TOXICOLOGY OF ORGANIC COMPOUNDS OF INDUSTRIAL IMPORTANCE[1]

By Ethel Browning, M.D.
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The toxic effects of practically all the organic substances used in industry have been repeatedly stressed in the literature for many years, and their external manifestations in the form of symptoms and signs of injury are well known. It is only during the last 30 or 40 years that attention has been focused more closely on the fundamental biochemical processes which underlie these effects, and which are believed to account for the widely varying results of exposure to different compounds, even those belonging to the same chemical group. These biochemical processes involve all the dynamic physicochemical systems of the living cell and, particularly, the intracellular enzymes which catalyze its reactions. Most of these enzymes are contained in the mitochondria within the cytoplasm of the cell. The electron microscope has revealed their highly complex structure, and the isolation of the enzymes has been accomplished *in vitro* especially by the method of differential centrifugalization [Schneider (130)]. Although the enormous amount of recent research has thus succeeded in unravelling the details of the *in vitro* structure and organization of these vital constituents of the cell, full elucidation of their physiological function *in vivo* has not yet been achieved.

The knowledge which is available has, nevertheless, shed a vivid light on the relation between the toxic action of certain organic compounds and their passage as foreign substances through the body, their elimination, partial retention, detoxication, and, particularly, their interrelationship with the enzyme systems. Any injury to the mitochondria, or any inhibition of any of the groups of enzymes, can bring about a disturbance of the whole organism leading, in severe intoxication, to death, and in less severe cases to symptoms characteristic of the site of action of the toxic metabolites formed in the body.

Among the inhibitory substances, an important member, in view of some recent investigations on the metabolism of carbon disulphide (see p. 426), was tetraethyldithiocarbamyl disulphide, derived from sodium diethyldithiocarbamate by an oxidation catalyzed by the oxidase cytochrome system.

[1] The survey of the literature pertaining to this review was concluded in April, 1960.

[2] Abbreviations used in this chapter include: DNP (dinitrophenol); DPN (diphosphorydine nucleotide); SGOT (serum glutamic oxalacetic transaminase); TCA (trichloracetic acid); TCE (trichoroethanol).

Since then, many other enzyme systems have been identified and isolated, and their importance in the nature and structure of the cell itself elucidated. A key system is the Krebs citric acid cycle (82), which catalyzes the synthesis of citric acid and, subsequently, its conversion to oxalacetic acid and the generation of energy-rich phosphate bonds. Many enzymes require high-energy phosphate, such as adenosine triphosphate, for their processes of oxidation and synthesis. The synthesis of the vital ribonucleic acid from ATP is now known to be an enzymatic reaction catalyzed by specific enzymes, some of which require the presence of bivalent metallic ions, especially magnesium [Lehman *et al.* (90)]. The phosphorylation of adenosine monophosphate is one of the chief functions of the mitochondria, and the synthesis of ribose- and diribosenucleic acid is a process on which the whole metabolism of the cell depends. Oxidative phosphorylation is, in fact, the mechanism by which the esterification of inorganic phosphate provides the major part of ATP, regarded as the primary source of energy for cellular function. The statement by Kalckar (74) in 1941 that "the classification of the coupling between triosephosphate and the uptake of inorganic phosphate represents one of the greatest advances in modern biology" still remains undisputed.

In addition to the nucleotides, linked together by organic phosphorus bonds, there are also coenzymes, so called because until recently they were believed to be tightly bound to an enzyme and inseparable from it by processes of enzyme purification. It is now known that they can function as catalysts and also, more frequently, as substrates in metabolic reactions, being transformed and then regenerated through a complex cyclic reaction [Strominger (145)].

Electron microscopy demonstration of certain enzymes bound to the mitochondria has succeeded in explaining to some extent how the very complex structure of the mitochondrion can integrate the numerous enzymatic reactions required of this extremely minute body, and how the enzymes exist as multienzyme systems, chains acting together as a team in the cycle of metabolic activity. But the extreme complexity of interrelationships between nuclear fractions, supernatant fluid, mitochondrial fractions, and microsomes is still not completely solved. Nevertheless, the investigations already carried out have undoubtedly shed much light on the mechanisms by which toxic foreign substances, during their passage through the body, can exert their toxic action by producing metabolites injurious to mitochondria or by inhibiting the enzymatic reactions which can produce nontoxic metabolites.

A special mechanism for dealing with exogenous toxins appears to exist in the liver, whose cells are rich in mitochondria. These mitochondria contain a number of enzymes specifically able to deal with foreign substances. Some observers have noted congestion of the liver in animals following exposure to solvents with no known specific toxic effect on the liver and have regarded this as a manifestation of its detoxicating capacity.

The reactions of foreign compounds in their progress through the body have been described as occurring in two stages: first, the toxic including oxidation, reduction, hydrolysis, or a combination of these; and, second, the nontoxic during which conjugation takes place, including the synthesis of substances such as ethereal sulphates, glucuronides, or hippuric acid. These can be excreted without causing toxic effects. If conjugation is adequate and sufficiently rapid, the toxic substance will be completely "detoxicated"; if it is slower than that of the oxidation phase, the toxic metabolites will be left free to exert their harmful action on the living cell and especially on the enzymes contained in it [Williams (157)]. The appearance of symptoms of intoxication depends largly upon the degree of detoxication; if this fails completely and the toxic phase exerts its full effect, the whole metabolic and enzymatic activity of the cell will be disturbed, resulting possibly in death of the whole organism.

This brief review of some of the factors involved in the potential toxicity of certain toxic organic compounds helps to explain the differences in intensity and character caused by members of different, or even of the same, chemical groups. Depending on the amount of retention in the tissues, on the production of metabolites of less or greater toxicity than the original substance, and on their attack on different types of cell and different enzymes, the symptoms produced will differ in kind, in intensity, and in time of appearance.

The Aromatic Hydrocarbons

Among the aromatic hydrocarbons there is an outstanding difference in the nature of the toxic effects of benzene, toluene, and xylene, all members of the same chemical group. With high short-term exposures all three are narcotic; benzene the least so. With chronic exposure, benzene is the only member with a specific effect on hemopoietic tissue. Neither toluene nor xylene, when pure, produces the syndrome of anemia, leucopenia, and thrombocytopenia characteristic of early benzene absorption, or a final aplastic anemia. When such a benzene-like blood picture does occur during chronic exposure to toluene or xylene, it has always been, in the author's experience, associated with the use of a commercial variety of these solvents which has contained from 7 to 15 per cent of benzene (146).

The reason for this specific hemopoietic effect of benzene lies in its metabolic transformation in the body. During the phase of oxidation, initiated by various enzymes including peroxidase, catalase, and indophenoloxidase [Gabor (47)], certain phenol metabolites—phenol, quinol, pyrocatechol, and hydroxyquinol—are produced, together with the minor metabolites, muconic and mercapturic acid [Williams (157)]. These, in conditions of complete detoxication, would become conjugated with sulphuric or glucuronic acid and appear in the urine as ethereal sulphates. In fact, the measure of the change in proportion of inorganic to organic urinary sulphate can be taken as a measure of the absorption of benzene, though not of its

toxic action. It is when the process of conjugation is inadequate or delayed that some of the phenol metabolites are left free to exert their toxic action. They are mitotic poisons and have a special predilection for cells such as those of the bone marrow that are actively proliferating.

A corollary to the hypothesis that disturbance of enzymes controlling the oxidation of benezene is its fundamental toxic effect has been described by Gabor in 1959 with an attempt to use the alteration in the blood level of certain enzymes as an early indication of chronic benzene poisoning. The enzymes investigated were peroxidase and catalase, which were present in the blood of rats exposed to inhalation of varying concentrations of benzene for three hours a day on alternate days over a period of 165 days. For the first 130 days the concentration was 0.1 mg. per liter, for the next 28 days 1.1 mg. per liter, and for the last seven days 2.5 mg. per liter. The peroxidase level increased during the first 42 days to a concentration 30 per cent greater than the original and maintained this level up to 126 days, then abruptly decreased to a level of 65 per cent below the original. In the same conditions, the catalase showed a decrease from the beginning, though with periods of recuperation when it reached normal levels temporarily, only to fall again, reaching a final level 50 per cent below the original. The temporary rises were regarded as evidence of a compensatory reaction, possibly a "detoxicating" effort of the liver in the production of more catalase. The indophenol oxidase showed much less disturbance, decreasing slightly in comparison with nonexposed animals only towards the end of the experiment. Blood samples taken at intervals during the period of exposure showed oscillations of the white and red corpuscles above and below the normal, with no notable change in the hemoglobin level.

These results, particularly the rise in activity of peroxidase at the same time as a fall in catalase, followed by a simultaneous fall in both when the concentration of benezene was increased, are interpreted as indications that the first reaction represents a functional disturbance, possibly the result of a stimulative action by the free phenol metabolites on the production of peroxidase; the second reaction may represent the exhaustion of the possibility of detoxication with the appearance of morphological changes. The indophenol oxidase, in which the alteration is slower and less marked than in the other two enzymes, may be regarded as an index of the increase in the blood of pyruvic acid resulting from disturbance of oxidation-reduction processes. This acid has been stated to have an inhibitory effect on indophenol oxidase [Mikhlin (104)]. The early measurement of enzymatic activity may, therefore, be a means of estimating injury from exposure to benzene before the classical signs of poisoning appear.

Toluene and xylene.—The failure of toluene and xylene to carry out the typical benzene attack on the bone marrow is attributable to the fact that their metabolites are not the toxic phenols [Porteous & Williams (118)]. Toluene is oxidized chiefly by way of its methyl group which is replaced by a —COOH group giving benzoic acid. This is conjugated with glycollic

or aminoacetic acid to form hippuric acid. (This conjugation is normally carried out in herbivorous animals as a blockage of COOH groups and is catalyzed by coenzyme A [Adler-Herzmark (2); Chantrenne (28)]. In carnivorous animals benzoic acid undergoes a different form of conjugation with glucuronic acid, giving benzoyl-glucuronic acid.) In man, it has been estimated that although an average amount of 53.3 per cent of toluene inhaled is retained by the body, 80 per cent of this is transformed into benzoic acid and eliminated as hippuric acid within 24 hours, with about 10 to 20 per cent in the form of benzoylglucuronic acid [Srbova & Teisinger (139)]. The amount of hippuric acid in the urine which corresponds to a human exposure of 200 p.p.m. has been estimated as 3 gm. per liter [Elkins (42)], and that of benzoic acid as 2.1 gm. [Teisinger & Srbova (148)].

Though benzoic acid has not definitely been proved to be toxic per se, it may be pointed out that this amount involves, during its conjugation with glycocoll to form hippuric acid, a considerable depletion of an essential amino acid. Some observers have reported enlargement of the liver at this level of exposure, but, as already mentioned, signs of congestion of the liver following exposure to solvents with no known hepatoxic action have been recognized as evidence of its performance as a detoxicating agent.

Undoubtedly, toluene, in addition to its acute narcotic effect, does cause symptoms indicating systemic disturbance of the central nervous system—fatigue, nausea, drowsiness, paraesthesia, in-co-ordination, and insomnia—but practically all observers are agreed that when pure, it does not produce the hemopoietic syndrome characterisitc of benzene, either in animals or human beings [Gerarde (50); Browning (unpublished observations)].

Xylene of the commercial variety is a mixture of the ortho-, para-, and meta-isomers (88), the *m*-isomer usually contributing 75 to 80 per cent, and, as already mentioned, the commercial xylene may contain considerable amounts of benzene. Although chronic exposure to xylene may produce some gastrointestinal disturbance, especially nausea, the pure compound does not cause the hemopoietic effect characteristic of benzene absorption.

The chief metabolite of *o*-xylene in animals is *o*-toluic acid, excreted partly as such and partly as the corresponding glucuronide which is assumed [Bray *et al.* (18, 19)] to be *o*-toluyl glucuronide. Only a very small proportion (0.3 per cent) of the *o*-toluic acid is excreted as the glycine conjugate, *o*-toluric acid. *o*-Xylene is also hydroxylated to some extent, and the metabolites so formed may be both xylenols and hydroxytoluic acids. Bray *et al.* (18, 19) have suggested that 15 to 20 per cent of the dose may be excreted as ether glucuronides, which might be derivatives of these metabolites, since they found in the urine or rabbits given *o*-xylene, 6 per cent of the dose in the form of ethereal sulphates.

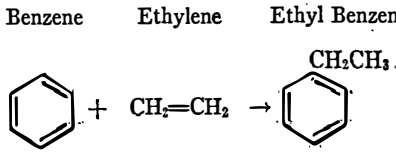
The *m*- and *p*-xylenes are more highly oxidized (80 to 90 per cent) to the corresponding toluic acids than is the *o*-isomer, and these are excreted almost entirely as glycine conjugates with practically no ester glucuronide formation. There is also a small amount of hydroxylation to xylenols; that

corresponding to *p*-xylene has been identified as 2,5-dimethylphenol.

Alkyl benzenes.—The alkyl benzenes most commonly used in industry—ethyl, propyl, butyl, triethyl, and trimethyl benzene (mesitylene)—are also devoid of hemopoietic effect. They are used as solvents for paints, dyes, inks, and lacquers, as pesticides, in protective coatings and resins, as well as constituents of automobile fuels, and as starting materials in the chemical industry.

All alkyl benzenes are irritants to skin and mucous membranes and cause increased dilatation and permeability of the capillaries that, in some cases, lead to hemorrhage into the surrounding tissues. These changes have been observed chiefly in the lungs of animals, where the most consistent post-mortem finding is a chemical pneumonitis with pulmonary edema and hemorrhage [Gerarde (50)]. They have also caused symptoms indicating depression of the central nervous system, in contrast to the initial excitatory and convulsant action of acute benzene poisoning. This depressant action is reversible when the hydrocarbons are eliminated, except in the case of one compound, *p-tert.* butyl toluene. This is a highly neuro-toxic compound causing paralysis in animals; its action is believed to be due to secondary hemorrhage in the grey matter of the cervical and thoracic spinal cord [Hine, Unger & Anderson (70)].

None of the alkyl benzenes, if pure, have a myelotoxic effect. It appears that any change in the benzene ring, whether hydrogenation, alkylation, or sulphonation, destroys the specific myelotoxicity of the benzene molecule [Scott, Cartwright & Wintrobe (133)]. Alkylation of benzene in this group is achieved by the addition of an alkyl group during the process of obtaining them from petroleum with the use of a catalyst, e.g.,



Gerarde has indeed urged that the name "alkyl benzenes," with its implied similarity to benzene, should be changed to "phenyl alkanes," which toxicologically they are.

When inhaled, these compounds are for the greater part eliminated unchanged by the lungs, and the small amount metabolized has a common pathway. The first phase of oxidation takes place on the carbon molecules of the side chains, forming alcohols or carboxylic acids. These are conjugated with glycine or glucuronic acid and excreted in the urine. These transformations are probably effected chiefly by the microsomes of the liver, though other tissues, such as the brain, spinal cord, bone marrow, kidney, and adrenal, may play a part. They differ from benzene not only in their innocuousness to the blood-forming organs with chronic exposure and their depressant rather than convulsive acute toxicity, but also in their narcotic

potency within their own group. This decreases with their chain length, falling off at four carbon atoms and diminishing steadily thereafter. The speed of onset of depression is related to the rate of absorption and transfer to the brain. Absorption depends on the water solubility, which in its turn diminishes rapidly with chain length, and multiplicity of alkylation. Thus, toluene and ethyl benzene are fast acting; *n*-propyl and *n*-butyl benzene are slow acting. The duration of the central nervous depression increases with length and branching of the side chain; this is probably related to the rate of removal of the hydrocarbon from the cells in which it is temporarily retained. Oxidation is slower with the branched side chain than the straight chain with the same number of carbon atoms. Thus, cumene and *n*-butyl benzene have a more prolonged narcotic effect than toluene and ethyl benzene. Gerarde believes that the narcotic effect of the alkyl benzenes is not structurally specific like those of the alcohols, esters, ketones, and ethers, but physical, the quality and intensity depending on the number of molecules present in the cell at the particular moment, rather than on their type. This would explain the rapid reversibility of the narcosis unless the dose is so heavy as to cause permanent injury to the brain from lack of oxygenation during prolonged unconsciousness.

The metabolism of the alkyl benzenes in general is that of hydroxylation to form carbinols which may then be conjugated with glucuronic acid and excreted as such, or further oxidized to yield benzoic or phenyl acetic acid, or of ring oxidation to phenyl fatty acids which are then further oxidized to these substances. These processes differ with the different compounds. Styrene, for example, which is more toxic than ethyl benzene, differs from it in not being converted to phenaceturic acid, though both give rise to phenyl carbonyls which are the precursors of hippuric and mandelic acids. *p*-Cymene (*p*-isopropyl toluene) apparently undergoes no hydroxylation of the ring, but oxidation only of its methyl group, giving cumic acid (*p*-isopropyl benzoic acid), and its glycine conjugate, while cumene (isopropyl benzene), is mainly converted to carbinols which are excreted as conjugated glucuronic acid, as also is *tert.*-butyl benzene.

Trimethylbenzene.—Trimethylbenzene has two isomers, pseudocumene (1-2-4-trimethylbenzene) and mesitylene (1-3-5-trimethylbenzene), of which the latter is the more widely used in industry as a thinner known as "Fleet X" which contains more than 80 per cent of it. Workers using this thinner at concentrations of 10 to 60 p.p.m. have shown some central nervous disturbance in the form of lassitude, giddiness, headache, and drowsiness [Bättig *et al.* (10)]. Animals exposed to chronic inhalation also showed some ataxia with narcosis which was reversible a few hours after cessation of exposure, but no significant variation in the blood picture. The chief metabolic product of mesitylene is dimethyl benzoic acid, which is excreted partly as the glycine conjugate, but Bättig & Grandjean found that prolonged exposure was also followed by an increased excretion of phenols, both free and bound, in the urine. Because of its resemblance to benzene in

this respect (although some blood changes found in these workers were attributed to dietary inadequacy, especially of ascorbic acid, and not to any specific hemopoietic effect of this compound), it is suggested that mesitylene cannot be regarded as entirely innocuous.

The Chlorobenzenes

Some of the chlorinated benzenes, such as *p*-dichlorobenzene and the tri-tetra- and hexa- compounds, are solids, but monochlorobenzene and *o*-dichlorobenzene are liquids and are used as industrial solvents (the former for ethyl cellulose, oils, and fats; the latter for resins and lacquers and in paint and varnish removers).

The toxicity of the chlorobenzenes is that of a central nervous system poison, and the degree of acute toxicity increases with the substitution of the chlorine atom in the benzene ring up to two and, thereafter, decreases. Thus, the maximum allowable concentration for monochlorobenzene is 75 p.p.m., and for *o*-dichlorobenzene 50 p.p.m. Above the di-compounds, the more chlorine the halogenated benzene compounds contain, the less readily are they metabolized. Thus, trichloro- and pentachlorobenzene are only slightly altered in the body, and hexachlorobenzene, used as a seed fungicide, appears to be metabolically inert [Parke & Williams (115)]. None of these three compounds form conjugated glucuronic acids, ethereal sulphates, or mercapturic acid.

Monochlorobenzene.—Although there have been no reports of monochlorobenzene causing any cases of industrial poisoning, its central nervous toxicity has been deduced from fatal cases following ingestion when it has been found to cause cyanosis, loss of reflexes, twitching of the facial muscles, and unconsciousness [Reich (123)]. When administered to animals, about 30 per cent is eliminated by the breath, but some is oxidized to phenols, especially chlorocatechol, which are excreted chiefly in conjugation with glucuronic and sulphuric acids, and also as mercapturic acid. It is in this quantitative excretion of mercapturic acid that there lies the main difference between the metabolism of monochlorobenzene and that of benzene. Whereas monochlorobenzene produces 20 per cent of mercapturic acid, benzene produces not more than 1 per cent of the administered dose [Azouz, Parke & Williams (6)].

o-Dichlorobenzene.—Used as a fumigant and insecticide as well as a solvent, *o*-dichlorobenzene has caused no industrial symptoms other than dermatitis [Downing (37)], but in animals it has produced renal damage and liver necrosis [Cameron *et al.* (25)]. Its metabolism is qualitatively similar to that of monochlorobenzene, in that it gives rise to dichlorocatechols and phenols which are excreted after conjugation with glucuronic and sulphuric acids, but it differs quantitatively in that catechol and mercapturic acid are formed in much smaller amounts and that the excretion of these metabolites is much slower [Bray *et al.* (17); Parke & Williams (114)].

Benzyl chloride.—Also known as α-chlorotoluene, benzyl chloride is

used extensively in the manufacture of pigments, resins, and perfumes, and in intermediates in the preparation of acid dyes. Its main toxic effect is a severe irritation of mucous membranes, especially of the eyes. It has, in fact, been classified as a powerful lachrymator, and this property may account for the relative lack of reports of systemic toxic effects. It is so unpleasant that 1 p.p.m. has been suggested as the maximum allowable concentration, and it therefore provides its own warning.

Its metabolic behavior is interesting in that it differs in one respect from that of the halogenated benzene compounds described above. Whereas these eventually produce mercapturic acid through intermediate precursor metabolites (sometimes known as premercapturic acids), benzyl chloride is converted directly to this substance, and at least 10 per cent of the dose can be eliminated in the urine as benzyl mercapturic acid [Knight & Young (78)].

The Halogenated Hydrocarbons

Some of the halogenated hydrocarbons are so volatile that, though their metabolites are appreciably toxic, so much is excreted by the lungs that with some compounds they are relatively small in amount. How far the toxicity of those which attack primarily the liver and kidneys is attributable to inhibition or destruction of enzymes or to actual injury of the mitochondria is not completely clear (20).

Tetrachloroethane.—The most toxic of them all, tetrachloroethane, is no longer of wide use in industry since the prohibition of its use as a "dope" for airplane fabrics during World War I. By 1916, 70 cases of jaundice with 12 deaths had occurred as the result of inhalation of its vapor [Wilcox (156)]. It still has, however, a variety of applications as a celluloid solvent in the film industry, in the impregnation of furs and skins, and, especially in Germany and France, as a solvent for a mixture of acetyl cellulose and "essence d'orient" in the manufacture of artificial pearls. In this industry a fatal case of acute yellow atrophy of the liver occurred in 1930 [Boidin, Rouques & Albot (16)]. The liver lesion is that of severe fatty degeneration of the central zones, progressing to necrosis which is frequently fatal. Tetrachloroethane intoxication may also produce a polyneuritic syndrome, which has occurred especially in the artificial pearl industry [Feil & Heim de Balzac (45); Léri & Breitel (92)].

Only 19.6 per cent of the dose inhaled by animals is excreted by the lungs; the amount retained is excreted slowly, partly in the urine, and the liver retains traces 24 hours after the last exposure [Gasq (48)]. Its actual metabolites have not been isolated, but it has been suggested that one of them may be oxalic acid and that this may be the determining toxic factor. Increased amounts of oxalic acid were found in a sample of urine of a person poisoned by tetrachloroethane [Lilliman (93)]. It has also been suggested [Williams (157)], that if it were metabolized by dehydrochlorination consisting of the loss of hydrogen chloride (a reaction which occurs in insects treated with the chlorinated hydrocarbon insecticide, DDT), it

might yield trichloroethylene and eventually trichloracetic acid, but this substance has not yet been proved to be a metabolite of tetrachloroethane [Barret, Cunningham & Johnston (7)].

Carbon tetrachloride.—Carbon tetrachloride is very widely used as a fire extinguisher, a solvent for rubber, a grease remover, and a dry-cleaning agent. Its toxic effects have been reported mainly from the point of acute toxicity when severe damage to the liver and kidneys has been observed (140).

The liver lesion following a large dose is a centrilobular necrosis. If it is not too massive, repair may begin within three or four days and may be complete in two or three weeks (38). With chronic exposure the symptoms are chiefly gastrointestinal, with nausea and vomiting, and also nervous, with headache, drowsiness, and excessive fatigue. Jaundice is rarely present.

There is some disagreement as to the actual mechanism by which carbon tetrachloride produces its hepatotoxic effect (136). Examination of the livers of rats to which it has been administered by stomach tube has shown the presence of hydropic globules in the cells. These globules contained succinoxidase and ribonucleic acid, suggesting that they were derived from mitochondria [Christie & Judah (29)], and many authorities consider that the primary biochemical lesion is the result of a direct structural attack on the mitochondria themselves. This disrupts their function, depriving them of their ability to retain the small amount of coenzyme which they possess and, thus, disorganizing their whole enzyme action.

The measurement of oxidative phosphorylation is a sensitive indicator of mitochondrial damage, and it has been found that this is inhibited *in vivo* in the liver of rats treated with carbon tetrachloride. It was also observed [Calvert & Brody (23)] that there was a definite time factor in the biochemical changes caused by carbon tetrachloride. The morphological changes in the liver occurred before the oxidative changes could be detected. It is interesting to note that oxidative phosphorylation survived a long time and that in a recent investigation by Alexander & MacDonald (3) in sheep, there was no significant change in the level of plasma alkaline phosphatase. This may lend support to the findings of Steward & Witts (143) in 1944 that the early changes in the liver are reversible. Indeed, more recent researches by Oberling (109) have shown that there is intense mitochondrial regeneration after carbon tetrachloride intoxication. On the other hand, a somewhat ominous suggestion has emerged from the investigations of Andervont (5) in his demonstration that carbon tetrachloride has a carcinogenic action on the liver of the mouse.

Another possibility of measuring slight changes in liver cells, not demonstrable by clinical examination or by the usual tests for liver function, is that of estimation of the level of serum glutamic oxalacetic transaminase (SGOT). Transaminases are widely present in animal tissues and in blood; they catalyze an amino-acid reaction, and when cells rich in these enzymes are injured, increased quantities of transaminase are released into the blood

stream. This is especially the case with injury by hepatotoxic agents such as carbon tetrachloride, and the increase of SGOT is regarded as a highly sensitive indication of its injury of liver cells. This has been confirmed in both animals [Block & Cornish (15)] and human beings [Wroblenski & La Due (160, 161)]. In men acutely poisoned by carbon tetrachloride the rise of activity of SGOT has been particularly striking. In two such cases examined by Wroblenski & La Due, the rise in SGOT level two days before admission to hospital was up to 27,840 and 12,340 units respectively as compared with the normal range of 8 to 40. In both cases there was a return to a normal level with a week. More recent work [Beaufay *et al.* (11)] on the influence of carbon tetrachloride on the bound enzymes of the liver indicates that the hepatotoxic action may be related to the release of intracellular enzymes segregated in a group of cytoplasmic particles, the lysosomes, distinct from the mitochondria. When the membrane of these particles, which is probably of a lipoprotein nature, is injured, they release a collection of soluble hydrolytic enzymes that may play an important part in the necrosis of liver cells. This extremely careful and complex investigation also revealed a time factor in morphological and biochemical manifestations; in this case the enzyme alterations were present as much as several hours before detectable microscopical liver lesions.

Trichloroethylene.—The acute toxic effect of trichloroethylene, as manifested by its use as a surgical anesthetic, is that of a powerful narcotic which when inhaled in high concentrations rapidly produces complete unconsciousness. With chronic exposure there is also some evidence of its predominant effect on the nervous system; it has been reported to cause vague nervous symptoms—headache, drowsiness, giddiness, fatigue, insomnia, and intolerance to alcohol (110). Whether it has a chronic toxic effect on the internal organs, especially the liver as in the case of carbon tetrachloride, has been much debated. On the whole, the consensus of opinion is that it has no such hepatotoxic action, but there has recently been one report from Italy [Capellini & Grisler (26)] suggesting that it may have caused slight functional disturbance of the liver. Among 12 women chronically exposed, some showed, in addition to dyspeptic symptoms, slight enlargement of the liver and an increased level of bilirubin in the blood. Estimations of the serum transaminase level, already described as an indication of liver injury, have, however, shown no increase such as that observed in carbon tetrachloride intoxication, and Grandjean (59) is of the opinion that it occurs only in persons with a predisposition to liver disorder.

Trichloroethylene has quite a remarkable metabolic performance. It forms two main metabolites, trichloracetic acid (TCA) and trichloroethanol (TCE), and a number of others which include urochloralic acid, monochloracetic acid, chloroform, and chloral [Marshall & Owens (101)]. Both TCA and the TCE fractions are excreted slowly and are demonstrable in the blood 15 days after exposure. The excretion of the other metabolites is also slow; they can be found in the urine two weeks after a single exposure,

and even longer with chronic exposure. It is suggested that this is caused by slow release of trichloroethylene retained in the tissues, where it may be bound to protein [Bartoniček & Souček (9)]. Acording to Rubino *et al.* (127), the chloral hydrate is the first to appear in the blood stream; the free TCE shows an initial decrease because of its glucuronic conjugation, but both fractions are recoverable from the blood for at least 15 days. TCA is the last metabolite to appear, its maximum concentration being present about 50 hours after inhalation and remaining for 15 days. The ratio of urinary elimination of TCA to TCE varies in man from 1: 1.5 to 1: 2, and the ratio of urinary TCA to atmospheric trichloroethylene is said to be from 6: 1 in young subjects to 2: 1 in older people.

A statistical analysis by Grandjean *et al.* (60) in 1955 indicated that subjective, vegetative, and neurological disturbances were more common in workers excreting an average amount of 67 to 180 mg. per liter of TCA than in those with an average excretion of 20 mg. per liter. Following the statement by Barrett & Johnston (8) in 1939 that the amount of TCA in the urine can be correlated with the amount of trichloroethylene inhaled, it was at one time thought that the best estimation of trichloroethylene exposure might be that of estimation of urinary TCA. More recent investigations of the relation between the urinary metabolities of trichloroethylene in workers in a dry-cleaning establishment in Italy [Rubino *et al.* (127)] have suggested that though urinary TCA is a good criterion of its own level in the blood, it is not strictly correlated with the amount of trichloroethylene itself, either in the blood or in the environment, and that TCE is a preferable criterion. TCE is present in the urine, both free and conjugated, in much larger amount than TCA. In both massive and chronic intoxication by trichloroethylene, 68 per cent of the metabolic excretion was represented by TCE as against 32 per cent by TCA, and there was a much closer correlation between the TCE in blood and urine with the level of trichloroethylene in the atmosphere and the blood. Also, there was a wide individual variation in the excretion of TCA, and it was considered a not wholly reliable guide to the amount inhaled [Butler (22)].

Dichloroethane.—Dichloroethane has two isomers, 1-1-, or ethylidene chloride, and 1-2- (symmetrical), or ethylene dichloride. The latter is the more important industrially, being a low-priced, efficient solvent for resins, rubber, cellulose acetate, and lacquers. It is also used in the production of ethylene glycol, as a degreaser for metal and wool, as an extraction solvent for soya bean and caffeine, and as an insecticide and fungicide. From the point of view of acute poisoning, it is a powerful narcotic and was at one time recommended as an anesthetic. Acute toxicity from its industrial use has not been frequently recorded considering its wide application, but four cases occurred in 1957 [Menschick (102)] in men painting the inside of a container. One man became unconscious 10 minutes after an attack of vomiting and on recovery of consciousness had muscle spasms and severe gastrointestinal disturbance; a second showed similar symptoms but with-

out loss of consciousness; the remaining two had a latent period of about one hour before developing gastric symptoms, giddiness, and weak heart action. Both these men showed some disturbance of liver function as evidenced by increased blood bilirubin and a positive Taka Ara reaction. Dichloroethane is generally regarded as having a much less toxic action on the liver than carbon tetrachloride, chloroform, and tetrachloroethane, but that it can affect the liver and also the kidneys is evident from fatal cases following accidental ingestion. Two such cases following ingestion of an antirheumatic external remedy containing sym. dichloroethane were reported by Weiss (153), in which both liver and kidneys showed parenchymatous lesions; two more [Morozov (105)], one fatal, occurred from swallowing several mouthfuls of a fluid which was later identified as dichloroethane, though no distinction between the two isotopes was made. In the fatal case there was toxic hepatitis and nephrosis.

In animals, undoubtedly, the liver damage caused by acute exposure to dichloroethane is less than with carbon tetrachloride [Heppel *et al.* (68)], but many develop necrosis and hemorrhage of the adrenal cortex and a temporary corneal opacity caused by infiltration of the cornea with lymphocytes and connective tissue cells. It is interesting to note that this effect was also observed in human beings as early as 1887 when its use as an anesthetic was suggested [Dubois & Roux (39)]. It is the only saturated chlorine compound of the ethane series which has this action [Hueper & Smith (69)].

Its metabolism has not been thoroughly elucidated. In animals it is mainly exhaled unchanged, and no metabolite has been definitely isolated. It has been suggested, however, that like other halogenated hydrocarbons it is dehalogenated *in vivo* by a hydrolytic enzyme occurring in the kidney, liver, and spleen, with formation of an oxidized hydrocarbon and a halogen ion. Other views are that oxalic acid may be one of its metabolites and that this may account for the difference in toxicity of the two isomers, the 1-1- form, yielding acetic acid, being allegedly less toxic than the 1-2- [Heppel (68)].

Trichloroethane.—Also known as methyl chloroform, trichloroethane has two isomers, 1-1-1- and 1-1-2-; according to Elkins (43), the latter is the more toxic. It has solvent properties similar to those of dichloroethane and is used for many similar purposes. Both isomers are narcotic. The 1-1-1- isomer was studied in detail by Adams *et al.* (1) in 1950; they found the principal toxic effect of single exposures in rats to be a depression of the central nervous system typical of an anesthetic agent. In such anesthetic dosage there was some relatively slight injury of the liver—fatty change and necrosis—and, as with carbon tetrachloride, the central portions of the lobules were more severely affected than the peripheral. With repeated dosage the liver damage was slight, and it appeared that animals could tolerate much greater exposure than to carbon tetrachloride without injury. As in the case of dichloroethane, the metabolism of trichloroethane has not been completely explored, but it is believed to be similar in its mechanism.

Perchloroethylene.—Perchloroethylene, or tetrachloroethylene, has sol-

vent properties similar to those of trichloroethylene and is used for similar purposes. Symptoms resulting from chronic exposure are also similar but have been less widely reported, probably owing to its lower volatility. It is eliminated chiefly unchanged through the lungs, but some metabolite is excreted in the urine. In 1939 Barrett & Johnston (8) stated that its composition was unknown, but according to Grandjean (59), it is trichloracetic acid, excreted slowly to the extent of about 20 per cent.

The blood alcohol content of animals and humans, even with inhalation of concentrations high enough to produce unconsciousness, is not raised [Paulus (117); Schleyer (129)].

The Alcohols

The primary aliphatic alcohols.—Methyl, ethyl, propyl, butyl, and amyl are the most widely used alcohols in industry. They all have irritant and narcotic properties, but, except for methyl alcohol which has a specific toxicity for the optic nerve and butyl alcohol which can cause an unusual form of keratitis, their systemic toxicity is not high.

Their narcotic potency when given by mouth increases with their molecular weight (111). It can also be correlated to some extent with their conjugation with glucuronic acid, the second of two reactions that the primary alcohols undergo. The first is the formation of an intermediate aldehyde followed by the production of carboxylic acid, which may be either completely oxidized to carbon dioxide, or excreted as such, or combined with glucuronic acid. There is little conjugation with the primary alcohols, not more than about 10 per cent of the dose, and depending on the nature and length of the carbon chain, with a peak of conjugation at about six carbon atoms. The sensitivity of rat-brain cortex respiration when it has been stimulated by potassium chloride also increases with the length of the carbon chain.

Beer & Quastel (12) have shown that *n*-pentanol has a much greater inhibitive potency than *n*-butanol, which in its turn is more potent than *n*-propanol and ethanol. The fact that these alcohols have little or no inhibitive effect on brain respiration unless it has been stimulated by potassium indicates that potassium is concerned with the activation of the citric-acid cycle of enzymes, and it is suggested that the site of action of the alcohols is the cell membrane rather than the mitochondria, which are respiring in media containing a high potassium ion concentration.

The enzyme concerned in the first step in the reaction is believed to be alcohol dehydrogenase, which catalyzes the transference of hydrogen from alcohol to diphosphorydine nucleotide (DPN) with the formation of acetaldehyde and reduced DPN. The results of Beer & Quastel (12) in their investigation of the effects of acetaldehyde on brain mitochondrial respiration have indicated that DPN has an alleviating action on the inhibitory effect of acetaldehyde and accelerates the removal of this from the system, either by oxidation or dismutation. Acetaldehyde is, for equivalent concentrations,

about 200 times more effective than ethanol in bringing about a suppression of potassium-stimulated brain respiration. There is still some controversy as to whether alcohol dehydrogenase is the enzyme of primary importance in the complete oxidation of ethanol; catalase has been suggested as a secondary mechanism [Gillespie & Lucas (53)]. Beer & Quastel state that their results do not support the view that ethanol exercises its inhibitive effect on brain respiration by preliminary conversion in the brain tissue to acetaldehyde, or that an active alcohol dehydrogenase exists in the brain.

Correlation of the toxic action of the primary alcohols with their metabolic fate is best exemplified in the difference between ethyl and methyl alcohol. Ethyl alcohol is metabolized chiefly in the liver; other organs play a subordinate part. As already described, its oxidation takes place in steps, acetaldehyde being an intermediate metabolite. This is rapidly oxidized in the organism to acetic acid (14), and in normal conditions is further oxidized to carbon dioxide and water. This reaction is not specific for ethyl

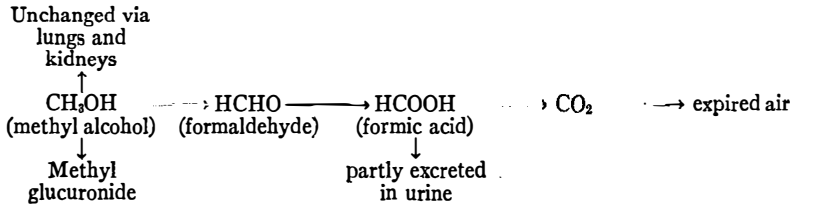


FIG. 1. Metabolism of methyl alcohol[3]

alcohol but occurs also with propyl, butyl, and amyl alcohol. Methyl alcohol, on the other hand, is oxidized in its first step to formaldehyde and then to formic acid (Fig. 1). This is believed to be effected more probably by the catalase than the dehydrogenase mechanism, and four to five times more slowly than ethanol [Keeser & Vincke (75)]. The characteristic toxic action of methanol is, therefore, believed to be attributable to the high toxicity of these metabolites and to the slowness of their elimination. Formaldehyde has been detected in the aqueous and vitreous humor of the eyes of rabbits poisoned with methanol, and formaldehyde is extremely active in inhibiting oxygen uptake and carbon dioxide production [Leaf & Zatman (89); Potts & Johnson (119)].

The site of this inhibition of retinal glycolysis is, possibly, the disturbance of phosphorylation. It appears that the primary retinal changes are followed by lesions of the optic nerve, ultimately developing into optic atrophy. In nearly all cases such optic atrophy has followed ingestion of methyl alcohol, but some have been reported following inhalation of wood alcohol [Robinson (125)]. Few investigations of the effects of chronic industrial exposure to the vapor of methyl alcohol have been made. One of these by Leaf & Zatman (89) was carried out in a factory where the air

[3] After Williams (157).

concentrations in a methanol synthesis, distillation, and stripping plant were analyzed. In the synthesis plant they were very low, less than 5 p.p.m. In the distillation plant in the late afternoon the level reached 64 p.p.m., and in the stripping plant in the early afternoon a maximum of 116 p.p.m.

The question whether ethyl alcohol, when given at the same time as methyl alcohol, can reduce the toxicity of the latter has been much discussed [Zatman (162)]. It is believed that ethanol may reduce the formation of formate by inhibiting the oxidation of methanol so that more is eliminated unchanged in the expired air and in the urine [Røe (126)]. This increased elimination was actually observed by Leaf & Zatman in their factory investigation, and they emphasize their belief that the enzymic removal of methanol is attributable to the competitive inhibitory effect of ethanol on the process of oxidation.

Among the other primary alcohols, *n*-propyl and *n*-butyl are the most predominant in industrial use, as solvents for gums, resins, oils, and cellulose ethers and esters. *n*-Butanol is also used extensively in the lacquer and dye industries. From the narcotic point of view, *n*-propyl alcohol is twice as potent as ethyl alcohol, and *n*-butyl even more so, but the only toxic effect reported from their industrial use is that associated with exposure to *n*-butyl alcohol. This is the development of an unusual form of keratitis described by Cogan & Grant (32) in 1945. It occurred in workers employed in applying a synthetic resin dissolved in *n*-butanol to Army greatcoats. They were exposed to a concentration varying from 15 to 200 p.p.m., and their symptoms consisted of irritation of the eyes with some blurring of vision. The lesions seen under transillumination consisted of clear, translucent vacuoles in the superficial layers of the cornea, especially numerous on the central portion where in the most severe cases they numbered up to 1000. They improved, and in some cases completely resolved, within 10 days of absence from work, but recurred a few days after further exposure.

The metabolism of *n*-propanol is characterized by its oxidation at a constant rate to propionic acid, which is then presumably further oxidized to carbon dioxide and water. Very little is conjugated, only large doses producing about 1 per cent of conjugated glucuronic acid. *n*-Butanol is also rapidly oxidized, about 2 per cent of the dose being conjugated.

Secondary alcohols.—Of the secondary alcohols, the most important industrially is iso-propyl alcohol; it has a wide use as a solvent in many industries. It is nearly twice as narcotic to animals as ethyl alcohol, but causes only a moderate fatty infiltration of the liver and kidneys, and no such visual disturbance as methyl alcohol. No ill-effects have been reported from its industrial use. Its greater narcotic potency in relation to ethyl alcohol is ascribed partly to its slower rate of metabolism, and partly to the fact that while it is conjugated (to a smaller extent than the other secondary alcohols) with glucuronic acid, it is oxidized in the body chiefly to acetone, the most likely catalyst being alcohol dehydrogenase. Williams (157) remarks that since acetone is slowly converted to acetate, formate, and carbon dioxide,

it seems reasonable to conclude that iso-propanol may form these compounds.

Used only to a small extent in the lacquer industry, iso-butyl alcohol is not of great industrial importance. Like the other alcohols it is narcotic to animals (to about the same extent as *n*-butyl alcohol), but has not been recorded as causing any eye injuries in man. It is metabolized by oxidation to ethyl methyl ketone, and conjugated to *sec.*-butyl glucuronide.

With the substitution of a chlorine atom, the toxicity and narcotic activity of the alcohols increases, and, again, there is more conjugation when two or more chlorine atoms are present. Thus, whereas ethanol is largely oxidized to acetic acid, trichloroethanol, a much more active narcotic, is converted in the body, partly, to the trichloroethyl glucuronide and partly to trichloroacetic acid. Both di- and trichloroethanol produce relatively nontoxic glucuronides, in contrast to the highly toxic monochloroethanol whose toxic properties are ascribed to its formation of chloroacetic acid and its failure to conjugate to the much less toxic glucuronides [El Masri, Smith & Williams (44)].

Monochloroethanol.—Also known as ethylene chlorohydrin, monochloroethanol is a colorless, mobile liquid used chiefly in the chemical industry in the manufacture of ethylene glycol and also as a solvent. In 1927 Koelsch (80) described two fatal cases resulting from considerable exposure to this compound. One man was using it for cleaning a machine, the other as a solvent for pigments. The symptoms preceding death were nausea, vomiting, headache, and giddiness. It was nearly 20 years later that a series of cases of poisoning, two fatal and nine nonfatal, occurred during the manufacture of ethylene chlorohydrin [Goldblatt & Chiesman (55)]. In one of the fatal cases death occurred 14 hours after exposure to a high concentration of a vapor which included ethylene chlorohydrin. Here, also, the preceding symptoms were those of gastrointestinal and central nervous disturbance. In neither fatal case did autopsy result in a clear definition of the cause of death. Animal experiments, however, showed that lethal concentrations (1120 p.p.m. for rats) caused renal hemorrhage and tubal degeneration, fatty degeneration and necrosis of the liver, and high nonlethal concentrations caused inhibition of cardiac action, respiration, and smooth muscle, and nerve transmission. These toxic effects are ascribed to its metabolic characteristics, especially to the formation of monochloracetic acid which is 20 to 40 times more toxic than acetic acid or the metabolites of di- and trichloroethanol (85).

The Glycols

The glycols and their ethers and esters are used to some extent in industry as solvents for nitrocellulose and cellulose acetate and as vehicles for pharmaceutical preparations and skin lotions.

Ethylene glycol.—Best known as an antifreeze mixture for motor vehicles, ethylene glycol is not sufficiently volatile to be an industrial hazard in

normal conditions, but there has been one report from Italy [Troisi (150)] of chronic poisoning from inhalation of the vapor from a mixture containing 40 per cent of ethylene glycol, 55 per cent boric acid, and 5 per cent ammonia, heated to 105°C. The chief symptoms were recurrent attacks of unconsciousness, and some of the affected women showed nystagmus. These effects were attributed to an action of the substance on the central nervous system.

When taken internally, ethylene glycol is extremely toxic; in fatal cases death has been due to renal failure, with crystals of oxalic acid deposited in the tubules. In fact, in poisoning by any of the glycols or their derivatives the outstanding toxic effect is exerted on the kidneys. Propylene glycol is about half as acutely toxic to animals as ethylene glycol and very much less with repeated administration. Injury of the kidneys, in the form of hemorrhage and casts, has been produced by fatal intravenous dosage, but continued feeding in concentrations of 2.45 to 4.9 per cent of the food did not cause the deposition of oxalic acid stones as with ethylene glycol. For neither propylene nor butylene glycol have there been any reports of ill-effects on human beings.

Diethylene Glycol.—Used in the lacquer industry, and in face creams and as a vehicle for medicinal preparations, diethylene glycol is less toxic than ethylene glycol judging from their respective minimal fatal doses for animals by intravenous injection (ethylene glycol, 8.54 g. per kg., diethylene glycol, 14.8). But, when mixed with food, 5 to 10 per cent has proved fatal to some animals within a few weeks. Some of these animals showed stones of calcium oxalate and phosphate in the bladder. Damage to the kidneys consisted of extensive injury to the tubules, causing obstruction and uremia. The toxicity of diethylene glycol by ingestion for human beings was strikingly illustrated by a series of deaths occurring in the United States in 1937 from the use of an "elixir" of sulphanilamide, with diethylene glycol as the vehicle [Ruprecht & Nelson (128)]. The initial symptoms included nausea, dizziness, pain in the kidney region and abdomen. These were followed in the course of a few days by oliguria and anuria, with a final development of uremic coma. The most characteristic changes, severe "chemical nephrosis," were found in the kidneys [Geiling & Cannon (49)].

Dipropylene glycol.—The higher homologue of diethylene glycol, dipropylene glycol is apparently less toxic but in large doses has produced similar, though less severe, kidney damage in animals by oral and intravenous administration.

The monoalkyl ethers.—The trade names of the monoalkyl ethers are Cellosolve (ethylene glycol monoethyl ether), Methyl Cellosolve (ethylene glycol monomethyl ether), and Butyl Cellosolve (ethylene glycol monobutyl ether). They are good solvents for cellulose esters and ethers, and are widely used in lacquers. Methyl Cellosolve has also been applied to the "fused collar" industry, where the collars are dipped in a solution of Methyl

Cellosolve and finished under heat and pressure. Butyl Cellosolve is used in detergent solutions, and with phosphoric acid in metal cleaners to prepare sheet metal for lacquering and enamelling.

Ethylene glycol diethyl ether (Diethyl Cellosolve) and diethylene glycol monoethyl ether (Carbitol) are other members of this group with smiliar solvent properties and similar potential toxicity, but in varying degree, for the kidneys in animals. Their industrial hazard is not great in normal conditions.

In animals Butyl Cellosolve has proved the most toxic, and Carbitol the least, the butyl compound producing symptoms of kidney inflammation [Werner *et al.* (154)]. Methyl Cellosolve has been reported to have an effect, unusual in this series, on the central nervous system and the blood picture. This "toxic encephalopathy" associated with slight blood changes was observed in the fused collar industry. The symptoms were a change of personality from liveliness and intelligence to dullness and apathy [Greenberg *et al.* (63); Parsons & Parsons (116)]. A case of nervous disturbance, with symptoms of irritability and insomnia, following the use of a paint containing Butyl Cellosolve has recently been brought to the notice of the author (unpublished observation).

The differences in toxicity of these chemically related compounds are probably to be found in differences in their metabolic progress, depending upon the occurrence of primary, secondary, or tertiary alcoholic groups and upon the relationship of these groups to each other. Where primary alcohol groups occur, oxidation might result in the corresponding acids which may exert their toxic action by inhibiting enzyme systems. Some of them undergo conjugation with glucuronic acid, but only if they contain six carbon atoms; even then the conjugation is less than 10 per cent. Ethylene glycol, for example, is oxidized through oxalic and glycolic acid to carbon dioxide and water with practically no conjugation [Gessner, Parke & Williams (51)], whereas propylene glycol, with considerably lower toxicity, is metabolized to the extent of two-thirds of the dose to lactic acid, which is then oxidized to carbon dioxide and water through the tricarboxylic acid cycle.

Carbitol, though potentially a kidney poison to animals, probably owes its relatively low toxicity to the fact that it is excreted as a glucuronide and, also, that its lower vapor pressure limits its toxicity to exposure by inhalation. Undoubtedly, the predilection of those members of the group for the kidney as the site of their toxic action is associated with their metabolic formation of oxalic acid and its tendency to block the tubules of the kidney in their efforts to eliminate it, but it is now believed that other metabolic intermediates, particularly glyoxal, may also be involved [Doerr (36)].

Cyclohexylamine and Dicyclohexylamine

These two liquids are used in the emulsification of soaps, as intermediates in the dying industry, as solvents for dyestuffs, and as vulcanizers in

the rubber industry. Neither has proved toxic to human beings, but in animals both act as neurotoxins, the toxic action being exerted on the motor centers of the spinal column and medulla [Flinn (46)].

The lethal oral dose of cyclohexylamine has not been established; repeated doses of 100 mg. per kg. body weight were not lethal after 82 days, and at autopsy there were no lesions of internal organs. With subcutaneous injection, of 0.5 gm. per kg. convulsions, after a delay of several hours, preceded death, but injections of 0.25 gm. per kg., repeated daily for 10 days, had no ill-effects. Application to the skin resulted in dermatitis but not absorption of lethal amounts.

Dicyclohexylamine is the more toxic of the two to animals. Convulsions, temporary paralysis of the hind quarters, and death occur sooner with a smaller dose. Like cyclohexylamine, if left on the skin, dicyclohexylamine will produce dermatitis. According to Flinn, unlike cyclohexylamine, lethal amounts can be absorbed. No detailed investigations of the metabolism of these compounds have been made, but Williams (157) states that cyclohexylamine appears to be readily oxidized in the dog, with no aromatization to aniline or its metabolites.

The Nitrophenols

The nitrophenols provide a striking example of the difference in toxic action of members of the same group of compounds. Even the isomers of the mononitrophenols show this difference, since the *o*-compound is relatively innocuous, while the *p*-nitrophenol is rapidly fatal to animals [Beutner & Block(13)].

Mononitrophenols.—Mononitrophenols, of which there are three isomers, are used chiefly in the synthesis of dyestuffs. None of the isomers have any such influence on basal metabolism as that exerted by dinitrophenol, and none increase the rectal temperature, but rather depress it [Cameron (24)]. (Cameron did not, however, regard rectal temperature as a reliable index of metabolic action.) With regard to methemoglobin formation, the mononitrophenols are not as effective as some of the dinitrophenols, though small inconstant amounts are found with 2-nitrophenol [Grant (61)]. *o*-Nitrophenol is almost completely excreted in the urine of rabbits, more so than any other phenol derivative [Cameron (24)]. The main excretory product of all the isomers is a nitrophenylglucuronide, with small amounts of dihydric phenols. According to Williams (157) the relatively higher toxicity of the *p*-derivative appears to be an intrinsic property of this compound per se, since all its metabolites, including 4-nitrocatechol appear to be nontoxic.

Dinitrophenols.—There are six isomers of the dinitrophenols which are also used in the synthesis of dyestuffs in the manufacture of explosives, and as preservatives of timber.

The most important isomer, 2,4-dinitrophenol, is not a former of methemoglobin as are, to a small extent, the 2,3-, 2,5- and 3,4- isomers, but

it has a powerful stimulatory effect on the basal metabolic rate, causing a rise of temperature as high as 110°C., loss of weight, profuse perspiration, and nervous disorders. Liver and kidney damage has also been observed by some authorities. Changes in the blood cholesterol level following administration to human beings have been reported [Grant & Schube (62)], but the evidence on this point from animal experiments is conflicting [Koch, Lee & Tainter (79); Tainter *et al.* (147)].

Similarly, with regard to kidney injury, Magne, Mayer & Plantefol (99) found some evidence in chronic poisoning, but Schulte & Tainter (131) found no change in the renal function of rabbits receiving repeated subcutaneous injections of dinitrophenol for as long as 77 days. In industry, the majority of cases of dinitrophenol poisoning have occurred from handling the finished product and in the centrifuging, drying, and packing operations [Koelsch (80)], and, during World War I, in the manufacture of explosives.

Acute poisoning is usually sudden in onset, with severe fatigue, thirst, profuse perspiration, oppression of the chest, extreme nervous agitation, a rapid irregular pulse, and raised temperature. In fatal cases colic and diarrhea, muscular cramps, excitement, and convulsions may precede coma and death. In less severe cases, gastrointestinal symptoms are also prominent, and there may be tenderness over the liver and jaundice.

It has been shown that the major excretory product in dog and man is probably 2-amino-4-nitrophenol [Magne, Mayer & Plantefol (99)] and its presence in the urine, becoming or remaining high, has been used as an indication of neglect of preventive measures in factories where dinitrophenol was used [Horner (71)].

Dinitrophenol has a delayed effect, the formation of cataracts, when taken, generally in the form of the sodium salt, as a remedy for obesity. In spite of warnings, its use for this purpose became extremely popular in the years following 1933; Horner states that during the first 15 months after its introduction in that year, 100,000 persons took the drug for weight reduction. In addition to cataracts, skin lesions were a frequent result—dermatitis, urticaria, and angioneurotic oedema. Otitis media, agranulocytosis, neuritis, and cardiovascular damage were also reported. The occurrence of cataract was first described by Horner, Jones & Boardman (72) in 1935, and in the same year Cogan & Cogan (31) reviewed 20 cases which had appeared in the literature. The cataracts have characteristic features distinguishing them from the senile or traumatic variety. Their onset may be delayed for months or years after taking the drug and is usually marked by blurring of vision. The most striking change occurs in the posterior part of the lens in the form of a dense granular lustrous deposit. The opacities gradually invade the cortex and, finally, the nucleus. In the late stages the whole lens is divided into radiating sections separated by dark bands; glaucoma is a not infrequent sequela.

Efforts to reproduce the cataractogenic effect in animals have been un-

successful in ordinary laboratory animals, but have succeeded in chicks; these, when kept on a diet containing 0.1 to 2.5 per cent of dinitrophenol (DNP) develop, after four to six hours, a fine opacity of the lens progressing to a marked opacity of the anterior portion within 24 hours [Robbins (124)]. Many attempts have been made to elucidate the mechanism of the toxic action of dinitrophenol, both with regard to its stimulatory effect on metabolism and its cataractogenic action; these were fully reviewed by von Oettingen (112) in 1949. The outcome of the numerous investigations is that it is an inhibitor of phosphorylations in biological systems and that its difference from the less toxic isomers is probably based on the different aminophenols which are their main metabolites. The relative potency of the dinitrophenols in stimulating respiration is related to their structure—nitro-groups being most effective in the ortho- and least in the meta- position—except in the case of 2,6-dinitrophenol, which is unique in this series in having side chains on both sides of the hydroxyl group [Grant (61)]. This compound also fails to conform to the correlation between potency as respiratory stimulants and the degree of acidity—the more strongly acid compounds stimulate respiration more powerfully. The increase in respiration rate in rats was found to be only 5 per cent, and the increase in total volume only 15 per cent for this isomer, as compared with 16 and 37 per cent, respectively, for 2,4-dinitrophenol; 12 and 31 per cent for 2,3-dinitrophenol; and 9 and 21 per cent for 2,5-dinitrophenol.

When Cameron (24) in 1958 compared the effects on oxygen consumption in the rat of the six dinitrophenols, she found a lack of correlation between increased carbon dioxide output and oxygen uptake with some of the compounds and suggested that some of the respiratory stimulation might be due to simple hyperventilation rather than to interference with metabolic pathways. Grant does not agree with this view; he states that if the excess liberation of carbon dioxide were caused by hyperventilation, the increased carbon dioxide production would vary in strict proportion to the effect on ventilation. The fact that in his experiments there was a complete absence of such correlation suggests that the CO_2 effect is due to an action on cell respiration. Ramsay (120, 121) has further suggested that a component, known as the "exercise stimulus," in the regulation of breathing may also be involved in the hyperventilation produced by 2,4-dinitrophenol. He postulated that the site of this stimulus may be the nerve receptors in the region of the muscles, since he found that after perfusion of the limb of a dog with blood into which 2,4-dinitrophenol had been injected, an increase of the metabolism of the limb caused an increase in pulmonary ventilation. He believed, therefore, that these receptors were sensitive to changes in metabolic activity.

Investigations of the *in vitro* mechanism of phosphorylation by Clifton (30) and others indicate that dinitrophenol acts on the basic mechanism in the cell by which phosphate bond generation is coupled to oxidative reactions. Loomis & Lipmann (95) have shown that uncoupling, which can be achieved by very small concentrations of DNP with no effect on, or

with stimulation of oxidation, is reversible. A more recent investigation by Wessels (155) suggests that DNP can also catalyze the synthesis of ATP by chloroplasts, which are also able to convert light energy into the pyrophosphate-bond energy of ATP. The mechanism of this action is not yet fully explained. Wessel puts forward several alternative hypotheses; one is that this ability of DNP may bear some relation to its uncoupling action in oxidative phosphorylation. Other nitrophenols capable of this reaction included 2,5- and 2,6-dinitrophenol and *m*- and *o*-nitrophenol.

Further correlation with the effect of DNP on oxidative phosphorylation is suggested by its depressant effect on the renal secretion of uric acid in chickens, on the basis that the tubular transport of uric acid may be dependent on energy supplied by oxidative phosphorylation [Nechay & Nechay (107)].

The mechanism by which 2,4-dinitrophenol exerts its toxic action specifically upon the lens and the variation in species response have been the subject of many hypotheses, but no explanation has been experimentally verified. In view of the investigations described above, the opinion expressed by Krause (81) in 1938 would seem to approximate most closely to the present view of DNP metabolic behavior. He stated that "if the reaction of the lens is comparable to other tissues, DNP cataract arises from the interference with the creatine and adenosine processes in the lens."

Dinitro-orthocresol.—Dinitrol-orthocresol (2-methyl 4,6-dinitrophenol) is used chiefly as an insecticide and has also been used for weight reduction. There were fatal results in at least one case [Goldman & Haber (57)] and cataract formation in many others [Gilbert-Dreyfus & Onfray (52); Vannas (152)]. Industrial poisoning, with symptoms similar to those following exposure to dinitrophenol, has also been reported [Jordi (73); Schwarz (132)]. Jordi emphasized that, unlike dinitrophenol, dinitrocresol was not included in the official list of agricultural poisons in Switzerland, although it was widely used as a spray.

It is eliminated slowly; after ingestion only about 1 per cent of a single dose of 75 mg. is excreted in 24 hours, and it may take several weeks for the blood level to fall to zero. Its metabolites have been investigated in the rabbit by Smith, Smithies & Williams (134). They found that about 5 to 6 per cent of an oral, near-lethal dose is excreted as free dinitro-orthocresol, and 1 per cent in conjugation, mainly with 6-acetamido-2-methyl-6-nitrophenol, together with small amounts of 3-amino-5-5-nitro salycilic acid and conjugates of 4-amino-2-methyl-6-nitrophenol. The 6-acetamido-4-nitro-0-cresol is 20 times less toxic than dinitro-orthocresol and may, therefore, be regarded as a true detoxication product.

The Nitriles

From the industrial point of view, the most important of the nitriles, or alkyl cyanides, is acrylonitrile. It is attaining increasing importance as one of the raw materials in the manufacture of synthetic rubber and thermo-

plastic resins, as an intermediate in chemical syntheses, and in the synthetic fiber industry. Other members of the group, such as acetonitrile, are used as industrial solvents, and some, such as trichloroacetonitrile, as fumigants and insecticides.

As a group, these compounds are a good example of the relation between toxic action and metabolism, since their toxicity depends almost entirely upon whether their metabolic progress within the body is accompanied by liberation of the free cyanide ion.

Acrylonitrile.—A liquid volatile at room temperature, acrylonitrile can cause toxic symptoms whether ingested, inhaled, or absorbed through the skin. Fatal poisoning has occurred from accidental ingestion of a preparation known as Ventox, used as a fumigant, and from its application as a pediculicide [Grunske (65); Lorz (96)]. In both cases death was preceded by vomiting, cyanosis, and loss of consciousness. In the case of a child to whose feet it had been applied, it was believed to have been absorbed in lethal amounts by the skin; the symptoms were, in fact, similar to those observed in cyanide poisoning.

In industry, the hazard is primarily that of inhalation of the vapor [Malette (100)], but few serious and no fatal cases of poisoning have been recorded. (67). Subacute symptoms, following exposures varying from 16 to 100 p.p.m. and including headache, slight anemia, irritation of the skin and mucous membranes, and sometimes jaundice, were described by Wilson (158), and Wilson, Hough & McCormick (159).

Animals fatally poisoned by acrylonitrile show convulsions and cyanosis. Dogs are particularly susceptible; in the experiments of Wilson *et al.*, rats were able to survive concentrations (100 p.p.m.) which were fatal to all the dogs exposed. Histological changes observed in the brain were characteristic of anoxia, and Dudley, Sweeney & Miller (41) also observed renal injury and bronchopneumonia. Many animals are able to convert sublethal doses of cyanide to thiocyanate; thiocyanate can, on the other hand, be converted to cyanide (58). Administration of thiocyanate can, therefore, produce symptoms resembling subacute cyanide poisoning. This is an example of the reversible action of an enzyme on the metabolism of certain substances. In normal metabolism the conversion of hydrocyanic acid to thiocyanic acid is regarded as a "detoxication" mechanism, but for abnormal quantities entering the body this mechanism would most probably fail to function adequately. In fact, a rise in the level of thiocyanate in the blood of animals which did not return to normal 12 hours after exposure has been regarded as an indication that the detoxication capacity was exceeded.

The enzyme chiefly responsible for the conversion of cyanide in the body to thiocyanate is known as thiosulphate transsulphurase, or rhodanase. It is widely distributed in animal tissues, with especially high activity in the liver (86). In view of the high susceptibility of the dog to poisoning by acrylonitrile, it is interesting to note that the tissues of the dog have been found to have a considerably lower concentration of rhodanase than those

of the less susceptible rat [Brieger, Rieders & Hodes (21)]. Rhodanase catalyzes the transfer of sulphur from thiosulphate to cyanide to form thiocyanate and sulphite. In rat-liver homogenates it is localized in the mitochondrial fraction, and its activity is low unless the mitochondria are caused to swell or disrupt, when its activity increases [De Duve *et al.* (35); Greville & Chappel (64)].

Another enzyme, also a transsulphurase (β-mercaptyruvate), may also be concerned in catalyzing the transfer of sulphur to cyanide. According to the recent work of Kun & Fanshier (83, 84) this enzyme is also widely distributed in the tissues, but in rat liver it appears to be present in highest concentration in the "nuclear" and "soluble" fractions rather than in the mitochondria themselves. The soluble fractions were found to contain microsomes, and the supernatant fluid represented only one-quarter to one-half of the total enzyme content, suggesting that it is bound by the nuclei and microsomes.

It has already been seen that thiocyanate, if formed in large amounts by enzymatic action, can, owing to the reversibility of this action, become potentially toxic [Anderson & Chen (4)]. The use of sodium thiosulphate as a therapeutic measure in acrylonitrile poisoning might, therefore, appear to be theoretically inadvisable, but as Brieger, Rieders & Hodes (21) point out, the formation of high concentrations of thiocyanate is not to be expected in man. An increase of thiocyanates in the urine of dogs exposed to the vapor of acrylonitrile was found by Lawton, Sweeney & Dudley (87). It has been suggested that this might be used as a measure of poisoning by acrylonitrile, but this is not accepted by all authorities.

Methacrylonitrile.—Also used in the manufacture of synthetic rubber and other types of polymers, methacrylonitrile's toxic effects in animals are similar to those of acrylonitrile, with a typical cyanide-like action and with about the same degree of toxicity. But whereas it has a less irritative action on the skin, it is more readily absorbed by this route than is acrylonitrile [McOmie (98)].

Trichloroacetonitrile.—Known also as Tritox, trichloroacetonitrile is used as a fumigant, pest controller, and insecticide. It is extremely irritating to the mucous membranes of animals, and lethal concentrations are followed by dyspnea and convulsions. Death is primarily attributable to lung injury, acute hyperemia, and edema. With slightly lower concentrations, acute degenerative changes develop in the heart, liver, and kidneys [Treon *et al.* (149)]. The urine of animals exposed to air containing 1.33 mg. per liter of trichloroacetonitrile contained a substance that was either trichloracetonitrile or some compound which responded to the same test. Its further metabolic features do not appear to have been investigated in detail.

Carbon Disulphide

Carbon disulphide has long been used in the rubber and viscose artificial silk industries; it is also used in extraction and pharmaceutical proc-

esses, in the manufacture of waterproof cements and transparent paper, and as an insecticide for vines and tobacco plants.

Its chronic toxic effects have been more widely recognized in industry than its acute narcotic effect. In high concentrations it may cause loss of consciousness preceded by delirium or acute mania and followed by death from respiratory failure. Table 1 represents a summary of the results of an investigation in 1939 by the Department of Scientific and Industrial Research, London, England, showing the toxic effects of various concentrations. The symptoms of chronic poisoning by carbon disulphide are pre-

TABLE I

TOXIC CONCENTRATIONS OF CARBON DISULPHIDE

Concentration of Vapor in Air		Duration of Exposure	Effect
parts by volume (approx.)	mg./l (approx.)		
1 in 300 to 1 in 500	10 to 6	About ½ hour	Serious illness and danger of mania and coma.
1 in 3000	1	Single exposure for a few hours	Severe headache and mental dullness or confusion.
		Daily exposure	Increasingly severe symptoms with neuritis, distorted vision, and mental disturbance.
1 in 15,000 to 1 in 30,000	0.2 to 0.1	Repeated daily exposure	General condition of ill health headache, drowsiness, and hysterical outbursts.

dominantly nervous, with peripheral effects in the form of polyneuritis, central effects consisting of a strio-pallidal, or Parkinsonian syndrome; psychic abnormality or mental derangement; and eye lesions including lesions of the optic nerve, and retina, and kerato-conjunctivitis.

In recent years much attention has also been focused on a possible long-term effect—the development of arteriosclerosis, sometimes associated with kidney lesions, occurring at a relatively early age but with a latent period of about 20 years. In cases of this kind, described by von Rechenberg (122) in 1957, the arteriosclerosis was found especially in the arteries and arterioles of the cerebrum, but also in those of the peripheral muscles where it affected the media with calcification of this coat. Hypertensive kidney injury, with high blood pressure, some albuminuria, slight microhematuria, and a raised residual nitrogen were also observed—a condition

designated as "CS_2 glomerulosclerosis." The whole syndrome, consisting of encephalopathy, hypertensive nephropathy, and medial sclerosis (a vascular sulphocarbotoxic late syndrome), does not always present itself in the complete form. Individual cases may show only some aspects; for example, diffuse vascular encephalopathy may be present without hypertensive nephritis.

With short-term exposures, even to high concentrations, CS_2 is rapidly removed from the body on cessation of exposure and, owing to the rapid drop in the blood level, the amount retained is normally small. With continued exposure, on the other hand, although blood saturation is reached within 1½ hours with concentrations of 25 to 50 p.p.m., entrance into the body fluids and tissues is much slower; even after two days the tissues are still taking up labelled CS_2 [McKee *et al.* (97); Srittmatter, Peters & McKee (144)]. It has been shown that when CS_2 is inhaled, 10 to 30 per cent is eliminated by the lungs, about 1 per cent is excreted in the urine, and the remaining 70 to 90 per cent undergoes metabolic transformation in the body. By parenteral routes, the proportion retained is much less—20 to 50 per cent [Souček (137)].

The actual mechanism of the metabolic transformation is not completely understood, but it appears to be related to the binding of the portion retained in the tissues with substances containing a nitrogen atom—peptides, proteins, and amino acids. These new products are decomposed by proteolytic enzymes with more difficulty than normal albumen. The enzymic reactions, especially with the amino-acid compounds, produce dithiocarbamates, which can be further decomposed by the desulphydrase system of enzymes, liberating H_2S. This may, in its turn, be rapidly oxidized to H_2SO_4 and excreted in the urine as sulphate. Some observers have, indeed, stated that exposure to CS_2 is followed by an increased excretion of inorganic sulphate [Strittmatter *et al.* (144)], and it has been shown that radioactive H_2S administered to animals is promptly oxidized and eliminated. Within five to ten minutes 50 per cent of the total sulphur in the urine is in the form of inorganic sulphates, and within 30 minutes these show a considerable rise [Gunina (66)].

Possibly, the dithiocarbamates may also give rise ultimately to isothiocyanates. Dithiocarbamate acids contain relatively strong acid sulphydryl groups, and, in fact, the serum of guinea pigs exposed to inhalation of 300 to 1800 µg. per liter of CS_2 has shown an increase of 88 per cent of sulphydryl groups. They tend to be statistically higher than normal in viscose workers exposed to an average concentration of 200 µg. per liter [Souček & Madlo (138)]. The dithiocarbamate in the serum was shown to decompose under the influence of heat and H_2SO_4 giving free CS_2. These facts indicate that the intramolecular reaction of CS_2 with albumen causes a marked alteration in the molecule.

The liver is, undoubtedly, an important organ in the metabolism of CS_2 and some recent investigations in Germany have suggested that signs of its

capacity for detoxication of CS_2 are shown by changes indicating a disturbance of capillary permeability. This endothelial disturbance, present probably in the general arterial system, and generally, as already remarked, associated with premature arteriosclerosis, is not, however, directly its cause. Other factors, such as hyperlipemia, and especially an increase in blood cholesterol, have been held responsible [Michalova, Bartoniček & Zastavá (103)].

Cholesterolemia is certainly a feature of CS_2 intoxication in animals, but it is not certain whether it is directly associated with the changes in the kidney. Von Rechenberg (122) believes, rather, that the nephropathy is associated with the brain lesions and with the specific sclerosis of the arterial media in the sense of a general vascular toxic effect of CS_2 or its metabolites. Nevertheless, cholesterolemia may be another manifestation of altered body metabolism intricately bound up with the enzyme systems of the body, and particularly with the metal complexes associated with them and required as activators (94). Alkaline phosphatase, for example, requires zinc and magnesium ions as activators, and the urinary excretion of both has been found increased in animals chronically poisoned with CS_2, with a simultaneous decrease in alkaline phosphatase activity. The chain of events in this metabolic disturbance appears to be that of a chelating effect by the dithiocarbamate and thiozolidone resulting from the reaction of CS_2 with free amino groups in the body. This chelation results in the formation of nonionized complexes with the polyvalent metal ions, so that these are prevented from performing their natural function in normal cell metabolism.

An interesting aspect of this hypothesis is the way in which it can be linked with the predominant nervous lesions of CS_2 toxicity, central or peripheral. Histological examination of the nerve tissues of animals subjected to prolonged and severe exposures to CS_2 has revealed degenerative changes in the cells of the brain and spinal cord amounting to almost complete destruction and loss of function. In the brain and spinal cord, copper is normally present as a structural part of cytochrome oxidase and coenzyme A dehydrogenase. If it is unavailable, the metabolism of these nerve cells may be severely disorganized. Copper is also known to be tightly bound by the thiocarbamate groups, and in animals with severe pathological lesions of the spinal cord and cerebral cortex, the copper content of these tissues is about half the normal [Cohen *et al.* (33)]. Thus, it appears that the specific toxic action of CS_2 on the nervous system can be directly associated with its metabolic behavior.

Tetraethyl Lead

Tetraethyl lead [$Pb(C_2H_5)_4$], in the form of "ethyl fluid," a mixture of 49 to 63 per cent of tetraethyl lead with ethylene bromide or dibromide or dichloroethane, is used chiefly as an antiknock additive to motor fuels. It is very volatile—at 18°C., air saturated with its vapor contains about 5 mg. per liter. It is, therefore, most commonly absorbed by inhalation, but

Kehoe & Thaman (76) have shown that in animals it can also be absorbed by the skin.

Its chief hazard arises during its manufacture, during mixing with motor fuel, and, especially, while cleaning storage tanks which have contained leaded petrol. Kitzmiller, Cholak & Kehoe (77) state that men exposed to air in tanks in which the sludge may contain 0.1 per cent or more of tetraethyl lead may inhale and absorb a lethal dose in half an hour. In less severe conditions, in a day or a number of successive days a dose sufficient to cause a serious or even fatal illness can be absorbed. It produces a syndrome of intoxication entirely different from that of inorganic lead (27).

The symptoms, whether acute and fatal, severe but not fatal, or mild in degree, are neurotoxic in character. In acute fatal cases, delirium, mania, hallucinations, convulsions, and coma may precede death occurring within a few days of exposure. In severe but not fatal cases, there is often mental confusion, excitement, restlessness, disorientation, sometimes depression, and nausea, abdominal pain, and vomiting. Such cases frequently progress towards the more acute mental disturbance, but if the critical stage is passed within a week or two of the onset, recovery usually occurs within four to 10 weeks, though relapses are not uncommon. In mild cases, such as those described by Müller (106) in men employed in the recovery of lead scrap used for the production of tetraethyl lead, the complaints include insomnia with bad dreams, restlessness, loss of appetite, and gastrointestinal disturbance—a picture difficult to distinguish from an anxiety state. These cases usually regress rapidly on cessation of exposure.

The distribution of tetraethyl lead in the tissues following absorption differs, at least in its initial stages, from that of inorganic lead, as well as in its chief site of deposition. It has been shown that inorganic lead is very sparsely deposited in the brain of animals, even when given intravenously, and that it is not accumulated there [Ginsberg & Weatherall (54); Goldblatt & Goldblatt (56)]. In fatal tetraethyl lead poisoning, both in animals and humans, "volatile and nonvolatile" lead compounds have been found in the brain in amounts up to 12 to 21 μg. per gm., and Goldblatt has suggested that the critical level in the brain at which signs of central nervous disturbance may appear is 2 to 3 μg. per gm. He suggests, also, that the volatile organic lead compounds have a special predilection for lipoid and nerve tissue, a view also suggested by Smusin (135) on the basis of his experiments on the relation between tetraethyl lead and cholinesterase (see below). More recent investigations on its metabolism by Cremer (34) and Stevens *et al.* (141, 142) have not supported the view that the reason for its predominant effect on the nervous system is essentially its fat-soluble character allowing selective localization in nerve tissue, or that its toxicity is a function of its lead ion content. It now appears that the metabolic products of tetraethyl lead are more toxic than the original compound.

"Volatile lead" has also been identified in the liver, though without certainty that this compound was in fact tetraethyl lead [Norris & Gettler (108); Kehoe & Thaman (76); and others]. An analysis of liver tissue concentrated by low temperature vacuum distillation and examined spectroscopically, with a confirmatory dithizone analysis, has, however, shown that it was present in small amounts. During its metabolism within the body, other water-soluble organo-compounds are formed, of which those most closely investigated are triethyl and diethyl lead. It is to the former of these, triethyl lead, that the toxic effects of tetraethyl lead are attributed [Cremer (34)].

The isolation of triethyl lead was achieved by Cremer and by Stevens, Feldhake & Kehoe (141, 142) using different methods. Cremer's method of estimation of triethyl lead was based on the formation of a triethyl lead-dithizone colored complex in a chloroform extract (tetraethyl lead does not form a colored complex with dithizone), and this complex was distinguished from diethyl lead by its absorption spectrum.

In the method of Stevens *et al.* (142) isolation of triethyl lead depended upon extraction of hemogenized liver with pentane, conversion to the benzoate salt, and examination of the isolated material by infrared radiation, melting point, and lead analysis. Crystals obtained by evaporating to dryness, leaching with iso-octane, and further decantation and evaporation, melted within a few degrees of the melting point of triethyl lead benzoate and over 60 degrees below that of diethyl lead benzoate. These results were considered to indicate that an appreciable part of the inhaled tetraethyl lead is covered to the trivalent lead ion, which then persists for a time in the body. This view was confirmed by Cremer, who gave 10 mg. per kg. of triethyl lead chloride intraperitoneally to rats and analyzed the tissues for triethyl lead at intervals between two hours and four days. It was found that the level of this compound remained almost unaltered during this time, so that any subsequent degradation to diethyl or inorganic lead takes place more slowly than the initial conversion to triethyl lead. It was also found that though the amount of triethyl lead was less in the brain than in other tissues, brain slices were metabolically highly sensitive to this compound as well as to tetraethyl lead. Inhibition of oxygen uptake, increase in lactate, and decrease in pyruvate were produced by both, but nearly double the dose of tetraethyl lead was required to cause effects equivalent to those of triethyl lead. Diethyl lead chloride had a much less marked effect, and the metabolic disturbances caused by all the organo-lead compounds differed from that following administration of lead acetate in that the latter caused no alteration in the oxygen consumption and only a small change in the lactate and pyruvate levels. The conversion of tetraethyl lead to triethyl lead appears to be brought about by enzymes in the liver. The exact location of the principles involved is not known, but, according to Cremer, 94.5 per cent of the activity of the whole hemogenate is found in the supernatant fluid which contains the microsomes and soluble material and

which remains after large particles, including nuclei and mitochondria, have been removed.

That the toxicity of tetraethyl lead is mainly attributable to its decomposition product, triethyl lead, and not to the diethyl compound, was suggested by the fact that rats receiving either tetra- or triethyl lead developed identical signs of poisoning—excitability, tremors, and convulsions—whereas those given diethyl lead showed only slight initial uneasiness and loss of appetite. Inhibition of cholinesterase has also been suggested as an important factor in the mechanism of poisoning by tetraethyl lead. Smusin (135) found that a dose of 1.07 gm. per kg. produced a severe depression of true cholinesterase in the cerebral cortex, medulla, and muscle tissue of mice, reaching its lowest level in four to eight hours. There was a much less marked depression of the pseudo-cholinesterase of these tissues. Preliminary treatment of the tissues with acetone in the cold had no obvious effect on the cholinesterase level, a fact which it is suggested, provides indirect evidence of the ability of tetraethyl lead to penetrate into every tissue, including lipoid.

Chelation treatment, effective with inorganic lead poisoning, does not appear to be so with tetraethyl lead. Kitzmiller, Cholak & Kehoe (77) did find that intravenous administration of calcium versenate caused a varying increase in the urinary excretion of lead but not in the blood level. In view of the facts that triethyl lead does not combine with either BAL or calcium versenate and that its ion, to quote Stevens *et al.* (142), "is a monovalent electrolyte that probably cannot be chelated," it is unlikely that chelating agents would prove effective as a therapeutic measure.

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