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EDITORIAL*

BIOCHEMICAL ASPECTS OF METHANOL POISONING

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INTRODUCTION

POISONING from methyl alcohol (wood alcohol) has been known since 1856, but despite an extensive literature on the subject (by 1904, 275 cases of blindness or death attributable to methanol had been reported), this toxic solvent was still being used in the early part of the twentieth century as a substitute for grain alcohol (ethyl alcohol) in liniments, toilet articles, perfumes, and patent medicines.[1, 2] Even Paul Ehrlich was using methyl alcohol as a solvent for arsphenamine in 1914. Although most of the cases of poisoning resulted from ingestion of methylated spirits, toxic effects attributable to inhalation or absorption through the skin were well documented; for example, Brown[3] described the case of a factory worker who spilled a gallon of methanol down his trouser leg, was dizzy on the following day, took a short nap, and woke up totally blind.

Despite the numerous reports of individual toxic responses to methanol, a survey of the immense literature on the subject reveals a high incidence of poisoning in epidemic form, generally resulting from the sale of bootleg liquor. Thus, for example, in one period of 7 months, during the years when the sale of spirits was prohibited in the United States, there were 400 fatalities.[4] A series of 323 cases of methanol poisoning resulted from the ingestion of adulterated liquor, which occurred in the area of Atlanta, Georgia, was described in 1953.[5] During war time, servicemen are prone to drink whatever alcohol is available, without regard to the length of the carbon chain, and the results of this practice are evident in the estimate that 6 per cent of all cases of blindness in the Armed Forces during World War II was caused by methanol.[6] It should be noted that this figure takes into account only nonfatal cases; consideration of the number of deaths that resulted from methanol would considerably enlarge this statistic.

CLINICAL CHARACTERISTICS OF METHANOL INTOXICATION

The clinical course in methanol toxicity in man is characterized both by a marked variation in response to size of dose and by an asymptomatic latent period between

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the ingestion of methanol and the onset of manifestations of poisoning. Ingestion of from 70 to 100 ml of methanol is usually fatal although cases are listed in the literature in which the consumption of 540 ml did not result in the development of any irreversible manifestations of toxicity.[7] On the other hand, Duke-Elder[8] has cited instances in which a teaspoonful of methanol caused blindness, and about 30 ml was lethal. Within 2 hours, or even as late as 72 hours, after ingestion of the methylated spirit, the patient becomes fatigued, and experiences such signs and symptoms as headache, dizziness, nausea, and moderate gastrointestinal distress, generally followed by visual disturbances. In more severe cases, intense upper abdominal pains are manifest, weakness develops, and the patient usually is comatose upon admission to hospital, with increased reflex hyperexcitability and even convulsions. Respiration may be rapid and shallow, or of the Kussmaul type, as in diabetic coma. The patient may have a lowered blood pressure, and if dyspnea and cyanosis are present, the prognosis is doubtful.

The most significant laboratory finding is the occurrence of a severe metabolic acidosis as determined by the CO_2-combining power of the blood, which falls to less than 20 volumes per 100 ml; only one record of measurement of blood pH has been found in the literature of methanol poisoning: a pH value of 7·08 was found.[9]

Retinal changes are characteristic of methanol poisoning. Total bilateral blindness may develop after a few hours or may be delayed by a few days. Observation soon after the onset of visual disturbances reveals considerable retinal edema; there may be papillitis with swelling and dilation of veins and some diminution of the pupillary light reflex. The degree of impairment may be of prognostic value, for most patients with fixed and dilated pupils succumb.[10] A dense central or paracentral scotoma usually develops and may precede retrobulbar neuritis or optic atrophy. Other ocular abnormalities noted by Duke-Elder[8] include ptosis, paresis of extraocular muscles, and an excavation of discs that results in deep glaucomatous cupping, despite the absence of elevated intraocular tension; residual ocular defects persist in up to 50 per cent of the nonfatal cases.

In summary, it is emphasized that ophthalmoscopically visualized changes, such as retinal edema and pupillary dilation, associated with delirium, coma, and severe abdominal pain, together with a lowering of the CO_2-combining power of the blood, are characteristic of methanol poisoning. Death usually occurs in inspiratory apnea as a result of failure of the respiratory center that is associated with severe damage to the central nervous system.

Pathology

The findings at autopsy in fatal cases of methanol poisoning have been described by various authors,[5, 7, 11–13] these include variable cerebral edema and hyperemia, edematous lungs congested with patchy atelectasis, and petechial hemorrhages. Additional changes, although these are not pathognomonic of methanol poisoning, are gastritis, epicardial hemorrhages, mild fatty infiltration of the liver, cloudy swelling in the cells of the spinal cord, and congestion of the glomerular tufts and cloudy swelling of the convoluted tubules of the kidneys. The bronchial passages may contain frothy debris, and sometimes desquamation of the bronchial epithelium occurs. Pancreatic necrosis, observed by Bennett *et al.*[5] in cases of methanol poisoning, has been attributed to generalized vascular injury and hemorrhage.

The significance of the pathological changes and the site of the primary lesion in the eye have been the subjects of considerable controversy. In the fatal cases described by MacDonald,[14] in which care was taken to ensure prompt fixation of the ocular tissues, the retinal changes were characterized by marked degenerative changes with cystic spaces in the layer of ganglion cells, irregular external nuclear layer, irregular rod and cone nuclei, migration of pigment granules and congestion of choroidal vessels. Much earlier, Pick and Bielschowsky[15] had noted histological changes in the ganglion cell layer. Recent studies have confirmed the cystic degeneration of the ganglion cells and associated eccentric placement of nuclei and tigrolysis, but few changes were observed in the optic nerves, except for edema and hyperemia with associated gliosis.[11, 16, 17]

Absorption and excretion

The gastrointestinal tract is the most common route of entry of methanol, although, as previously mentioned, the literature includes reports of poisonings from inhalation or absorption through the skin. Methanol is distributed uniformly in tissues, in proportion to their water content, and is highest in muscles, blood, the gastrointestinal tract, and the liver, in that order;[18, 19] cerebrospinal fluid was not analyzed in these experiments, which were done on dogs and rats. In clinical cases of poisoning, Bennett *et al.*[5] have observed consistently higher levels in the cerebrospinal fluid, as compared with those in the blood. Of methanol ingested, up to 50 per cent is eliminated unchanged through the lungs,[20] and, in experiments with dogs and rabbits, approximately 10 per cent is excreted unchanged in the urine.[21] In addition to excretion of methanol through the pulmonary and renal routes, methanol is secreted into the gastric juice in concentrations five to twelve times greater than those in the blood, even 10 days after poisoning;[5] this circumstance suggests that gastric lavage may be a useful adjunct to therapy.

Metabolism

A large part of the ingested methanol is oxidized to formaldehyde, and this, in turn, is oxidized to formic acid; the latter is either excreted in the urine or further oxidized to carbon dioxide and water. There is considerable variation among animal species with respect to the contribution of renal excretion to the elimination of formic acid. The experiments of Lund[21] in rabbits, showed only a slight increase in urinary excretion of formic acid after the administration of methanol through an esophageal tube, whereas Bastrup[22] observed that up to 8 per cent of ingested methanol may be excreted as formic acid. In dogs, however, Lund and Bastrup independently demonstrated that up to 20 per cent of the methanol administered could be excreted as formic acid. The excretion of formic acid in man follows an intermediate course and, within 24 hours, the amount eliminated by the kidneys may be equivalent to as much as 5 per cent of the methanol ingested.[23] In all these experiments, however, maximal blood and urinary levels of formic acid were reached from 2 to 3 days after ingestion of methanol.

As indicated previously, the formation of formic acid from methanol in the animal organism proceeds through the intermediate formation of formaldehyde. Although the production of formaldehyde from methanol by liver tissue can be readily

demonstrated,[24] attempts by various workers[25, 26] to isolate formaldehyde from autopsy material obtained from victims of methanol poisoning have failed; this circumstance is attributable to the rapid reaction of formaldehyde with the tissue proteins;[27] on the other hand, Keeser[28] found demonstrable amounts of formaldehyde in the vitreous humor of the calf's eye and Benton and Calhoun[10] have reported the presence of a "trace of formaldehyde" in the vitreous humor of one patient who died after poisoning with methanol. These reports must await further substantiation before it can be concluded unequivocally that formaldehyde can be isolated from tissues in clinical cases of methanol poisoning.

The mechanism of oxidation of methanol to formaldehyde is a subject of much lively debate. Lutwak-Mann[29] observed that partially purified alcohol dehydrogenase from horse liver could catalyze the oxidation of methanol to formaldehyde, an observation confirmed by Zatman,[30] who found that ethanol competitively inhibited this reaction. The crystalline enzyme, however, was found to be incapable of promoting this reaction.[31–33] These observations led to the postulate that methanol is oxidized to formaldehyde by a peroxidative mechanism mediated by catalase and a hydrogen peroxide-generating system such as hypoxanthine and xanthine oxidase. According to Chance, the kinetics of the disappearance of methanol from the blood of rabbits agreed with such a postulate.[34] Using the undefined system, Tephly *et al.* found that ethanol is a competitive inhibitor of the oxidation of methanol;[35] however both 3-amino-1,2,4-triazole, a specific inhibitor of liver catalase, and sodium tungstate, which inhibits hepatic xanthine oxidase, were without effect on the disappearance of methanol from the blood of rats to which the alcohol had been administered.

In this laboratory we have recently investigated this problem with material from monkeys, since it has been firmly established that a unique type of methanol poisoning occurs in primates.[36, 37] We found that the ratio of the rate of oxidation of methanol, as compared with that of ethanol, remained almost constant over a 90-fold range of purification of an enzyme system isolated from the liver of the rhesus monkey. Ethanol competitively inhibited the oxidation of methanol. The enzyme was subsequently crystallized from horse liver. From this information, as well as studies with inhibitors, it was concluded that the enzyme involved is alcohol dehydrogenase. Studies on the rate of elimination of methanol in the blood of monkeys given methanol by intubation agreed with its rate of oxidation as observed *in vitro*. All these observations led Kini and Cooper[38] to conclude that it is alcohol dehydrogenase, and not the catalase system, that is responsible for the physiological oxidation of methanol. The inability of previous investigators to show that methanol is a substrate for crystalline liver alcohol dehydrogenase is attributable to the low concentration of methanol used in their experiments. The K_m for methanol is about $1{\cdot}7 \times 10^{-2}$ M, but most of these previous workers employed the alcohol at a level of about 1×10^{-3} M. Although it is unnecessary, at the present time, to invoke the participation of the catalase system in the oxidation of methanol, the experiments of Kini and Cooper do not exclude the participation of this mechanism. It is possible that, with low levels of methanol in the body, the peroxidative action of catalase could operate.

At least seven different enzymes capable of catalyzing the conversion of formaldehyde to formate are present in animal tissues: aldehyde dehydrogenase, xanthine oxidase, glyceraldehyde-3-phosphate dehydrogenase, catalase, peroxidase, and aldehyde oxidase; in addition, Strittmatter and Ball[39] have obtained from extracts of beef

and chicken liver a specific DPN-dependent formaldehyde dehydrogenase that requires glutathione as an additional cofactor. The presence of this enzyme has also been demonstrated in bovine, monkey, and human retinas.[40] Since the half-life of formaldehyde in the body is short, it is most logical to assume that the formaldehyde that acts on the retina is actually formed *in situ* by alcohol dehydrogenase; this enzyme occurs in the retina and apparently is normally concerned with the oxidation of vitamin A alcohol to retinene.[41]

Although, as mentioned earlier, formic acid is excreted in the urine, a portion of it is oxidized to carbon dioxide and water; the mechanism of this oxidation has been shown recently to involve the peroxidative action of catalase and a hydrogen peroxide-generating system.[42]

Treatment

The rationale for the treatment of methanol poisoning is based upon the inhibition of the metabolism of methanol, combined with alkali therapy to combat acidosis. Gastric lavage, using either saline solutions or tap water, is usually recommended only in the early stages of poisoning, before the onset of the delayed characteristic symptoms and signs. Elimination of already assimilated methanol through extracorporeal dialysis has been successfully employed in methanol poisoning in dogs[43] and peritoneal dialysis has been successfully used by Stinebaugh,[44] who concluded that this method is effective in withdrawing methanol from the tissues. Acidosis must be treated by an early and massive administration of sodium bicarbonate given orally or intravenously (500 ml of a 5% solution); such treatment must be controlled by careful estimations of the bicarbonate and pH levels of the blood, in order to prevent the occurrence of hypokalemia or tetanic convulsions.

The use of the simultaneous administration of ethanol as an adjunct to bicarbonate treatment in methanol toxicity has been championed by Röe;[25] it was found that patients who imbibed ethanol along with a dose of methanol often were protected from the toxic effects of the latter. A concentration of 100 mg of ethanol per 100 ml of blood is recommended by Röe as a means of preventing the recurrence of acidosis. This form of therapy has been made use of by Chew *et al.*,[7] who administered an ounce of whiskey every 4 hours and found no fatalities in their treated group. Although it has been shown that ethanol delays the oxidation of methanol, thereby increasing its excretion in rabbits,[45] monkeys,[46] and man,[47] it should be remembered that administration of ethanol would enhance the degree of depression of the central nervous system in an already comatose patient, and a fatal outcome could result for this reason.

The toxic agent in methanol poisoning

It is now generally accepted that a metabolite of methanol, formaldehyde, forms the proximal toxic agent in methanol poisoning. This belief is based upon (i) the presence of a characteristic latent period prior to the onset of the clinical manifestations of poisoning; (ii) the beneficial effects of ethanol in both experimentally poisoned animals and clinical cases of poisoning, whereby the oxidation of methanol to formaldehyde is inhibited; and (iii) the experimental demonstrations *in vitro* of the greater toxicity of formaldehyde to the retina than of methanol or formic acid. In a comparative

study of the toxicity of methanol, formaldehyde, and sodium formate on bovine retinal homogenates *in vitro*, Potts and Johnson have found formaldehyde to be the most toxic to retinal glycolysis and respiration;[48] their studies were confirmed by Leaf and Zatman.[47] Praglin *et al.*[49] extended these investigations by examining the effects of this series of compounds on the electroretinogram (ERG) of monkeys and found that a concentration of formaldehyde, which approximates that reasonably assumed to be present in methanol poisoning (0·0007 mole/kg of body weight), produced by intravenous administration, abolished the b-wave of the ERG, while this was affected by formate and methanol only at 0·025 and 0·03 mole/kg, respectively. Acetaldehyde did not affect the ERG in doses 50 times higher than the effective dose of formaldehyde. A detailed study by Kini and Cooper[50] on the effects of methanol and its metabolites on the bovine retina *in vitro* have essentially confirmed the observations of Potts and Johnson; in addition, Kini *et al.*[51] found that formaldehyde, administered to rabbits intraocularly in order to avoid the principal sites of metabolic alteration, affected both the morphology of the retina and the adenosine triphosphate (ATP) production, as inferred from the incorporation of ^{32}P-labeled inorganic phosphate into phospholipids. In contrast, neither methanol nor sodium formate had any effect on the labeling of phospholipids or on the histology of the retina (*vide infra*).

From all these observations the conclusion is almost inescapable that the toxic agent in methanol poisoning is formaldehyde. It should be pointed out, however, that the ingestion of large amounts of methanol will give rise to manifestations that can most correctly be ascribed to a nonspecific narcotic effect of the alcohol itself, an effect seen with the intake of many alcohols.

Species difference

One of the major stumbling blocks in the elucidation of the biochemical events that occur in methanol poisoning was the inability of earlier investigators to discern the difference between laboratory animals and humans in their response to methanol ingestion. It was primarily due to Röe,[25] and subsequently to Gilger and Potts,[37] that the effect of methanol on nonprimates was attributed to a narcotic effect similar to that seen with various alcohols and completely different from the effect seen in primates —namely, the production of blindness and of metabolic acidosis.

A critical evaluation of the extensive literature of the histopathological changes in the eyes of laboratory animals poisoned with methanol is beyond the scope of this review. Many of the changes described in the earlier work on the histology of the retinas of methanol-poisoned animals could not be obtained by Friedenwald[52] or by de Schweinitz.[53] Friedenwald regarded these earlier findings as artifacts of fixation and embedding. Subsequent investigations by Alder *et al.*[54] and Röe,[17] who worked with rats and rabbits, and by Potts *et al.*[55] and Cooper and Felig,[56] who worked with rhesus monkeys, have failed to demonstrate histological changes in the retina analogous to those seen in clinical cases of methanol poisoning. Potts and co-workers observed cyst formation in the external nuclear layer, edema, and nuclear pyknosis in the putamen and caudate nucleus, but saw no other major change in these methanol-poisoned monkeys, despite marked edema ophthalmoscopically observed, and alteration in the electroretinogram. In conclusion, it appears that any histological changes seen in the retinas of nonprimate species of animals are attributable to the narcotic effect of toxic doses of methanol and differs from the changes characteristic of man.

In addition, acidosis, another common manifestation of methanol poisoning in man, is not present in experimental animals, with the exception of the monkey.[57]

The reason for this peculiar species difference in the response to the ingestion of methanol is still unknown. In studies in our laboratory on oxidative phosphorylation, and in a recent investigation by Lowry *et al.*[58] on the activity of enzymes associated with glycolysis and the hexose monophosphate shunt, no significant biochemical differences were noted between retinas obtained from nonprimates and those obtained from the monkey. Thus, although the biochemical lesion in blindness attributable to methanol intoxication may be ascribed to an inhibition of ATP-generating systems in the retina (*vide infra*), these systems do not appear to account for the species difference in methanol poisoning; there are, however, at least three possible factors that could contribute to an explanation of this curious phenomenon.

One of the possibilities that must be considered is that the metabolism of formaldehyde is different in these two groups. It may be that in the nonprimate retina, oxidation of methanol to formaldehyde is very slow, and the further metabolism of formaldehyde is rapid. Thus, the concentration of formaldehyde in the retina at any one time would be too low to cause damage. In contrast, formaldehyde may be generated rapidly from methanol by the primate retina, but its subsequent elimination may be slow; thus, formaldehyde could achieve a concentration high enough per unit time that its inhibitory properties could be exerted. Unfortunately, no data are available that bear on this hypothesis. With respect to the second striking sign of methanol intoxication, metabolic acidosis, the same hypothesis outlined above may apply, with the liver being the tissue of paramount importance.

A second possibility that could be involved in the species difference is referable to an anatomical consideration. It is well known that the retina of the rabbit and the guinea pig are essentially avascular, in contrast to that of man and monkey.[59] Accordingly, it is conceivable that the toxic agent may have difficulty in reaching the retinal cells of the nonprimate. A third possibility to be considered is that, for some reason not yet evident, the sensitivity of the retinal cells of primates to formaldehyde could be much greater than that of nonprimates.

The metabolic lesion in blindness

Despite the large number of case reports and studies of methanol poisoning, as noted in the introduction, no theory that is compatible with present knowledge of retinal metabolism has been offered to account for the retinal damage. Although in recent times both Potts and Johnson[48] and Leaf and Zatman[47] have observed the inhibitory properties of formaldehyde on retinal respiration and glycolysis, neither group has proffered a hypothesis that explains the mechanism of the blindness caused by the administration of methanol.

In this laboratory our initial hypothesis relating to metabolic lesion in the eye to methanol poisoning was based on the premise that formaldehyde interferes with the generation of ATP, a compound assumed to be intimately related to the visual process; the resultant deficiency of this compound would then lead to a degeneration of certain retinal cells, with blindness as the end result. The authors are aware of the "easy virtue" of interference with ATP-generation as a hypothesis to explain drug action, and of the fact that in every case in mammalian tissues in which the detailed

biochemical mechanism of action of a drug is known (e.g., physostygmine, tetraethylthiuram disulfide (Antabuse), acetazolamide) the inhibited enzyme system may be classed as an "accessory" enzyme, rather than one involved in energy production. Nevertheless, in this situation we feel justified in asserting this thesis. After working for over two years on the problem we have yet to encounter any evidence to negate this hypothesis; indeed, much evidence to support it has been amassed.

If the criteria of Welch and Bueding[60] or Lowry and Hunter[61] are adopted, to demonstrate that an effect of a drug *in vitro* fully accounts for the situation *in vivo*, we can at least assess the reasonableness of our hypothesis.

With respect to the concentration of formaldehyde used in our experiments, we never exceeded the concentration of the agent that one might reasonably find in a typical case of methanol poisoning. Thus, the ingestion of 100 ml of methanol is generally considered to be toxic; if an even distribution of the alcohol in the body be assumed, and in the usual 70-kg man, the maximal concentration of formaldehyde that could be derived from the methanol would be 0·04 M. In our experiments *in vitro* we were impressed with the results that were obtained at concentrations of formaldehyde of only 0·0005–0·002 M. These concentrations are not only well within the maximal expected level in body tissues, including the retina, but also are compatible with the level of formaldehyde that could be expected in a reported individual who drank 4 ml of methanol, with ensuing loss of vision.[62] In experiments *in vivo* in which formaldehyde was injected directly into the eye, it was necessary (for reasons to be discussed below) to produce higher concentrations of the agent, 0·01 to 0·02 M but, from a pharmacological standpoint, these concentrations are still reasonable.

Using intact retina or mitochondria prepared from beef, we have studied the effect of formaldehyde on glycolysis, respiration, the conversion of ^{14}C-glucose to $^{14}CO_2$, the incorporation of ^{32}P-phosphate into retinal phospholipids, oxidative phorphorylation, and electron transport.

Although in intact retina an inhibition of 50% of anaerobic glycolysis was observed at the low concentration of formaldehyde of 0·0005 M, it is difficult to assess this finding, in view of the vital dependence of the retina on oxygen, with the probability that anaerobiosis does not exist in the normal retina. In contrast, formaldehyde in concentrations of 0·0005–0·005 M did not inhibit aerobic glycolysis, but actually produced a slight stimulation of this process.[50] Both Potts and Johnson[48] and Leaf and Zatman[47] have shown that formaldehyde inhibits glycolysis in retinal homogenates. These studies were confirmed and extended by Cooper and Marchesi,[63] and hexokinase was implicated as the sensitive enzyme in the glycolytic chain; more recent studies by Kini and Cooper,[50] however, have failed to demonstrate an effect of formaldehyde when testing up to a level of 0·01 M. The reason for these conflicting results is not yet apparent; it may be that, since hexokinase is in a particulate form in the retinal homogenate, the kinetics of this enzyme and of glycolysis may be dependent upon a critical factor in the preparation of the homogenate.

In intact cells, respiration, as measured by oxygen uptake or by the conversion of ^{14}C-glucose to $^{14}CO_2$, was not especially sensitive to inhibition by formaldehyde, nor was electron transport in retinal mitochondrial preparations.

The most striking finding observed in our laboratory is the marked sensitivity of oxidative phosphorylation in the retinal mitochondria to the toxic agent. At a

concentration of 0·0005–0·001 M, formaldehyde uncoupled oxidative phosphorylation by more than 50 per cent when either pyruvate, α-ketoglutarate, or succinate was used as a substrate. By way of control, acetaldehyde, even when used at a concentration of 0·005 M, had no effect. The interesting observation was made that, whereas formaldehyde at low concentrations uncoupled oxidative phosphorylation in retinal mitochondria, formaldehyde was actually a substrate for coupled phosphorylation in mitochondria prepared from liver, and yielded a P/O ratio of approximately 2·0. Similar results were obtained with both beef and monkey preparations.[64] This finding is a rare example of a qualitative difference in mitochondria prepared from different tissues.

Although the inhibition of coupled phosphorylation in retinal mitochondria is effected by pharmacological concentrations of formaldehyde, and although this inhibition is not merely a nonspecific aldehyde effect as evidenced by the lack of inhibition of acetaldehyde on this process, it is still necessary to demonstrate that this effect *in vitro* truly reflects events *in vivo*. That is to say, there are many drugs that can uncouple oxidative phosphorylation (e.g., barbiturates), but it has not been demonstrated that the pharmacological activity of these agents is a result of an interruption of oxidative phosphorylation. Unfortunately, it is impossible to measure oxidative phosphorylation in intact mammalian cells: one can assay this system only by an indirect approach. The method that we used in intact retina was the incorporation of ^{32}P-labeled inorganic phosphate into phospholipids, a process known to be dependent upon oxidative phosphorylation.[65, 66] With this technique it has been shown that formaldehyde, at a level in the eye of 0·001 M, inhibits by 50 per cent the incorporation of ^{32}P into phospholipids.[51] Thus there is some support, albeit indirect, that energy production in whole retinal cells is diminished by the toxic agent.

In order to demonstrate this effect on phospholipid synthesis *in vivo*, formaldehyde and ^{32}P-phosphate were injected intraocularly into rabbits; the final concentration of the toxic agent in the eye was approximately 0·01 M. When the rabbits were sacrificed 24 hours later and the retinas were removed and assayed for ^{32}P-labeled phospholipids, a 50 per cent inhibition of the incorporation of the isotope was observed in the formaldehyde-injected eyes, as compared with that of the control eyes, which were injected with ^{32}P-phosphate alone. Correlative studies also were performed in order to assess the effect of the formaldehyde injection on the histology of the rabbit retina. At a concentration of 0·02 M, formaldehyde caused a loss of ganglion cells, cyst formation in inner nuclear and inner plexiform layers, blurring of the rods and cones, and swelling and edema of the optic nerve fibers. These histological changes, which could be seen on occasion with the toxic agent at a concentration of 0·01 M, are strikingly similar to changes observed in retinal sections from fatal cases of human methanol poisoning. In these experiments the injection of methanol, sodium formate, or acetaldehyde, when tested at final concentrations of 0·05 M in the eye, had no significant effect either on the incorporation of ^{32}P-phosphate into phospholipids or on the histology of the retina.[51] The necessity for the higher concentration of formaldehyde required *in vivo*, as compared with experiments *in vitro*, may be attributable to nonspecific binding of this reactive compound to other components of the eye; thus, the actual concentration of formaldehyde in the eye would be considerably lower than that estimated without taking such a factor into consideration. Furthermore, it is conceivable that some of the formaldehyde injected into the eye is eliminated either

by diffusion or by enzymic metabolism with a resultant diminished concentration. Although the role played by ATP in the transmission of the visual impulse is still unclear, the close topographical arrangement between the centers involved in visual excitation and the mitochondria in the rods and cones, as shown by Sjostrand,[67] suggests that this "high-energy" compound may be intimately concerned with this process.

To summarize our investigations on the biochemical lesion in blindness caused by methanol poisoning, we postulate that in the retina, methanol is oxidized to formaldehyde, which inhibits ATP-generation primarily through the uncoupling of oxidative phosphorylation and, perhaps secondarily, through an inhibition of anaerobic glycolysis. The net result of this deficiency of ATP would then be a degeneration of those retinal cells that are concerned with vision and the ultimate production of blindness.

Metabolic acidosis

In addition to the loss of vision, the second striking characteristic of methanol poisoning is the development of a metabolic acidosis. This condition closely parallels the occurrence of amblyopia, particularly in severe cases of methanol intoxication. Acidosis can be so severe as to lead to a plasma CO_2-combining power of zero. Although earlier workers assumed that formic acid was the causative agent in this condition, it soon became apparent, with the increasing number of reports of metabolic acidosis in patients who drank very little methanol, that even if all the methanol they had consumed was converted to formic acid, the body buffers could easily take care of the acid. Harrop and Benedict[68] reported in 1920 a large increase in the urinary organic acids of methanol-poisoned patients (2200 ml of 0·1 N acid/l of urine). In the same year Van Slyke and Palmer[69] titrated the organic acids in the urine of a patient who subsequently survived methanol ingestion; they accounted for approximately 25 per cent of the organic acids in the urine as lactic, formic, or acetoacetic acid, but the remaining 75 per cent was unidentified. In 1955, Potts[57] observed an increased urinary excretion of organic acids in monkeys to which methanol had been administered, but no attempt was made to identify the acids.

In this laboratory,[56] attempts were made to repeat the work of Potts, with a view to the subsequent identification of the unknown acid(s) excreted in the urine. However, despite numerous attempts and despite methanol administrations up to the point of fatal reactions in monkeys, we have been unable to show any increase in urinary organic acids or, for that matter, any significant manifestations of toxicity, other than narcosis, as seen with many alcohols. In addition, the oral LD_{50} for methanol in our monkeys was over twice as high as that reported by Potts in his series with the same species of monkey (rhesus). We have no explanation at this time for these discordant reports. It appears, however, that monkeys do not always emulate man with respect to their response to methanol, and that the ultimate answer to the identification of the acid or acids, that appear in 20- to 40-fold excess over normal organic acid excretion in urine, will have to await human material. At this time, with no evidence available, it would be idle to speculate on the nature of the acidic material.

CONCLUDING REMARKS

The many-faceted problems of methanol poisoning, most of which have been recognized since the turn of the century, have only begun to be explored in the light of

modern biochemical knowledge. Definite answers are still to be obtained to the questions of the long latent period before the onset of symptoms, the nature of the organic acid or acids that are responsible for the metabolic acidosis, and the reason for the curious species difference that is observed in this poisoning. It is unfortunate that most of these problems can be solved only with the use of human material after exposure to this toxic agent that produces such unusual and unfortunate biochemical lesions.

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