Lung-excretion.
Protection

THE METABOLISM AND TOXICITY OF METHANOL

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A. *Metabolism of Methanol*

Absorption and distribution in the organism. Although poisoning with methanol is usually caused by oral intake, poisoning after inhalation of methanol vapor has been described several times (52).

Animal experiments have shown that considerable amounts of methanol can be absorbed through the lungs. Absorption from the intestinal tract takes place within a few hours after ingestion. Haggard and Greenberg (17) found absorption complete within 6 hours, even after high doses. Agner and Behrang (1) found a maximal blood concentration of methanol 4 hours after administration of 2.2 g. of methanol per kg. to rabbits.

Several experiments have proven that methanol is distributed evenly in the tissues according to their water content. The lowest concentrations were found in bone marrow and fat (17, 53). Recent investigations with C^{13}-labelled methanol showed the highest concentrations in the liver, the kidneys and the gastro-intestinal tract, and only small concentrations in the muscles, the fat and the brain (3).

Elimination in expired air. A much larger proportion of methanol than of ethyl alcohol is excreted by the lungs. However, various authors differ in their statements about the proportion of methanol excreted in this way. In dogs given 5 g./kg. more than 50 per cent (2), and in rats given 1 to 5 g./kg. more than 70 per cent (17) was eliminated in the expired air during several days after administration. In rats given 1 g. of C^{13}-labelled methanol per kg. body weight, Bartlett (3) found only 14 per cent excreted unchanged in the expired air during 48 hours. Haggard and Greenberg (17) showed that elimination through the lungs is a simple diffusion process dependent upon the concentration in the blood and the respiratory volume. They found that up to 25 per cent of the expired methanol might condense on the fur of the experimental animal. To avoid this source of error, they collected the expired air through a tracheal cannula. This technique may partly explain the high percentage of methanol found in the expired air by Haggard and Greenberg, because the tracheal cannula may have caused some irritation with consequent hyperventilation. In contrast to ethyl alcohol they did not find Widmark's β factor (decrease in blood concentration per minute expressed in mg. per ml. of blood) constant. Instead, it varied almost proportionately to the blood concentration. This effect could not be entirely explained by the dependence of pulmonary excretion on concentration. The authors concluded that the rates of elimination by routes other than pulmonary excretion and by tissue oxidation were not constant, but were also dependent on blood concentration. However, Bartlett's recent experiments with labelled methanol have demonstrated that the rate of oxidation to CO_2 is independent of concentration. (See below.) The

decrease in blood concentration was similarly variable in rabbits. Agner and Beifrage (1) found that β was 0.0017 with a blood concentration of 2 mg. /ml., but only 0.0006 when the concentration was below 1 mg. /ml.

Methanol is also excreted by the kidneys in amounts up to 10 per cent of the administered dose (27, 28).

Elimination by means of oxidation. Since Pohl's investigations in 1893 (36) it has been clear that a part of the administered methanol is oxidized to formic acid, and that part of the formic acid is further oxidized to carbon dioxide and water. After administration of 4.3 g. /kg. a dog excreted 4 per cent as formate. In similar experiments, Asser (2) recovered 5 to 8.7 per cent after 2 g. /kg. Pohl reported that when methanol was given orally or intravenously the maximal amount excreted on the second and third day was independent of the route of administration. Lund (28) recovered somewhat higher amounts, about 20 per cent, when the bladders of the experimental animals were emptied frequently so as to prevent reabsorption through the bladder epithelium. The highest concentration of formic acid in the blood (50 mg. per cent) was not reached until 40 to 50 hours after the administration of 2 g. /kg. methanol to a dog.

Several experiments on the capacity of the organism to oxidize formate have been made. After the administration of 2 g. of formate to dogs, Asser recovered 15 to 20 per cent in the urine (2). In similar experiments Pohl recovered between 5.8 and 18 per cent. A dose of 1 g. was almost completely oxidized (36). In dogs, Lund (28) found nearly half of a dose of 2 g. excreted unchanged if reabsorption through the bladder was prevented. Determination of the renal clearance made it seem likely that formic acid is not a threshold substance. Rabbits have a higher capacity to oxidize formate than dogs, since practically no formate is excreted in the rabbit's urine after doses of 0.676 g. /kg. (27).

Pohl gave an experimental subject 25 ml. of methanol and found the highest amount of formic acid excreted in the urine on the second day, which is when the symptoms of methanol poisoning are usually most severe. About 3 per cent of the methanol was excreted as formic acid.

It is generally assumed that both *in vivo* and *in vitro* methanol is first oxidized to formaldehyde. Apparently the formaldehyde is further oxidized to formic acid very rapidly, since most investigators have not been able to demonstrate even traces of formaldehyde in the organs during the oxidation of methanol (36). Neither is it found in blood, urine or expired air (3). Pohl reported that the liver was especially active in the oxidation of methanol and formaldehyde, but that it was practically unable to oxidize formate. More recent experiments, however, indicate that the liver has a considerable capacity to oxidize formate. Bartlett (3) measured the production of CO_2 in Warburg experiments and found that the relative capacities of rat tissue slices to oxidize methanol were: liver 100, kidney 34, intestine 8, heart 1, diaphragm 1 and brain 0.

During methanol poisoning in man the concentration of formic acid in the blood is quite variable. In 5 lethal cases it ranged from 9 to 68 mg. per cent (29). In three other patients who also died it ranged from 5.7 to 19 mg. per cent (40). When it is added that the methanol concentration in the blood in 23 lethal cases

varied between 51 and 274 mg. per cent (40); it becomes obvious that the mere concentrations of these substances are not the only deciding factors in the clinical course of the poisoning. This question will be considered further during the discussion of the mechanism of poisoning.

The mechanism of methanol oxidation. No details are known about the enzymatic processes responsible for the oxidation of methanol. Lutwak-Mann (31) showed in 1938 that *partly* purified alcohol dehydrogenase from the livers of a variety of animals is capable of oxidizing methanol, although more slowly than ethyl alcohol. However, Theorell and Bonnichsen (46) and Theorell and Chance (47) have found that methanol is not oxidized by *crystalline* alcohol dehydrogenase from horse liver. They suppose that the oxidation takes place by means of catalase-peroxide. With this mechanism of oxidation the rate of oxidation is increased when the concentration of substrate increases. Chance (10) considers that the shape of the elimination curve of methanol found by Agner and Bellringe (1) provides support for the catalase-peroxidase mechanism. However, the shape of the elimination curve is largely determined by pulmonary excretion, and can therefore hardly be a sound basis for estimating the rate of oxidation. The fact that the catalase-peroxide oxidation proceeds very rapidly *in vitro* while methanol oxidation is rather slow *in vivo* might also seem to argue against the assumption of a catalase-peroxide oxidation of methanol according to Bartlett (5). Recently Hevesy (20) has shown that treatment of white mice with massive doses of roentgen rays (1,500 r) provokes some increase in the rate of methanol oxidation and simultaneously increases the activity of catalase.

Bartlett's experiments with labelled methanol indicate that the complete oxidation of methanol proceeds at a constant rate, since the amount of $C^{14}O_2$ excreted in the expired air per unit of time was not dependent on the concentration of methanol in the organism. He administered 1 g. of methanol per kg. of body weight to rats, but it would have been desirable to carry out similar experiments with considerably higher doses, *e.g.*, 3 to 4 g. kg. From his experiments, Bartlett concluded that methanol is probably oxidized by the same type of enzymes as are effective in the oxidation of ethyl alcohol (3, 5).

The rate of metabolism of methanol is very important clinically. It is generally assumed that the symptoms of methanol poisoning are due to the oxidation products formaldehyde or formic acid. However, it is beyond doubt that the symptoms develop much more rapidly after high doses of methanol than after low doses. If one of the aforementioned substances is primarily responsible for the symptoms, we must conclude that the rate of oxidation of methanol to the toxic intermediate increases with increasing concentration of methanol in the organism. The influence of ethyl alcohol on the rate of metabolism of methanol will be discussed in a subsequent section.

B. The Toxicity of Methanol

A study of the literature on methanol poisoning leaves a distinct impression that experimental investigations have not contributed much to answer the many questions connected with poisoning in humans. The results of animal experiments

are frequently in direct contradiction to clinical experience. Therefore, it is necessary to evaluate critically the question of whether or not the effect of methanol is the same in man and animals. For this reason the toxic and lethal doses, the symptoms, the most important pathological findings, and the effects of different modes of treatment in man and animals will be compared and discussed.

Toxic and lethal doses. The many animal experiments made at the beginning of this century all demonstrated that ethyl alcohol is more toxic than methanol. Reid Hunt (21) found that experimental animals died earlier after administration of ethyl alcohol than after equal doses of methanol. According to Schieck (42), Loevy and v. d. Heyde showed that ethyl alcohol is much more toxic to mice and dogs than is methanol; the same was shown by Hammarsten in rabbits. Neymark (33) states that Nicloux and Placet in 1912 demonstrated that the lethal dose of methanol for rabbits and dogs was about twice that of ethyl alcohol.

All these investigations show that in experimental animals Richardson's rule is valid: in a homologous series of alcohols toxicity increases as the number of carbon atoms increases. The lethal dose of methanol is high. Hunt found it to be 9 ml. kg. for dogs and 10 ml. kg. for rabbits (21). Several other investigators have found about the same lethal doses for these and other experimental animals.

It is understandable that the results of these and similar investigations contributed to the conception that methanol is but slightly toxic. Today we know that this conception is true only for experimental animals. It is beyond doubt that in man the toxicity of methanol is an exception to Richardson's rule since 1 g. kg. of body weight or less can produce blindness or even death.

Symptoms of methanol poisoning. The symptoms of methanol poisoning in various experimental animals are rather uniform and indicate decisively that methanol produces nothing more than a general anesthetic effect. After doses of 3 ml. kg. practically no effect is seen in rabbits. After 7 ml./kg. or more these animals exhibit almost immediate ataxia, loss of righting reflexes, and coma. The animals that recover after the anesthesia are already much more alert on the second day and start eating (8). The symptoms provoked by various doses of methanol administered to mice are as follows (15): After 4 g./kg. slight ataxia lasting less than one hour is seen; with 5.5 g./kg., the ataxia is more pronounced and in some animals slight anesthesia is produced; 7.5 g./kg. of methanol quickly produces deep anesthesia in some animals, only slight anesthesia in others; 10 g./kg. always induces deep general anesthesia beginning a few minutes after the injection. Some animals die, but those which recover show practically normal activity 48 hours after the injection. Everybody who has administered methanol to experimental animals will agree with Hunt that: "The symptoms of acute poisoning of animals with methyl alcohol are, in general, similar to those observed in cases of poisoning by ethyl and other alcohols of this series" (21).

The symptoms mentioned have little in common with those seen in man. Those who drink methanol are quite disappointed in the slight intoxicating effect. Except when ethyl alcohol has been taken together with methanol and occasional instances where exceedingly large amounts of methanol have been consumed (*e.g.* half a liter) 12 to 18 hours pass between the time of ingestion and the onset

of symptoms. Frequently patients have been able to continue their normal activities for several hours before symptoms appear. The first symptoms are a feeling of weakness, anorexia, headache and nausea. Then follow in rapid sequence vomiting, dyspnoea of Kussmaul's type, pain in the back and in the extremities, and in many cases exceedingly violent abdominal pain. The patient presses his hands against his abdomen, cries loudly, or throws himself out of bed and hunches up on hands and knees. Simultaneously with or shortly after the onset of severe symptoms, amblyopia appears which may develop rapidly into amaurosis. The pupils dilate and become partially or totally reactionless. The skin develops a reddish cyanotic colour. Sopor and coma then relieve the patient of the violent pains, the dyspnoea and the anxiety. The respiration is slowed and cyanosis becomes more pronounced. Usually the heart continues to beat for some time after cessation of respiration, up to two hours during artificial respiration in the iron lung (38). During the development of the symptoms the alkali reserve in the blood decreases steadily, and serious symptoms are seen only when pronounced acidosis is present (7, 11, 39, 40). Immediately before death, the alkali reserve, measured as the CO_2 content of the blood, is about 10 to 15 vol. per cent or sometimes even less. If no treatment is given, the same symptoms are seen in patients who have taken 100 ml. as in patients who have taken several times as much. The only difference is that when large amounts have been taken, the signs and symptoms develop much sooner, and the symptom-free interval may be reduced to a couple of hours.

Even after lethal doses of methanol most animals do not develop acidosis. However, a moderate acidosis can sometimes be observed (19, 26, 41). The cause of this difference between man and animals is unknown.

In humans, amaurosis and abolished pupillary reactions are seen regularly during methanol poisoning with severe acidosis (less than 20 vol. per cent CO_2). No clear-cut impairment of vision is demonstrable in animal experiments (8, 12, 22, 43). Obviously, the pupillary reactions are abolished during the period of anesthesia, but they reappear with the disappearance of the anesthesia (11).

The most important pathological findings. Severe changes in the ganglion cells of the retina are found in patients who have died of methanol poisoning. The changes are so severe that they must be irreversible. Pick (34) has stressed that the picture is the same as that described by Nissl. The most pronounced changes are seen in the nuclei of the cells. They are always placed at the extreme periphery of the cells; it often looks as if the nucleus protrudes outside the limit of the cell. The nucleus is flattened, polygonal and irregular, and the nucleolus is displaced to the outer limit of the nucleus. Sparse remnants of the tigroid substance are found in a circle peripherally in the cytoplasm. Many of the cells are enlarged and globoid. The dendrites are difficult to see, even after silver staining (12, 32, 35, 41, 48).

There are widely divergent opinions about whether or not pathological changes are present in the retinal ganglion cells of animals poisoned with methanol. Birch-Hirschfeld (8), Schwartzkopf (43) and others claim that such changes are present. However, they found the most pronounced changes in the tigroid substance,

while apparently the nucleus usually remained normal. The technique used by these investigators has been criticized by several authors. Igersheimer and Verzár (22) found that the administration of methanol in doses so high that the animals remain in a prolonged coma was a source of error because the consequent anoxemia possibly could cause changes in the ganglion cells. Therefore, they gave only moderate, not anesthetic, doses during weeks and months. In chickens they did not find any sign of decreased vision and no changes in the ganglion cells. De Schweinitz (44) and Friedenwald (13) also disagreed with Birch-Hirschfeld's findings. Friedenwald considered that the changes described were artefacts since he was able to produce a similar histological picture in eyes from normal animals by using certain methods of fixation and imbedding. He examined a series of animals including dogs, cats, rabbits, mice, rats, and chickens, but in no instance did he find changes in the retinal ganglia (14). The fact that impaired vision cannot be demonstrated in animals with any degree of certainty provides additional evidence that, in contrast to man, severe retinal changes are not present in animals.

Pathologic changes in other organs after methanol poisoning are not usually pronounced. No changes which can be regarded as irreversible are found in the ganglion cells of the cerebrum (35). Necrosis of the liver is not found in man although it may occur in animals (32). On the other hand a marked contraction of the lower ileum and of parts of the colon is almost always found at autopsy of patients (40), but is never present in animals. Bennett *et al.* (6) among others have described hemorrhagic pancreatitis in fatal human cases which they suppose is the cause of the severe abdominal pain. In the author's opinion it is more likely, however, that the pain is due to the very pronounced intestinal spasms since the pain disappears immediately after administration of alkali.

In summary, it must be stressed that there is a fundamental difference between the toxic effects of methanol in man and the toxic effects in animals. This fact has been, and is still far too little realized.

C. The Course of the Intoxication

The effects of ethyl alcohol. Hitherto, the symptomatology of poisoning with methanol alone has been described. As mentioned, a symptom-free period of 12 to 18 hours is generally seen. Only after very high doses (*e.g.*, half a liter) of methanol is the latent period shorter.

However, when several persons in the same party drink methanol, the variations in their clinical course are frequently much greater than can be accounted for by differences in the amount of methanol ingested. For example, the patient who has consumed the smallest amount may rapidly become ill and die, while another patient may remain without symptoms in spite of the fact that he has consumed much more; a third who has also consumed a large amount may not become ill for one and a half to two days, but may then rapidly develop the most severe symptoms and die within a few hours.

The explanation of this fact is that while consuming the methanol, many patients have also been drinking ethyl alcohol, which inhibits the development of

the acidosis (39, 40). It is the author's experience that if ethyl alcohol is taken simultaneously or almost simultaneously with methanol, the symptoms of methanol poisoning will not appear until most of the ethyl alcohol is eliminated. If at that time a toxic amount of methanol is still present in the body poisoning will develop as described. Generally the only effect of a *single* dose of ethyl alcohol is a prolongation of the latent period. If ethyl alcohol is taken repeatedly at such intervals that it is constantly present in the body together with methanol, the patient will not develop symptoms provided that the concentrations of methanol and ethyl alcohol are not too much out of proportion.

Bennett and coworkers (6) found that some patients with methanol poisoning developed acidosis in spite of a rather high concentration of ethyl alcohol in the blood. However, it cannot be excluded that some of their patients had taken ethyl alcohol after the development of their acidosis. All but one recovered. The patient who died (case No. 17) had no methanol in the blood when admitted to the hospital, but an extremely low CO_2 (4 m.eq. /l.), a high concentration of ethyl alcohol (340 mg. per cent) and *normal pupils*. After treatment with 220 g. sodium bicarbonate he developed a severe alkalosis (46 m.eq. /l.) and died on the third day. It seems justified to conclude that neither the symptoms, the course of the disease nor the biochemical tests indicate that this patient died of methanol poisoning.

The hypothesis that ethyl alcohol is able to inhibit the oxidation of methanol (39) has been confirmed by later experiments. Zatmann (54) found that the oxidation of methanol *in vitro* by impure alcohol dehydrogenase was considerably inhibited by ethyl alcohol. Inhibition was seen even when the concentration of ethyl alcohol was about 1/16th that of methanol. Bartlett (4) administered C^{14}-labelled methanol to rats and measured the excretion of $C^{14}O_2$ as an index of the amount of methanol oxidized. In these experiments, as well as in experiments with liver tissues incubated with methanol for 2 hours *in vitro*, there was a marked decrease of methanol oxidation in the presence of ethyl alcohol. The inhibitory effect was a linear function of the log of the ethyl alcohol concentration, with 72 per cent inhibition at 0.01 molar. The experiments clearly demonstrate that ethyl alcohol is a very effective inhibitor of methanol oxidation.

D. The Treatment of Methanol Intoxication

We owe to Harrop and Benedict (1920) the demonstration that acidosis occurs during methanol poisoning and the proposal to treat this poisoning with alkali (18). The same year Isaacs (23) observed good results with this treatment. It is indeed deplorable that about 30 years elapsed before the good effect of this treatment became commonly known, and unfortunately some still doubt its value. It seems that the authors of medical textbooks have paid more attention to the results of animal experiments than to clinical observations. Animals only rarely develop a decrease in the alkali reserve and never a severe acidosis (19, 26). Some authors advise against alkali treatment because the animals treated with alkali generally die (19, 25) doubtless from the resulting alkalosis. In contrast, clinical investigations have shown that the degree of acidosis decides the outcome of the

poisoning, and for this reason treatment with alkali is absolutely indicated (7, 11, 39, 40). The alkali reserve must be determined at intervals of a few hours, because acidosis can reappear rapidly even when the patients have received the amount of bicarbonate advised by Van Slyke (40, p. 253). This fact is also most strikingly emphasized in the comprehensive report by Bennett *et al.* (6).

As previously mentioned, ethyl alcohol inhibits the metabolism of methanol to toxic products. For this reason it has been proposed to administer ethyl alcohol in order to prevent the recurrence of acidosis (39, 40). A concentration of 100 mg. of ethyl alcohol per 100 ml. of blood is sufficient, particularly in view of Bartlett's finding (4) that in rats a concentration of 46 mg. per cent can decrease the rate of methanol metabolism by 72 per cent. Ethyl alcohol must be given often enough to keep the ethyl alcohol concentration reasonably constant, and due account must be taken of the rate of disappearance of ethyl alcohol which is about 7 g./hour.

Just as treatment with bicarbonate does more harm than good in animal experiments, so also no beneficial effect of ethyl alcohol can be expected in animal experiments because experimental animals do not develop acidosis even when oxidation of methanol is allowed to proceed at a normal rate. It is therefore not surprising that Gilger *et al.* (15) found that ethyl alcohol increased the toxicity of methanol. They reported that the LD_{50} of methanol in mice was 10.5 to 11.0 g./kg. If in addition 2 g. of ethyl alcohol per kg. was given all the mice died. Since the anesthetic potency of ethyl alcohol is almost twice that of methanol, the animals undoubtedly died of the combined anesthetic effect of the two alcohols. It is incomprehensible why Gilger *et al.* on the basis of these and similar experiments, discourage the use of ethyl alcohol in the treatment of human methanol poisoning. They themselves (see ref. 15, p. 115) refer to the use of ethyl alcohol and bicarbonate in the treatment of methanol poisoning in the United States Navy. Chew *et al.* (11) have given an interesting report of the above-mentioned patients in the Navy. The 26 patients had drunk pure methanol in an average amount of about 220 ml., varying between 540 and 90 ml. Moreover, most of them had taken an unknown amount of ethyl alcohol. Besides treatment with alkali, the patients were given 30 ml. of whisky every 4 hours. The results were excellent, as only 4 of the patients had decreased vision when discharged from the hospital, and 2 of these regained normal vision after a short time. The dose of alkali employed (in cases No. 3, 6, 9, 14 and 15) was not much more than half the dose recommended by Van Slyke. Apparently, the tendency to recurrence of the acidosis was only slight. To anyone who has had some experience with the treatment of methanol poisoning, it is quite inconceivable that a patient (case No. 3) who presumably drank as much as 360 ml. of methanol, and whose alkali reserve was reduced to 25 vol. per cent, could escape a fatal recrudescence of the acidosis after the administration of only 28 g. of sodium bicarbonate in 24 hours. Such a patient ought to have been treated with about 60 g. of sodium bicarbonate *initially*. It is more than likely that the good results obtained in spite of inadequate doses of bicarbonate can be attributed to the retardation of methanol oxidation caused by the repeated administration of small amounts of ethyl alcohol.

If we are to avoid unnecessary losses of vision or lives in the future, it is essential to recognize that all patients with methanol poisoning must be treated intensively with alkali, unless ethyl alcohol is given repeatedly after the acidosis has been first corrected. To illustrate what close supervision is needed for successfully combatting the tendency to reappearance of the acidosis, two case histories are presented (40). Both patients were given about 80 g. of sodium bicarbonate during the first hours of treatment. This turned out to be far too small a dose, and they developed a severe exacerbation of the acidosis within the following 24 hours. One of the patients died; the other had a narrow escape.

Case No. 57: A 39 year old man drank about 600 ml. of antifreeze during 2 days. The first day, some time before he started to drink the antifreeze he also took 1 bottle of aquavit (corresponding to 360 ml. ethyl alcohol). Not until the morning of the third day after he had started his work did he experience the first symptoms of severe acidosis. He was admitted to the hospital at 10 a.m. The alkali reserve was 23 vol. per cent of CO_2 and he complained of blurred vision. He was given 71 g. of sodium bicarbonate, partly intravenously, partly orally, and 40 ml. of ethyl alcohol. The latter was presumably eliminated after about 4 hours. At 3 p.m. the alkali reserve was 36 vol. per cent CO_2 and an additional 13 g. of sodium bicarbonate was administered intravenously. In all he received 84 g. of sodium bicarbonate this day. The morning of the fourth day the alkali reserve was 27 vol per cent of CO_2 and an additional 66 g. of sodium bicarbonate was administered intravenously and orally. At 3 p.m. the alkali reserve was 48 vol. per cent and 30 g. of sodium bicarbonate was given. Recovery was complete.

Case No. 73: A 33 year old man took a considerable amount of methanol during a two day period. The first day he also drank about 220 ml. of ethyl alcohol. The second day he was completely free from symptoms. The morning of the third day he was dizzy and had a typical "black-out". Some hours before his admission to the hospital he drank about 60 ml. of ethyl alcohol. The alkali reserve was 30 vol. per cent of CO_2 at 1.30 p.m. His vision was normal. He was given 55 g. of sodium bicarbonate partly intravenously and partly orally. The alkali reserve was 39 vol. per cent at 3 p.m. and an additional 25 g. of sodium bicarbonate was given, making a total of 80 g. No further determination of the alkali reserve was made that day. The fourth day at 7.30 a.m. the patient was restless and dyspnoeic. The alkali reserve was now 16 vol. per cent. At 9 a.m. the patient collapsed and further treatment was without effect. The concentration of methanol in the urine was about 0.2 per cent. The blood concentration of volatile reducing substances calculated as methanol was 0.192 per cent.

The disproportion between the long symptom-free period and the later persistent tendency of the acidosis to recur in these two patients is striking. The large amounts of ethyl alcohol taken by the patients had presumably not been eliminated in less than 24 hours, and the symptom-free period was about one and a half days. The principal error in treatment was too infrequent determination of the alkali reserve. Undoubtedly more frequent determinations would have shown that more than 100 g. of sodium bicarbonate during the first 12 hours was required in both cases.

Many physicians must treat patients with methanol poisoning under conditions where determination of the alkali reserve is impossible. Here ethyl alcohol may provide very valuable protection against recurrence of the acidosis, although it must be admitted that some authors (*e.g.*, Bennett *et al.*) still express some doubt concerning the beneficial effect of ethyl alcohol, and strongly advise

against the use of ethyl alcohol alone in treatment. Even in a hospital with a well equipped laboratory, however, it may be difficult to obtain a sufficient number of determinations, if a great number of patients are admitted at the same time. In every case, combined treatment with alkali and ethyl alcohol is the safest and easiest treatment.

Is it possible to use animal experiments in the investigation of methanol poisoning in man? It is a waste of time to attempt to investigate the mechanism of the toxic effects of methanol in man by means of animal experiments until it is clear why animals do not develop more than a moderate degree of acidosis. To my knowledge no experiments in this direction have been carried out. There is considerable evidence that the oxidation of methanol proceeds in the same manner in man and in at least some animals, *e.g.*, dogs. The difference in the toxic effect may perhaps be due to a greater capacity of dogs to maintain the pH of the body fluids when a large load of organic acid is suddenly imposed. A single investigation seems to indicate that this is true. Kröhl found in 1913 (24) that the excretion of ammonia in the urine of a dog poisoned with methanol considerably exceeded the corresponding amount of formic acid. The concentrations of the two substances were proportional, but not equimolecular.

It is conceivable that the clinical picture seen in man might be produced in experimental animals by giving them enough acid to cause severe acidosis and simultaneously administering enough methanol to produce light anesthesia. If such an experiment were successful, it would be permissible to draw analogies from animal experiments.

The pathogenesis of the poisoning. Only further biochemical investigations of methanol poisoning in man can provide a better understanding of the mechanism involved. Some of the symptoms indicate the lines along which such investigations must be conducted.

Cyanosis. The cyanosis is not a pure cyanosis, but rather a mixture of cyanosis and rubeosis. This symptom appears during the severe acidosis *before* the onset of failure of respiration or circulation. At present it is uncertain whether or not the hemoglobin is partly inactivated so that complete oxygen saturation of the blood is impossible. Two facts speak in favor of this hypothesis. a) By spectroscopic examination Isaacs found absorption lines similar to, but not identical with, methemoglobin (23). Rabinovitch has definitely shown that this substance is not methemoglobin (38). b) In a cyanotic patient, the same investigator found an oxygen deficit of only 9 vol. per cent in the venous blood (38). According to Lundsgaard (30), if the arterial blood has been saturated with oxygen, cyanosis should not appear until a deficit of 13 vol. per cent is reached. Possibly, therefore, the cyanotic colour during methanol poisoning is not caused solely by an increased amount of reduced hemoglobin.

Thus there seems to be reason to investigate whether some abnormal substance is combining with the hemoglobin iron and to try to isolate the substance in this hypothetical compound. If this were successful we would be well on the way towards an explanation of the mechanism of the serious metabolic disturbances found in methanol poisoning. It is, furthermore, possible that the same substance

TABLE 1

Methanol concentration, formic acid, and alkali deficit in the blood 40 to 60 minutes prior to death in three patients with methanol poisoning

Case No.	Volatile reducing substances in the blood, calculated as methanol	Formic acid in the blood		Alkali deficit* in the blood	
	per cent	mg. per cent	m.eq./l.	CO_2 vol. per cent	m.eq./l.
11	0.163	19	4.1	51	23
15	0.115	5.7	1.2	52	24
20	0.238	17	3.7	14	20

* Calculated as the difference between the observed values and the normal values (60 vol. per cent, or 27 m.eq./l). Note that the alkali deficit (decrease of the alkali reserve) can by no means be ascribed to formic acid alone.

or substances could combine with the pyrrol iron enzymes in the tissues, *e.g.*, cytochrome oxidase, peroxidase, and catalase, and thus produce tissue anoxia.

Acidosis. In some cases a high concentration of lactic acid is found in the blood (9, 39, 48) and in the urine (18). A single investigator (49) has demonstrated a very large difference amounting to 31.15 m.eq. per liter between the total amount of inorganic base and acid in the blood which indicates the presence of a high concentration of organic acids in the blood. According to Bennett *et al.*, Van Slyke noted an increase in the urinary excretion of lactic, as well as formic acid, in a patient with methanol intoxication, but he showed that most of the urinary acid was in the form of unidentified organic acids which were not formic, lactic or acetic acids.

As shown in table 1, the concentrations of formic acid and methanol are not the only factors determining the outcome of poisoning.

The determination of the alkali reserve is an important indicator for clinical treatment, but it does not provide information about the pH if the CO_2 tension is unknown. An isolated determination in one case gave a blood pH of 7.08 (49).

Hypotheses concerning the pathogenesis of methanol intoxication. As a tentative hypothesis, let us assume that the metabolic changes are caused by the combination of some hitherto unidentified toxic substance or substances with one or more important enzymes in the organism. The relation demonstrated between the degree of acidosis and the severity of symptoms seems to indicate that the pH might be a deciding factor in such enzymatic reactions. It is possible that at a low pH the enzymes might be inactivated by a smaller concentration of the toxic substance than when the pH is nearer to the normal value. Only when we know with certainty what substance is primarily responsible for the toxic effects and what enzymes are inhibited, can we expect to find a precise explanation for the influence of pH.

About 60 years ago Pohl demonstrated in animal experiments (36) that formaldehyde is far more toxic than either formate or methanol. He emphasized that formaldehyde intoxication produced symptoms unlike those of methanol poisoning and for this reason he concluded that formaldehyde was probably not the

primary cause of the symptoms of methanol poisoning. On the other hand he found the largest amount of formate in the urine when the symptoms were at their peak. Since Pohl's investigations, many have supported the theory that formic acid was the real toxic agent, but even more have assumed that the active substance is formaldehyde. Recently this latter concept has been stressed by Potts and Johnson (37) who investigated respiration and glycolysis in retina cells by the Warburg technique. They found that formaldehyde inhibited oxidation and especially glycolysis to a much greater extent than did formate, and still more than did methanol. However, it is doubtful whether it can be concluded from these experiments that formaldehyde is the primary toxic agent in methanol poisoning. Formaldehyde is very rapidly oxidized to formic acid in the cells. Many experiments have shown that formic acid is incompletely, or at least very slowly, oxidized to carbon dioxide and water. When sodium formate is added to the Warburg vessels, it will probably not provoke a decrease of the pH of the cells. On the other hand, when formaldehyde is added so that formic acid is produced within the cells, the buffer capacity of the cells may be insufficient to prevent a decrease of the pH. Thus it is possible that formic acid formed intracellularly will be more toxic than a corresponding amount of sodium formate which diffuses into the cells. Theoretically, the toxic effect of formaldehyde may be caused by three factors, the formaldehyde *per se*, the formic acid and the low pH, or any combination of these three factors. For this reason no conclusion can be drawn about the toxic effect of formaldehyde *per se* until experiments can be performed in which the oxidation of formaldehyde to formic acid in the cells is inhibited.

The author has earlier put forward the hypothesis that in man methanol, or one of its metabolites, inhibits oxidative processes, and that this inhibition causes an accumulation of organic acids in the tissues, presumably in the first place lactic acid (39, 40). As long as the pH remains normal, this process is moderate and does not give clinical signs of anoxia. However, if the compensatory mechanisms are overwhelmed by the continued production of acid, the pH decreases. This in turn provokes an increased inhibition of the enzymatic processes and acid production is further increased. In this way a vicious circle is established which can be broken by the administration of alkali. The fundamental effect of ethyl alcohol may be that it has a higher affinity for the enzymes which oxidize methanol than has methanol itself, and thus inhibits the oxidation of methanol considerably. Since formic acid easily forms compounds with ferrous and ferric iron (16) it is possible that formic acid is the substance which inhibits iron-containing enzymes. In proportion to its content of iron, retina has a greater oxygen consumption than any other normal tissue (51). This might explain why patients become blind before they die, and why irreversible damage may be done to the retina, even if treatment has been started in time to save the life of the patient.

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