems to have been made on the rather slender grounds of absence of other

causes and the finding of evidence of solvent in tissues. The cause of death was assumed to be ventricular fibrillation.

An extensive review and survey of experience with trichloroethylene degreasers in the United States has been published by Hargarten *et al.* (71). In the period from 1948–1957, only ten deaths were found—most of these were from entering or falling into tanks. The injury frequency rate was only 0.3 injuries per million man hours of exposure. A study of exposures in 323 degreasers showed that 90 per cent were under 100 ppm and 50 per cent under 50 ppm. This evidence was thought to be reassuring in view of the nearly total coverage of experience.

Hexachlorobenzene.—One of the most interesting and new toxicologic reactions to be reported in recent years is the massive outbreak of cutaneous porphyria in Turkey which first appeared about 1955 to 1956, the probable etiology of which was first suggested by Cam (30) and reviewed by Schmid (116). The clinical picture consisted of a hyperpigmentation (especially on exposed surfaces), hypertrichosis, epidermolysis, and vesiculation, with marked sensitivity of the skin to light and trauma. The cutaneous lesions were slow to heal, became infected, and in some cases gave use to a suppurative arthritis and osteomyclitis of the digits.

The urine showed intense red fluorescence and increased porphyrins. The number of cases was estimated to be as high as 5,000 in three districts, with 135 cases and 8 deaths occurring in one township of 3,600 people. Children and adolescents were more sensitive, and there were marked exacerbations in the summer months. Many cases had repeated episodes and sustained severe scarring of the skin.

The relationship to hexachlorobenzene (this is C_6Cl_6, and not $C_6H_6Cl_6$ or "benzene hexachloride" or hexachlorocyclohexane) was established when dietary histories showed that these patients had virtually all consumed some treated seed wheat containing about 200 ppm of this fungicide in addition to the usual mercuric or mercurous chloride. The epidemiologic evidence seemed indisputable, and the relationship to the fungicide was established even more firmly by the experiments of Ockner & Schmid (95) who fed rats a diet containing 0.2 per cent of the compound causing a delayed onset of marked increases in porphyrin excretion in the urine and feces which became irreversible if feeding was continued. Hepatomegaly was common, with increased liver porphyrins, central necrosis, and in some cases deaths with central nervous symptoms. This finding was confirmed by DeMatteis *et al.* (43) who studied a variety of species (rabbits, guinea pigs, rats, and mice). The rabbit most nearly resembled the human in that uroporphyrin was the predominant urinary porphyrin as the authors also showed in analyses of urines from clinical cases. Porphobilinogen was not present. The increase in glucuronic acid previously reported by Parke & Williams (96) was not found. In the latter experiments, no evidence could be found for metabolic products in the urine, none was excreted in the expired air, and the majority of it was found in the feces after oral dosing or at the site of an injection. The neurologic disturbances seen in animals are not seen in man, and deserve further study.

An additional report on the toxicity in rats (57) is in agreement with other studies. Large amounts of uroporphyrins were found in the urine and feces and in tissues. Centrilobular necrosis of the liver resulted, and neurologic symptoms were produced. A group of the treated animals was given daily subcutaneous injections of 10 to 12 mg of adenosine-5-monophosphoric acid with improvement in skin and liver but not central nervous system symptoms.

Hexachlorocyclohexane.—This substance, frequently referred to as "benzene hexachloride" should not be confused with hexachlorobenzene just discussed. Its widespread use as an insecticide, either as a mixture of its alpha-beta-gamma-delta isomers, or as its gamma isomer, has not been accompanied by serious occupational disease. Barnes (5, 6) has reviewed its properties and pointed out that the isomers may cause quite different symptoms in mammals—the gamma isomer causing excitement, while the delta isomer is a depressant. Convulsive-type symptoms have been seen in man under conditions of excessive exposure or from its ingestion. There have been occasional reports of allergic eczema. Behrbohm & Brandt (14) discuss 26 cases of dermatitis and patch test studies which were said to show no reaction to some of the highly purified samples. Another study of a skin sensitization reaction in a worker preparing the compound indicated that of the 10 isomers known to be present in the particular product, only one (delta-heptachlorocyclohexane) caused a reaction in the subject (73). The metabolism of the various isomers has been reviewed by Williams (140). It is evident that the variations in metabolism and toxicity of the various isomers provide an interesting subject for study.

Fluorocarbon compounds.—These are of interest to the toxicologist because of their industrial importance but more so because of the great variety of their physiological effects. Two excellent reviews have appeared. The first by Pattison (97) deals with the compounds related to monofluoroacetic acid and interprets their action in relation to the probable metabolic pathway. These compounds are of interest because they include some of the most potent of all known compounds. Another review by Clayton (39) discusses the toxicology of these materials in reference to their chemical structure, comparing the activity of mono and polyfluorinated compounds, effects of chain length, fluoroalkanes vs. fluoroalkenes, fluoroalcohols, nitrogen-fluorine and sulfur-fluorine compounds, and fluoropolymers.

In general, the polyfluorinated materials were of lower toxicity than monofluorinated, and toxicity tended to decrease with increasing chain length. The fluoroalkenes were associated with a higher toxicity than the unsaturated compounds, and a number of them were pulmonary irritants in high concentrations. By and large, the fluorinated alkanes were less apt to cause liver damage than other halocarbons.

The fluoropolymers have been investigated extensively and have been found to have an extremely low order of toxicity when given in the diet or placed in contact with skin or within body tissues. Like other halogen-containing materials, pyrolysis products may be irritating under certain conditions, and the effect will vary with temperature of pyrolysis. A syn-

drome resembling metal fume fever, marked by a temporary attack of fever, chills, and increased white count with prompt recovery has been reported to have occurred a few hours after exposure (72). Attempts to reproduce this in animals have not been entirely successful. However, some progress has been made in this direction as a result of studies by Pernis *et al.* (100) on metal fume fever. While rabbits do not show a delayed fever after inhalation of zinc oxide fumes, if they were pretreated with an irritant such as an acetic acid aerosol, the typical delayed onset was apparent. It was assumed that the fever was caused by liberation of an endogenous pyrogen from leucocytes in the lung resulting from contact with zinc oxide.

This type of experiment was repeated using instead of zinc oxide, fumes from pyrolysis of a fluorocarbon polymer at 400–500°C. A somewhat similar response was noted with a delayed fever. Transfer of serum from febrile to normal rabbits caused a febrile response in the latter. Filtration of all solid particles from the fume before inhalation abolished the fever and only a pulmonary irritant response was seen, due to gaseous fluorine-containing compounds. It was concluded that, as in the case of the zinc oxide experiment, the particles penetrate into the lung capillarics, are phagocytosed, and liberate from them an endogenous pyrogen (35).

Further evidence in support of this theory was obtained by using a technique similar to that of Hirsch & Cohn (74) in their studies of the degranulation of leucocytes following phagocytosis. Rabbit polymorphonuclear leucocytes were incubated with particles of zinc oxide or the polymer and the number of granules counted. A marked decrease resulted in both cases. Because of the similarity of this response to other pyrogen responses, it seems likely that this could be the mechanism of the reaction (99). Other clinical and experimental observations on fluorocarbon compounds have appeared (31, 42, 101) which are similar in nature to older observations.

Alcohols and Glycols

There are two substances in this group which continue to be of especial interest to investigators—methanol and ethylene glycol. In each case the hazard is principally from ingestion, although under conditions of very high exposure, such as are rarely met with at present, methanol may also present a serious hazard by inhalation. Both substances present interesting problems of mechanism of action; and although great progress has been made, unsolved problems remain, and opinions differ as to the mechanism by which their toxic effects are produced.

Methanol.—The extraordinary effects of methanol by ingestion have been noted in many studies and reports dealing with the effect on the eye, the gross and microscopic pathology, the remarkable acidosis, and in recent years the metabolism and nature of the biochemical lesion produced. The comprehensive reviews by Røe (112, 113) should be consulted for general background on the subject. The review by Treon (134) gives emphasis to the effects on man and animals by inhalation. The report by Bennett *et al.* (16) is particularly useful, not only as a description of the clinical and laboratory

findings in one of the largest known epidemics of poisoning, but also for its lucid discussion of the complex nature of the human response to methanol and of the relationship of symptoms to the biochemical abnormalities produced.

It has been known for many years (16) that exposure to or ingestion of methanol would give rise to small amounts of formates in human urine and that this could be decreased by the ingestion of ethanol. For example, Leaf & Zatman (87) showed that only a small fraction (two per cent) of low doses of ingested methanol was normally excreted as such in expired air or urine and that the concentration in urine could be increased by simultaneous ingestion of ethanol, indicating an inhibition of the oxidation of methanol. There appears to be a species difference in reaction to methanol, and Gilger *et al.* (62) believe that the syndrome of delayed onset of acidosis and amblyopia with damage to the retina, optic nerve, and central nervous system is characteristic of primates. The typical picture of the human response in these respects has been reproduced in monkeys, and ethanol has been demonstrated to delay the disappearance of methanol from the blood, as well as to reverse the acidosis and retinal damage. A variety of experiments [reviewed by Williams (140)] had shown that formaldehyde and formates were present in various tissues, including vitreous humor after exposure to methanol, and that formaldehyde was more potent than formate or methanol in inhibiting retinal respiration (87, 103). Kendal & Ramanathan (80) had also found that horse liver alcohol dehydrogenase could convert methanol into methyl formate (presumably by means of a hemi-acetal-dehydrogenase mechanism) and suggested that the lipoid solubility of the latter might enable it to produce special effects. A specific retinal formaldehyde dehydrogenase was found which converted the formaldehyde to formic acid (82). Electroretinogram studies also demonstrated that methanol was less active than its oxidation products (104).

In the meantime an electron microscopy study of the fine structure of the retinal receptors of the eye showed that the inner segments of the rods and cones contained dense aggregations of mitochondria (119), suggesting the possibility of a very active metabolism of substances such as methanol in these vital structures in the visual process. This possibility has now been studied intensively by Kini & Cooper (81) who used fresh intact ox retinas and also mitochondria obtained by centrifugation. Methanol, formaldehyde, sodium formate, and methyl formate were studied for their effect on oxygen uptake and carbon dioxide production using ^{14}C glucose. Formaldehyde was far more potent than methanol in inhibiting these functions. For example, methanol at a concentration of 2,000 mM caused only a 0.9 per cent inhibition of $^{14}CO_2$ formation, while 5.0 mM formaldehyde caused this to drop by 84 per cent. Formaldehyde at 0.5 mM still caused a 27 per cent inhibition. Methyl formate was active in the range of 5–100 mM, while sodium formate was slightly less active.

Anaerobic glycolysis was inhibited 50 per cent by 0.5 mM formaldehyde, while aerobic glycolysis was stimulated. Studies with ox retinal mitochondria

showed that when succinate was used as a substrate, as low as 1m*M* formaldehyde would abolish phosphorylation completely.

As a result of these and other experiments, the authors conclude that the dominant effect of formaldehyde on retinal metabolism is to decrease the synthesis of ATP and that it affects coupled phosphorylation rather than electron transport. They consider that their demonstration of the respiratory effects of formaldehyde on the retina is added proof that the production of this agent *in situ* from methanol is the true cause of the retinal lesion; also that while the role of ATP in the visual process is not known, the close arrangement of mitochondria of the inner segments to the photochemically active molecules of the outer segments (such as rhodopsin) suggests some intimate connection of high energy intermediates of the phosphorylating mechanism to the visual process.

They also consider the concentrations used in their *in vitro* experiments to be comparable to those existing in body fluids in cases of human poisoning. If the lethal dose of methanol in man is considered to be about 65 g, this may be estimated to be about 0.042*M* in body fluids. This seems reasonable since marked *in vitro* effects were found at concentrations of formaldehyde at 1–5 m*M*. It is perhaps of interest that if one accepts the estimate of Treon (134) that inhalation of 50,000 ppm of methanol for one hour is about the acutely dangerous level for man, then the respiratory intake in this time would be on the order of 65 g. The approximate lethal concentration of methanol for various species in exposures of from one to six hours is also of the same order of magnitude, although the relative respiratory intake would vary somewhat. Since most species studied show evidence of formate in body fluids or urine, it seems probable that formaldehyde must have been produced also and probably in the same orders of magnitude at lethal concentrations. Since the retinal structures and functions are similar in most mammals, the resistance to retinal damage in subprimate species does not exactly fit in with the formaldehyde theory of methanol toxicity.

Furthermore, humans have ingested quite large amounts of formaldehyde daily for long periods with no effects on vision (141). It might be, of course, that the doses were not large enough, the metabolism different, or the concentrations lower than when produced *in situ*. Fatal human cases have been seen from doses as low as 6 ml of methanol (16) which would mean that the possible concentrations of formaldehyde *in situ* must have been extremely small.

Perhaps another reason to question the formaldehyde theory lies in the failure of other solvents which are converted into C_1 fragments to cause retinal damage. A good example of this is the toxicologically innocuous acetone which has been shown by Price & Rittenberg (105) to be split into C_2 and C_1 fragments, with the latter being found to have been incorporated in the methyl groups of methionine, choline, etc. It would seem essential to know something about the effects of other C_1 producing materials before final conclusions could be made. Since formaldehyde is known to be normally

produced or utilized in the course of many reactions, such as in the conversion of serine to glycine [see Fruton & Simmonds on the role of C_1 fragments in metabolism (56)], quantitative data on concentrations under *in vivo* conditions would seem desirable. Finally, it does not seem possible at this point to entirely rule out other types of reactions such as methylol formation, transmethylation reactions, effects of formic acid or methyl formate, etc.

As has been stated by Bennett (16), there are other clinical problems to be solved such as the mechanism of the tremendous acidosis. It hardly seems possible to account for this by the simple production of formic acid in relatively small amounts. Røe (113) has discussed the high concentrations of organic acids in blood and the unidentified organic acids in urine. The pancreatic necrosis with rise in serum amylase also needs more study. While the therapy with ethanol seems to be on a sound theoretical basis, it probably needs to be started earlier than the normal time of appearance of such cases in the wards (62). The occasional rapid clearance of visual symptoms with bicarbonate alone raises a question as to a special local etiologic action of formaldehyde.

As far as industrial exposures by inhalation are concerned, there is a virtual absence of serious difficulty in recent times. Only one recent report could be found of amblyopia from inhalation of methanol (70). While this was a clinical report with no air analyses, it was evident that massive exposures had occurred to methyl formate as well as methanol. A review of some of the older literature makes it clear that the exposures causing amblyopia must generally have been very high (probably on the order of 10,000 ppm or more), since it is apparent that methanol can scarcely be detected by odor at 2,000 ppm (115). The present threshold limit of 200 ppm appears to be ample to protect against injury (94).

Ethanol and other alcohols.—The inhibiting effect of tetraethyl thiuram disulfide on the oxidation of acetaldehyde to acetate is well known. The possibility that some of the sulfonyl ureas being used as oral antidiabetic drugs may also produce such an effect has been discussed by Larsen & Madsen (86). Previous studies were reported to have shown that tolbutamide inhibited several DPN-dependent enzymes including alcohol dehydrogenase and acetaldehyde dehydrogenase, and clinical observations indicated that some patients taking alcohol during this treatment experienced symptoms similar to the typical ethanol-antabuse type reaction (29). In the present studies, attempts were made to investigate this under carefully standardized conditions using constant ethanol infusions in nembutal anesthetized cats. The effect of a tolbutamide metabolite and of another compound, carbutamide, were also studied. Under these conditions, tolbutamide was found to cause a reduction in rate of disappearance of ethanol from serum of about 34 per cent. The metabolite (*N*-(4-hydroxymethyl-benzol sulphonyl)-*N'*-n-butylurea) was also found to inhibit the metabolism of ethanol but without exerting any hypoglycemic effect. Carbutamide was less effective in reducing ethanol metabolism than tolbutamide.

It was apparent that the ethanol effect bore no relation to the hypoglycemic action. These effects on the ethanol disappearance rate have not been entirely borne out by Büttner and others in studies on normal human subjects (29), and the clinical importance of this effect seems to require confirmation. Nevertheless, the subject seems worthy of more study and might have some importance under conditions of industrial exposure to ethanol or possibly other alcohols.

While ethanol is largely metabolized to carbon dioxide, it is known that a minor percentage is excreted in the urine as a glucuronide (140). It has now been shown in several studies by Boström & Vestermark that alcohols and polyols can also undergo esterification with sulfate (19, 20, 135 to 137). While *in vitro* techniques using $^{35}SO_4$ (to aid in determining the esters) were used in most cases, the reaction has been shown to occur *in vivo* in the rat after oral or intraperitoneal doses. Partial excretion as sulfate esters has been shown to occur for the aliphatic alcohols C_1 to C_5 in length and for a number of polyols. In the latter case the diols with a greater distance between the OH groups were more easily esterified. The quantitative importance of this type of metabolite is not clear at present, but the finding is of considerable interest.

2-Fluoroethanol is known to be a highly toxic substance and to be converted to fluoroacetate. However, it is less apt to produce the typical tonic convulsions of the latter. Treble (133) has found that the conversion to fluoroacetaldehyde is probably not accomplished by the same alcohol dehydrogenase as in the case of ethanol. Guinea pig kidney particles were not affected by fluoroethanol, although fluoroethanol can be converted by liver to a compound capable of inducing citrate accumulation in such particles. Fluoroethanol, also, is not a substrate for horse liver alcohol dehydrogenase and may actually inhibit the oxidation of ethanol.

Glycols.—The interesting features of the toxicity and metabolism of glycols have been reviewed by Browning (25), and Williams (140). As a class, they vary widely in their toxicity and hazard, and show considerable variation in their action on different species. The hazard from oral ingestion may differ greatly from that by inhalation; ethylene and diethylene glycol are quite hazardous by ingestion, but are practically devoid of hazard by inhalation. Their metabolism, therefore, has been of considerable interest, especially as concerns the mechanism of the renal damage which appears in man and other species after ingestion of large doses.

While it has been known for more than 60 years that ethylene glycol ingestion would cause increases in urinary oxalate (41, 102), and considerable attention given more recently to the details of its metabolism (61), it is still not clear whether the deposition of oxalate in the renal tubules is cause or effect. The recent work of Gershoff & Andrus (59) is of considerable interest in this respect and suggests new possibilities for both mechanism of action and therapy. Previous reports by these authors had been concerned with endogenous oxalate production, renal oxalate deposition, and pyridoxine deficiencies in the rat. It had been found that a lack of pyridoxine would

cause deposition of renal oxalate from endogenous sources, and further that the inhibition of transaminase activity in pyridoxine deficiency could result in increased accumulation of glyoxylic acid with resultant increased oxalate production. Diets high in magnesium were found to protect rats against renal oxalate deposition even though the urinary oxalate levels were not decreased by this procedure. It was, therefore, of obvious importance to relate these effects to the ethylene glycol-type of renal oxalate formation.

Weanling male rats were given 0.25 per cent ethylene glycol in drinking water for four weeks, with other groups getting 0.2 or 20 mg pyridoxine HCl per 100 mg diet in combination with magnesium, or magnesium alone, 40 or 400 mg per 100 gm diet. At the end of the experiment, the kidneys and lower urinary tracts were examined carefully under magnification for apical papillary encrustations and histologically. Based on anatomical changes, the use of excessive pyridoxine gave partial protection, while excessive magnesium could completely prevent the renal lesions. When both were used, the growth depression was also reversed, and essentially normal growth resulted. Of interest in the histologic sections was the finding that in animals protected against oxalate deposition by high pyridoxine supplements, the typical tubular dilatation and loss of normal eosinophilic staining was still present, even though oxalate crystals were absent.

The relation of the magnesium-pyridoxine effect to metabolism of ethylene glycol was studied using intraperitoneal injections of 54 μg. of ethylene-1,2-C^{14} glycol per 100 g of body weight. $C^{14}O_2$ output in expired air was used as the measure of the over all per cent oxidation of ethylene glycol. The possible effect of pyridoxine on oxidation of glycerol-1, 3-C^{14} was also measured. The use of 0.4 mg per cent pyridoxine in the diet about doubled the rate of $C^{14}O_2$ production from ethylene glycol compared to that where pyridoxine was absent, but showed no change in the case of glycerol. Magnesium alone or combined with pyridoxine did not change the $C^{14}O_2$ output appreciably. While the mechanism of action of excess pyridoxine is not clear, a marked inhibition of oxidation of ethylene glycol to CO_2 is seen when it is deficient, and a partial protection from the renal effect results when it is provided.

The magnesium effect is probably on a different basis, and while it does not prevent the ethylene glycol-induced oxaluria (69), it may have altered the solvent properties of the urine in such a way as to prevent deposition of oxalate. The possible antidotal action of these substances is suggested. However, more work would seem necessary on the magnesium-pyridoxine effect at high acutely toxic levels before the probable benefits of the therapy can be determined.

The recent detailed study of Gessner *et al.* (61) using C^{14} ethylene glycol has added much new information and has provided a sound basis for some older concepts.

Marked species differences were shown, with the cat and rat being especially liable to oxalate formation and the rabbit and guinea pig being insensitive. The proportion excreted by expired air compared to urine varies

considerably with dose. Unchanged ethylene glycol is the principal urinary substance, and both glycolaldehyde and glyoxylic acid are formed in the course of the oxidation. A previous study by these authors should be consulted for information on the relation of chemical structure to metabolism in the glycol series (60).

Silbergeld (117) has examined the effects of long-term feeding of glycolic acid and glycolates in rabbits with particular reference to renal oxalate deposition and renal function. Doses up to 0.5 gm per kg per day for seven months produced a tenfold increase in renal oxalate content, but no change in kidney function in terms of phenolsulfonphthalein excretion, blood nonprotein nitrogen, or creatinine. There were no signs of toxicity or of gross pathologic changes in the kidneys. Since the rabbit produces little oxalate in the urine after ethylene glycol (61), it would seem that the glycolic acid formed from this source may not have acted in the same manner as when given by mouth.

Blood *et al.* (18) have studied the chronic toxicity of ethylene glycol in monkeys, feeding up to 0.5 per cent in the diet for three years. No evidence was seen of renal calculi or of any type of oxalate deposit. Some granular eosinophilic material was apparent in the renal tubules, but no other histologic changes or evidence of toxicity was noted. Ethylene glycol produced by ethylene oxide sterilization of pine shavings being used as bedding for a particular strain of mice (SWR/J) was thought to be associated with a peculiar hemorrhagic syndrome in the animals. No other reports of this reaction were found and further studies are said to be in progress (2).

While little new information could be found on diethylene glycol, an intensive study has been made of metabolism of triethylene glycol (93). Previous reports had indicated that it had a low acute and chronic toxicity and that renal oxalate formation was not present in the rat, but its metabolism was unknown. Using a C^{14}-labeled sample, it was shown that the rat does not metabolize it to CO_2, that only small amounts of activity were found in the feces, and that the major portion was excreted in the urine. Virtually no labeled oxalate was seen in the urine. About 30 to 60 per cent was estimated to be present in the unchanged form; and while the structure of the metabolites was not identified, their properties suggested that either one or both end-hydroxyl groups had been oxidized to the carboxylate. Work with unlabeled material in the rabbit suggested somewhat similar results. This appears to be in line with the fate of certain other glycols—for example, the mono-n-butyl ether of ethylene glycol is said to form butoxy acetic acid (33). The absence of breakdown to ethylene or diethylene glycol is in accord with its low toxicity.

Zavon (143) has reported the occurrence of temporary central nervous system symptoms in 28 workers exposed to relatively high levels of the monomethyl ether of ethylene glycol in a printing operation. Concentrations as high as 3,000 ppm were found, and most values were well over the current threshold limit of 25 ppm. The symptoms were similar to those reported by Greenburg *et al.* (65). The use of proper industrial hygiene controls was

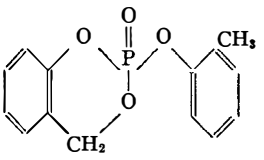
successful in controlling the hazard, which probably involved skin absorption as well as inhalation. Goldberg *et al.* (63) have reported an interesting experiment which seems to confirm the ability of this compound to cause certain central nervous system effects. Using rats, they showed that repeated daily inhalation at levels of about 150 to 500 ppm would produce a specific inhibition of conditioned avoidance-escape behavior. Such levels produce no signs of motor imbalance.

Except for these instances, the use in industry of glycols in large quantities does not seem to have given rise to reports of serious injury.

Neurotoxic Phosphate Esters

Since the classical studies of M. I. Smith and co-workers (121) on the neurotoxic properties of tri-o-cresyl phosphate (TOCP) and related esters in the "Jamaica Ginger" epidemic, the mechanism of action of this group of compounds has been of intense interest to toxicologists and pharmacologists. In spite of the knowledge of the action of TOCP, a number of subsequent epidemics occurred, one as recently as 1959. The delayed onset of a flaccid paralysis was found to be associated with a typical demyelination of the spinal cord (7). It was found by Smith (121) that the cat and chicken were especially sensitive to such effects, and some type of paralytic symptoms have been reproduced with trimethyl, triphenyl, and tri-o-cresyl phosphate and with triphenyl and tri-o-cresyl phosphite (51). While some of these are known to have effects on choline esterases, there has been no clear relationship of this to the demyelination process (36). While there had been some conjectures that these compounds might have penetrated the central nervous system and hydrolyzed to active phenol products, a study of P^{32} labeled TOCP by Hodge & Sterner (75) showed that the distribution of P^{32} in tissues was not that which would have been expected if phosphate had been liberated by hydrolysis. Large amounts were found in the brain and very little in bone.

During the past year, evidence has been produced for the presence of a highly active neurotoxic metabolite in the liver of rats and from rat microsomes exposed to TOCP (9, 34). The structure of the active metabolite was established to be as follows:



2-(o-Cresyl) 4H-1,3,2-benzodioxaphosphoran-2-one

This compound is thought to arise from methyl hydroxylation and cyclization. Production of the typical delayed paralysis in chickens occurred at much smaller doses than with TOCP, but the symptoms came on and disappeared more rapidly. Some effects were seen as low as 4 mg per kg intraperitoneally. It is said to be a potent esterase inhibitor. Attempts were made

to prevent or reduce the ataxia in hens with TOCP or its metabolite with thiamine, tocopherol, cortisone, and aldoximes without effect. Another recent report states that pyridine aldoxime dodecyl iodide did delay the onset of TOCP ataxia in hens but that the microsome inhibitor, SKF, 525A, was not effective. Diacetyl monoxime was of questionable benefit (17).

Amides and Related Compounds

The amides and their derivatives are becoming of increasing importance in industry, not only as intermediates for the manufacture of organic chemicals, but in some instances as useful solvents or as monomeric materials for polymers. Some of them have received intensive study, while for many others only fragmentary information seems to be available. Fassett (51) has recently reviewed some of the available information. A considerable amount of work has been done on the metabolism of various amides, for the details of which the papers by Bray *et al.* (22), Fiske (53), and the text by Williams (140) should be consulted. The simple unsubstituted fatty acid amides such as acetamide, propionamide, oleic acid amide, and stearamide have presented no toxicologic problems in their handling in industry. They are thought to be readily hydrolyzed *in vivo* to the corresponding acid and ammonia. The acid produced then undergoes the normal metabolic fate of a fatty acid of the corresponding type. Some toxicologic information has appeared on certain *N*-substituted amides which are used principally as solvents.

Dimethyl formamide has been studied by Massmann (91, 92). While it does not have a high acute toxicity by oral or parenteral injection, it is fairly readily absorbed through the intact skin, and exposure of cats and rats for 8 hr per day for a period of 65 days at concentrations of 100 to 450 ppm resulted in some fatalities and loss of weight, especially in cats. Rats appeared to be less affected, but on post-mortem examination they were found to have some central necrosis of the liver, and in the case of cats a fatty degeneration but no necrosis was found. There was some hyperemia of the brain and some cloudy swelling of the tubules in the kidney. There was no apparent effect on the blood picture. It was of interest that the cat showed a rather striking rise of blood sugar following intraperitoneal injection with 2 ml per kg. This returned to normal at 72 hours. The cause of this was not explainable, since there was no obvious primary disturbance of carbohydrate metabolism and no signs of pathology in the pancreas.

Attempts were made to study its metabolism after the repeated injection of small doses intravenously into fasting, anesthetized cats. Under these circumstances, small quantities of unchanged dimethyl formamide were found in the urine, blood, and stomach but practically none in the expired air. A total of 10 to 15 per cent of the dose was found to be excreted in the urine within 24 hours. A search was made for the possible metabolites, formic acid and dimethylamine, but none were detected. Examination of workers exposed to concentrations averaging less than 20 ppm showed no evidence of liver injury, and neither dimethyl formamide nor its decomposition products

could be detected in the urine of workers exposed to these levels. There were some nonspecific symptoms of headache and digestive disturbances, but these did not appear to be very significant.

Tolot *et al.* (132) have reported that eleven cases of gastric irritation were seen in a synthetic textile plant over a period of about 15 months with a majority of cases occurring in either spinners or winders. The symptoms were relatively nonspecific, consisting of some nausea, anorexia, and occasional burning pain in the epigastric region. There were no signs of liver dysfunction nor of x-ray abnormalities of the GI tract. No albuminuria was noted. There were three cases of dermatitis and itching of the skin and one case of conjunctivitis. No air analyses were made of the levels encountered in this process. There appear to be no reports of significant toxic reactions thus far in its use in the United States. A Hygienic Guide is available for dimethyl formamide (45), and the recommended threshold limit is about 20 ppm.

The toxicity of dimethyl acetamide has been investigated by Horn (76). Repeated dermal applications of 0.1–4.0 ml per kg in dogs resulted in severe symptoms or death at the 1 and 4 ml per kg levels in a period of about two weeks. Symptoms were loss of appetite, depression, ataxia, and abdominal tenderness. Some diarrhea and jaundice were present in the highest dose level. No major changes were seen at the 0.1 ml per kg level after a six-month period of exposure. On autopsy the liver showed fatty degeneration at the high level but only minor changes at low dose levels.

Rats and dogs were exposed to levels of 40 to 195 ppm of dimethyl acetamide using a six-hour daily exposure and continuing the exposure for a period of approximately six months. No obvious signs of toxicity were seen except at the highest dose level in the rat where some reddish discharge occurred around the eyes. There was also some difference in weight gain from the control animals. On post-mortem examination, the dogs exposed to 100 or 195 ppm showed some degeneration of the liver cells and some periportal fatty changes. The rats also demonstrated some focal necrotic changes along with some fatty degeneration at the two higher levels of exposure. There was little evidence of effect on the blood-forming organs. There have been no reports of toxic effects in humans, and a tentative threshold limit value of 10 ppm has been suggested (44). More specific metabolic information on the *N*-substituted amides would be helpful since it appears likely that any toxic effects which might be encountered during use would arise from skin absorption as well as from inhalation. It would be of interest to know, for example, whether demethylation occurs since formamide itself appears to be absorbed readily through the guinea pig's skin and has a dermal LD_{50} of less than 5 ml per kg. (51). The relative lack of irritation by skin contact does not provide a good warning that exposure of the skin has occurred.

Thioacetamide has previously been reported to be a liver carcinogen in the rat. Rees & Rowland (108) have studied the correlation between early histologic changes and rate of incorporation of orotic acid and adenine into liver nuclei. Both show a sharp drop in contrast to butter yellow where orotic acid rises and adenine drops.

Acrylamide and some of its N-substituted derivatives are of some interest toxicologically because of the rather striking neurotoxic effects that can be produced in certain animal species. In the case of acrylamide the syndrome is characterized (especially in the cat) by signs of motor incoordination and weakness of the hind quarters with normal or hyperactive reflexes and sensory responses. Some general changes in behavior take place in these animals. There is a characteristic delay in onset of symptoms for 24 to 48 hr even after large doses, and as the dose decreases, the time for onset of symptoms becomes longer. Kuperman (83) has made a careful study of the neurologic changes and has stated that the location is probably sub-cortical or in the midbrain. A few human cases of a somewhat similar syndrome have been noticed which recovered on cessation of exposure. While there appear to be no details regarding the mechanism of action and metabolism of this compound, the conversion to a toxic metabolite seems probable in view of the characteristic delay in onset of symptoms. Polymers made from this material are considered to be completely inert.

Attempts to induce this syndrome in cats with methacrylamide were unsuccessful, even after relatively large doses intraperitoneally for a period of three weeks. While no metabolic data are available, it would be of interest to know why the substitution of an alpha-methyl group completely removes this unusual effect (51). N-substitution also appeared to alter this effect. Cats were given N-demethyl acrylamide and N-isopropyl acrylamide intraperitoneally; and while some ataxia, tremors, and weakness of the hind quarters appeared at large doses, the typical syndrome was never reproduced (51).

Dialkyl Nitrosamines

Probably one of the most significant advances in toxicology in recent years has arisen from the study of the hepatotoxic and carcinogenic action of dimethylnitrosamine. The first inkling of its hepatotoxic action arose from the investigation of the causal relationship of dimethylnitrosamine to the appearance of cirrhosis of the liver in two out of three men in a research laboratory who had been using the compound for approximately 10 months (8).

A high degree of acute toxicity by various routes of administration in rats, rabbits, mice, guinea pigs, and dogs was shown to be associated with a severe central necrosis of the liver and with a hemorrhagic ascites. This finding, of course, suggested the possibility that it might have been associated with the cases of human liver cirrhosis, although its relatively low volatility and the failure to demonstrate rapid penetration of the skin of experimental animals made the causal relationship somewhat uncertain. It was soon established by these authors to be a potent liver carcinogen in experimental animals and subsequent investigations by Magee, Barnes, and others have given rise to some new theoretical concepts regarding a possible mechanism of carcinogenic action and possibly of hepatotoxic effects.

Magee & Hultin (90), acting on the theory that one of the break-down products could be diazomethane, conducted studies of the methylation of the proteins of rat liver slices *in vitro* by dimethylnitrosamine labeled with C^{14}. The results showed that C^{14} formaldehyde was formed but that this also entered the normal metabolic pathway for C-1 fragments and that in all probability any abnormal labeled tissue constitutents would not have arisen directly from formaldehyde production. Radioactivity was found to be incorporated into total protein of both liver and kidney; subsequent hydrolysis to the component amino acids demonstrated that activity was present in association with serine and methionine (as would be expected from the normal metabolism of a C-1 fragment), but some new methylated histidines were also found.

The theory of a diazomethane-induced methylation was strengthened when Druckrey *et al.* (50) reported that only those compounds expected to yield diazo alkanes produced tumors. Magee & Farber (89) have now found that rats injected intraperitoneally with C^{14}-labeled dimethylnitrosamine incorporate radioactivity into the DNA of liver and kidney and also in the lipid fraction of the liver. Radioactivity was found in RNA and in protein of liver, kidney, spleen, and pancreas. A search for the site of the labeling in the liver RNA finally resulted in the isolation of a new methylated derivative, 7-methylguanine. Whether diazomethane itself is responsible for the methylation or whether it is some other reactive intermediate such as a monomethyl nitrosamine is uncertain. Methylation of kidney RNA has also probably taken place, and while the severe degree of cell damage seen in the liver is not present in the kidney, nevertheless some rats have been shown to develop kidney tumors about a year after exposure.

The important findings from these studies are: (*a*) the simple generation of C-1 fragments, such as formaldehyde, probably bears no relation to pathological processes; (*b*) reactive methylating agents, such as diazomethane, may be produced *in vivo;* and (*c*) strong evidence is produced that in some cases carcinogenesis may be an extrachromosomal event, induced by an abnormal RNA.

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