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Methanol Metabolism and Toxicity

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INTRODUCTION

Methanol is commonly used in industry for organic synthetic procedures or as a solvent. As a result, it is accessible to the general public in a variety of products such as antifreeze, fuels (Sterno), duplicating machine fluids, and in gasoline as a fuel extender. Methanol and other alcohols have been employed as sources of energy or fuel for many years, particularly in times of war. Methanol's use as an automobile fuel, as well as other proposed uses for energy production, will increase human methanol contact from a limited laboratory or industrial exposure to a general environmental exposure. Although methanol theoretically represents a "clean" substance capable of oxidation to water and carbon dioxide, in humans biochemical reactions produce metabolites that are clearly toxic.

A consideration of the toxicity of methanol, especially in species which demonstrate signs and symptoms, seems appropriate for several reasons. First, humans are sensitive to methanol poisoning, and limits of tolerance must be considered. Second, nutritional factors may play an important role (e.g., folate deficiency) in determining susceptibility. Our current understanding of the mechanisms involved in methanol toxicity is described.

CHARACTERISTICS OF POISONING IN MAN

The toxicity of methanol in humans has been appreciated since the early part of the twentieth century. In 1855 MacFarlan (1) proposed that a mixture of 1 part

of impure methanol ("wood naphtha") to 9 parts ethanol would be a cheap substitute for the use of ethanol in dissolving resin or in chemical synthesis. This "methylated spirit" did not affect the eyes as the wood naphtha was known to do and soon became widely used in industry. Because of the toxicity of the vapors, wood naphtha itself was not used a great deal in industry, and since it was a foul-smelling and vile-tasting liquid, it was not consumed. After 1896, when methanol purification processes improved, the use of methanol increased dramatically, both as an industrial solvent and as an inexpensive substitute for ethanol. Concurrent with this increased use, reports of blindness and death followed. By 1904 Buller and Wood had collected 235 cases of blindness or death connected with exposure of the victims to methanol (2,3), including 10 cases involving inhalation or absorption of methanol through the skin. Although the dissemination of knowledge concerning methanol toxicity to industrial users decreased the number of industrial poisonings, cases of severe methanol poisoning continued to be observed due to the ingestion of methanol as a substitute for ethanol. Epidemics were not uncommon, especially in areas where extreme poverty existed, where prohibition was the rule, or where war was waged.

Since the initial report by Buller and Wood (2), numerous accounts of individual toxic responses to methanol and epidemics of methanol poisoning have appeared in the literature (4-6). The pattern of signs and symptoms of methanol poisoning is clear. A central nervous system depression is observed similar to that produced by ethanol, but to a much lesser degree. The inebriating effects of methanol have been described as disappointing by numerous users (7). The initial depressant period is followed by an asymptomatic latent period which occurs about 8-24 hr after ingestion of the alcohol and during which patients describe no overt symptoms or signs. Then headache, dizziness, weakness, and nausea are reported, followed in more severe cases by intense vomiting and excruciating abdominal and muscular pain. The abdominal pain has been described by Roe (8) to be so intense as to cause the patients to throw themselves out of bed. Patients may be disoriented and may display severe mental disintegration. With more severe cases, classic respiratory difficulties of metabolic acidosis are noticed, that is, Kussmaul breathing (severe dyspnea characterized by both marked increases in depth and rate of respiration). Coincident with the onset of the respiratory problems, patients complain of visual defects ranging from blurred vision to complete loss of vision. Prior to death, coma deepens, respiration becomes shallower, and convulsions may occur. Death is attributed to respiratory failure, and patients are usually blind prior to death (8). Often a patient may not die and may be left partially or totally blind.

Visual impairment in methanol poisoning is a characteristic feature. Upon ophthalmoscopic examination the optic fundus displays distinctive characteristics which appear in a defined sequence with various intensities (9). Initially, when symptoms of visual disturbance are reported, hyperemia of the optic disk is observed. This may disappear within 1-7 days and may be succeeded by peripapillary

edema characterized by a whitish, striated edema which blurs the margin of the optic disk and extends over the adjacent retina. The optic nerve head appears swollen, as are the retinal veins. Generally, patients who exhibit marked degrees of edema suffer permanent visual loss. Optic disk edema may persist for 10-60 days, and if the damage is sufficiently severe, pallor of the optic disk, indicating optic nerve atrophy, will eventually ensue. This is seen as a contraction of the retinal vessels, a lesion that has been reported by Buller and Wood (2) and by Benton and Calhoun (9).

Harrop and Benedict (10) first reported metabolic acidosis in a patient in whom the plasma carbon dioxide binding power was 36.4 vol % of CO_2 (equivalent to 15.8 mEq/liter bicarbonate), and, in this case, the titratable organic acids in the urine were increased markedly. These findings have been confirmed by other workers (11,12). However, because metabolic acidosis was generally not seen following methanol administration to lower animals, acidosis was not accepted as a major feature of methanol poisoning until Chew et al. (13) and Roe (8) showed the beneficial effect of alkali therapy in methanol poisoning. The administration of sodium bicarbonate intravenously provided rapid relief from dyspnea, abdominal pain, and visual disabilities, with a rapid return of normal metabolic and mental function. Indeed it has subsequently been shown many times that methanol intoxication leads to marked depletion of bicarbonate, with plasma bicarbonate levels reaching as low as 4.0 mEq/liter, with a blood pH as low as 7.04 (14). Recently we have observed patients with blood pH values as low as 6.9 (5). The plasma CO_2 combining power of four moribund patients examined by Bennett et al. (15) was 0, and urinary pH had declined to 4.5. The concurrent decrease in blood pH and blood pCO_2 indicates that the acidosis produced by methanol is an uncompensated metabolic acidosis (16), as suspected by earlier workers such as Roe (8). In some studies the degree of metabolic acidosis closely parallels the severity of the ocular symptoms (8,9).

One would expect that the identification of the characteristic signs and symptoms of methanol poisoning should be confirmed by appropriate laboratory tests which would indicate the presence of methanol. Whereas the methods employed for the measurement of blood methanol have been accurate and useful, one must remember that there is no correlation between the blood methanol and the methanol toxicity syndrome. The most severe toxicity occurs many hours following the peak blood level or tissue level of methanol, and the identification of methanol in blood or tissues, while important, does not necessarily provide an accurate indication of the toxicity.

Other observations have led to confusion concerning the methanol poisoning syndrome. For example, there are large individual differences in the duration of the latent period, and there may be great variations in the amount of methanol needed to produce toxicity in individuals. According to Bennett et al. (15), as little as 15 ml of 40% methanol produced death, whereas as much as 500 ml did not induce permanent damage in other patients. Symptoms of methanol poison-

ing appeared within a few hours or were delayed up to 72 hr. The severity of the disease was not related to the length of the latent period or to the amount of methanol consumed, observations which have led some authors (2,15) to suggest that, within the general population, different susceptibilities to methanol exist.

The apparent variability in sensitivity of humans to methanol ingestion may have several causes. The variation could be due to the inability to obtain exact information from patients who were relatively disoriented. Roe (8) proposed another theory, that the variance in reaction to methanol could be explained by the different amounts of ethanol consumed with the methanol. In his studies those patients who had consumed ethanol either before or after methanol had a longer latent period before the appearance of poisoning than those who had ingested only methanol. Furthermore, the ethanol consumers were more apt to demonstrate no toxic effects of methanol. In uncomplicated methanol poisoning (where ethanol was not a factor), those patients who had ingested more methanol generally presented with symptoms sooner and of much greater severity than those who had consumed less methanol. Death occurred more quickly in those who drank more methanol. Although the amount of methanol ingested was not the only factor determining the degree of acidosis, individual predisposition was thought not to play a major role (8).

Recent information suggests that another explanation for the individual variation may account for the variable sensitivities observed among humans who have ingested methanol. Formate is an intermediate produced during the oxidation of methanol to carbon dioxide and water, and is thought to be responsible for many of methanol's toxic effects. Susceptibility to methanol poisoning may depend on the activity of folic acid-requiring metabolic reactions which are involved in formate metabolism. Nutritional differences among individuals, such as folic acid deficiency, may play an important part in the ability of an individual to metabolize formate. Different degrees of nutritional deficiency may be observed in debilitated and inebriated persons who have not had an adequate diet. In monkeys we observed variability in the metabolism of methanol to formate and carbon dioxide when the animals were studied at different times. Some laboratories have been unable to duplicate results obtained by others (17). This failure may not be due to differences in experimental design or differences in the procedures of those individual laboratories. Instead, it is possible that animals maintained on the best nutritional regimens may be less susceptible to methanol poisoning, owing to a better hepatic capacity to metabolize methanol and formate to carbon dioxide. This will be discussed further under the role of folic acid in formate metabolism.

CHARACTERISTICS OF POISONING IN ANIMALS

Nonprimates

One of the obstacles that has retarded our understanding of the mechanism by which methanol produces its toxicity in humans has been the difficulty in extrap-

olating results obtained from experiments with certain common laboratory animals to humans. A fundamental difference exists between the characteristics of methanol poisoning in humans and those in nonprimate animals (18,19). Metabolic acidosis and ocular toxicity, the usual symptoms of methanol poisoning, are not observed in lower species. The effect of methanol in nonprimate animals is manifested almost exclusively as a central nervous system depression such as that observed with other aliphatic alcohols. The species differences in susceptibility to methanol poisoning have not been recognized by some authors and have contributed to certain misleading statements when results from experimental animals have been applied to human methanol poisoning. For instance, despite clinical evidence of the effectiveness of ethanol therapy in methanol poisoning, Gilger et al. (20) once recommended that the use of ethanol therapy be discontinued, since it significantly increased the toxicity of methanol in mice.

In 1902 Hunt (21) observed that the signs of acute poisoning with methanol in rabbits were similar to those observed with ethanol poisoning and that ethanol was more toxic than methanol. The lethal dose for a single oral dose of methanol in dogs was about 8-9 g/kg (18,22), 7-9 g/kg for rabbits (18,21), and 10 g/kg for mice and rats (18,20). Gilger and Potts (18) reported that in most nonprimate laboratory animals, methanol intoxication was observed as ataxia, a loss of righting reflex, and other symptoms consistent with central nervous system depression.

Despite the ingestion of lethal doses of methanol, nonprimate species generally do not develop significant metabolic acidosis. Haskell (22) concluded from studies of 14 dogs poisoned with methanol that, although acidosis was sometimes observed, the severity of intoxication was not correlated with the degree of acidosis. Roe (23) found no decrease in the alkali reserve or any signs of acidosis in rats or rabbits that had received toxic oral doses of methanol. Gilger and Potts (18) found little effect on the CO_2 combining capacity of blood after oral administration of very high doses of methanol in rats, rabbits, and dogs.

No impairment of vision has been observed in methanol-poisoned nonprimate animals. Although the production of clinical visual impairment with methanol has been reported in nonprimates (21,24,25), Gilger and Potts (18) pointed out that these claims were often based on four common sources of confusion not related to the typical visual disturbances seen in humans: (a) interpretation of ataxic manifestation following methanol treatment as blindness, (b) nonspecific visual impairment following exposure keratitis which results from the eyelids remaining open for extended periods of time during methanol-induced coma, (c) alteration of pupillary size and reaction due to the anesthetic action of methanol in nonprimates, and (d) interpretation of the lack of response of comatose animals to visual stimuli as blindness. Furthermore, in well-conducted studies of ocular effects of methanol in rabbits (18,23,26), chickens, dogs (18,26,27,28), and rats (18,23), there was neither evidence of visual disturbances nor any changes in the appearance of the fundus.

No consistent histopathology has been demonstrated in nonprimate species. Roe (23) showed no histological changes in the retina, particularly those lesions

that have been reported on human autopsy specimens. Although Fink (28) did not observe any ophthalmoscopic or clinical evidence of ocular dysfunction in rabbits and dogs poisoned with methanol, he reported retinal ganglion cell degeneration and occasional edema within the optic nerve. Cooper and Kini (29) pointed out that any histological changes in the retina of nonprimates were probably the result of narcotic effects of high doses of methanol.

Monkeys

Although the differences between the effects of methanol in humans and in laboratory animals were known prior to 1955 (19), there had been few investigations of the toxicity of methanol in the nonhuman primate. In a study of the ocular effects of methanol in three rhesus monkeys as well as in various nonprimates, Birch-Hirschfeld (26,27) reported clinical and ophthalmoscopic evidence of ocular damage in only one animal, a monkey. Tyson and Schoenberg (25) observed ocular changes in one monkey poisoned with methanol; however, these results were considered by Gilger and Potts (18) as an artifact of their experimental methods. Scott et al. (30) reported histopathological evidence of retinal ganglion cell degeneration in monkeys, but they did not discuss their ophthalmoscopic findings.

In 1955 Gilger and Potts (18) reported the first of a series of studies which centered on the toxicity of methanol in the rhesus monkey. The monkey was much more sensitive to methanol than were other laboratory animals. They reported a minimum lethal dose of 3 g/kg body weight for the monkey. Clinically, the signs observed in the monkey were similar to those noted in humans. There was a slight initial central nervous system depression followed by a latent period, a progressive weakness, coma, and death usually in about 20-30 hr. All four monkeys given a lethal dose became severely acidotic (plasma bicarbonate less than 6.5 mEq/liter) within 24 hr. Two of the monkeys showed signs typical of methanol amblyopia observed in humans including dilated, unresponsive pupils and changes of the retina on ophthalmoscopic examination. One monkey showed evidence of optic disk hyperemia and retinal edema.

Potts (31) examined the efficacy of alkali treatment following the administration of lethal doses (6 g/kg) of methanol to the monkey. If adequate bicarbonate was initially given to reverse the metabolic acidosis, four of six animals survived. However, despite correction of the acidosis, two animals eventually died and both of these monkeys showed severe optic disk and retinal edema. Data from one representative monkey indicated that the excretion of organic acids in the urine was markedly elevated, but only a minor part of the acid excretion was accounted for as formic acid. Since only a slight excretion of formic acid was found in this monkey, formaldehyde was suggested to be the toxic agent in the methanol poisoning syndrome in monkeys.

Gilger et al. (32) evaluated the efficacy of ethanol therapy in methanol poisoning in the monkey. Five monkeys were given oral doses of methanol (4-6 g/kg)

together with small repeated doses of ethanol (initially 0.75 g/kg then 0.5 g/kg every 4 hr for about 2½ days). All five survived; however, four animals died when administered methanol only. No monkey displayed visual impairment during or after the methanol plus ethanol exposure; when given methanol alone, two demonstrated eye changes. Four of five monkeys did not develop acidosis when administered both ethanol and methanol; the first monkey was given ethanol for only 1½ days and became moderately acidotic when the ethanol was stopped. Monkeys administered only methanol became severely acidotic prior to death.

Although ethanol could prevent methanol poisoning in the monkey, ethanol therapy was known to be ineffective in some clinical cases when administered late in the course of the poisoning (15). When ethanol therapy was delayed as much as 12 hr, the lethality of methanol could be fully prevented (33), but when ethanol was administered 16 hr or more after methanol, monkeys died. In all monkeys, ethanol reversed or slowed the development of acidosis. In four of five monkeys in which ethanol treatment was delayed no eye changes were observed; the fifth showed minor peripapillary edema which lasted until death. Ethanol therapy decreased the rate of disappearance of methanol from the blood, which is further evidence that ethanol inhibits the metabolism of methanol in vivo. This study showed that there is a period beyond which ethanol is not effective as a therapy for methanol poisoning in monkeys. The explanation for this lack of effect beyond a given time relates to damage caused by a methanol metabolite. Once the metabolite concentration is high enough to induce irreversible damage, inhibition of the metabolism of methanol to the metabolite producing the damage cannot alter the course of the toxicity.

Although the studies by Potts, Gilger, and co-workers appeared to establish that the rhesus monkey is a model for methanol poisoning in man, their results could not be reproduced by Cooper and Felig (17). They administered methanol orally to rhesus monkeys, reporting a failure to produce the typical symptoms observed in man or in the monkey by Gilger and Potts (18). Cooper and Felig (17) observed inebriation, narcosis, coma, and death within 24 hr (usually without a latent period). However, no obvious visual impairment was observed. The minimal lethal dose was 7 g/kg (17), with 16 animals surviving on 6 g/kg or less. Acidosis (plasma bicarbonate value of 4.9 mEq/liter) was reported in only one of three cases, and in the other two monkeys the results were equivocal.

Results obtained by McMartin et al. (34) and Clay et al. (35) agreed with the earlier studies done by Potts and his co-workers (18,31-33,36). The administration of methanol (3 g/kg) to monkeys produced a syndrome similar to that described for humans. An initial slight central nervous system depression was followed by a latent period of 12-16 hr, during which time the monkeys displayed no obvious signs of toxicity. This was followed by a progressive deterioration in their condition characterized by anorexia, vomiting, weakness, hyperpnea, and tachypnea. Then they went into coma with shallow and infrequent respiration, followed by death due to respiratory failure 20-30 hr after methanol administra-

tion. When an attenuated but prolonged syndrome was produced by the administration of an initial 2 g/kg body weight of methanol with subsequent supplemental doses of methanol, a profound ocular toxicity was observed approximately 40-60 hr after the initial dosage (37-39).

These studies of methanol poisoning in the monkey have allowed for the examination of several features which would not be apparent in human cases. First the latent period appears to represent a period of compensated metabolic acidosis when an increase in formic acid levels and a decrease in plasma bicarbonate levels (Fig. 1) follow in an inverse relationship. When the compensatory mechanisms become exhausted and acidity increases further, the blood pH begins to drop (uncompensated metabolic acidosis). In monkeys the ocular toxicity takes time to become obvious, and when high doses were used (3 g/kg), animals died rapidly, probably owing to metabolic acidosis (34). Clinically evident ocular toxicity developed in methanol-poisoned monkeys after only 40 hr of exposure

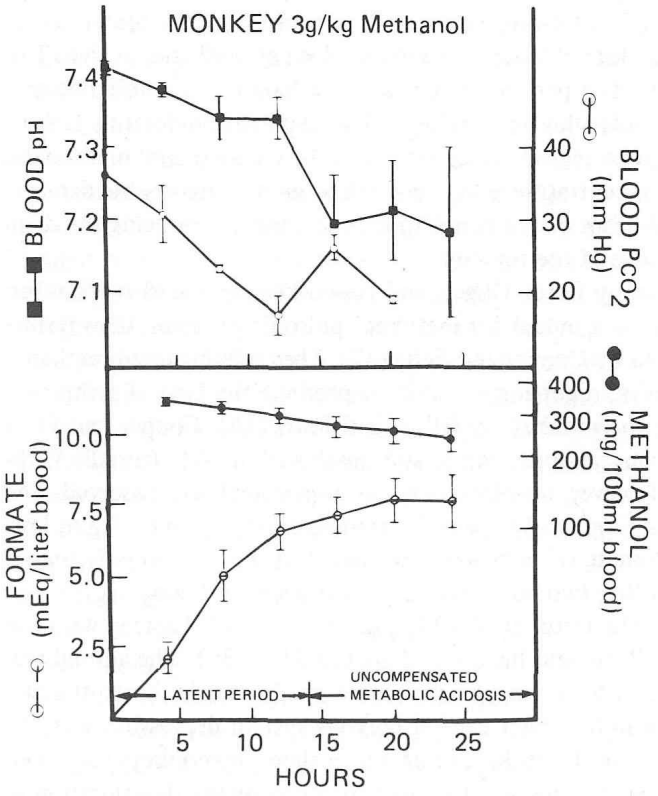

Figure 1 Metabolic acidosis, formate accumulation, and methanol metabolism in the monkey. Each point represents the mean ± SEM of at least three animals. (From T. R. Tephly, *Fed. Proc.* 36, *5*: 1627-1628, 1977.)

to methanol and its metabolites (39). More subtle changes in the ocular system probably occur earlier, but these changes were not assessed by objective methods.

Potts (31) found that only a small fraction of the increased urinary excretion of organic anions which occurred during metabolic acidosis was present as formate. McMartin et al. (34) demonstrated that the depletion of bicarbonate in methanol-poisoned monkeys occurred coincident with an accumulation of formate in the blood. Clay et al. (35) also showed that the increase in blood formate levels in monkeys poisoned with methanol was completely accounted for by a decrease in bicarbonate. Thus formate accumulation appears to be responsible for the generation of metabolic acidosis in the monkey, at least during the early phases of metabolic acidosis.

Potts suggested (31) that formaldehyde may be responsible for the development of methanol toxicity. Studies by McMartin et al. (40) showed that in methanol-poisoned monkeys with profound metabolic acidosis, no formaldehyde could be detected in either tissues or body fluids. The lack of accumulation of formaldehyde should be expected because, when formaldehyde was injected intravenously into monkeys, it disappeared from the blood with an extremely short half-life (1 min). Blood formate levels rose accordingly (40). The failure to detect formaldehyde after methanol administration does not necessarily preclude a role for formaldehyde in methanol toxicity, since formaldehyde may be formed in situ and still interfere with normal cellular functions (41) in a "hit and run" fashion. However, direct evidence for formaldehyde involvement is lacking, and formate accumulation is important, at least in the metabolic acidosis seen in the monkey after methanol administration.

In humans the accumulation of formic acid following methanol ingestion is marked (5), ranging from 11 to 26 mEq/liter. Acidosis with blood pH values of 6.9 and plasma bicarbonate concentrations of about 3 mEq/liter has been a common observation. Decreases in bicarbonate coincide well with the increase in formate concentration, and, under many conditions, formate accumulation is a major factor in the acidosis observed in methanol-poisoned humans. Hemodialysis, as an integral part of the treatment of methanol toxicity has been shown to rapidly decrease the levels of formate as well as methanol (5).

The accumulation of formate in monkeys and humans, but not in rats, after methanol exposure indicates that either methanol oxidation to formate occurs at a faster rate in monkeys and humans, or that formate oxidation to carbon dioxide proceeds at a slower rate in monkeys and humans. Watkins et al. (42) and Clay et al. (35) observed that the rate of methanol oxidation to carbon dioxide is approximately the same in the rat and monkey. Studies conducted in our laboratory have determined that methanol disappearance from blood occurs at the same rate in both rats and monkeys. At a dose of 1 g/kg methanol, methanol disappeared at a rate of 3.7 mg/dl per hour in rats and at 3.9 mg/dl per hour in monkeys. Thus the species difference in blood formate accumulation seen between rats and monkeys cannot be explained by different rates of renal clearance in these species.

Furthermore, differences in the renal disposition of formate do not appear to account for the species difference in blood formate levels. Clay et al. (35) found, in rats, that during a 24-hr period after the administration of methanol (6 g/kg), the urine collected from these animals contained only 0.2% of the administered dose as formate. In contrast, urine collected from a monkey for 23 hr after methanol administration (4 g/kg) contained more than 2% of the dose as formate. Thus the renal excretion of formate appears to be directly related to the systemic formate concentrations, and differences in excretion probably do not account for the observed species differences in blood formate accumulation.

The accumulation of formate in monkeys after methanol administration is probably related to the fact that monkeys metabolize formate to CO_2 slower than rats. McMartin et al. (43) administered various doses of [^{14}C] formate (ranging from 0.5 to 15 mmol/kg) to rats and monkeys. They noted that the rate of formate oxidation to CO_2 was dose dependent in both species. More importantly, however, the rate of formate metabolism in the monkey was at least 50% slower than that found in rats at all doses of formate administered. The results of these studies confirmed those of Clay et al. (35), who determined the half-life of formate elimination in both rats and monkeys. In the rat doses of sodium formate up to 100 mg/kg were eliminated with a half-life of 12 min, whereas in the monkey doses of 50 and 72 mg/kg yielded half-lives of 31 min. These values for the monkey are in agreement with those reported by McMartin et al. (43). Thus the slower rate of formate metabolism to CO_2 in the monkey explains why formate accumulates and produces acidosis in the methanol-poisoned monkey but not in rats.

METHANOL METABOLISM

Absorption and Distribution of Methanol

The absorption and distribution of methanol have been characterized by numerous workers (44,45) and are similar to the absorption and distribution of ethanol. Methanol, like ethanol, is rapidly absorbed from the gut and distributes uniformly to body water (45). Methanol may also be absorbed by inhalation, and this property has resulted in several cases of poisoning in humans (2,3). Concentrations of methanol over 300 ppm in the air are considered hazardous (46). Percutaneous absorption of methanol occurs and has been shown to lead to poisoning in children (47).

No differences exist in capabilities for absorption of methanol between various animal species, and blood levels are entirely predictable based on the concept that methanol distributes uniformly to body water. This is not true for the metabolites of methanol. Thus a direct comparison of the toxicity of certain metabolites of methanol to that of methanol itself is difficult when these substances are administered systemically. In other words, metabolites generated in situ within a par-

ticular cell locus or within a given organ may have a different toxic potential than those same substances administered parenterally or enterally.

Methanol Elimination

The elimination of methanol from the blood appears to be very slow in all species, especially when compared to ethanol. Where studies of methanol disappearance from the blood have been reported, some reports indicate zero-order disappearance from blood, and some studies indicate first-order kinetics. Bildsten (48) and Widmark and Bildsten (49) reported that, at a dose of 0.8 g of methanol per kilogram body weight, rabbits eliminated an average of 0.76 μg of methanol per gram of animal per minute. Bernhard and Goldberg (50) reported an average elimination rate of 0.67 μg/g per minute when 0.56-2.10 g of methanol per kilogram body weight was administered to rabbits. The elimination curves in those studies were linear and it was noted that 4-6 hr after administration there was a temporary increase in the blood methanol concentration. On the other hand, Koivusalo (51) found that when methanol was administered to rabbits at doses of 0.2-3.4 g/kg body weight, the elimination rate of methanol from the blood was dependent on the blood concentration of methanol. Haggard and Greenberg (44) also concluded that the rate of elimination of methanol from the blood is dose dependent and proportional to the concentration of methanol in the blood. We recently found that the rate of elimination of methanol from the blood is linear with time in monkeys given 1 g/kg of methanol where peak blood levels are about 110 mg/dl in blood. In contrast, first-order decay curves are obtained in monkeys administered 3 g of methanol per kilogram body weight (peak blood methanol levels over 300 mg/dl). A half-life of about 24 hr has been reported (34) for the higher dose. We also (34) observed that, when inhibitors of methanol oxidation are administered to monkeys receiving 3 g of methanol per kilogram body weight, the half-life of methanol disappearance from the blood increases from 24 hr to about 49 hr. Thus at doses of 3 g of methanol per kilogram body weight, first-order disappearance from the blood is established in monkeys.

Metabolism of Methanol

Two enzymes are important in the oxidation of methanol to formaldehyde, alcohol dehydrogenase, and catalase (Fig. 2). The existence of relatively selective inhibitors for each enzyme has made it possible to test their importance in methanol oxidation in animals. It had been known for many years that the metabolism of methanol was blocked by the administration of ethanol and that methanol toxicity was attenuated by ethanol. Roe (8) suggested that humans who had taken ethanol simultaneously with methanol had less severe toxicity than when methanol was ingested alone. The assumption had existed for years that alcohol dehydrogenase was the major enzyme involved in methanol oxidation. Studies on alcohol dehydrogenase by Lutwak-Mann (52) showed that a partially

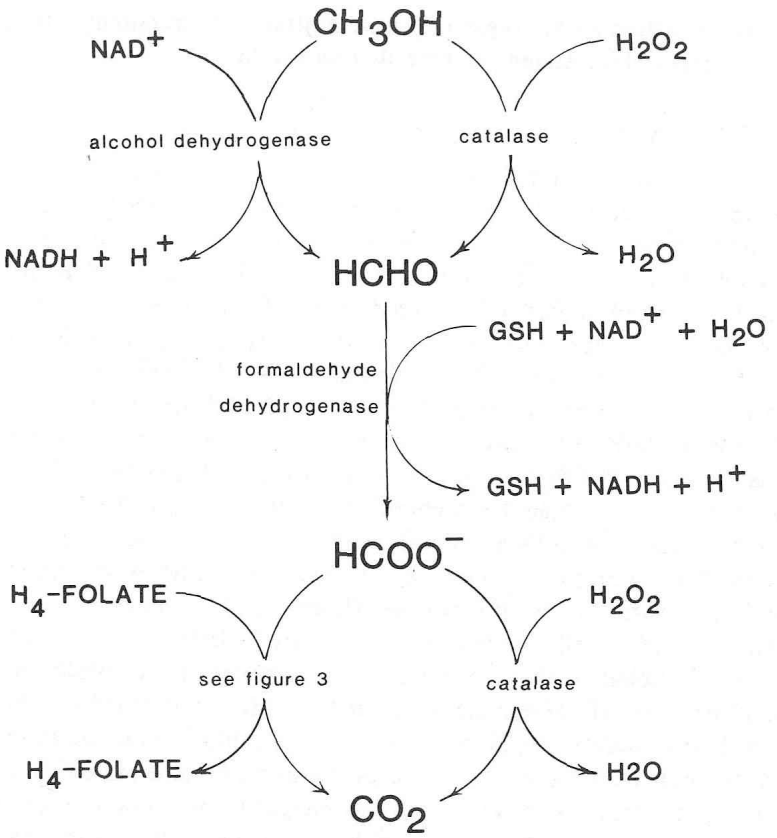

Figure 2 Biochemical reactions in the oxidation of methanol to carbon dioxide.

purified preparation of horse liver alcohol dehydrogenase oxidized methanol, although at a slower rate than ethanol. However, when crystalline horse liver alcohol dehydrogenase was prepared, it appeared to be incapable of catalyzing the oxidation of methanol (53,54), an observation that directed the attention of investigators to the catalase-peroxidative system as a mediator of the metabolism of methanol.

Interest returned to alcohol dehydrogenase and its role in methanol oxidation when Kini and Cooper (55) showed that, at high substrate concentrations, methanol was metabolized by crystalline horse liver alcohol dehydrogenase. Kini and Cooper (55) also showed that it was possible to copurify ethanol and methanol dehydrogenase activities from monkey liver. Their results conclusively demonstrated that alcohol dehydrogenase from monkey liver was capable of catalyzing methanol oxidation in vitro. Makar and Tephly (56) repeated these studies and showed that monkey liver alcohol dehydrogenase catalyzes methanol oxidation in vitro and that this activity is inhibited by the alcohol dehydrogenase

inhibitors pyrazole and 4-methylpyrazole. They reported that the Michaelis constant for methanol was about six times higher than that observed for ethanol, results similar to those found by Kini and Cooper, who had reported a K_m of 17 mM for methanol and 2.7 mM for ethanol. Makar and Tephly (56) observed K_m values of 20 mM for methanol and 3.2 mM for ethanol with the monkey liver enzyme. Pyrazole and 4-methylpyrazole were found to be competitive inhibitors when methanol and ethanol were utilized as substrates for monkey liver alcohol dehydrogenase. 4-Methylpyrazole yielded a K_i value of 9 μM which was about one-fourth that observed for pyrazole. Makar and Tephly (56) also showed that 4-methylpyrazole had no inhibitory properties toward catalase activity of rat liver homogenates in vitro or in vivo. Pyrazole, on the other hand, inhibits hepatic catalase activity when injected in vivo (57). Other studies have shown that purified hepatic alcohol dehydrogenase from rats (58) and humans (59,60) catalyze methanol oxidation. Although the Michaelis constant of methanol for alcohol dehydrogenase appears to be relatively high (10-100 mM), concentrations of this magnitude (20-30 mM) can be achieved in vivo after drinking a sizable quantity of either methanol or ethanol.

The inhibition of methanol oxidation by ethanol does not necessarily mean that the alcohol dehydrogenase system functions for methanol oxidation in a given animal species. Catalase can mediate the oxidation of a variety of alcohols to their corresponding aldehydes in the presence of a hydrogen peroxide-generating source (61). A study performed by Keilin and Hartree (62), using purified catalase and various peroxide-generating systems, showed that methanol and ethanol were metabolized at similar rates. Both rates were more rapid than those obtained with alcohols possessing higher molecular weights. Thus methanol and ethanol had about equivalent reactivities with the catalase peroxidative systems, whereas propanol and butanol appeared to display lower substrate reactivity. In fact, Keilin and Hartree suggested (62) that the physiological function of catalase might be involved with the metabolism of certain alcohols. Previously, it had been presumed that the exclusive function of catalase in the living organism was to decompose hydrogen peroxide. An important understanding of how alcohols such as ethanol or methanol might react with catalase in the presence of hydrogen peroxide was provided by Chance (63). He showed that substrates for catalase peroxide (complex I) react with substrates such as methanol and ethanol and promote the decomposition of the catalase peroxide complex, the rate of which was dependent upon the rate of reactivity with the substrate and the catalase-hydrogen peroxide complex. Chance postulated that catalase could conceivably account for most of the metabolism of methanol in the animal organism in vivo (63).

Definitive studies on methanol oxidation in vivo began with the use of selective inhibitors. Heim et al. (64) discovered that the herbicide 3-amino-1,2,4-triazole could inhibit hepatic and renal catalase activity in rats when injected intraperitoneally. This provided a means to test the direct participation of hepatic

catalase in the oxidation of methanol in vivo. Aminotriazole has since been a very useful and effective substance for studying the role of hepatic or renal catalase in the oxidation of agents in vivo. It does not inhibit erythrocyte catalase activity, nor does it affect liver cytochrome c content, blood hemoglobin levels, or urobilinogen excretion (64).

Nelson et al. (65) showed that aminotriazole had no effect on ethanol elimination in the dog, although hepatic catalase activity was markedly reduced. Mannering and Parks (66) showed that aminotriazole inhibited hepatic catalase activity in rats in vivo and that, in livers from rats whose hepatic catalase activity had been reduced by 90%, a marked inhibition of methanol oxidation to formaldehyde was observed in vitro. When crystalline beef liver catalase was added to reaction mixtures employing homogenates of rat liver obtained from aminotriazole-treated animals, methanol-oxidizing capacity was restored to control values. These results indicated that hepatic catalase activity was important for methanol oxidation in vitro and, furthermore, that the rate-limiting step in the process was likely to be the capacity of the liver to generate hydrogen peroxide (66). Thus, when peroxide-generating systems were added to hepatic homogenates in addition to crystalline liver catalase, a marked stimulation of activity beyond control values was observed. Mannering and Parks (66) also employed aminotriazole in order to determine whether catalase participated in the metabolism of methanol by rats in vivo. However, they found that aminotriazole had no effect on the rate of disappearance of methanol from the blood of rats. This apparent discrepancy was later explained (67) on the basis that considerable amounts of methanol are eliminated via excretory routes, as well as by metabolism, at the high doses of methanol which were employed in their studies (3 g/kg). When [^{14}C]methanol oxidation was studied by measuring $^{14}CO_2$ formation in vivo in rats, aminotriazole treatment markedly inhibited the oxidation of methanol to CO_2 (67).

Further evidence for a catalase-peroxidative system functioning in the metabolism of methanol in rats was provided in studies where ethanol and 1-butanol were employed as alternate substrate inhibitors of methanol oxidation. Ethanol and methanol have about equal reactivities with catalase peroxide complex I, while ethanol is 6-10 times more reactive than methanol with alcohol dehydrogenase (68). Thus if catalase was functioning in the oxidation of methanol by the rat, one would have expected a 50% inhibition of methanol oxidation by ethanol, and, if alcohol dehydrogenase were functionary, a 90% inhibition would have been expected. Tephly et al. (67) showed that when equimolar doses of ethanol and methanol were injected into rats, a 50% decrease in the rate of methanol oxidation occurred. When 1-butanol, which has only a slight reactivity with the catalase-hydrogen peroxide complex I, was injected, only a very slight inhibitory effect on methanol oxidation in the rat occurred. These results are consistent with the concept that the catalase-peroxidative system is the major catalyst of methanol oxidation in rats. Similar conclusions have been reached in isolated perfused rat liver experiments (69).

Although the role of a catalase-peroxidative system for the metabolism of methanol in rats was clear, different results were obtained with monkeys. Makar et al. (70) showed that pretreatment of monkeys with 1 or 3 g/kg body weight of aminotriazole 1 hr prior to methanol injection did not inhibit the rate of methanol metabolism, although hepatic catalase activity in livers from monkeys was reduced to 10% of control values. Studies were also performed using substrate inhibitors. When equimolar doses of ethanol and methanol were injected in monkeys, an 80% inhibition of the rate of methanol oxidation was observed (70). When 1-butanol, which produced only a slight effect on methanol oxidation in the rat, was injected into monkeys along with [^{14}C]methanol, a 90% inhibition of methanol oxidation was observed. Butanol is a highly reactive substrate for alcohol dehydrogenase, and, if alcohol dehydrogenase were functioning, one would have expected a 90% inhibition of methanol oxidation by 1-butanol. These results support the concept that the catalase-peroxidative system is not functional in methanol oxidation in the primate and that the metabolism of methanol in the monkey is dependent on the activity of alcohol dehydrogenase.

Other evidence for a major role of alcohol dehydrogenase in methanol oxidation in the monkey was provided by Watkins et al. (42), who showed that pyrazole markedly inhibited methanol oxidation in the rhesus monkey. Although pyrazole rapidly inhibited methanol metabolism in vivo, there was a possibility that inhibition of hepatic catalase activity by a pyrazole metabolite could be responsible for the inhibition of methanol oxidation in the monkey. Thus 4-methylpyrazole, a more potent inhibitor of alcohol dehydrogenase activity than pyrazole and one which does not inhibit hepatic catalase activity (56), was tested in the monkey (34). 4-Methylpyrazole was found to be a potent inhibitor of methanol oxidation with little or no effect on hepatic catalase activity.

Thus a major role of alcohol dehydrogenase in the metabolism of methanol in vivo in the monkey has been established. McMartin et al. (34) also showed that 4-methylpyrazole prevents the development of methanol poisoning in the monkey.

The question of why the peroxidative system does not function in the monkey has been examined. It should be recalled that Mannering and Parks (66) showed that when a peroxide-generating system was added to rat hepatic homogenates, peroxide generation appeared to be a rate-limiting factor. When a glucose and glucose oxidase preparation was added, marked stimulation of methanol oxidation occurred. When catalase activity had been reduced markedly, such as from aminotriazole-treated rats, glucose and glucose oxidase addition did not stimulate methanol oxidation (66). Goodman and Tephly (71) suggested that the monkeys may not metabolize methanol through a catalase-dependent system owing to decreased activity levels of peroxide-generating enzymes. Since peroxide-generating systems appear to be rate limiting for methanol oxidation via a catalase-dependent system in the rat, these workers proposed that this system should be rate limiting in the monkey, perhaps to an even greater degree (71) than noted in the rat. It is well known that urate oxidase activity is essentially absent in human liver, and

Goodman and Tephly (71) have shown that urate oxidase activity was also very low in monkey liver. Furthermore, glycolate oxidase activity, xanthine oxidase activity, and other peroxide-generating enzyme activities are also very low in monkey and human liver (72). This could account for why methanol oxidation in the monkey via a catalase-peroxidative system is difficult to demonstrate. Makar and Mannering (58) also suggested that the catalase distribution in the cell may be a consideration.

A third possible mechanism by which methanol could be oxidized to formaldehyde has been suggested by Rietbrock et al. (73) and Teschke et al. (74). This system, the hepatic microsomal mixed-function oxidase system, employs the hepatic endoplasmic reticulum, NADPH, and molecular oxygen.

METABOLISM OF FORMALDEHYDE

Formic acid was considered as the toxic agent in the acidosis seen in methanol poisoning until Van Slyke and Palmer (11) discredited the toxic role of formate. They failed to account for the increased organic acid excretion observed in methanol toxicity as due to formate. Potts (31) also failed to account for the organic acids excreted in the urine as due to formate following methanol poisoning in monkeys. Thus formaldehyde became a candidate as a causative agent in the toxicity of methanol poisoning (41,75,76), even though no one had demonstrated the presence of elevated formaldehyde levels in body fluids or tissues following methanol administration. Keeser (77) appeared to demonstrate the presence of formaldehyde in the cerebrospinal fluid, vitreous humor, and peritoneal fluid of rabbits which had been administered methanol. However, these studies were rather incomplete, lacked appropriate controls, and the method employed to measure formaldehyde lacked sensitivity and specificity. No formaldehyde could be detected in blood, urine, or tissues obtained from methanol-intoxicated animals in studies performed by Koivusalo (51) and Scott et al. (30) or from methanol-poisoned humans (8,78).

There are several ways by which formaldehyde can be disposed of in biological systems. First, formaldehyde has a high degree of reactivity with proteins and other endogenous compounds containing active hydrogen atoms (79). Formaldehyde can combine with any number of functional groups found in proteins or nucleic acids. Thus it may immediately form adducts with cellular constituents, leading to the formation of stable intermediates.

Strittmatter and Ball (80) isolated a formaldehyde-specific, NAD-dependent formaldehyde dehydrogenase from beef liver in 1955 and pointed out that this enzyme required reduced glutathione (GSH). This enzyme, which appears to be quite specific for formaldehyde, is often isolated with glutathionine thiolase (81, 82). In the reactions catalyzed by this enzyme (Fig. 2), formaldehyde combines with reduced GSH to form S-formyl glutathione, and in the presence of the thiolase, the product hydrolyzes to form formic acid and reduced glutathione. Reduced glutathione is therefore a key agent in the generation of formate from

formaldehyde. The first reaction appears to be freely reversible, but the second reaction is not, a feature which explains the apparent irreversibility of the two-step reaction as described by Strittmatter and Ball (80). Formaldehyde dehydrogenase activity is present in rat liver, human brain, and a number of other species and tissues such as retina (83). These tissues have not been examined adequately for the presence of S-formyl glutathione hydrolase. The specific activity of this enzyme in crude preparations appears to be quite high, and its presence would be expected in other tissues (81).

Formaldehyde oxidation can also occur in liver mitochondria through an aldehyde dehydrogenase activity (or activities) which is likely to be similar to the aldehyde dehydrogenases of mitochondria that have been described previously (84-86). Aldehyde dehydrogenase activity of mitochondria appears to be very high and is capable of reacting nonspecifically with many aldehyde substrates. Thus it is likely that formaldehyde-oxidizing capabilities of liver are extremely high, either through the formaldehyde dehydrogenase-S-formyl glutathione hydrolase system or through aldehyde dehydrogenase activities in mitochondria or cytosol. Goodman and Tephly (87) have shown that the formaldehyde dehydrogenase activity of human liver is, in fact, higher than that of rat liver. Thus one cannot explain, at this time, the fact that methanol poisoning is uniquely present in humans or monkeys on the basis of an inability to metabolize formaldehyde, since the conversion of formaldehyde to formate can apparently proceed as readily in humans as it does in rats.

Formaldehyde can be metabolized through the tetrahydrofolic acid-dependent one-carbon pool which is capable of utilizing one-carbon units at various oxidation levels and transferring these one-carbon moieties to various endogenous acceptors. Apparently, free formaldehyde enters these reactions by combining with tetrahydrofolate nonenzymatically (88) or through the formaldehyde-activating enzyme to form N^5,N^{10}-methylenetetrahydrofolate. This enzyme has been demonstrated in pigeon liver by Osborn et al. (89) and has been found to be present in a number of mammalian tissues (90).

The metabolism of formaldehyde has been studied by Malorny et al. (91) in dogs and cats in vivo. These investigators administered formaldehyde intravenously and orally to dogs and showed that there was a rapid appearance of formic acid in blood plasma and the presence of only negligible levels of formaldehyde in blood. Experiments in vitro with human blood showed that formaldehyde was oxidized to formic acid (92,93). Rietbrock (94) showed that in dogs, cats, rabbits, guinea pigs, and rats the infusion of formaldehyde resulted in a rapid disappearance of formaldehyde from the blood with a half-life of approximately 1 min. Malorny et al. (91) found that when equimolar amounts of formaldehyde, formic acid, or sodium formate were infused in dogs, the peak concentrations of formic acid in the plasma were equivalent in all three cases, indicating that formaldehyde was rapidly metabolized to formic acid.

Although it is possible that formaldehyde may be responsible for certain of

the toxic findings in methanol poisoning, it would be unlikely that it could account for the metabolic acidosis, since formate appears to be the major factor in the metabolic acidosis seen in monkeys and humans poisoned with methanol. It is also unlikely that formaldehyde can be generated in the liver and delivered to the optic nerve in an intact state. Therefore either formaldehyde forms a product with some endogenous acceptor which is responsible for the ocular toxicity, or formaldehyde is generated in situ in the eye, where it may exert an effect on the ocular system. Although these possibilities cannot be ruled out at this time, the responsibility of formaldehyde for the ocular toxicity of methanol is unlikely, since formate itself can produce ocular toxicity in the monkey (95). In studies where blindness in monkeys was produced from formate, no formaldehyde could be detected in body fluids or tissues (95). In any case, more studies need to be performed on the fate of formaldehyde in the organism in order to disregard it as a toxic agent in the methanol poisoning syndrome in man.

FORMATE METABOLISM

Nonprimates

The ability of animal tissues to oxidize formate into CO_2 was first reported by Batelli (96) and Battelli and Stern (97), who observed that tissues obtained from a variety of animals, such as the horse, cow, sheep, dog, and rabbit, were capable of oxidizing formate into CO_2 in the presence of hydrogen peroxide (Fig. 2). More that 40 years later, Chance (63) studied the kinetics of the catalase-hydrogen peroxide system with different substrates and showed that formate reacts with the hydrogen peroxide catalase complex (complex I).

In subsequent years, a number of in vitro investigations strongly indicated a key role of the catalase-hydrogen peroxide system in the oxidation of formate. Some of the experimental results leading to this conclusion are the following:

1. There is a good correlation between the formate-oxidizing ability and the catalase activity in liver preparations of different species (98), in different tissues within one animal species (99), and in the subcellular compartments from tissue preparations (100).
2. Administration of aminotriazole to guinea pigs greatly lowered the formate-oxidizing ability of liver fractions in vitro (98).
3. Certain types of neoplasms in rats (101), mice (102), and humans (103) lead to a marked lowering of both catalase activity and the formate-oxidizing ability in vitro.
4. Folate-deficient rats possess a marked impairment in formate-oxidizing ability (104) and lowered hepatic catalase levels.
5. Decreased formate oxidation in vitro results from decreased hydrogen peroxide generation caused by factors such as a decreased hepatic xanthine oxidase activity, vitamin B_6 deficiency (105), or thyrotoxicosis (100). On the

other hand, factors that increase hydrogen peroxide generation stimulate formate oxidation. This can be accomplished by supplementing liver preparations with hypoxanthine, a known substrate of xanthine oxidase.

Another path of formate oxidation to CO_2 is the folate biochemical pathway (88,90,106,107). Formate enters into the folate pool by combining with tetrahydrofolate (THF) to form 10-formyl-THF, a reaction catalyzed by 10-formyl-THF synthetase, an enzyme widely distributed among mammalian tissues (108). Kutzbach and Stokstad (109) showed that 10-formyl-THF oxidoreductase catalyzes the oxidation of the formyl group directly to CO_2. Thus there is a two-step conversion of formate to CO_2.

Rietbrock et al. (73) suggested that exogenously administered formate, or formate arising from methanol metabolism in vivo, is oxidized via the folate-dependent pathway. They found an inverse correlation between plasma levels of folate in different animal species and the half-life of exogenously administered formate. They also reported that dogs accumulated formic acid to a small extent (2 mEq/liter) in their blood following methanol administration. Pretreatment of dogs with folic acid prior to methanol produced a lower blood formate level, whereas methotrexate (an inhibitor of dihydrofolate reductase) had the opposite effect (110).

Palese and Tephly (111) measured $^{14}CO_2$ formation following [^{14}C] formate administration to rats and showed that folate deficiency resulted in a greatly diminished rate of formate oxidation. In contrast, administration of aminotriazole, the potent catalase inhibitor, did not inhibit the rate of formate oxidation to CO_2. Administration of ethanol in molar ratio of 22:1 (ethanol:formate) did not alter the rate of formate oxidation in the rat. However, in folate-deficient rats, the catalase-hydrogen peroxide system may serve as an alternate pathway, since, in folate-deficient rats aminotriazole or ethanol administration did result in some inhibition of the rate of formate oxidation (111).

The knowledge that formate is being metabolized in vivo via a folate-dependent system has been utilized to advantage in order to produce metabolic acidosis in rats after methanol treatment. Rats, made folate deficient, oxidize formate at a markedly slowed rate (111,112), and administration of methanol (4 g/kg) to folate-deficient rats leads to high formate levels and severe metabolic acidosis (113). Blood formate levels reached values as high as 18 mEq/liter in these animals. This value is higher than blood formate levels noted in methanol-poisoned monkeys (34).

Monkeys

In monkeys the folate-dependent pathway is also the major route of formate oxidation to CO_2. Makar et al. (70) showed that aminotriazole had no effect on methanol oxidation to CO_2 in the monkey, and McMartin et al. (114) demonstrated that neither the rate of formate oxidation nor the half-life of formate in

the blood was altered by aminotriazole. However, the rate of formate metabolism in folate-deficient monkeys was approximately 50% lower than that observed in control monkeys. Formate oxidation was stimulated in monkeys by the administration of either folic acid (114) or 5-formyl-THF (115).

McMartin et al. (114) also showed that the sensitivity of monkeys to methanol was related to folate, since folate-deficient monkeys became especially sensitive to the toxicity of methanol relative to the amounts of formate produced. Thus, when 0.5 g/kg of methanol was given to either folate-deficient or control monkeys, the level of blood formate in the folate-deficient animals was more than two times greater than that observed in the control animals.

Noker and Tephly (115) then showed that methanol toxicity can be modified considerably in monkeys by the administration of folate derivatives. These workers followed the course of methanol toxicity in monkeys administered [^{14}C] methanol (2 g/kg) or [^{14}C]methanol with repetitive doses of 5-formyl-THF. In monkeys treated with 5-formyl-THF (2 mg/kg at 0, 4, 8, 12, and 18 hr after methanol), blood formate levels were significantly decreased (by at least 50%) from those observed in the untreated animals. Similar results were obtained when sodium folate was employed instead of 5-formyl-THF. In both treated and untreated monkeys, the elimination of methanol from blood followed zero-order kinetics and proceeded at a rate of 7.9 mg/dl per hour in the 5-formyl-THF-treated animals, and at 7.1 mg/dl per hour in the untreated animals. Therefore the clearance of methanol was not altered by folate administration. In addition, the distribution and route of metabolism of [^{14}C]methanol did not appear to be changed by 5-formyl-THF treatment, since the total amount of ^{14}C label recovered in urine as either expired [^{14}C]methanol or $^{14}CO_2$ was the same for both treated and untreated monkeys. However, the rate of methanol oxidation to CO_2 was significantly increased in those animals treated with 5-formyl-THF, and folate treatment was effective in reducing blood formate levels by increasing the rate of formate metabolism to CO_2. Blood pH and blood bicarbonate levels remained within the normal range in animals treated with 5-formyl-THF, in contrast to the marked bicarbonate depletion, high blood formate levels, and metabolic acidosis observed in animals not given 5-formyl-THF.

Noker and Tephly (115,116) have also shown that 5-formyl-THF (when given in repetitive doses) is effective in reversing methanol toxicity in the monkey once it has developed. The accumulation of blood formate in monkeys could be markedly altered by 5-formyl-THF, even when administered after toxicity became apparent. A rapid decline in blood formate levels was observed in methanol-poisoned animals several hours after the initiation of 5-formyl-THF treatment. In monkeys not given 5-formyl-THF, formate levels continued to climb. The decline in formate concentrations in monkeys treated with folate was coupled to an increase in the rate of CO_2 formation from methanol.

The results demonstrate that the severity of methanol toxicity in monkeys is correlated with accumulation of formate in the blood and that this can be sig-

nificantly modified by procedures which provide the monkey with more folate. These results suggest that there is a reciprocal relationship between the formate oxidation rate and the hepatic folate level of the animal. They suggest the possible use of folates for the treatment of human methanol toxicity.

Regulation of Formate Oxidation Through Regulation of Tetrahydrofolate

Since the folate biochemical pathway is primarily involved in the metabolism of formate, the regulation of the rate of formate metabolism is governed by the regulation of the hepatic tetrahydrofolate concentrations in liver. This concept has been advanced recently by studies which have explored the role of 5-methyl-THF:homocysteine transmethylase (methionine synthetase). This cytosolic enzyme is reponsible for the methylation of homocysteine to form methionine as well as for the conversion of 5-methyl-THF to THF (Fig. 3). It requires methyl-

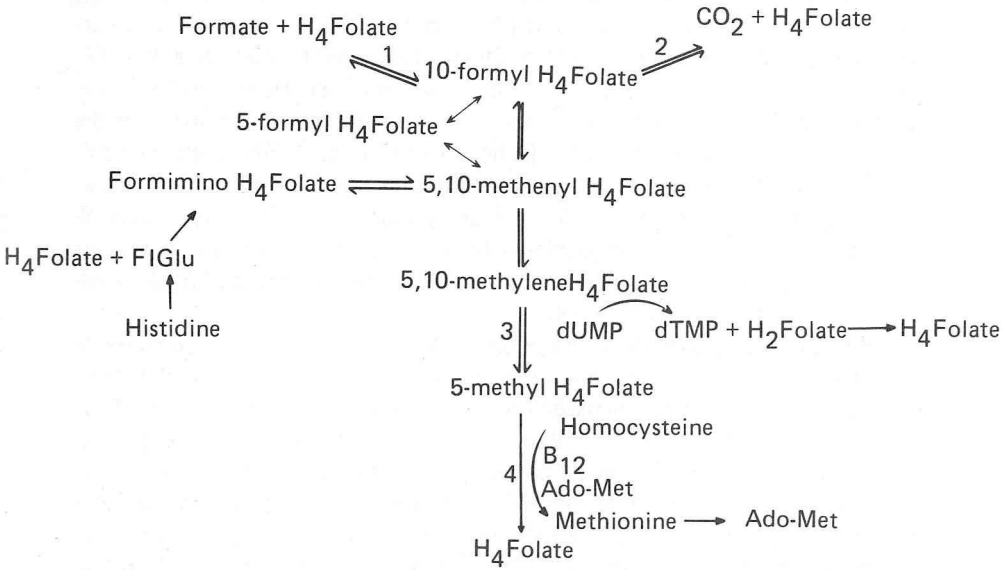

Figure 3 Pathway of folate-dependent formate metabolism (H_2 folate, dihydrofolate; H_4 folate, tetrahydrofolate; B_{12}, vitamin B_{12}; Ado-Met, S-adenosylmethionine). Reaction 1 is catalyzed by formyl-tetrahydrofolate synthetase and requires activation of formate by ATP. Reaction 2 is catalyzed by formyl-tetrahydrofolate dehydrogenase and utilizes NADP+. Reaction 3 is catalyzed by methylene-tetrahydrofolate reductase and is thought to be irreversible. Reaction 4 is catalyzed by methyl-tetrahydrofolate homocysteine methyltransferase (methionine synthetase) and is dependent upon vitamin B_{12} and catalytic amounts of adenosylmethionine, a reducing system.

cobalamin and S-adenosylmethionine for maximal activity. As far as we know, methionine synthetase is the only methlycobalamin-dependent enzyme in the mammalian organism. The anesthetic gas nitrous oxide has been reported to react with transition methyl complexes, such as the cobalt-ligand complex in vitamin B_{12}, and oxidizes the coenzyme from the active cob(I)alamin form to the inactive cob(III)alamin form (117). Deacon et al. (118) have shown the inhibition of hepatic and brain methionine synthetase activity in vivo by nitrous oxide, and Eells et al. (119,120) demonstrated that, following nitrous oxide treatment of rats, there was a significant decrease in hepatic levels of tetrahydrofolate forms and an increase in hepatic 5-methyl-THF. Rats treated with nitrous oxide also exhibited a marked decrease in the rate of formate oxidation to carbon dioxide. When methanol (4 g/kg) was administered to rats which were exposed to nitrous oxide:oxygen (50:50) for 2 hr, there was a marked metabolic acidosis in these animals, with accumulation of blood formate, a decrease in blood pH to 7.2, and a depletion of blood bicarbonate. This metabolic acidosis produced after the administration of methanol to rats had not been demonstrated previously, except where rats were made folate deficient (104). Hepatic methionine synthetase activity was reduced to 10% of control levels in animals treated with $N_2O:O_2$ (50:50), a finding which accounts for the depletion of hepatic tetrahydrofolate. Recently, Eells et al. (120) demonstrated an excellent correlation between the rate of formate oxidation in rats with hepatic tetrahydrofolate levels. Since S-adenosylmethionine levels are also dependent upon hepatic methionine levels, one would expect alteration of S-adenosylmethionine concentrations in liver. S-Adenosylmethionine levels are depleted by the treatment of rats with nitrous oxide, and a good correlation between tetrahydrofolate levels and S-adenosylmethionine was also recorded (120).

Methionine administration to rats which have been treated with nitrous oxide leads to a reversal of the depletion of tetrahydrofolate levels in liver and a reversal of the inhibition of formate oxidation produced by nitrous oxide (120). However, the mechanism by which methionine is capable of reversing the depletion of tetrahydrofolate brought on by nitrous oxide treatment is still unexplained; that is, although nitrous oxide inhibits methionine synthetase activity and depletes tetrahydrofolate levels, methionine administration does not reverse the inhibition of methionine synthetase activity, although it restores tetrahydrofolate in liver. Therefore methionine cannot be exerting its effect by a direct action on methionine synthetase activity. It is possible that methionine exerts its effect through the elevation of S-adenosylmethionine concentrations in liver. Following methionine treatment, there is a marked elevation of S-adenosylmethionine levels in rat liver (120) and S-adenosylmethionine acts as an inhibitor of 5,10-methylene-THF reductase (121). More work is needed in order to determine the mechanism by which methionine exerts its reversal of the nitrous oxide depletion of hepatic tetrahydrofolate.

Recent studies in our laboratory have shown that treatment of monkeys with a nitrous oxide:oxygen (50:50) mixture leads to marked sensitization of the monkey to methanol toxicity. Following a dose of 1 g/kg of methanol (a dose which produces only a slight increase in blood formate in monkeys), there was a marked accumulation of formate (4 mEq/liter) 12 hr after methanol. These values are greater than blood formate levels observed when 2 g/kg of methanol were given to air-breathing monkeys.

A great deal more work is needed in order to understand which step of the many enzymatic reactions in the folate biochemical pathway regulates the regeneration of tetrahydrofolate in monkeys. However, it is important to realize that primates are at some risk with respect to their folate regulation; and it would appear to be important for future work to determine that step or process which is deficient and which places the primate at a distinct liability when it comes to the disposition of one-carbon moieties.

REFERENCES

1. MacFarlan, J. F. (1855). On methylated spirit, and some of its preparations. *Pharm. J. Trans.* 15, 310-315.
2. Buller, F., and Wood, C. A. (1904). Poisoning by wood alcohol: Cases of death and blindness from Columbian spirits and other methylated preparations. *J. Am. Med. Assoc.* 43, 1117, 1132, 1289-1296.
3. Wood, C. A., and Buller, F. (1904). Poisoning by wood alcohol: Cases of death and blindness from Columbian spirits and other methylated preparations. *J. Am. Med. Assoc.* 43, 972-977.
4. Gonda, A., Gault, H., Churchill, D., and Hollomby, D. (1978). Hemodialysis for methanol intoxication. *Am. J. Med.* 64, 749-757.
5. McMartin, K. E., Ambre, J. J., and Tephly, R. T. (1980). Methanol poisoning in humans: Role for formic acid accumulation in the metabolic acidosis *Am. J. Med.* 68, 414-418.
6. Naraqi, S., Dethlefs, R. F., Slobodniuk, R. A., and Sairere, J. S. (1979). An outbreak of acute methyl alcohol intoxication. *Aust. N.Z. J. Med.* 9, 65-68.
7. Kobro, M. (1946). Methanol poisoning. *Acta Pharmacol.* 2, 95-108.
8. Roe, O. (1948). The ganglion cells of the retina in cases of methanol poisoning in human beings and experimental animals. *Acta Ophthalmol.* 26, 169-182.
9. Benton, C. D., and Calhoun, F. P. (1953). The ocular effects of methyl alcohol poisoning: Report of a catastrophe involving 320 persons. *Am. J. Ophthalmol.* 36, 1677-1685.
10. Harrop, G. A., and Benedict, E. M. (1920). Acute methyl alcohol poisoning associated with acidosis. *J. Am. Med. Assoc.* 74, 25-27.
11. Van Slyke, D. D., and Palmer, W. W. (1920). Studies of acidosis XVI. The titration of organic acids in urine. *J. Biol. Chem.* 41, 567-585.
12. Ziegler, S. L. (1921). The ocular menace of wood alcohol poisoning. *J. Am. Med. Assoc.* 77, 1160-1166.

13. Chew, W. B., Berger, E. H., Brines, O. A., and Capron, M. J. (1946). Alkali treatment of methyl alcohol poisoning. *J. Am. Med. Assoc.* 130, 61-64.
14. Kane, R. L., Talbert, W., Harlan, J., Sizemore, G., and Cataland, S. (1968). A methanol poisoning outbreak in Kentucky. *Arch. Environ. Health* 17, 119-129.
15. Bennett, I. L., Jr., Cary, F. H., Mitchell, G. L., Jr., and Cooper, M. N. (1953). Acute methyl alcohol poisoning: A review based on experiences in an outbreak of 323 cases. *Medicine* 32, 431-463.
16. Keyvan-Larijarni, H., and Tannenberg, A. M. (1974). Methanol intoxication. Comparison of peritoneal dialysis and hemodialysis treatment. *Arch. Intern. Med.* 143, 293-296.
17. Cooper, J. R., and Felig, P. (1961). The biochemistry of methanol poisoning II. Metabolic acidosis in the monkey. *Toxicol. Appl. Pharmacol.* 3, 202-209.
18. Gilger, A. P., and Potts, A. M. (1955). Studies on the visual toxicity of methanol V. The role of acidosis in experimental methanol poisoning. *Am. J. Ophthalmol.* 39, 63-86.
19. Roe, O. (1955). The metabolism and toxicity of methanol. *Pharmacol. Rev.* 7, 399-412.
20. Gilger, A. P., Potts, A. M., and Johnson, L. V. (1952). Studies on the visual toxicity of methanol II. The effect of parenterally administered substances on the systemic toxicity of methyl alcohol. *Am. J. Ophthalmol.* 35, 113-126.
21. Hunt, R. (1902). The toxicity of methyl alcohol. *Johns Hopkins Hosp. Bull.* 13, 213-225.
22. Haskell, C. C., Hileman, S. P., and Gardner, W. R. (1921). The significance of the acidosis of methyl alcohol poisoning. *Arch. Intern. Med.* 27, 71-82.
23. Roe, O. (1948). The ganglion cells of the retina in cases of methanol poisoning in human beings and experimental animals. *Acta Ophthalmol.* 26, 169-182.
24. Holden, W. A. (1899). The pathology of the amblyopia following profuse hemorrhage and of that following the ingestion of methyl alcohol, with remarks on the pathogenesis of optic-nerve atrophy in general. *Arch. Ophthalmol.* 28, 125-134.
25. Tyson, H. H., and Schoenberg, M. J. (1914). Experimental researches in methyl alcohol inhalation. *J. Am. Med. Assoc.* 63, 915-922.
26. Birch-Hirschfeld, A. (1901). Experimentelle Untersuchugen uber die Pathogenese der Methylalkoholamblyopie. *Arch. Ophthalmol.* 52, 358-383.
27. Birch-Hirschfeld, A. (1902). Weiterer Beitrag zur Pathogenese der Alkoholamblyopie. *Arch. Ophthalmol.* 54, 68-98.
28. Fink, W. H. (1943). The ocular pathology of methyl-alcohol poisoning. *Am. J. Ophthalmol.* 26, 694, 802-815.
29. Cooper, J. R., and Kini, M. M. (1962). Biochemical aspects of methanol poisoning. *Biochem. Pharmacol.* 11, 405-416.
30. Scott, E., Helz, M. K., and McCord, C. P. (1933). The histopathology of methyl alcohol poisoning. *Am. J. Clin. Pathol.* 3, 311-319.
31. Potts, A. M. (1955). The visual toxicity of methanol VI. The clinical aspects of experimental methanol poisoning treated with base. *Am. J. Ophthalmol.* 39, 86-92.

32. Gilger, A. P., Potts, A. M., and Farkas, I. S. (1956). Studies on the visual toxicity of methanol IX. The effect of ethanol on methanol poisoning in the rhesus monkey. *Am. J. Ophthalmol.* 42, 244-252.
33. Gilger, A. P., Farkas, I. S., and Potts, A. M. (1959). Studies on the visual toxicity of methanol X. Further observations on the ethanol therapy of acute methanol poisoning in monkeys. *Am. J. Ophthalmol.* 48, 153-161.
34. McMartin, K. E., Maker, A. B., Martin-Amat, G., Palese, M., and Tephly, T. R. (1975). Methanol poisoning I. The role of formic acid in the development of metabolic acidosis in the monkey and the reversal by 4-methylpyrazole. *Biochem. Med.* 13, 319-333.
35. Clay, K. L., Murphy, R. C., and Watkins, W. D. (1975). Experimental methanol toxicity in the primate: analysis of metabolic acidosis, *Toxicol. Appl. Pharmacol.* 34, 49-61.
36. Potts, A. M., Praglin, J., Farkas, I., Orbison, L., and Chickering, D. (1955). Studies on the visual toxicity of methanol VIII. Additional observations on methanol poisoning in the primate test object. *Am. J. Ophthalmol.* 40, 76-83.
37. Baumbach, G. L., Cancilla, P. A., Martin-Amat, G., Tephly, T. R., McMartin, K. E., Makar, A. B., Hayreh, M. S., and Hayreh, S. S. (1977). Methyl alcohol poisoning IV. Alterations of the morphological findings of the retina and optic nerve. *Arch. Ophthalmol.* 95, 1859-1865.
38. Hayreh, M. S., Hayreh, S. S., Baumbach, G. L., Cancilla, P., Martin-Amat, G., Tephly, T. R., McMartin, K. E., and Makar, A. B. (1977). Methyl alcohol poisoning III. Ocular toxicity. *Arch. Ophthalmol.* 95, 1851-1858.
39. Martin-Amat, G., Tephly, T. R., McMartin, K. E., Makar, A. B., Hayreh, M., Heyreh, S., Baumbach, G., and Cancilla, P. (1977). Methyl alcohol poisoning II. Development of a model for ocular toxicity in methyl alcohol poisoning using the rhesus monkey. *Arch. Ophthalmol.* 95, 1847-1850.
40. McMartin, K. E., Martin-Amat, G., Noker, P. E., and Tephly, T. R. (1979). Lack of a role for formaldehyde in methanol poisoning in the monkey. *Biochem. Pharmacol.* 28, 645-649.
41. Koivusalo, M. (1970). Methanol. In *International Encyclopedia of Pharmacology and Therapeutics*, Vol. 2 (Tremolieres, J., ed.), Pergamon, New York, Section 20, pp. 465-505.
42. Watkins, W. D., Goodman, J. I., and Tephly, T. R. (1970). Inhibition of methanol and ethanol oxidation by pyrazole in the rat and monkey *in vivo*. *Mol. Pharmacol.* 6, 567-572.
43. McMartin, K. E., Martin-Amat, G., Maker, A. B., and Tephly, T. R. (1977). Methanol poisoning: Role of formate metabolism in the monkey. In *Alcohol and Aldehyde Metabolizing Systems*, Vol. 2 (Thurman, R. G., Williamson, J. R., Drott, H., and Chance, B., eds.), Academic, New York, pp. 429-439.
44. Haggard, H. W., and Greenberg, L. A. (1939). Studies in the absorption distribution and elimination of alcohol IV. The elimination of methyl alcohol. *J. Pharmacol. Exp. Therap.* 66, 479-496.
45. Yant, W. P., and Schrenck, H. H. (1937). Distribution of methanol in dogs after inhalation and administration by stomach tube and subcutaneously. *J. Ind. Hyg. Toxicol.* 19, 337-345.

46. Leaf, G., and Zatman, L. J. (1952). A study of the conditions under which methanol may exert a toxic hazard in industry. *Br. J. Med.* 9, 19-31.
47. Gimenez, E. R., Vallegjo, N. E., Roy, E., Lis, M., Izurieta, E. M., Rossi, S., and Capuccio, M. (1968). Percutaneous alcohol intoxication. *Clin. Toxicol.* 1, 39-48.
48. Bildsten, N. V. (1924). Mikrobestimmung von Methylalkohol im Blute. *Biochem. Z.* 146, 361-369.
49. Widmark, E. M. P., and Bildsten, N. V. (1924). Die Elimination des Methylalkohols and die Bedingungen fur die Akkumulation desselben. *Biochem. Z.* 148, 325-335.
50. Bernard, C. G., and Goldberg, L. (1934). Uber die Einwirkung der durch Kohlensaure gesteigerten atmung auf die Ausscheidung des Methylalkohols beim Kaninchen. *Skand. Arch. Physiol.* 67, 117-128.
51. Koivusalo, M. (1956). Studies on the metabolism of methanol and formaldehyde in the animal organism. *Acta Physiol. Scand. Suppl.* 131,
52. Lutwak-Mann, C. (1938). Alcohol dehydrogenase of animal tissues. *Biochem. J.* 32, 1364-1374.
53. Bonnichsen, R. K., and Wassen, A. M. (1948). Crystalline alcohol dehydrogenase from horse liver. *Arch. Biochem.* 18, 361-363.
54. Theorell, H., and Bonnichsen, R. (1951). Studies on liver alcohol dehydrogenase I. Equilibria and initial reaction velocities. *Acta Chem. Scand.* 5, 1105-1126.
55. Kini, M. M., and Cooper, J. R. (1961). Biochemistry of methanol poisoning III. The enzymatic pathway for the conversion of methanol to formaldehyde. *Biochem. Pharmacol.* 8, 207-215.
56. Makar, A. B., and Tephly, T. R. (1975). Inhibition of monkey liver alcohol dehydrogenase by 4-methylpyrazole. *Biochem. Med.* 13, 334-342.
57. Lieber, C. S., Rubin, E., De Carli, L. M., Misra, P., and Gang, H. (1970). Effects of pyrazole on hepatic function and structure. *Lab. Invest.* 22, 615-621.
58. Makar, A. B., and Mannering, G. J. (1968). Role of the intracellular distribution of hepatic catalase in the peroxidative oxidation of methanol. *Mol. Pharmacol.* 4, 484-491.
59. Blair, A. H., and Vallee, B. L. (1966). Some catalytic properties of human liver alcohol dehydrogenase. *Biochemistry* 5, 2026-2034.
60. von Wartburg, J.-P., Bethune, J. L., and Vallee, B. L. (1964). Human liver-alcohol dehydrogenase. Kinetic and physicochemical properties. *Biochemistry* 3, 1775-1782.
61. Keilen, D., and Hartree, E. F. (1936). Coupled oxidation of alcohol. *Proc. R. Soc. London Ser. B.* 119, 141-159.
62. Keilen, D., and Hartree, E. F. (1945). Properties of catalase. Catalysis of coupled oxidation in alcohols. *Biochem. J.* 39, 293-301.
63. Chance, B. (1947). An intermediate compound in the catalase hydrogen peroxide reaction. *Acta Chem. Scand.* 1, 236-267.
64. Heim, W. G., Appleman, D., and Pyfrom, H. T. (1956). Effects of 3-amino-1,2,4-triazole (AT) on catalase and other compounds. *Am. J. Physiol.* 186, 19-23.

65. Nelson, G. H., Kinard, F. W., Aull, J. C., and Hay, M. G. (1957). Effect of aminotriazole on alcohol metabolism and hepatic enzyme activities in several species. *Q. J. Stud. Alcohol* 18, 343-348.
66. Mannering, G. J., and Parks, R. E., Jr. (1957). Inhibition of methanol metabolism with 3-amino-1,2,4-triazole. *Science* 126, 1241-1242.
67. Tephly, T. R., Parks, R. E., Jr., and Mannering, G. J. (1964). Methanol metabolism in the rat. *J. Pharmacol. Exp. Ther.* 143, 292-300.
68. Sund, H., and Theorell, H. (1963). Alcohol dehydrogenases. In *The Enzymes*, 2nd ed., Vol. 7 (Boyer, P. D., Lardy, H., and Myrback, eds.), Academic, New York, pp. 25-83.
69. Van Harken, D. R., Tephly, T. R., and Mannering, G. J. (1956). Methanol metabolism in the isolated perfused rat liver. *J. Pharmacol. Exp. Ther.* 149, 36-42.
70. Makar, A. B., Tephly, T. R., and Mannering, G. J. (1968). Methanol metabolism in the monkey. *Mol. Pharmacol.* 4, 471-483.
71. Goodman, J. I., and Tephly, T. R. (1968). The role of hepatic microbody and soluble oxidases in the peroxidation of methanol in the rat and monkey. *Mol. Pharmacol.* 4, 492-501.
72. Goodman, J. I., and Tephly, T. R. (1970). Peroxidative oxidation of methanol in human liver: The role of hepatic microbody and soluble oxidases. *Res. Commun. Chem. Pathol. Pharmacol.* 1, 441-450.
73. Rietbrock, N., Stieren, B., and Malorny, G. (1966). Beeinflussung der Methanolstoffwechsels durch Folsaure. *Klin. Wochenschr.* 44, 1318-1319.
74. Teschke, R., Hasumura, Y., and Lieber, C. S. (1975). Hepatic microsomal alcohol-oxidizing system: Affinity for methanol, ethanol, propanol and butanol. *J. Biol. Chem.* 250, 7397-7404.
75. Cooper, J. R., and Marchesi, V. T. (1959). The possible biochemical lesion in blindness due to methanol poisoning. *Biochem. Pharmacol.* 2, 313-315.
76. Potts, A. M., and Johnson, L. V. (1952). Studies on the visual toxicity of methanol I. The effect of methanol and its degradation products on retinal metabolism. *Am. J. Ophthalmol.* 35, 107-113.
77. Keeser, E. (1931). Uber die Ursache der Giftigkeit des Methyl Alkohols. *Dtsch. Med. Wochenscr.* 57, 398-399.
78. Alha, A. R., Raekallio, J., and Mukula, A.-L. (1958). Detection of methanol poisoning: With special consideration of the estimation of formic acid in solid viscera, blood and urine; investigation of 11 fatal cases. *Ann. Med. Exp. Biol. Fenn.* 36, 444-451.
79. French, D., and Edsall, J. T. (1945). The reactions of formaldehyde with amino acids and proteins. *Adv. Protein Chem.* 2, 277-335.
80. Strittmatter, P., and Ball, E. G. (1955). Formaldehyde dehydrogenase, a glutathionine-dependent enzyme system. *J. Biol. Chem.* 213, 445-461.
81. Uotila, L., and Koivusalo, M. (1974). Formaldehyde dehydrogenase from human liver: Purification, properties, and evidence for the formation of glutathione thiol esters by the enzyme. *J. Biol. Chem.* 249, 7653-7663.
82. Uotila, L., and Koivusalo, M. (1974). Purification and properties of S-formylglutathione hydrolase from human liver. *J. Biol. Chem.* 249, 7664-7672.

83. Kinoshita, J. H., and Masurat, T. (1958). Effect of glutathione on formaldehyde oxidation in the retina. *Am. J. Ophthalmol.* 46, 42-46.
84. Cinti, D. L., Keyes, S. R., Lemelin, M. A., Denk, H., and Schenkman, J. B. (1976). Biochemical properties of rat liver mitochondrial aldehyde dehydrogenase with respect to oxidation of formaldehyde. *J. Biol. Chem.* 251, 1571-1577.
85. Koivula, T., and Koivusalo, M. (1975). Different forms of rat liver aldehyde dehydrogenase and their subcellular distribution. *Biochim. Biophys. Acta* 397, 9-23.
86. Siew, C., Deitrich, R. A., and Erwin, V. G. (1976). Location and characteristics of rat liver mitochondrial aldehyde dehydrogenases. *Arch. Biochem. Biophys.* 176, 638-649.
87. Goodman, J. I., and Tephly, T. R. (1971). A comparison of rat and human liver formaldehyde dehydrogenase. *Biochim. Biophys. Acta* 252, 489-505.
88. Blakley, R. L. (1969). *The Biochemistry of Folic Acid and Related Pteridines*, Wiley, New York.
89. Osborn, M. J., Hatefi, Y., Kay, L. D., and Huennekens, F. M. (1957). Evidence for enzymic deacylation of N^{10}-formyl tetrahydrofolic acid. *Biochim. Biophys. Acta* 26, 208-210.
90. Huennekens, F. M., and Osborn, M. J. (1959). Folic acid coenzymes and one-carbon metabolism, *Adv. Enzymol.* 21, 370-446.
91. Malorny, G., Rietbrock, N., and Schneider, M. (1965). Die Oxydation des Formaldehyde zu Ameisensaure im Blut, ein Beitrag zum Stoffwechsel des Formaldehyde. *Naunyn-Schmiedebergs Arch. Exp. Pathol. Pharmakol.* 250, 419-436.
92. Matthies, H. (1957). Untersuchungen uber eine Aldehydededehydrogenase in kernlosen Erythrocyten. *Biochem. Z.* 329, 421-527.
93. Mattheis, H. (1958). Vergleichende untersuchungen uber die Aldehydededehydrogenase kernloser Erythrocyten. *Biochem. Z.* 330, 169-173.
94. Rietbrock, N. (1965). Formaldehydroxydation bei der Ratte. *Naunyn-Schmiedebergs Arch. Exp. Pathol. Pharmakol.* 251, 189-201.
95. Martin-Amat, G., McMartin, K. E., Hayreh, S. S., Hayreh, M. S., and Tephly, T. R. (1978). Methanol poisoning: Ocular toxicity produced by formate. *Toxicol. Appl. Pharmacol.* 45, 201-208.
96. Battelli, F. (1908). Oxydation de l'acide formique par les extraits des tissus animaux en presence de peroxyde d'hydrogene. *Compt. Rend. Soc. Biol.* 138, 651-665.
97. Battelli, F., and Stern, L. (1908). Uber die Peroxydasen der Tiergewebe. *Biochem. Z.* 13, 44-51.
98. Aebi, H., Fiei, E., Knab, R., and Siegenthaler, P. (1957). Utersuchungen uber die Formiatoxydation in der Leber. *Helv. Physiol. Acta* 15, 150-167.
99. Nakada, H. I., and Weinhouse, S. (1953). Studies of glycine oxidation in rat tissues. *Arch. Biochem.* 42, 257-270.
100. Venkataraman, S., and Sreenivasan, A. (1966). Formate oxidation in rat liver. *Enzymologia* 30, 91-96.
101. Stein, A. M., and Mehl, J. W. (1955). Reduction of total formic acid oxi-

dase and liver catalase in leukemic and tumor bearing mice. *Fed. Proc.* 14, 286.
102. Aebi, H., and Portwich, F. (1959). Formiatoxydation und Katalase-aktivitat bei Ratten mit Walker Sarkom. *Helv. Physiol. Acta* 17, 189-201.
103. Portwich, F., and Aebi, H. (1960). Erfassung der Peroxydbildung tierischer gewebe Mittels peroxydatischer Umsetzungen. *Helv. Physiol. Acta* 18, 1-16.
104. Friedmann, B., Nakada, H. I., and Weinhouse, S. (1954). A study of the oxidation of formic acid in the folic acid-deficient rat. *J. Biol. Chem.* 210, 413-421.
105. Schulman, M. P., and Rickert, D. A. (1959). The oxidation of glycine and formate to CO_2 by rat liver homogenates. *J. Biol. Chem.* 234, 1781-1783.
106. Krebs, H. A., Hems, R., and Tyler, B. (1976). The regulation of folate and methionine metabolism. *Biochem. J.* 158, 341-353.
107. Stokstad, E. L. R., and Koch, J. (1967). Folic acid metabolism. *Physiol. Rev.* 47, 83-116.
108. Whitely, H. R. (1960). The distribution of the formate-activating enzyme and other enzymes involving tetrahydrofolic acid in animal tissues. *Comp. Biochem. Physiol.* 1, 222-247.
109. Kutzbach, C., and Stokstad, E. L. R. (1968). Partial purification of a 10-formyltetrahydrofolate: NADP oxidoreductase from mammalian liver. *Biochem. Biophys. Res. Commun.* 30, 111-117.
110. Rietbrock, N., Stieren, B., and Malorny, G. (1966). Beeinflussung der Methanol-Stoffwechsels durch folsaure. *Klin. Wochenschr.* 44, 1318-1319.
111. Palese, M., and Tephly, T. R. (1975). Metabolism of formate in the rat. *J. Toxicol. Environ. Health* 1, 13-24.
112. Plaut, G. W. E., Betheil, J. J., and Lardy, H. A. (1950). The relationship of folic acid to formate metabolism in the rat. *J. Biol. Chem.* 184, 795-805.
113. Makar, A. B., and Tephly, T. R. (1976). Methanol poisoning in the folate-deficient rat. *Nature* 261, 715-716.
114. McMartin, K. E., Martin-Amat, G., Makar, A. B., and Tephly, T. R. (1977). Methanol poisoning V. Role of formate metabolism in the monkey. *J. Pharmacol. Exp. Ther.* 201, 564-572.
115. Noker, P. E., and Tephly, T. R. (1980). The role of folates in methanol toxicity. In *Alcohol and Aldehyde Metabolizing Systems*, Vol. 4 (Thurman, R. G., Williamson, J. R., Drott, H., and Chance, B., eds.), Plenum, New York, pp. 305-315.
116. Noker, P. E., Eells, J. T., and Tephly, T. R. (1980). Methanol toxicity: Treatment with folic acid and 5-formyltetrahydrofolic acid. Alcoholism. *Clin. Exp. Res.* 4, 378-383.
117. Banks, R. G. S., Henderson, R. J., and Pratt, J. M. (1968). Reactions of gases in solution. Part III. Some reactions of nitrous oxide with transition-metal complexes. *J. Chem. Soc. A*, 2886-2898.
118. Deacon, R., Lumb, M., Perry, J., Chanarin, I., Minty, B., Halsey, M., and Nunn, J. (1980). Inactivation of methionine synthetase by nitrous-oxide. *Eur. J. Biochem.* 104, 419-422.
119. Eells, J. T., Makar, A. B., Noker, P. E., and Tephly, T. R. (1981). Meth-

anol poisoning and formate oxidation in nitrous oxide-treated rats. *J. Pharmacol. Exp. Ther.* 217, 57-61.

120. Eells, J. T., Black, K. A., Makar, A. B., Tedford, C. E., and Tephly, T. R. (1982). The regulation of one-carbon oxidation in the rat by nitrous oxide and methionine. *Arch. Biochem. Biophys.* 219, 316-326.
121. Kutzbach, C., and Stokstad, E. L. R. (1967). Feedback inhibition of methylenetetrahydrofolate reductase in rat liver by S-adenosylmethionine. *Biochim. Biophys. Acta* 139, 217-220.