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CHAPTER 17

METHANOL

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17.1. INTRODUCTION

The biological behavior of methanol is markedly different from that of the next homologous alcohol, ethanol. Its toxicity for man with the typical visual disturbances has been known about one hundred years and considerable attention has been devoted to methanol in toxicological and clinical literature. (For a detailed history see Orthner, 1950.) Methanol poisonings have occurred quite frequently, especially during times of war, shortages and prohibition. During the Second World War about 6% of the total analyzed cases of blindness in the United States Armed Forces were due to methanol poisoning (Greear, 1950). A large number of cases of methanol poisoning have been described in the literature, including reports of several mass poisonings like those in Berlin during Christmas 1911 with 163 cases and 72 deaths (Pinkus, 1912; Stadelmann and Magnus-Levy, 1912) and in Atlanta, Georgia, 1951 with 323 victims and 41 deaths (Bennett *et al.*, 1953; Benton and Calhoun, 1952). The biochemical basis of methanol toxicity, however, is still largely unknown in spite of a considerable amount of experimental work. In the following chapter a survey is given of the toxicology and biochemistry of methanol and especially of the metabolic aspects which are of prime importance in the understanding of methanol toxicity. More detailed discussions and additional references can be found in the reviews listed in the first part of the bibliography, especially in those of Röe (1946, 1955), Bennett *et al.* (1953), Oettingen (1943), Orthner (1950), Cooper and Kini (1962), Grant (1964) and Koivusalo (1956a).

The following abbreviations are used: ATP, adenosine triphosphate; AMP, adenosine monophosphate; NAD (H), oxidized (and reduced) form(s) of nicotinamide adenine dinucleotide; NADP(H), oxidized (and reduced) form(s) of nicotinamide adenine dinucleotide phosphate.

17.2. PHYSICAL AND OTHER PROPERTIES OF METHANOL

Methanol (CH_3OH) is also called methyl alcohol, carbinol. wood alcohol, wood spirit and Columbian spirit. The molecular weight of methanol is 32.04, density at 20° 0.7915 and boiling point at 760 mm Hg 64.7°. It is a flammable colorless liquid which is freely miscible with water, ethanol, ether and most other organic solvents. Pure methanol has only a slightly alcoholic odor but crude material can have a repulsive pungent odor. Methanol was originally obtained by distillation of wood, now it is usually manufactured in large quantities of high purity from hydrogen and carbon monoxide or carbon dioxide, also by oxidation of hydrocarbons. It is widely used as an industrial solvent and raw material, as an antifreeze fluid and solvent for shellac and some paints and varnishes and in paint removers, and solid canned fuels. It is also used to denature ethanol in many countries. Analytical methods which are suitable for the determination of methanol and its metabolites in biological material even in the presence of ethanol have been described by Agner and Belfrage (1947), Koivusalo (1956a), Bonnichsen and Linturi (1962), Hindberg and Weith (1963), Bastrup (1947a) and Nash (1953).

17.3. ABSORPTION AND DISTRIBUTION

The absorption and distribution of methanol in the organism is similar to that of ethanol. Methanol is easily absorbed from the digestive tract (Haggard and Greenberg, 1939), and most poisonings with methanol are due to oral intake. Methanol is also absorbed by inhalation as has repeatedly been shown in animal experiments (Haggard and Greenberg 1939) and several cases of poisoning in man have been described due to the inhalation of methanol vapor (e.g. Wood and Buller, 1904, Humperdinck, 1941; Hansohm, 1952). Concentrations of methanol over 200 ppm in the air have been considered hazardous (Leaf and Zatman, 1952, Moeschlin, 1964). Some methanol has also been shown to be absorbed through the skin of experimental animals (Eulner and Gedicke, 1955) and some cases of human poisoning due to possible percutaneous absorption of methanol have been described (Eulner, 1954). Subcutaneously or intraperitoneally administered methanol is also easily absorbed.

Like ethanol, methanol is distributed in the tissues according to their water content, with minor modifications (Yant and Schrenck, 1937; Neymark, 1936; Harger *et al.*, 1938; Haggard and Greenberg, 1939; Lund, 1948; Bartlett, 1950a; Koivusalo *et al.*, 1958b).

17.4. ELIMINATION

The elimination of methanol is much slower than that of ethanol and a much larger proportion of methanol than of ethanol is excreted unchanged by the lungs and kidneys. In dogs given 1.6–5.0 g/kg methanol 22–27% of the dose was eliminated in the expired air during the two days following the administration (Völtz and Dietrich, 1912; Asser, 1914) but considerable amounts of methanol were still exhaled during the 3rd–5th days; in five days about 50% of the dose was exhaled (Asser, 1914), while only 3% was found as such in the urine. In his experiments with dogs Lund (1948b) recovered 7–10% of the dose unchanged in the urine. In rabbits 8–10% of the dose (0.8–2.4 g/kg) has been found as methanol in the urine (Lund, 1948a; Koivusalo, 1956a). Haggard and Greenberg (1939) have reported in rats that an average of 76% of the amount eliminated during the first 6 hr was lost in expired air. However, only 6% of the total dose was eliminated during that time.

In experiments with rats using radioactive methanol 14% of the dose (1 g/kg) was eliminated unchanged in exhaled air and 3% in the urine during 48 hr (Bartlett, 1950a). Similar figures (10–15% in exhaled air and 7–19% in the urine) were obtained by Aebi *et al.* (1957b) with rats and guinea pigs. Tephly *et al.* (1964) reported that during 36 hr 24% of the dose (1 g/kg) was eliminated unchanged and that about equal quantities were excreted by the pulmonary and renal routes.

The rate of disappearance of methanol from the blood has been studied by several authors as a measure of the total elimination rate from the organism. Nicloux and Placet (1912) studied the elimination of methanol in dogs and rabbits after peroral administration of 3 to 5 ml of methanol. Traces of methanol were still present in the blood after more than 100 hr. The elimination curves were practically linear in both dogs and rabbits.

Several studies have been made using Widmark's (1922) method for blood ethanol determination as adapted by Bildsten (1924) for the determination of methanol. The elimination of intravenously administered methanol from the blood of rabbits was studied by Bildsten (1924) and Widmark and Bildsten (1924). In rabbits given about 0.8 g methanol per kg the elimination rate varied from 0.00057 to 0.00117 mg per g per minute, averaging 0.00076 The elimination occurred at a constant rate which was not dependent on the methanol concentration in the blood. In most curves a temporary increase in the methanol concentration was found 4–6 hr after the administration. It was regarded possible that this increase could be due to some substance other than methanol which accumulated temporarily during the metabolism of methanol and which was determined as methanol by the dichromate oxi-

dation method used. In the experiments of Bernhard and Goldberg (1934) the average elimination rate was 0.00067 mg per g per minute when 0.56 to 2.10 g methanol per kg was administered to rabbits. In general, the elimination curves were linear and the temporary increase 4–6 hr after administration was seen in most curves. The elimination rate was found to vary considerably even in the same animal at different times. The elimination rate of methanol in dogs was studied by Neymark (1936) and Neymark and Widmark (1936) and was found to be 0.00018 mg per g per minute and independent of the dosage. The temporary increase seen in the experiments with rabbits was not noted in these experiments.

Haggard and Greenberg (1939), differing from the above, concluded that in rats and dogs the rate of elimination is proportional to the concentration of methanol in the body. In their opinion most of the ingested methanol is excreted in the expired air as a result of simple diffusion and, therefore, the elimination rate is directly proportional to the concentration of methanol in the blood and the blood concentration curve is consequently exponential.

The elimination rate of methanol from blood was also dependent on the blood concentration of methanol in the experiments with rabbits conducted by Koivusalo (1956a). The methanol determinations were made by the specific chromotropic acid method. Methanol was given both orally and intravenously in doses of 0.2 to 3.4 g per kg. The rate of elimination from blood was about 0.00042 mg per ml per minute at blood methanol concentrations of less than about 0.8 mg per ml, and about 0.00120 at blood methanol concentrations of 2.5–0.8 mg per ml. Similar although slightly higher values have also been obtained by Agner and Belfrage (1947). The temporary increase in the blood methanol concentration seen in the experiments of Bildsten (1924), Widmark and Bildsten (1924), Bernhard and Goldberg (1934) and Orthner (1950) was not found.

The elimination of small amounts of methanol has also been studied in human subjects (Leaf and Zatman, 1952). The decline in the concentration of methanol in the body was determined in these experiments by following the concentration of methanol in the urine. Small variable doses of 2.5–7.0 ml methanol per subject were employed. The rate of elimination was at all times found to be proportional to the concentration of methanol in the body. Only a very small fraction of the ingested methanol—about 2%—was eliminated as such by the respiratory and urinary routes.

17.5. METABOLISM OF METHANOL

17.5.1. OXIDATION OF METHANOL

The earliest systematic investigations on the metabolism of methanol in animals were made near the end of the last century by Pohl (1893), who

demonstrated that methanol is converted in the organism to formic acid. There were, however, earlier reports in the literature on the excretion of formate in the urine after administration of methanol to animals (see for example, Husemann and Husemann, 1862). Pohl found that when methanol was administered to dogs the urinary excretion of formate was markedly increased for several days. The maximum increase occurred during the 2nd to 4th days after the administration of methanol, when toxic symptoms were also most severe. The excretion of formate in the urine was also found to be increased in humans to whom small amounts of methanol were administered. Furthermore, Pohl demonstrated the formation of formic acid from methanol in experiments *in vitro* with dog liver preparations.

Pohl's results have since been confirmed by several investigators. The increased urinary excretion of formate after the administration of methanol has been found in dogs (Bongers, 1895; Asser, 1914; Harrop and Benedict, 1920; Leo, 1925; Bastrup, 1946; Lund, 1948b), cats (Rost and Braun, 1926) and humans (e.g. Autenrieth, 1920; Kobro, 1946; Kendal and Ramanathan, 1953; Erlanson *et al.*, 1965). The determination of the formate concentration in the urine in cases of suspected poisoning has also been used as a diagnostic aid (Alha *et al.*, 1958).

Apparently, there are quite large differences between different species of animals in the relative amounts of formic acid excreted in the urine after the intake of methanol. Although increased values have been found in rabbits they have not been present in all cases (Asser, 1914; Pohl, 1922; Rost and Braun, 1926; Koivusalo, 1956a). In comparison to humans and dogs the increase in the excretion of formate by methanol-treated rabbits is much lower. Only 1% or less of the administered methanol can be recovered from the urine as formate in rabbits; in dogs the value is 14–20% (Bastrup, 1947b; Lund, 1948a,b,c). This difference is apparently due to the greater capacity of rabbits to metabolize formate (Bastrup, 1947b; Lund, 1948a,b).

These findings have clearly demonstrated that methanol is at least partly converted to formic acid in the organism. The logical mechanism for this conversion is, of course, oxidation, with formaldehyde as intermediate. Pohl (1893) tried to demonstrate formaldehyde in the urine and tissues of dogs poisoned with methanol, with negative results. Despite several attempts to detect formaldehyde in cases of methanol poisoning, it was not until 1931 that this was successful for the first time. Keeser (1931a, b) then demonstrated by qualitative tests the presence of formaldehyde in the cerebrospinal fluid, vitreous humour of the eye and peritoneal fluid of rabbits which had been poisoned with methanol. Usually no formaldehyde can be found in tissues, blood or urine in human or experimental poisoning (e.g. Koivusalo, 1956a; Alha *et al.*, 1958).

The formation of formaldehyde can be more easily demonstrated in experiments *in vitro*. The first reliable reports were apparently those of Keeser (1931a, b) and Keeser and Vincke (1940), who obtained positive tests for formaldehyde after incubation of methanol with fresh vitreous humour of the ox or with ground horse liver. When various tissue preparations are incubated with methanol formaldehyde is accumulated in the medium and the amount of accumulated formaldehyde has been used as a relative measure of the oxidation of methanol (e.g. Mackenzie, 1950, 1955; Koivusalo, 1956a, 1958a, b; Mannering and Parks, 1957; Tephly *et al.*, 1961). The enzyme system responsible for the conversion of methanol to formaldehyde is located mainly in the supernatant fraction of the rat liver homogenate (Mackenzie *et al.*, 1953).

The oxidation of carbon-labelled methanol to carbon dioxide in the intact rat occurs at a constant rate of 25 mg per kg per hour (Bartlett, 1950a). In similar experiments ethanol is oxidized at a constant rate of 175 mg per kg per hour (Bartlett and Barnet, 1949). Ethanol is thus oxidized seven times as rapidly as methanol. Of the ingested methanol 60% was directly oxidized to carbon dioxide, 14% was eliminated unchanged in the expired air, and 3% was recovered unchanged in the urine. The elimination of unchanged methanol in the expired air was dependent on the blood methanol concentration. The tissues fixed appreciable amounts of methanol carbon, and liver, kidney and intestine were especially active in this respect. Similar results are reported for rats by Aebi *et al.* (1957b) and Tephly *et al.* (1964), while somewhat higher oxidation rates are found in guinea pigs (Aebi *et al.*, 1957b) and in mice (Hevesy, 1953).

In experiments with rat tissue homogenates (Koivusalo, 1956a) the liver tissue was most active in the utilization of methanol; kidney, spleen, lung, testis and heart muscle tissues also utilized methanol, the activity diminishing in this order. Homogenates from skeletal muscle and brain had no activity. The utilization of methanol in rat liver homogenates was inhibited by monoiodoacetate, malonate, cyanide and disulfiram. Similar results on the abilities of different tissues to oxidize methanol to carbon dioxide have been obtained in rat tissue slices (Bartlett, 1950a). Considerable amounts of methanol carbon were fixed by the slices in these experiments, the proportion in the liver being 25% of the amount oxidized to carbon dioxide.

Our knowledge of the enzymic details of the oxidation of methanol to formaldehyde is limited. Two enzymes are known to exist in the animal organism which may be implicated in the oxidation of methanol to formaldehyde *in vivo*, namely alcohol dehydrogenase and catalase, but there has been very much controversy on their relative importance.

Alcohol dehydrogenase catalyses the following reaction:

$$\text{alcohol} + \text{NAD}^+ \rightarrow \text{aldehyde} + \text{NADH} + \text{H}^+.$$

This enzyme is widely distributed in plant and animal tissues and its properties have been reviewed earlier in this volume. Best known are the crystalline enzymes from yeast (Negelein and Wulff, 1937) and horse liver (Bonnichsen and Wassen, 1948). The kinetics of the horse liver alcohol dehydrogenase have been extensively studied by Theorell and his associates and there is quite convincing evidence that alcohol dehydrogenase is the main enzyme responsible for the physiological oxidation of ethanol (Theorell and Bonnichsen, 1951). The oxidation of methanol by liver alcohol dehydrogenase, on the other hand, has been the subject of many controversial observations. Yeast alcohol dehydrogenase in high concentrations is capable of oxidizing methanol, although slowly (Barron and Levine, 1952; van Eys and Kaplan, 1957).

Of the earlier authors, Pohl (1893) and Battelli and Stern (1910a, b) had found that liver preparations were able to oxidize methanol to formic acid. The oxidation of methanol was found to be more labile than the oxidation of ethanol by the same preparations (Battelli and Stern, 1910a, b). The partly purified horse liver alcohol dehydrogenase preparations of Lutwak-Mann (1938) also oxidized methanol, although very slowly. Zatman (1946) used similar preparations and found methanol to be oxidized at one-ninth of the rate of ethanol oxidation, in the presence of nicotinamide-adenine dinucleotide. The crystalline horse liver alcohol dehydrogenase does not, however, react with methanol and NAD to any measurable extent according to several authors (Bonnichsen and Theorell, 1951; Theorell and Bonnichsen, 1951; Winer, 1958; Merritt and Tomkins, 1959; Theorell, 1965; Wratten and Cleland, 1965; Koivusalo, unpublished observations). In their studies Wratten and Cleland (1965) used crystalline horse liver enzyme and found no activity with methanol using methanol concentrations between 10^{-5}M and 10^{-1}M. Methanol (10^{-1}M), however, inhibited partially the oxidation of ethanol or the reduction of acetaldehyde in the presence of NAD^+ or NADH, respectively. It has been demonstrated, however, that liver alcohol dehydrogenase catalyses the reverse reaction, namely the reduction of formaldehyde by NADH, but this reaction is much slower than the reduction of acetaldehyde by NADH (Theorell and Chance, 1951). As a possible explanation for the failure of the crystalline enzyme to oxidize methanol Theorell and Bonnichsen suggested (1951) that the oxidation of methanol may require some additional catalyst besides alcohol dehydrogenase and NAD.

Contrary to the observations of the aforementioned authors Kini and Cooper (1961) have reported that crystalline horse liver alcohol dehydrogenase had an appreciable activity with methanol as substrate. No clear explanation is available for this controversy; according to Cooper and Kini (1962) the low concentrations of methanol used by some of the previous

investigators could be the reason because the K_m for methanol is according to Kini and Cooper (1961) rather high, 1.7×10^{-2}M. This cannot, however, explain the negative results of Theorell and Bonnichsen (1951) and Wratten and Cleland (1965). Kini and Cooper (1961) have also purified alcohol dehydrogenase from rhesus monkey and human livers and found their preparations to be active with methanol and that the ratio of oxidation of methanol as compared with that of ethanol was about the same as the ratio they obtained with crystalline horse liver enzyme. Their preparations could use both NAD and NADP as coenzyme.

Human liver alcohol dehydrogenase has also been purified by von Wartburg *et al.* (1964, 1965) and by Blair and Vallee (1966), who found it to resemble the horse liver enzyme except that it had broader substrate specificity, also oxidizing methanol and some other alcohols, which were not substrates for the horse liver enzyme. The K_m values for ethanol and methanol were 1×10^{-3}M and 3×10^{-2}M and the relative maximal velocities 1.0 and 0.11, respectively. In one preparation the maximal velocity for methanol was approximately equal to that of ethanol (von Wartburg *et al.*, 1964), which may denote individual variability in the capacity to oxidize methanol. Isoenzymes of the human enzyme were also described by these authors. Similar results were obtained in studies on the alcohol dehydrogenase isoenzymes from rhesus monkey liver (Papenberg *et al.*, 1965), which were also active on methanol.

The presence of alcohol dehydrogenase activity in retina has been demonstrated histochemically (e.g. Pearse, 1961), but we know very little of the substrate specificity of this enzyme. The retina contains also retinene reductase (or vitamin A dehydrogenase), which is present in visual cell outer segments and can use NADH or NADPH for the reduction of retinene to vitamin A or NAD or NADP for the reverse reaction (Bliss, 1948; Wald and Hubbard, 1949; Futterman, 1963). A special role in vision is proposed for this enzyme, which has been assumed to be identical with alcohol dehydrogenase. Liver alcohol dehydrogenase oxidizes vitamin A to retinene in the presence of NAD (Bliss, 1949, 1951; Hubbard and Wald, 1951), but Koen and Shaw (1966) have recently presented evidence for separate alcohol and vitamin A_1 dehydrogenases both in liver and in retina. The retinal and hepatic alcohol dehydrogenases were also different isoenzymes. Retinal preparations from pig oxidize methanol to formaldehyde in the presence of NAD, when estimated by the formation of both NADH and formaldehyde (Kos and Szybinski, 1962). The preparation also oxidized ethanol, but more slowly. The alcohol dehydrogenase preparation from pig liver obtained by the same method oxidized methanol but much more slowly than ethanol.

Keilin and Hartree (1935, 1945, 1955) have demonstrated that *catalase*

can oxidize ethanol and methanol in *in vitro* systems comprising a primary oxidizing system generating hydrogen peroxide, and alcohol and catalase. The hydrogen peroxide formed reacts with the alcohol, oxidizing it to the corresponding aldehyde. The latter reaction is catalyzed by the catalase, which functions as a peroxidase in these coupled oxidations.

The primary oxidizing system is formed by oxidizing enzymes such as xanthine oxidase, uricase, D-amino acid oxidase, tyraminase, glucose oxidase and others, which catalyze the oxidation of their respective substrates by molecular oxygen, reducing the latter to hydrogen peroxide.

In a model system composed of xanthine oxidase, aldehyde, catalase and alcohol, the hydrogen peroxide formed in the primary oxidation of aldehyde to acid is utilized by catalase for the oxidation of alcohol to aldehyde (Keilin and Hartree, 1945). Thus the product of the secondary oxidation supplies the substrate for the primary oxidation system and this cyclic oxidation reaction will proceed as long as alcohol is present and the enzymes are active. The reactions in the case of methanol and formaldehyde can be written as follows:

$$\begin{aligned} HCHO + H_2O + O_2 &\rightarrow HCOOH + H_2O_2 \\ H_2O_2 + CH_3OH &\rightarrow HCHO + 2\,H_2O \\ \hline CH_3OH + O_2 &\rightarrow HCOOH + H_2O \end{aligned}$$

Chance (1947) has demonstrated that catalase very rapidly forms an intermediate compound in the presence of hydrogen peroxide and this intermediate compound then reacts with different donors, e.g. with alcohols yielding the corresponding aldehyde. He has calculated that the peroxidatic activity of the intermediate catalase-hydrogen peroxide compound is adequate to account for the results of the experiments of Agner and Belfrage (1947) on the kinetics of the disappearance of methanol from the blood in rabbits. Because of the inability of horse liver alcohol dehydrogenase to oxidize methanol he suggested that the oxidation of methanol *in vivo* may be ascribed to the peroxidatic functioning of catalase.

There has been much discussion in the literature whether or not the peroxidative mechanism is responsible for the oxidation of methanol *in vivo*. Jacobsen (1952a, b) considers catalase a very possible factor in the oxidation of methanol and also partly in that of ethanol. The different oxidation rates of methanol and ethanol *in vivo* have been presented as evidence against the action of catalase which oxidizes both methanol and ethanol at the same rate (Bartlett, 1952); however, the possibility that methanol and ethanol might be metabolized by two different pathways with differing specificities was not taken into account. Detailed discussions on the physiological role of catalases

are presented by Lemberg and Legge (1949), Hunter and Lowry (1956) and Nicholls and Schonbaum (1963).

Considerable evidence has been reported, which shows that catalase apparently can take part in the oxidation of methanol in animal tissues. The distribution of catalase and the oxidation capacity for methanol are similar (Koivusalo, 1956a; Aebi *et al.*, 1957b). Very little catalase, however, is present in the retina (Kos and Szybinski, 1962). On the other hand, only low alcohol dehydrogenase activities have been found in the livers of rats and guinea pigs (Nyberg *et al.*, 1953).

Administration to experimental animals of 3-amino-1,2,4-triazole (AT), which effectively inhibits liver and kidney catalase activity (Heim *et al.*, 1955) also inhibits the oxidation of methanol *in vivo*, measured as $^{14}CO_2$ formation (Tephly *et al.*, 1964; Aebi *et al.*, 1957b), although no clear effect is seen in the rate of methanol disappearance from the blood (Mannering and Parks, 1957; Tephly *et al.*, 1961). Liver slices from AT treated rats have a decreased capacity to oxidize methanol (Aebi *et al.*, 1957b; Mannering and Parks, 1957; Smith 1961; Tephly *et al.*, 1961) and the oxidation of methanol is inhibited in perfused livers from AT treated animals (van Harken *et al.*, 1965). Administration of another catalase inhibitor, isopropylallylacetureide, to rats, likewise reduces both the hepatic catalase and the oxidation of methanol (Aebi *et al.*, 1957b). Administration of AT has no effect on the oxidation of ethanol (Kinard *et al.*, 1956; Smith, 1961; Tephly *et al.*, 1964).

The competitive inhibition of the oxidation of methanol by ethanol will be discussed in detail later in this chapter. The inhibition by equimolar amounts of ethanol in animal experiments is about 50% (Koivusalo, 1956a, 1959b, 1963b; Smith, 1961; Tephly *et al.*, 1961, 1964), which fits the catalase better than the alcohol dehydrogenase mechanism. In contrast to ethanol, n-propanol and n-butanol have only a slight or no inhibitory effect on the oxidation of methanol (Koivusalo, 1959b, 1963b; Tephly *et al.*, 1961, 1964; see also Aebi *et al.*, 1957b). The known animal alcohol dehydrogenases are very active also with n-propanol and n-butanol (e.g. Winer, 1958) but the rate of reduction of mammalian catalase-hydrogen peroxide compound I, which is the same for methanol and ethanol, is about 60 fold lower for n-propanol and 500-fold lower for n-butanol (Keilin and Hartree, 1945; Chance, 1947, 1951). Both methanol and ethanol protect *in vivo* and in model systems liver catalase from the inhibition by AT with equal ability (Tephly *et al.*, 1961), n-propanol and n-butanol have no or slight protective activity, paralleling their activity as donors for the catalase compound I. Methanol has no effect on the rate of oxidation of ethanol in the intact rat (von Wartburg, 1959; Tephly *et al.*, 1964), in rat liver slices (Smith, 1961) or in perfused rat liver (van Harken *et al.*, 1965) even with high methanol

to ethanol ratios, which also speaks for the different metabolic pathways for these two alcohols.

Molybdenum deficiency can be induced in rats by feeding them sodium tungstate. The activity of the molybdenum-containing xanthine oxidase and possibly also that of aldehyde oxidase is very low in the livers of these animals (Higgins *et al.* 1956). Liver homogenates from molybdenum-deficient rats have a much-reduced capacity to oxidize methanol (Koivusalo, 1958a; Tephly *et al.*, 1961). If molybdenum is added to the diet or purified xanthine oxidase to the homogenates the activity to oxidize methanol is restored. Xanthine and aldehyde oxidases can both serve with their respective substrates as a primary oxidizing system in the coupled oxidations by catalase and apparently in the molybdenum-deficient rats the formation of H_2O_2 by other systems is not enough to sustain the coupled oxidations. Feeding of tungstate has no effect on liver catalase activity.

On the basis of their experiments on human and monkey liver alcohol dehydrogenases Kini and Cooper (1961) and Cooper and Kini (1962) have concluded that alcohol dehydrogenase is responsible for the physiological oxidation of methanol but that with low levels of methanol the peroxidative action of catalase could operate. The basis of these conclusions has, however, been criticized by Mannering *et al.* (1962). Comparative studies on the metabolism of ^{14}C-methanol in rats and monkeys have implicated alcohol dehydrogenase in monkeys and the peroxidative system in rats as the main pathways for the oxidation of methanol (Mannering *et al.*, 1965). The recent results of Forsander (1967) also speak against the participation of alcohol dehydrogenase in the metabolism of methanol in rat liver. In these experiments ethanol strongly increased the lactate/pyruvate concentration ratio of rat liver slices but methanol had no effect.

On the basis of the present evidence it seems to be established that in the experimental animals, with the exception of the monkey, methanol is oxidized mainly by the coupled oxidations with catalase, at least in the *in vitro* experiments with various liver preparations. On the other hand, alcohol dehydrogenase may be responsible for the oxidation of methanol by humans and monkeys and also in the retina. However, the evidence does not exclude the possible participation of alcohol dehydrogenase in methanol oxidation by the experimental animals, or the operation of the peroxidatic system in humans and monkeys. It is possible that both systems are operative and that their relative importance may differ, according to the species and also to the experimental conditions and tissues used.

17.5.2. METHANOL AS A SOURCE OF LABILE METHYL GROUPS

Du Vigneaud and Verly (1950) and Arnstein (1951) demonstrated in

isotopic tracer experiments *in vivo* that methanol can be utilized by the rat in the formation of biologically labile methyl groups. In earlier nutritional experiments methanol had failed to support the growth of rats on a diet which was free of biologically labile methyl groups and which contained homocysteine (du Vigneaud *et al.*, 1939). The methyl group of methanol is apparently not directly utilized to any great extent in transmethylation reactions (du Vigneaud *et al.*, 1951). This was demonstrated in experiments where rats were administered methanol which was doubly labelled with isotopic carbon and deuterium in the methyl group. The ratio D:^{14}C in the methyl groups of choline isolated from the carcass was found to be lower than in the administered methanol; this indicated that oxidation and subsequent reduction had occurred to produce the labile methyl group from methanol. In transmethylation reactions the methyl group is transferred with its three hydrogen atoms intact (Keller *et al.*, 1949).

Folic acid and vitamin B_{12} are apparently both necessary in these reductions of methanol, which is well in accordance with the recent concept of the functions of these vitamins in the metabolism of one-carbon compounds (e.g. Mudd and Cantoni, 1964). Folic acid and leucovorin have been found to increase the incorporation of ^{14}C from methanol into the choline methyl groups in rats which were deficient in folic acid (Verly *et al.*, 1957, 1952; Verly and Cathey, 1955). On the other hand, the impairment of creatine metabolism in folic-acid deficient mice has been reported to be partially restored by the administration of methanol, which speaks against the direct participation of folic acid in the methyl group syntheiss from administered methanol (Fatterpaker *et al.*, 1953). Rats which were deficient in vitamin B_{12} incorporated less isotope from methanol into the choline methyl groups than the control rats which were given vitamin B_{12}. (Verly and Cathey 1955).

17.5.3. FACTORS INFLUENCING THE METABOLISM OF METHANOL

The effect of ethanol on the metabolism of methanol has in addition to its theoretical interest also a considerable clinical and toxicological importance since most persons who drink methanol consume it with ethanol, as in various denatured spirits. Ethanol has also been used in the therapy of methanol poisoning.

Most statements in the older literature concerning the effect of ethanol on methanol poisoning are purely speculative. Most authors were of the opinion that ethanol increases the toxicity of methanol, although early in the present century ethanol was advocated as a "stimulant" in the therapy of methanol poisoning (Wood and Buller, 1904). According to von Fellenberg (1918) the low urinary excretion of methanol in man found after consumption of pectin-rich food is considerably increased when ethanol is also consumed. He suggested that the oxidation of methanol becomes more difficult when the organism must oxidize large amounts of ethanol at the same time.

In extensive clinical studies Röe (1943, 1946) observed that in certain cases of methanol poisoning in which ethanol had also been consumed the poisoning was less severe than when only methanol was ingested and he regarded the principal cause of the great tolerance to methanol shown by some individuals to be the consumption of ethanol just before or during the methanol poisoning. In cases of uncomplicated methanol poisoning it was never noted that a person drinking less methanol than a companion suffered more severely than the latter, as might be expected to happen if individual predisposition were a factor of decisive importance, as was the opinion earlier. Ethanol, when drunk at the same time as methanol, seemed to prolong the latent period by the same interval as that required for the oxidation of ethanol. Ethanol taken after methanol but before the onset of symptoms was able to prolong the latent period and severe symptoms were found to vanish after the consumption of ethanol late in the course of the poisoning. Röe explained the effect of ethanol by its prevention of the adsorption of methanol on to the oxidative enzymes and consequently of its conversion to formate. On the basis of these observations he advocated the use of ethanol in addition to bicarbonate in the therapy of methanol poisoning.

Orthner (1950) takes an entirely opposite standpoint in regard to the effect of ethanol. He states that ethanol taken simultaneously with methanol dilates the blood vessels and raises the permeability thus increasing the special toxic effects of the metabolic products of methanol.

In white mice the median lethal dose (LD_{50}) for a single intraperitoneal injection of methanol is significantly decreased by ethanol (Gilger *et al.*, 1952). Similar results have also been obtained in experiments with guinea pigs (Moeschlin and Garson, 1955). However, these short term experiments have dealt mainly with the acute toxicity of methanol, which is due to its narcotic effects and is much smaller than that of ethanol, and cannot be used as argument against the therapeutic use of ethanol in the methanol poisoning.

Asser (1914) found in experiments on dogs and rabbits that when he gave ethanol simultaneously with methanol the increased excretion of formate in the urine decreased appreciably. Acetone and amyl alcohol had a similar effect. As explanation he considered the possible increase in the oxidation of formate due to an increased permeability of the cells. This decrease in the urinary excretion of formate after the simultaneous intake of ethanol and methanol has since been confirmed in experiments with dogs and rabbits

(Bastrup, 1947b) as well as in man (Kendall and Ramanathan, 1953). In the latter it was found that when the dosage of methanol was 4 ml, about 10 ml of ethanol per hour were required to suppress totally the increased urinary excretion of formate. Simultaneous administration of acetone had no effect on the metabolism of methanol in rats *in vivo* or *in vitro* (Koivusalo *et al.*, 1958a; Koivusalo, 1958a).

Human experiments were conducted by Leaf and Zatman (1952). In these experiments ethanol was taken at various times during the elimination of a fixed dose, 4 ml, of methanol. A single dose of 15 ml ethanol taken simultaneously caused a marked elevation in the peak concentration of methanol attained in the urine. When ethanol was taken after methanol it effectively arrested the decline in the urinary methanol concentration. After about 2 hr the decline occurred at the original rate. In all of their experiments it was seen that when ethanol was administered during the elimination of methanol, the decline in the methanol concentration in the urine was very slow. The authors considered that this effect is due to inhibition of the oxidation of methanol by alcohol dehydrogenase.

The elimination of methanol from the blood of rabbits (Agner and Belfrage, 1946; Koivusalo, 1956a) and monkeys (Gilger *et al.*, 1959) is distinctly retarded by simultaneous administration of ethanol, as is also the oxidation of carbon-labelled methanol to carbon dioxide in intact rat (Bartlett, 1950b) and in perfused isolated rat liver (van Harken *et al.*, 1965). This inhibition can also be demonstrated in liver slices from rats and guinea pigs (Bartlett, 1950b; Aebi *et al.*, 1957b; Smith, 1961; Tephly *et al.*, 1964) and in rat liver and kidney homogenates (Koivusalo, 1956a, 1959b, 1963b; Tephly *et al.*, 1961). By using a Lineweaver-Burk plot, it has been shown that this inhibition by ethanol of the oxidation of methanol is competitive (Koivusalo, 1956, 1963; Smith, 1961; Tephly *et al.*, 1961).

In the experiments of Zatman (1946) the oxidation of methanol by a partly purified horse liver alcohol dehydrogenase was inhibited by the presence of ethanol as judged from the decreased amount of formaldehyde formed. The inhibition can also be demonstrated in several model systems with a coupled oxidation mediated by catalase (Smith, 1961; Tephly *et al.*, 1961).

Disulfiram retards the disappearance of methanol from the blood of rabbits (Koivusalo, 1956a, b) but no formaldehyde can be found in the blood. Disulfiram also inhibits the utilization of methanol in rat liver homogenates and when given *in vivo* reduces liver catalase activity (Koivusalo, 1956b, 1959c). The lethal dose of methanol is decreased by disulfiram in rats and rabbits (Way and Hausman, 1950) and in mice (Gilger *et al.*, 1952).

Large doses of isoniazid retard the elimination of methanol from blood in rats and rabbits (Koivusalo, 1963a); however, it does not inhibit the *in vitro* oxidation of methanol. Semicarbazide in small concentrations is an effective trapping agent for formaldehyde in *in vitro* experiments, but higher concentrations were inhibitive (Koivusalo, 1963a), possibly due to their effect on catalase (Margoliash *et al.*, 1960).

Added adenosine triphosphate increases the oxidation of methanol in liver homogenates and slices (Koivusalo, 1958b; Smith, 1961); this effect may be mediated through the action of xanthine oxidase on the purine part of ATP. Addition of dinitrophenol inhibits, and pyruvate and lactate increases the oxidation of methanol in rat liver slices (Aebi *et al.*, 1957b). Administration of fructose increases the rate of disappearance of methanol from the blood of rats (Stuhlfauth *et al.*, 1952).

17.5.4. METHANOL IN THE NORMAL HUMAN URINE AND BREATH

Fellenberg (1918) studied the methanol content of different nutrients and came to the conclusion that pectin-rich food, e.g. certain fruits, contains considerable amounts of ester-bound methanol, which can be liberated in the digestive tract and absorbed. He demonstrated that after pectin-rich food there is a clear increase in the urinary excretion of methanol, which normally is almost zero. The presence of small amounts of methanol in normal human urine (Bonnichsen and Linturi, 1962) and breath (Eriksen and Kulkarni, 1963) has been confirmed by sensitive gas chromatographic methods. It is not known whether this methanol is of metabolic origin or comes entirely from the food or from the activity of intestinal flora.

17.6. METABOLISM OF FORMALDEHYDE

17.6.1. FORMATION OF FORMALDEHYDE IN THE METABOLISM

The formation of formaldehyde from methanol has already been discussed. Formaldehyde can also be formed in the animal organism from other and in some cases more physiological compounds than methanol. When various N-methylated compounds are demethylated by animal tissue preparations, it has been found that formaldehyde is formed from the methyl group. Sarcosine is oxidatively demethylated by sarcosine oxidase to glycine and formaldehyde (Handler *et al.*, 1941; Mackenzie, 1950; Mackenzie and Abeles, 1956; Dac and Wriston, 1958). N-methyl-L-amino acids are oxidized to formaldehyde and the corresponding amino acid by a specific demethylase (Ling and Tung, 1948; Moritani *et al.*, 1954). A microsomal demethylase forms formaldehyde from the N- and O-methyl groups of several foreign substances (for a review see Gillette, 1963) and S-methyl compounds can

also form formaldehyde by the action of microsomal enzymes (Mazel *et al.*, 1964). Formaldehyde is also obtained from dimethylaminoethanol, dimethylglycine, serine and from the methyl groups of choline and methionine and from the α-carbon of glycine and β-carbon of serine after incubation with rat liver preparations (Mackenzie *et al.*, 1953; Blakley, 1954; Alexander and Greenberg, 1955; Siekevitz and Greenberg, 1950).

17.6.2. OXIDATION OF FORMALDEHYDE

Formaldehyde is rapidly oxidized to carbon dioxide in experiments *in vivo*. When radioactive formaldehyde was given intraperitoneally to rats 82% of the dose was found in the expired air as carbon dioxide. The urine contained 13–14% of the isotope in the form of methionine, serine and an adduct formed from cysteine and formaldehyde (Neely, 1964). Intravenous or oral administration of formaldehyde to dogs leads to rapid appearance of increased formate in the plasma while only very little formaldehyde can be recovered (Malorny *et al.*, 1965). Infused formaldehyde is also rapidly eliminated from the blood of rats (Campos *et al.*, 1961).

There is very little information concerning the direct oxidation of formaldehyde in animal tissue preparations. Rat liver mitochondria are able to oxidize formaldehyde to formate in small concentrations, but concentrations over 1 mM are inhibitory (Walkenstein and Weinhouse, 1953; Kini and Cooper, 1960; Frisell and Sorrell, 1967). There is also a coupled esterification of inorganic phosphate giving a P/O ratio about 2. Formaldehyde is oxidized to formate by beef retinal extracts in the presence of NAD and reduced glutathione (Kinoshita and Masurat, 1958), but no formaldehyde is oxidized by beef or monkey retinal mitochondria (Kini and Cooper, 1962). Homogenates from various rat tissues are able to utilize added formaldehyde and liver was the most active tissue (Koivusalo, 1956a). Addition of disulfiram had only s slight inhibitory effect.

It has generally been assumed that the enzymes responsible for the oxidation of formaldehyde are the same which oxidize other aldehydes and the rather meagre information we have regarding them has mostly been obtained from the specificity studies of acetaldehyde-metabolizing enzymes. The enzymes of animal origin which are known to catalyze *in vitro* the oxidation of formaldehyde are aldehyde dehydrogenase, alcohol dehydrogenase, xanthine oxidase, aldehyde oxidase, glyceraldehyde-3-phosphate dehydrogenase, catalase and formaldehyde dehydrogenase. From these enzymes only the formaldehyde dehydrogenase is relatively specific for formaldehyde.

Battelli and Stern (1910a,b) demonstrated that aminal tissues catalyze the dismutation of two moles of aldehyde to one mole of alcohol and one mole

of fatty acid. The soluble enzyme responsible for this reaction was named aldehyde mutase (Parnas, 1910) and NAD was found to be required for this reaction (von Euler and Brunius, 1928). Formaldehyde was also rapidly dismutated to methanol and formic acid in the presence of NAD (Adler *et al.*, 1938). The isolation by Racker (1949) of NAD-dependent *aldehyde dehydrogenase* from liver has led to the conclusion that the aldehyde mutase action is probably due to the combined action of both alcohol dehydrogenase and aldehyde dehydrogenase and that there apparently exists no such enzymic entity as aldehyde mutase. The liver aldehyde dehydrogenase reacts also with formaldehyde, although only at one-half of the rate as with acetaldehyde (Racker, 1949).

Kendal and Ramanathan (1952) have studied the dismutation reaction of formaldehyde catalyzed by the alcohol dehydrogenase preparation of Lutwak-Mann (1938) in the presence of NAD. When methanol or ethanol was also added, the disappearance of formaldehyde was greatly accelerated and a volatile ester was formed. The reaction was quite insensitive to iodoacetate and this made the participation of aldehyde dehydrogenase in this reaction doubtful. It was therefore suggested that alcohol dehydrogenase may be capable of catalyzing the complete dismutation. Abeles and Lee (1960) have studied further the formaldehyde dismutation reaction and found that highly purified horse liver *alcohol dehydrogenase* catalyzes the formation of formate from formaldehyde and the formation of methyl formate from formaldehyde and methanol. It was suggested that it is the hydrated form of formaldehyde and the formaldehyde-methyl-hemiacetal which are oxidized to formate and methylformate, respectively. All attempts to separate the formaldehyde dehydrogenase activity from alcohol dehydrogenase failed. Gupta *et al.* (1966) and Gupta and Robinson (1966) have also reported on similar coupled oxidoreductase activity of crystalline horse liver alcohol dehydrogenase.

Most animal tissues contain an enzyme, *xanthine oxidase*, which oxidizes both purines and aldehydes, reacting very rapidly with formaldehyde (Corran *et al.*, 1939). It is a metalloflavoprotein which contains molybdenum (Mackler *et al.*, 1954). Another flavoprotein enzyme acting on aldehydes was found in pig liver by Gordon *et al.* (1940) and named *aldehyde oxidase*. It contains molybdenum and is very similar to xanthine oxidase but has no activity towards purines (Mahler *et al.*, 1954), and can also oxidize formaldehyde to some extent (Carpenter, 1951).

Glyceraldehyde-3-phosphate dehydrogenase catalyzes the oxidative phosphorylation of D-glyceraldehyde-3-phosphate to 1,3-diphosphoglyceric acid (Warburg and Christian, 1939; Cori *et al.*, 1948). It has also been found to catalyze the oxidation of other aldehydes like acetaldehyde and formaldehyde in the presence of NAD (Harting, 1951; Harting and Velick, 1954;

Nygaard and Sumner, 1952). The affinity of the enzyme for acetaldehyde has been found to be low and the physiological significance of this enzyme in the oxidation of these aldehydes is uncertain.

It has also been suggested that *catalase* takes part in the oxidation of formaldehyde *in vivo* (Chance, 1951) since the catalase-hydrogen peroxide complex reacts *in vitro* rapidly with formaldehyde in its methylene glycol form (Chance, 1948).

Strittmatter and Ball (1955) have identified and partly purified a specific NAD-dependent *formaldehyde dehydrogenase* in chicken and beef liver preparations. Reduced glutathione is a specific cofactor in this system and it is suggested that formaldehyde reacts first with reduced glutathione to form S-hydroxymethyl-glutathione, which is then attached to the enzyme and oxidized. The product can be released from the enzyme either directly as formate, or as a free thiolester, which is subsequently hydrolyzed to formate and glutathione. Kinoshita and Masurat (1958) have presented evidence which suggests the occurrence of this enzyme in cattle, rabbit and human retinas. It has also been purified from rat brain and liver, and was found to react with methylglyoxal and some other α-ketoaldehydes as well as with formaldehyde (Koivusalo and Uotila, 1968).

Matthies (1957a, b, 1958) has described a NAD-dependent *aldehyde dehydrogenase* in red blood cells, which is very active with formaldehyde but reacts also with other aldehydes. The enzyme is inhibited by disulfiram (Matthies, 1956) and is apparently different from the formaldehyde dehydrogenase of Strittmatter and Ball (1955) and from the aldehyde dehydrogenase of Racker (1949). Rapid adsorption and subsequent oxidation of formaldehyde to formate by human erythrocytes *in vitro* has been also demonstrated by Jung and Onnen (1955) and Malorny *et al.* (1965).

17.6.3. CONDENSATION REACTIONS OF FORMALDEHYDE

As a very reactive aldehyde, formaldehyde can readily undergo various condensation reactions with other substances. These reactions are of interest in the field of intermediary metabolism and may have considerable influence on the further metabolism of formaldehyde formed in the tissues from methanol and other sources.

The addition of pyruvate or other α-ketoacids has been found to enhance greatly the rate of disappearance of formaldehyde in rat liver homogenates and mitochondrial preparations (Mitchell and Artom, 1951; Mitchell and Artom, 1952). The reaction was interpreted as a condensation between formaldehyde and pyruvate. The responsible enzyme is probably identical with the *formaldehyde-pyruvic acid carboligase* of Hift and Mahler (1952),

which catalyzes an aldol condensation between formaldehyde and pyruvic acid. This enzyme is present in rat kidney and liver. According to Meyerhof *et al.* (1936) *aldolase* can catalyze the condensation of formaldehyde with dihydroxyacetone phosphate. Charalampous and Müller (1953) studied this reaction with the aid of isotopic formaldehyde and found that a soluble enzyme system from rat liver catalyzes the formation of erythrulose-1-phosphate by the condensation of formaldehyde with a triose phosphate. This activity was found in several rat tissues and the enzyme has been partly purified (Charalampous, 1954). The enzyme was thought to be distinct from the Meyerhof-Lohmann aldolase and was called phosphoketotetrose aldolase. Peanasky and Lardy (1958) have reported, however, that crystalline aldolase from bovine liver or rabbit muscle can condense dihydroxyacetone phosphate and formaldehyde. A soluble enzyme system, which catalyzes the condensation of formaldehyde and erythrulose-1-phosphate to ribose phosphate has also been described in rat tissues (Charalampous, 1955). All these aldol condensation type reactions may partly aid in the utilization of accumulated formaldehyde but probably do not have much physiological significance.

The oxidation of formaldehyde in various rat liver preparations is enhanced by the addition of aminoguanidine (Bernheim, 1950; Michels *et al.*, 1962), but the oxidation of acetaldehyde is not affected. The oxidation of the formaldehydeaminoguanidine complex formed is inhibited by the addition of a nitrogen mustard, a phosphoramide, or by aminopterin treatment (Miller, 1953, 1954). Aminoguanidine decreases the acute toxicity of formaldehyde to mice but has no effect on the acute toxicity of methanol (Zauder, 1950). This effect can be due to increased oxidation of formaldehyde or also to a lower toxicity of the formed complex, which has been found to be a five-membered heterocyclic compound and is oxidized by rat liver mitochondria to a resonance form of 3-amino-1,2,4-triazole (Michels *et al.*, 1962).

The accumulation of formaldehyde formed from sarcosine when incubated with mitochondria is reduced when homocysteine is added and a new compound is formed, which was concluded to be 1,3-thiazane-4-carboxylic acid. Added D-thiazane-4-carboxylic acid was easily metabolized but the L-form was metabolically inert (Wiston and Mackenzie, 1957). Formaldehyde and cysteine can also condense in mitochondrial systems to form thiazolidine carboxylic acid, which is then dehydrogenated by an apparently specific oxidase to yield the corresponding thiazoline, which in turn is hydrolyzed to N-formylcysteine. Thus, in the course of its oxidation the formaldehyde carbon undergoes a transfer from the sulphur to the amino group of cysteine (Mackenzie and Harris, 1957).

Formaldehyde can also under certain conditions combine with the guanidine and amide groups and with the indolyl ring. It also reacts rapidly in

neutral or alkaline solutions with amino groups forming aminomethylol groups (French and Edsall, 1945; Fraenkel-Conrat and Olcott, 1946).

17.7. METABOLISM OF FORMATE

It has been known for a long time that formate can readily be oxidized in animal tissues to carbon dioxide (Battelli and Stern, 1908). Plaut *et al.* (1950) demonstrated that injected formate-^{14}C was oxidized to $^{14}CO_2$ and that a major portion of the formate was incorporated into serine. They postulated that formate oxidation occurs by incorporation into serine followed by oxidation of serine to pyruvate and the degradation of pyruvate to carbon dioxide. The direct oxidation of formate to carbon dioxide without the intermediate formation of serine was demonstrated later by Plaut and Lardy (1950) and Kruhoffer (1951). Mathews and Vennesland (1950) have shown that in pea extracts formate is oxidized by a NAD-specific formate dehydrogenase, whereas in rat liver extracts the formate oxidation required AMP or ATP, and NAD was not necessary.

Formate can also be oxidized *in vitro* by the catalase-hydrogen peroxide complex (Chance, 1948 1950) and Nakada and Weinhouse (1953) and Weinhouse (1955) have presented evidence that the oxidation of formate *in vivo* is due to a coupled oxidation by catalase and suggested that the findings of Mathews and Vennesland (1950) can be explained by a coupled oxidation resulting from the combined action of xanthine dehydrogenase and catalase. Aebi *et al.* (1957a) and Oro and Rappoport (1959), with several other authors have studied further the oxidation of formate to carbon dioxide by different animal tissue preparations and also came to the conclusion that it is the result of a peroxidatic reaction of catalase.

17.8. FORMALDEHYDE AND FORMATE IN THE METABOLISM OF "ONE-CARBON" COMPOUNDS

Formaldehyde and formic acid are important in the metabolism of the so-called "one-carbon" compounds, where one-carbon units at the oxidation levels of methyl, hydroxymethyl and formyl groups can be converted from one oxidation level to another and transferred enzymically to various acceptors. Folic acid is the common coenzyme for all these reactions. These complex interrelationships are presented in the scheme of Fig. 1. For a detailed discussion of the metabolic reactions of the one-carbon compounds the reader is referred to the several recent reviews by Mudd and Cantoni (1964), Huennekens and Osborn (1959) and Jaenicke and Wilmanns (1963).

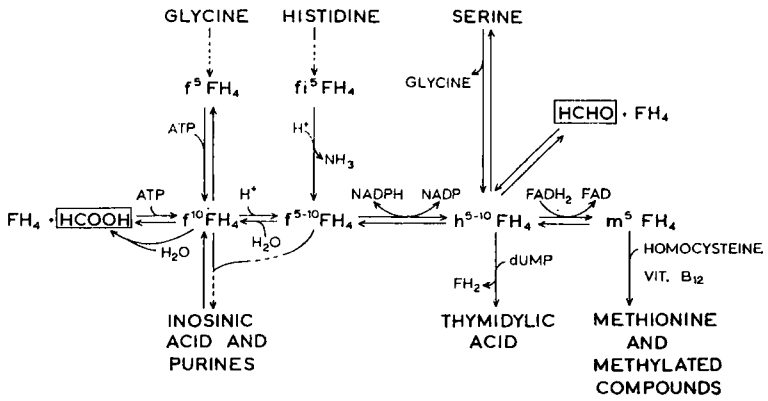

FIG. 1. The metabolic relationships of formate, formaldehyde and tetrahydrofolic acid. Modified after Mudd and Cantoni (1964). FH_4, tetrahydrofolic acid; $f^{10}FH_4$, N^{10}-formyltetrahydrofolic acid; f^5FH_4, N^5-formyltetrahydrofolic acid; $f^{5-10}FH_4$, N^5,N^{10}-metheynyltetrahydrofolic acid; fi^5FH_4, N^5-formiminotetrahydrofolic acid; $h^{5-10}FH_4$, N^5,N^{10}-methylenetetrahydrofolic acid.

Free formaldehyde and formate can enter these reactions after attachment to tetrahydrofolic acid catalyzed by the specific activating enzymes.

A *formaldehyde activating enzyme* obtained from pigeon liver has been described by Osborn *et al.* (1957). This enzyme catalyzes the formation of N^5, N^{10}-methylene-tetrahydrofolate (active hydroxymethyl) from formaldehyde and tetrahydrofolate, and no ATP is needed. It can be detected in a number of avian and mammalian tissues (Huennekens and Osborn, 1959) but its extreme lability has hindered any extensive purification and we have little information of its possible physiological significance. A *formate activating enzyme* has been purified from the same source (Jaenicke and Brode, 1961); it catalyzes the ATP-dependent formation of N^{10}-formyltetrahydrofolate from formate and tetrahydrofolate.

Through this group of reactions the carbon atom of formaldehyde can be incorporated into the methyl groups of choline in the intact rat and so be a precursor of labile methyl groups (du Vigneaud *et al.*, 1950; Jonsson and Mosher 1950; du Vigneaud *et al.*, 1951). Formaldehyde can also be used in rat liver homogenates for the synthesis of the methyl group of methionine (Siegel and Lafaye, 1950). Also the ureido carbon atoms 2 and 8 of the purine ring can be derived from formaldehyde (Marsh, 1951; Greenberg, 1951). Various liver preparations utilize formaldehyde for the synthesis of the *beta*-carbon atom of serine (Siegel and Lafaye, 1950; Kisliuk and Sakami, 1954, 1955; Blakley, 1954; Alexander and Greenberg, 1955).

17.9. TOXIC AND LETHAL DOSES

The lethal dose of methanol for most experimental animals is relatively high, in acute experiments higher than that of ethanol. The approximate lethal single dose for dogs and rabbits is about 8 g/kg (Hunt, 1902; Haskell *et al.*, 1921), for rats 10 g/kg (Gilger and Potts, 1955; Alder *et al.*, 1938). LD_{50} for a single intraperitoneal injection is 10.5–11.0 g/kg for white mice (Gilger *et al.*, 1952). Much lower values for monkeys than other experimental animals were reported by Potts (1955) and Gilger and Potts (1955). The minimum lethal dose of methanol was 2–3 g/kg and almost all animals died at dose levels higher than 3 g/kg. However, Cooper and Felig (1961) have reported also for monkeys a minimum lethal dose of about 7 g/kg, while the LD_{50} was within the range of 7–9 g/kg, which is about the same as that reported for lower experimental animals. In contrast to the results of Potts (1955) no monkeys in the experiments of Cooper and Felig died after 3 g/kg methanol.

The widely-varying statements in the literature concerning the minimum toxic and lethal doses of methanol in human cases of poisoning partly reflect the uncertain information regarding the consumed amounts of methanol and ethanol, which usually is obtainable in these cases, and the different time intervals between the ingestion of methanol and coming under observation. Even a dose of 4 ml of methanol has been reported to have caused visual disturbances and even blindness and about 30 ml can be fatal (Wood and Buller, 1904; Ziegler, 1921; Bennett *et al.*, 1953; Soehring *et al.*, 1954); on the other hand ingestion of much larger amounts of methanol, up to 500 ml or more (Soehring *et al.*, 1954) has not necessarily proved fatal. A remarkable case is the experiment of Francheschi (Orthner, 1950) in which he himself allegedly had ingested 32 g of pure methanol daily for 274 days without any toxic symptoms. In untreated methanol poisoning the dose generally accepted as lethal is about 0.8 to 1.5 g/kg.

17.10. PATHOGENESIS OF METHANOL POISONING

Since the end of the last century, when the first cases of human methanol poisoning were described, the cause of the specific toxicity of methanol has been extensively debated. Although many different hypotheses, some purely speculative and others based on clinical or experimental observations, have been presented, we still do not know exactly how the specific toxic action of methanol is brought about. It seems, however, to be rather well-established that formaldehyde is the compound actually responsible for these effects.

It was earlier a rather generally accepted opinion that methanol itself is not very toxic and that the toxicity depends on various impurities in the methanol (Stadelmann and Magnus-Levy, 1912; Igersheimer and Verzar, 1913; Hämäläinen and Teräskeli, 1928; and others). This theory, however, had to be abandoned after it was repeatedly shown that chemically pure, synthetic methanol was the cause of numerous poisonings (e.g. Reif, 1923; Alder *et al.*, 1938).

The acute toxicity of alcohols increases in the homologous series with the number of carbon atoms, as has been shown to apply to methanol and ethanol (Fuhner, 1905; Weese, 1926). However, some authors have considered methanol itself to be responsible for its specific toxicity. Pohl (1893) had shown that administered methanol remains in the organism for a long time and this accumulation was regarded as the basis of the toxicity by Egg (1926).

At a quite early date the oxidation products of methanol, formaldehyde and especially formic acid were held responsible for its toxicity. Pohl (1893) had demonstrated that the administration of methanol increased the urinary excretion of formate and many authors attributed the poisoning to formic acid (e.g. Harnack 1912; Leo, 1925). Röe (1943, 1946, 1955) has presented the hypothesis that formic acid combines with the iron in hemoglobin and in oxidative enzymes of the cells and the consequent hypoxia is the primary factor in the toxicity of methanol in man, and that retina would be the most sensitive tissue. However, there is no direct experimental evidence to support this theory and we know that formate in the concentrations produced in methanol poisoning is not very toxic for the cell metabolism.

Formaldehyde was regarded as the primary toxic agent by many authors before there was any evidence for its formation in the organism from methanol (e.g. Rabinowitch, 1922). Its great reactivity was regarded as the reason for the failure to demonstrate formaldehyde in human and experimental cases of methanol poisoning. After Keeser (1931a) obtained definitely positive tests for formaldehyde in methanol poisoning many authors became inclined to think that the specific toxicity of methanol is due to the slow formation of formaldehyde inside the cells (Flury and Wirth, 1936; Orthner, 1950). Kendal and Ramanathan (1952) have suggested the possibility that after primary conversion of part of the methanol into formaldehyde a secondary conversion of the latter into methyl formate may occur by the semiacetal dehydrogenase mechanism which they had observed *in vitro*. The preferential fat-solubility of the ester would then result in the localization and specific effects of methanol poisoning. Methyl formate is, however, a much less effective inhibitor of retinal metabolism than formaldehyde (Kini and Cooper, 1962).

The characteristic latent interval before the onset of clinical symptoms of poisoning and the good therapeutic effects obtained by inhibiting the methanol oxidation by treatment with ethanol, are also evidence that one of the metabolites of methanol is the actual toxic agent, but do not differentiate them. There is, however, much accumulated evidence that formaldehyde is more toxic than formate or methanol itself for many enzyme systems in the cell. Formaldehyde inhibits the oxidation of glucose and succinate in rat liver preparations in much smaller concentrations than formate or methanol (Watts, 1951). Similarly, formaldehyde is most toxic to retinal glycolysis and respiration *in vitro* (Potts and Johnson, 1952; Leaf and Zatman, 1952; Kini and Cooper, 1962). Potts and Johnson (1952) and Cooper and Marchesi (1959) have concluded that formaldehyde acts as a inhibitor of retinal hexokinase, which would account for the inhibition of glycolysis, but in later experiments Kini and Cooper (1962) could not reproduce this effect. Administration of formaldehyde *in vivo* to rabbits also abolishes the b-wave in the electroretinogram at a much smaller dose level than formate, methanol, ethanol or acetaldehyde (Praglin *et al.*, 1955). In rat liver mitochondria low concentrations of formaldehyde inhibit mainly the energy transfer pathway between the respiratory chain and the site of adenosine triphosphate synthesis and has little effect on uncoupled respiration (Van Buskirk and Frisell, 1967). According to the recent experiments of Tyler (1968) this effect may, however, be due to inhibition by formaldehyde of the entry of phosphate into mitochondria.

To account for the specific toxic action of methanol on retina Cooper and Kini (1962) have proposed the following hypothesis. Methanol is oxidized in the retina to formaldehyde by the retinal alcohol dehydrogenase and formaldehyde then uncouples oxidative phosphorylation in retinal mitochondria and may also inhibit anaerobic glycolysis. Both these effects lead to a deficiency of adenosine triphosphate, which then causes degeneration of the retinal cells concerned with vision and thus produces visual disturbances or blindness as the end result. They have reported inhibition of coupled phosphorylation in retinal mitochondria of beef and monkey (Kini and Cooper, 1960, 1962) by 0.5–2 mM formaldehyde, with little effect on electron transport. Formaldehyde at concentrations which reduced phosphorylation in mitochondria also markedly lowered the incorporation of radioactive phosphorus into the phospholipids of the intact retina, which is a process known to be adenosine triphosphate-dependent (Kini *et al.*, 1962). Acetaldehyde in much higher concentrations did not have similar effects. In mitochondria from beef and monkey liver, formaldehyde had no uncoupling effect but was actually metabolized (Kini and Cooper, 1960). No difference was found between the retinal mitochondria of monkey and beef to account for the known species difference in the toxicity of methanol. However, this difference may reside in the relative activities of the methanol- and formaldehyde-metabolizing enzymes in the retinas of the different species. On the other hand it is known that recognized uncoupling agents like dinitrophenol do not have effects on retina like those of methanol. The possible role of adenosine triphosphate in the visual process is also not known. Further experimental evidence is needed on the effects of methanol and its metabolites on the retinal metabolism before the actual point of attack in methanol intoxication can be ascertained.

The acidosis which is constantly observed in human cases of methanol poisoning apparently plays an important role in the development of the typical manifestations of the poisoning (Harrop and Benedict, 1920; Rabinowitch, 1922; Röe, 1943, 1946). The specific toxic effects cannot, however, be due to acidosis as such, because no similar effects are seen in other types of severe acidosis, and adequate correction of the acidosis does not always stop the evolution of the specific symptoms of methanol poisoning. In experimental animals only slight or no acidosis has been observed (Haskell *et al.*, 1921; Loewy and Münzer, 1923; Leo, 1925). On the basis of a critical review of earlier literature and their own experiments Gilger *et al.* (1955) have concluded that the typical acidosis is seen only in Primates. There are, however, reports that no acidosis developed in monkeys severely poisoned with methanol (Cooper and Felig, 1961). The acidosis is often regarded as being directly due to formic acid, but the maximum amounts of formic acid which can possibly be formed are not sufficient to account for the marked diminution in the blood alkali reserve which is often seen (Egg, 1927; Röe, 1946). Although a considerable increase in lactate concentration has been observed in blood (Tonning, 1945; Branch and Tonning, 1945; Röe, 1946) and urine (Harrop and Benedict, 1920; van Slyke and Palmer, 1920) in methanol poisoning, the majority of the increase in the titratable organic acids in the urine was not due to lactic, formic or acetoacetic acids, and the nature of the major acid(s) is unknown. The methylenation of amino groups in amino acids and proteins has also been suggested as a factor in the development of the acidosis (Rabinowitch, 1922; Orthner, 1950).

17.11. SPECIES DIFFERENCES

There is, apparently, a fundamental difference between the toxic effects of methanol in man and the toxic effects in non-primate animals as has been pointed out by Röe (1946, 1955) and Gilger and Potts (1955). On the basis of a critical review of the literature and their own experiments they concluded that acidosis and eye disturbances, the typical symptoms of human

poisoning, are usually not found in lower animals intoxicated with methanol. In a series of investigations Gilger, Potts and their coworkers demonstrated that the picture of methanol poisoning in the monkey, however, resembles very much that of human poisoning with acidosis and visual disturbances (Gilger and Potts, 1955; Potts, 1955; Potts *et al.*, 1955; Gilger *et al.*, 1956, 1959). The effect of methanol on non-primate animals is according to them mostly due to a narcotic effect of methanol similar to that of ethanol. This difference was not realized by the earlier workers and may have contributed to the controversial statements in the literature, which were made when results from animal experiments were compared to results obtained from human cases of poisoning by methanol. The reason for this species difference is still unknown. No significant biochemical differences are found between the retinas from primates and lower animals. There seems to be a difference, however, in the pathways of methanol oxidation in primates and non-primates, but it is difficult to see how that could produce the difference in the toxic effects.

17.12. HUMAN METHANOL POISONING

17.12.1. SYMPTOMS AND SIGNS

Three stages can be differentiated in primate methanol poisoning according to Gilger *et al.* (1956): (1) An initial narcotic stage, which is seen in all animals and is similar to that produced by ethanol. (2) The second stage which is manifested after a latent period is seen only in primates and is connected with an uncompensated metabolic acidosis caused indirectly by an oxidation product of methanol (formaldehyde). (3) The third stage may become manifest somewhat later also only in primates and is characterized by visual disturbances and central nervous system lesions and is also a result of poisoning by the oxidation product of methanol. The following description of the methanol poisoning is mainly based on the clinical reviews by Röe (1946), Bennett *et al.* (1953) and Benton and Calhoun (1952); the reader is referred to them for a detailed discussion.

There is, characteristically, a marked variation in the response to a given dose and in the manifestations of the symptoms in the human methanol poisoning. This may be due partly to the difficulty of getting exact anamnestic facts and partly to the simultaneous ingestion of different amounts of ethanol. If pure methanol has been ingested the subjective inebriating effect is much less than with ethanol. There is a typical asymptomatic latent period before the manifestation of toxic symptoms, of from 1 to 72 hr; usually it is about 10–15 hr. The length of the latent period is somewhat dependent on the amounts of methanol and ethanol ingested. Then follow in rapid sequence, nausea, vomiting, dizziness, headache, failing eyesight and deep respiration

concomitant with the severe metabolic acidosis. The skin may by cyanotic and there is often violent abdominal pain accompanied by rigidity of the abdominal muscles and tenderness. In severe poisoning the patient becomes rapidly soporous and coma develops. Death usually occurs as a result of apnoic respiratory arrest. Terminal convulsions are also common. There may be lethargy, confusion or maniacal reactions.

Visual disturbances, which are typical of methanol poisoning, are seen in most patients and range from mild photophobia and blurred or indistinct vision to total loss of light perception. The pupils are dilated and often react sluggishly or not at all to light. Ophthalmoscopic examination reveals typical changes in most patients, although a small number of fatal cases have been reported to have had normal eyegrounds. The visible retinal changes are hyperemia of the optic disc and superficial edema of the papilla and surrounding retina and later a simple atrophy of the optic nerve. The earliest central field defect is usually a cecocentral scotoma and later more complex types of visual field defects appear. Excavation of optic discs resulting in glaucomatous cupping with normal intraocular pressure has been described (Lasco, 1958). Partial or complete recovery of the initially reduced visual acuity is observed in most patients but residual ocular defects up to total blindness persist in up to 50% of the non-fatal cases.

17.12.2. PATHOLOGICAL AND LABORATORY FINDINGS

Pathologic changes in the eye in acute methanol poisoning have been described by several authors but in many instances the noted changes can equally well be explained by *post mortem* degeneration. Very few morphological abnormalities have been found in specimens from patients who died in the early stages of the poisoning (Benton and Calhoun, 1952), but a severe retinal edema is described by Muller (1950). At a later stage degeneration of retinal ganglion cells has been generally noted (Pick and Bielschowsky, 1912; Macdonald, 1930; Menne, 1938; Fink, 1943; Röe, 1949; Tonning, 1945).

Pathologic changes in other organs are mostly unspecific and not pronounced. However, there is usually cerebral edema and Orthner (1950) has described a seemingly specific symmetric putamen necrosis in 41 from 124 cases. It has been seen also in monkeys (Potts *et al.*, 1955). A marked contraction of the lower ileum and of parts of the colon is often found on autopsy (Röe, 1946) and the presence of a hemorrhagic pancreatitis has been described in several cases (Bennett *et al.*, 1952; Buchans, 1930).

The most typical laboratory findings are evidence of severe metabolic acidosis which is often uncompensated (low plasma standard bicarbonate value, low blood pH) and increased amounts of methanol and formate in

blood and urine. There may be moderate ketonemia, acetonuria and glycosuria, and albuminuria is often found. Hypokalemia has been present in some cases (Erlanson *et al.*, 1965; Tonning *et al.*, 1956). Cerebral blood flow and oxygen consumption are reduced (Battey *et al.*, 1956). Elevated cerebrospinal fluid pressure has been found in some cases (Reiner, 1950). Small abnormalities in the electrocardiogram have also been noted. Serum amylase is often elevated as a result of pancreatitis (Bennett *et al.*, 1952).

17.12.3. TREATMENT OF METHANOL POISONING

Correction of the severe acidosis, which is almost always present in human methanol poisoning, by alkali therapy is of prime importance in the treatment of the poisoning. It was first suggested by Harrop and Benedict (1920) and several reports have since made it clear that the toxic symptoms and the mortality could be reduced greatly by prompt use of parenteral alkali (Röe, 1943, 1946, 1950; Chew *et al.*, 1946; Kobro, 1946; Keeney and Mellinkoff, 1951; Bennett *et al.*, 1953; Erlanson *et al.*, 1965). The alkali therapy, however, is only symptomatic and adequate correction of acidosis in patients with severe methanol poisoning will not invariably save life or vision (Benton and Calhoun, 1952). It seems that no therapy can cure severe cerebral and visual disturbances after they have fully developed and it is important that the treatment must be started as soon as possible if methanol poisoning is suspected. The patients should be kept under careful observation for some days because the acidosis tends to reappear.

The theoretical basis and the history of the treatment with ethanol has been described earlier in this chapter. The use of ethanol was already described in 1904 by Wood and Buller but was not much used until Röe (1943, 1946, 1950) on the basis of his extensive clinical studies advocated its use as adjunct to the alkali therapy. Although there has been some negative opinions on the use of ethanol in the treatment of methanol poisoning (e.g. Orthner, 1950; Moeschlin and Garson, 1958) it seems at the present time to be generally accepted that the administration of ethanol is a very important measure in the treatment of methanol poisoning (Chew *et al.*, 1946; Moeschlin, 1964; Erlanson *et al.*, 1965; Wieth and Berthelsen, 1964). It is recommended to use small repeated doses of ethanol in amounts which would keep the blood level at about 0.1 %, which will almost totally block the oxidation of methanol to its toxic metabolites and give time for its elimination via the lungs and in the urine (Röe, 1950; Erlanson *et al.*, 1965). It must be pointed out, however, that the narcotic effects of both alcohols are synergistic and many patients have taken also considerable amounts of ethanol together with methanol.

If possible the blood ethanol and methanol levels should be checked during the therapy.

Hemodialysis or peritoneal dialysis has been used with some success in several cases of human methanol poisoning to hasten the elimination of methanol as suggested by Marc-Aurele and Schreiner (1960) on the basis of experiments with dogs. It is usually used as a supplement to the treatment with ethanol and alkali (Stinebaugh, 1960; Shinaberger, 1961; Wieth and Jörgensen, 1961; Erlanson *et al.*, 1965; Feltes *et al.* 1962; Cowen 1964; Wieth and Berthelsen, 1964).

In addition to the above therapy nutrition and the water and electrolyte balance should be maintained. It is often recommended to keep the eyes protected from strong light although there is no clear basis for it. Very many other forms of treatment have been employed clinically and recommended for use with little or no evidence to establish their value. More detailed schemes of the treatment can be found in the articles by Röe (1950), Chew *et al.* (1946), Erlanson *et al.* (1965), Wieth and Berthelsen (1964) and in the book by Moeschlin (1964).

17.13. REFERENCES

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