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

high doses of methanol, as described in an earlier review. Permanent impairment of vision has not been demonstrated with any degree of certainty.[43]

Since 1955 several investigations on methanol poisoning have been performed in monkeys. These results should be of special interest since it has been suggested that the monkey can serve as a valid model for the toxic syndrome seen in man.[30]

The minimal lethal dose of methanol in the rhesus monkey *(macaca mulatta)*, as determined by Gilger and Potts,[13] was 3 g/kg. The animal was slightly intoxicated for 1 to 2 hr. Then there was a symptomless latent period of about 12 hr, followed by "sickness and death". Narcosis appeared as a terminal manifestation.

With 1 g/kg of methanol, there was no drop in plasma CO_2. With 2 g/kg, it decreased from 53 vol% to 16 vol% by 24 hr. The monkey recovered soon. Four monkeys getting 3, 4, 6, and 8 g methanol per kilogram, respectively, died. The last monkey died after a few hours; in the others the plasma CO_2 decreased within 24 hr to less than 15 vol%. It should be noted, however, that the control plasma CO_2, tested just prior to the start of the poisoning, was between 32 and 41 vol%. In the two surviving monkeys, the control plasma CO_2 was about 50 vol%. The lowest plasma CO_2, 32 vol%, was found in the monkey having received 3 g methanol per kilogram.

Two out of six monkeys showed changes in the fundus oculi. The monkey given 3 g/kg presented slight blurring of the optic disc margins in both eyes after 25 hr. Another developed a small monocular retinal hemorrhage 29 hr after administration of 6 g methanol per kilogram. No sign of blindness was described. In the opinion of the authors, the "clinical picture" resembled that described in man.

A completely different picture of methanol poisoning in rhesus monkeys was described by Cooper and Felig 6 years later, in 1961.[7] A total of 12 monkeys were employed, 8 of which were reused 1 to 5 times. All animals given 1 to 6 g/kg of methanol survived. Death occurred in 50% of those monkeys having been given 7, 8, and 9 g/kg of methanol. There were no symptoms like those seen in man, such as vomiting or deep and irregular respiration. Varying degrees of intoxication and ataxia occurred, sometimes progressing to a narcotic state which usually lasted from 5 to 12 hr. All these animals were alert by 24 hr after poisoning. In fatal cases, the picture was one of narcosis followed by death.

In three monkeys given 6 g/kg of methanol, the serum bicarbonate was, respectively, 4.9, 4.6, and 8.6 mmol/ℓ 48 hr after methanol administration. The control serum bicarbonate, determined at the start of the experiments, was, respectively, 16, 9.9, and 13.8 mmol/ℓ. Again it seemed as if some of the blood bicarbonate had been displaced before methanol was given.

In spite of the high doses of methanol given in these experiments, no case of permanent blindness was seen. In one monkey having received 9 g/kg of methanol, there was no response to gross visual stimuli on the 4th day, but this was transient. From the following day the reaction was normal and remained so.

The animals were evidently allowed to be relatively unrestrained in both the experimental series referred to here. In both cases, methanol diluted to about 20% was given through a gastric tube. Both groups of investigators used the rhesus macaque. Why then the great differences in the reactions to methanol? This difficult question has to be discussed later.

In experiments performed by McMartin et al.[31] in 1975, the intention was to review the position of whether the rhesus macaque or the pig-tailed macaque could serve as models for human methanol poisoning. Through an indwelling catheter in the femoral artery, blood was obtained for determination of methanol, formate, pCO_2, pO_2, pH, and for electrolyte analysis. In addition, the rate of ^{14}C-methanol oxidation was also followed. The monkeys were restrained in a small chair that formed a part of the metabolism chamber, and their limbs were tied.

It is important to note here that Oluf had no idea that McMartin, Tephley, Makar, Martin-Amat, Mannering,Hayreh and Filer were being paid huge sums to prove that the monkey would indeed be an acceptable model for methanol poisoning in man.

The minimum lethal dose 3 g/kg of methanol, as determined by Gilger and Potts 20 years earlier, was given in 20% solution through a naso-gastric tube. Slight intoxication appeared for 1 to 2 hr, then the condition of the monkey progressively deteriorated with signs of anorexia, photofobia, weakness, restlessness, and profound hyperpnea. After 16 hr arterial blood pH was 7.15, pCO_2 (av.) 20 mm Hg. Plasma bicarbonate decreased from 21 mmol/ℓ to 8 mmol/ℓ. Maximum blood formate was 7.5 mmol/ℓ, about 50% of the increased anion gap. Death occurred from 12 to 33 hr after methanol administration.

The authors were probably right in suggesting that the severe restraint contributed to the evolution of metabolic acidosis and death.

Some of the monkeys used by Makar et al.[26] in studies on the methanol oxidation rate were given 6 g/kg which was tolerated well. However, the experimental period was only 4 hr in each of these cases.

McMartin et al. did not find any retinal edema in their cases within 24 hr.

Neither in the experiments mentioned nor in some others[29,31] has amaurosis or optic nerve atrophy been demonstrated. This represents the most fundamental difference between the toxic effects of methanol in man and that in the rhesus monkey and lower mammals. The cause of the species differences is as yet unknown. Most likely, the answer is to be found in differential metabolic processes.

From the beginning of this century, the oxidation products of methanol, formaldehyde, and formic acid have alternately been regarded as being the proximal toxic agent.[39] We can hardly discuss the possible cause of the species differences in methanol poisoning without considering the metabolism of the oxidation products in some detail. However, it would be appropriate first to give some data concerning the accumulation of formate, which so far have been presented only from experiments in monkeys.

II. SPECIES DIFFERENCES IN ACCUMULATION OF FORMATE

A. Animals

In *rats* no significant accumulation of blood formate was demonstrated after a methanol dose of 6 g/kg body weight had been given intraperitoneally.[31] *Rabbits* are able to oxidize formate almost completely, regardless of whether it has been given as methanol or as sodium formate.[23] In *dogs* varying results have been obtained. In two dogs given 2 g/kg of methanol intravenously, the maximal blood formic acid levels were, respectively, 2.6 and 3.2 mmol/ℓ.[26,38] In two other dogs given 1.7 and 1.9 g/kg of methanol orally, the blood formic acid concentration was 8.7 and 11 mmol/ℓ, respectively.[24] These discrepancies in results will be mentioned later in connection with a discussion on the anomalous purine catabolism in the Dalmation dog.

Animal experiments generally are based on the administration of a single dose of methanol. If accumulation of blood formate in monkeys has to be compared to that in other animals or in man, the same technic should be used. This demand was fulfilled in the experiments by McMartin et al.[31] in *rhesus monkeys*. After a single dose of 3 g/kg the maximal blood formate was 7.5 mmol/ℓ after about 16 hr.

B. Man

Two patients described by Lund[25] in 1948 died within 24 hr after methanol consumption. The diagnosis was made at autopsy. In the first case blood formic acid was 14.8 mmol/ℓ and liver formic acid 13.2 mmol/kg liver tissue. In the second case liver formic acid was 21.3 mmol/kg (blood formic acid probably about 23 mmol/ℓ).

In 1965 Erlanson et al.[11] reported three cases in which blood formic acid was determined. The patients were all in a hopeless situation, deeply comatose with

respiratory paralysis and very low blood pressure. Blood formic acid: 22.8 mmol/ℓ, 14.8 mmol/ℓ, and 8.3 mmol/ℓ *(vide infra)*.

The first two patients had developed severe acidosis with coma followed by respiratory paralysis shortly before they were transferred to a dialysis clinic from another hospital. The third patient had been treated with alkali and ethanol intravenously a few hours before the blood formic acid test was made. This treatment most probably lowered the blood formate level. Ethanol inhibits formate formation[43] and correction of severe acidosis increases the tissue respiration in methanol poisoning.[33,34]

Table 1 presents some data from experiments as well as from clinical cases. The data are collected over a period of about 30 years or more.

It will be seen from the table that only in man is the blood formate concentration high enough to provoke severe acidosis per se. It also seems evident that in rodents (rat and rabbit) there is no specific toxic action of methanol. It is felt that the low lethal dose of methanol for monkeys in some cases[13,31] is not due to the action of methanol alone. In order to get more knowledge on the pathogenesis of the acidosis in monkeys, it would be necessary to analyze the acids beside the formic which account for the increase in the anion gap.

The accumulation of formate evidently parallels the toxicity of methanol. Therefore we will return to the problems of the elimination of formate after having studied the formaldehyde, which, until recently, has been regarded by some authors as being the proximal toxic agent in methanol poisoning.[8,37]

III. FATE OF FORMALDEHYDE

A. Formaldehyde as a Normal Metabolite

Formaldehyde is an obligatory intermediate in the metabolism of the one-carbon compounds. The organism is able to synthetize formyl groups. The normal biological source of formyl carbon is the beta-carbon of serine.[22]

Formaldehyde formed by methanol oxidation can be partly utilized as a substitute for formyl groups formed in the normal metabolism.[18] Such groups combine with tetrahydrofolic acid(THFA) nonenzymatically. The N^{10}-formyl-THFA is a central compound in the one-carbon metabolism and is the form utilized for formyl transfer in many synthetic pathways. By a two-step reduction it is transferred to N^5-methyl-THFA, which affords a pathway for *de novo* genesis of methyl groups of substances as methionine, thymine, choline a.o. Vitamin B_{12} acts as a carrier of methyl groups from N^5-methyl-THFA to homocysteine to form methionine.

Besides contributing to the pool of labile methyl groups, formaldehyde (and formate) take part in the purine synthesis. Accordingly, carbon atoms no. 2 and no. 8 of the purine nucleus derive from formate in those organisms which can activate formate.[51]

It has earlier been assumed that direct conversion of the carbon of a one-carbon-THFA derivative to CO_2 does not occur.[51] However, later investigations in vitro, in which homogenates from pig liver were used, have led to the finding of an oxidative deformylation of N^{10}-formyl-THFA with production of CO_2.[21]

B. Formaldehyde Oxidation

The direct oxidation of formaldehyde to formate occurs even when the one-carbon pathway is closed by folic acid inhibition.[32] Most probably the specific NAD-dependent formaldehyde dehydrogenase found by Strittmatter and Ball[47] plays the major rôle in the oxidation. Glutathione is a necessary cofactor. Maximal activity occurs at formaldehyde concentration as low as 1×10^{-5} mol/ℓ.

Some years ago Uotila and Koivusalo[48] purified this enzyme from human liver (EC

Table 1

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

	Man	Monkey	Dog	Rabbit	Rat	Remarks
Minimal lethal dose, g/kg	1	3 (13, 31)* 7 (7)	8 (13)	7 (13)	9.5 (13)	
Metabolic acidosis at						
1 g/kg	Severe	None	None	None	None	Monkey (13): $PL.CO_2$ 32 vol% at start of exp., (31): severe restraint, long-lasting exp.
3 g/kg	Severe	Severe (13, 31)	None	None	None	
6 g/kg		Severe (7)	None	None	None (31)	
8 g/kg			X (13)			X: $PL.CO_2$ 23 vol% after 30 hr, blood formate not tested; recovery; respiratory acidosis?
Optic nerve atrophy	Yes	No	No	No	No	Monkey (13): slight blurring of optic disc margins
Retinal gangl. cells	Degenerated (41)	Normal (29)		Normal (41)	Normal (41)	Monkey (31): no retinal edema
						Monkey (30): prolonged acidotic state produced opt. disc edema
Blood formate, mmol/ℓ	15–22.8 (11)	7.5 (31)	2.6 (28)	0 (23)	0 (31)	Dogs, meth. g/kg: 2, 2, 1.7, and 1.9
	15–23 (25)		3.2 (38)			Man, blood meth. 0.275 and 0.277 g/ℓ (11)
			8.7 (24)			Rabbit, methanol 3.9 g/kg
			11.0 (24)			Rat, methanol 6 g/kg
Blood formate percent of anion gap increase		50% (31)				

* Reference numbers are in parentheses.

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.

Respiratory acidosis (hypercapnia) may develop, which soon is followed by a flow of cations from the cells to the extracellular fluid.

Brown and Mowlem[5] have demonstrated a constant rise in blood plasma potassium levels in dogs during the hypercapnic period. Moreover, 5 min after returning to air breathing, there was a significant loss of potassium from the heart muscle. This may be accompanied by severe cardiac irregularities, often resulting in death.

Respiratory acidosis always is accompanied by more or less hypoxia and lactic acid accumulates. When blood formate concentrations do not account for the increase in anion gap, analysis of blood pyruvate and lactate concentrations should be done.

C. Can the Rhesus Monkey be Blinded by Methanol?

Some investigators have regarded the monkey as a valid model for the toxic syndrome seen in man, including the ocular toxicity.[29-31] The appearance of more or less edema in the fundi of the eyes have, in their opinions, been a sign of ocular toxicity.

The investigations on the toxicity of formate, as well as of methanol in monkeys, have led to the conclusion that "formate produces its toxicity apart from the acidotic state which accompanies and complicates the methanol-poisoning syndrome in monkeys and humans."[29] In contrast to these results, clinical investigations have shown the severe acidosis to play the major role.[39,40]

The investigations by Nicholls tally best with the clinical experiences: "The inhibition of cytochrome c oxidase by formate increases by decreasing pH, indicating that HCOOH is the inhibitory species."[13,14] The enzyme catalase is inhibited by formate with the same affinity as cytochrome-oxidase. The toxic action of formic acid to biological membranes also runs parallel to the concentration of free acid.[16]

In conclusion, the toxicity of methanol seems to be a function of the two variables, the pH and the formate concentration in the tissues. In humans, amblyopia and amaurosis do not appear before severe, i.e., uncompensated acidosis, has developed. It seems very unlikely that in rhesus monkeys (blood pH about 7.2 (av.) and blood formate about 7.5 mmol/ℓ) amaurosis and atrophy of the optic nerve can be caused by methanol.

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