SPECIES DIFFERENCES IN METHANOL POISONING

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I. MINIMAL LETHAL DOSES, SYMPTOMS, AND TOXIC SEQUELAE OF METHANOL POISONING IN HUMANS AND EXPERIMENTAL ANIMALS

The minimal lethal dose of methanol in humans has not been determined. It has been suggested that about 1 g/kg can cause death if the patient is untreated and has not consumed ethanol.[43]

In some clinical cases, the blood methanol content is low in the last phase of the poisoning. In three such cases[11] blood methanol concentration was 0.275, 0.277, and 0.194 g/ℓ, respectively. On the assumption that the body water in diffusion equilibrium with the blood represents about 70% of the body weight, it has been calculated that 0.19, 0.19, and 0.14 g/kg, respectively, was present in the body.

Data on the rate of methanol oxidation in humans probably do not exist. In rhesus monkeys given 1 g/kg of methanol, Makar et al.[26] showed that 37 mg/kg/hr was oxidized. Provided that the rate of methanol oxidation is the same in man, the amount of methanol oxidized during 18 hr (the average time needed for development of severe acidosis in clinical cases) would be 0.666 g/kg. It seems reasonable still to regard 1 g/kg of methanol as the approximate minimal lethal dose in man.

The severe symptoms appearing after about 18 hr are well known: vomiting, Kussmauls respiration, pain in the back and the extremities, and often exceedingly violent abdominal pains. Simultaneously, or shortly after the onset of severe symptoms, amblyopia appears which may develop rapidly into amaurosis. The pupils are dilated and do not react to light. Sopor and coma follow. The next alarming symptom is a reddish-cyanotic color of the skin. Now the cessation of respiration is not far away. When respiratory arrest occurs it seems too late to save the patient.[4,11,44,52]

By ophthalmoscopy, a slight injection of the optic disc occurs in many cases, with or without some blurring of the disc margins. Extensive retinal edemas were not seen in our cases.[39,40]

Those patients who have regained full vision (i.e., V ≧ 6/6 with no central relative scotoma) in the course of a week after treatment, retain it. In patients whose vision partly returns, a decline is observed in the course of some weeks or a few months. The first clinical sign of optic nerve atrophy, the pale papilla, is seen from 4 to 6 weeks after the poisoning. A very marked atrophy of the retinal blood vessels follows.[40] Post-mortem examination shows some large and many small hemorrhages in the brain and both ganglion cells and glia cells are degenerated.[11] Degeneration of retinal ganglion cells has been observed.[31]

The minimal lethal dose of methanol in the rat, rabbit, and dog is, 9.5, 7, and 8 g/kg, respectively.[13] The symptoms are evidently produced by the general anesthetic effect of

1.2.1.1.). It was found that *S*-formyl-glutathione was an intermediate in the reaction mediated by this enzyme. Moreover, a new thiol esterase was found which hydrolyzes the *S*-formyl-glutathione.[49]

In vitro formaldehyde is easily oxidized by the catalase-H_2O_2 complex.[6] If the reaction works in vivo it would mean an increased defense against accumulation of free formaldehyde in the body.

C. Formaldehyde in Condensation Reactions

Formaldehyde can condense with a number of metabolites with formation of other physiological compounds.[18] Of special interest are the reactions of formaldehyde with sulfhydryl compounds such as -SH enzymes, cofactors as cysteine, glutathione, and CoA-SH. Aldehydes are generally regarded as SH reagents, and formaldehyde is a stronger reagent than other aldehydes of the homologous series. Thus in the reaction with the -SH group of glutathione, formaldehyde reacts about ten times as rapidly as acetaldehyde and propionaldehyde. The binding of aldehyde substrates to enzymes seems to involve reactions with sulfhydryl groups.[51]

In several investigations no free formaldehyde has been detected in blood and tissues of animals given methanol.[18,31,32]

IV. ELIMINATION OF FORMATE

A. Oxidation of Formate in vitro

Keilin and Hartree[17] described in 1945 experiments which showed that catalase can utilize hydrogen peroxide formed by a primary oxidation system such as xanthine oxidase and hypoxanthine. The catalase-H_2O_2 complex catalyzed secondary oxidations of ethanol, methanol, and some related compounds. They regarded this reaction to be a more likely biological property of this enzyme than the decomposition of hydrogen peroxide into oxygen and water.

In 1947 Chance[6] studied the coupled reaction spectroscopically and found that the catalase peroxide complex was formed in a reaction whose velocity was higher than that required for catalytic activity. Ethanol, methanol, formaldehyde, and formate were the compounds of biological interest which served as substrates.

Formate oxidation was studied in rat liver and jejunum extracts by Oro and Rappoport in 1959.[35] They concluded that formate is oxidized to carbon dioxide by the catalase-peroxide complex, and that dehydrogenases do not participate in the oxidation. The hydrogen peroxide is produced by the reactions between flavin-linked oxidases and their respective substrates, viz. the xanthine oxidation:

$$\text{Xanthine} + O_2 + H_2O \xrightarrow[\text{oxidase}]{\text{xanthine}} \text{Uric acid} + O_2^- \qquad (1)$$

$$2\,O_2^- + 2\,H^+ \xrightarrow[\text{dismutase}]{\text{superoxide}} H_2O_2 + O_2 \qquad (2)$$

Both the superoxide radical and hydrogen peroxide are very toxic. They are detoxicated by the dismutase and the catalase.

B. Oxidation of Formate In Vivo

Certain investigations seem to indicate that in rodents both formate and methanol are oxidized by the catalase-peroxide system. In the monkey, however, the liver alcohol dehydrogenase possibly plays a major role in the first step of methanol oxidation.[26]

It has been suggested that the species differences in the activity of the peroxidatic system might be due to differences in distribution of catalase between the microbodies (peroxisomes) and the cell sap. When compared on a per gram liver basis, the hepatic catalase from rodents showed a higher activity — both a peroxidatic and a catalatic activity — than that of the monkey.[27]

However, in a third investigation, it was found that the peroxidatic capacity of both microbody and cell sap catalase greatly exceeded the capacity of hydrogen peroxide generation in rat and monkey liver. Therefore, the control in vivo most likely would be the substrate levels for the oxidases generating hydrogen peroxide.[14]

C. An Alternative Pathway for Formate Oxidation

Kutzbach and Stokstad[19] have shown that formate can be oxidized to CO_2, when observed in a system in which N^{10}-formyl-THFA was formed *in situ* by the formylase reaction (formylase = formate:tetrahydrofolate ligase = formyl-tetrahydrofolate synthetase) (EC 6.3.4.3.). Very little oxidation of formate to CO_2 was observed in the absence of either the folate compound or the complete formylase system.

D. Elimination of Formate

Elimination of formate by its utilization in syntheses in the one-carbon metabolism occurs in nonprimates which possess the formate-activating enzyme formyltetrahydrofolate synthetase (EC 6.3.4.3.) This enzyme probably is lacking in primates, in which the formyl-C is derived from serine.[51]

E. Elimination in the Urine

In two dogs Lund[24] found maximal blood formate 48 hr after start of the experiments: 8.7 and 11 mmol/ℓ, respectively (see Table 1). The formic acid concentration of the urine corresponded fairly closely to the course of the blood formate curve. In two fatal clinical cases in which no treatment had been given,[25] the concentration of formate in the urine was 101 and 170 mmol/ℓ, respectively.

V. SPECIES DIFFERENCES IN PURINE METABOLISM

During evolution, man and anthropoid apes have been deprived of the liver enzyme urate oxidoreductase (uricase) which oxidizes uric acid to allantoin. Hence, uric acid is the main terminal compound of purine metabolism in primates and allantoin in nonprimates.[1]

Both in primates and in nonprimates, a part of the purines formed in the organism is reused in nucleotide and nucleic acid biosynthesis. The enzyme acting in this process is guanine-hypoxanthine-phosphoribosyl transferase. In man on purine-free diet, and excretion of uric acid is only 3 mmol/24 hr, while 27 mmol/24 hr of the purines are recycled.[22]

Total purine excretion in the urine of dog and rabbit, when compared to their body weight, exceeds by far the value found for man. The rat excretes 1.5 mmol/kg/24 hr of allantoin, while daily urate excretion in man is in the range of 0.06 mmol/kg/24 hr.[15]

In the Dalmatian dog, Benedict[2] found an anomalous purine excretion. On purine-free diet, the uric acid nitrogen was more than double that of allantoin. The anomaly is inherited according to Mendel's laws.[20] Friedman and Byers found that the uric acid was excreted by this animal at the level of glomerular filtration, without subsequent reabsorption or excretion. The total production and excretion of both purine end products was the same in the Dalmatian as in the non-Dalmatian dog.[12] Some authors have suggested that the liver uricase is less active in the Dalmatian than in an ordinary dog.[15]

VI. THE PATHOGENESIS OF HUMAN METHANOL POISONING

Clinical investigations during World War II showed that symptoms and signs late in the course of methanol poisoning indicated a state of tissue hypoxia. The great affinity known to exist between formic acid and iron salts formed the basis for the hypothesis that the acid might bind the iron of the respiration ferment.[39] Both the amblyopia and the cyanosis developed only during very severe acidosis, i.e., the symptoms evidently were dependent on low pH.[40]

During the last decade, some investigations have thrown more light on formate toxicity and its dependence on pH. In 1969 Herken et al.[16] found increasing toxicity by decreasing pH. A damaging action to biological membranes appeared, resulting in hemolysis and increased penetration of formic acid from blood vessels to the cerebrospinal fluid. The toxicity is determined essentially by the pH, and runs parallel to the amount of undissociated formic acid.

Some years ago Nicholls[33,34] demonstrated that "formate inhibits cytochrome c oxidase both in intact mitochondria, in submitochondrial particles, and in isolated cytochrome aa_3. The inhibition increases by decreasing pH, indicating that HCOOH is the inhibitory species."

Formate is bound at the sixth coordination position of ferric haem iron in cytochrome a_3. This has a hemoglobin-like structure with weak or no ligands at sixth position.

Cytochrome a has hemochromogen structure, with protein ligands at both fifth and sixth iron coordination points. Cytochrome aa_3 has a pattern of inhibition closely resembling that of catalase and peroxidases.[34]

In experiments on the effect of formate on cytochrome aa_3 and on electron transport in the intact respiratory chain, it was demonstrated that also under the new assay conditions formate was an effective oxidase inhibitor. The affinity of formate for catalase was equal to that for oxidases.[34] Both reactions are reversible and show the same dependency on pH.

Formate also inhibits succinate-cytochrome c reductase, and in the intact mitochondrion the glutamate-oxaloacetate transaminase and malate dehydrogenase.[34]

The K_i for formate inhibition of respiration is varying between 30 and 1 mmol/ℓ at pH 7.4 and 30°C.[34] It has been calculated that undissociated HCOOH by pH 7.4 is 0.022%, and by pH 6.9 is 0.071% of the total acid. When arterial blood contains 20 mmol/ℓ of formic acid, the concentration of undissociated HCOOH by pH 7.4 is $0.44 \cdot 10^{-5}$ M/l and by pH 6.9 $1.4 \cdot 10^{-5}$ M/l (Eldjarn). In the tissues, the decrease of pH is greater than in blood.

Hypoxia, which probably is the most serious symptom of formate toxicity, accelerates the adenine nucleotide catabolism of the cells. After a few minutes of anoxia, the purine metabolites are irreversibly lost, and hypoxanthine accumulates. Normally 90% of hypoxanthine is resynthetized to inosine monophosphate (IMP). In severe hypoxia or anoxia, little or no energy is available for this reaction.[45]

Formate inhibition of the catalase must contribute to accelerate the rise in blood formate concentration if formate oxidation in man is mediated by catalase. H_2O_2. Moreover, inhibited catalase means less capability to combat the toxic action of H_2O_2.

The biochemical investigations explain why acidosis is the dominant symptom in human methanol intoxication. There is every reason for pointing out that maximal chance of saving sight and life in severe methanol acidosis is a quick correction of the low blood pH to normal values. In addition, reapperance of acidosis must be prevented by administration of ethanol which strongly inhibits methanol oxidation.[39,40,43]

VII. DISCUSSION

A. Two Hypotheses on the Cause of Species Differences in Methanol Intoxication

For a long time the species differences in urate handling have been used in the classification of mammals into primates and nonprimates. Those animals which possess liver uricase and excrete allantoin as the main end product of purine catabolism have been regarded as nonprimates.[15] The group of Old World monkeys, to which *macaca mulatta* and *macaca nemestrina* belong, excrete allantoin and present a low blood urate level (30 μmol/ℓ), typical for nonprimates. In the present publication, the monkey will be classified as a nonprimate, being aware that some writers have regarded this animal as a primate.[13]

The great toxicity of methanol to man seems to be related to the loss of two enzymes during evolution: (1) the lack of uricase (EC 1.7.3.3.) and the high degree of recycling of purines (9/10) provides very little hydrogen peroxide from the purine catabolism, i.e., a small capability for peroxidatic formate oxidation; and (2) lack of formyl-tetrahydrofolate synthetase (EC 6.3.4.3.) probably is the reason why man cannot utilize formate in many syntheses via the folate pathway, and not be oxidized in an alternative oxidation process depending also on the synthetase.

For nonprimates, this pathway probably is very important. Rietbrock et al. found that in dogs a rise in blood formate from 3 to 6.7 mmol occurred after administration of a folate inhibitor. Administration of folate to the dog depressed blood formate concentration considerably.[38] Vitamin B_{12} had a similar but weaker effect.[38]

In the Dalmatian dog, only one third of produced uric acid is oxidized to allantoin. If the first hypothesis holds true, we should expect higher formate accumulation in the Dalmatian than in an ordinary dog. This is a matter pending further investigations.

B. The Animal Experiments in Methanol Poisoning

Gilger and Potts[13] found the rabbit to be a very poor subject for acidosis studies because the normal variation of plasma CO_2 was from 19 to 56 vol%. In dogs the normal range was 12 vol% only. Later, low plasma bicarbonate has also been demonstrated in most monkeys *before* methanol administration.[7,13,36]

It seems difficult to regard these variations as being normal. The differences in the instability of the acid base balance in the anxious rabbits and monkeys on the one side and in dogs — the best friends of man — on the other, seem to indicate a psychic cause of this "acidosis".

Severe psychic disturbances, as a long-lasting agonizing fear, can be the cause of pulmonary hyperventilation which results in respiratory alkalosis or hypocapnia. The studies in dogs by Eichenholz et al.[10] have shown that reduced pCO_2 precipitates a loss of bicarbonate, most of which can be accounted for by the rise in blood lactate and pyruvate. The bicarbonate deficit becomes evident within 60 min after pCO_2 reduction, and is progressive in nature. The production of lactic and pyruvic acid *does not* terminate at pH compensation by sustained low pCO_2. When suddenly normal pCO_2 is restored after partial or complete compensation, ventricular arrhythmias and sudden deaths have sometimes occurred.[10]

The severity of this metabolic acidosis depends on the degree and duration of the hypocapnia. The rhesus monkeys employed by Gilger and Potts were wild male monkeys which probably reacted more violently to the change to life in the laboratory than those monkeys which had been held in captivity for a longer time and had been used to being handled by laboratory personnel.

A large dose of methanol might cause decreased sensitivity of the respiratory centers.