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METHANOL POISONING. V. ROLE OF FORMATE METABOLISM IN THE MONKEY[1, 2]

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ABSTRACT

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After the administration of methanol to monkeys, a pronounced metabolic acidosis with marked increases in blood formic acid concentrations and decreases in arterial blood bicarbonate values is observed. Metabolic pathways for formate metabolism were studied in cynomolgus monkeys. The rate of [^{14}C]formate oxidation to $^{14}CO_2$ increased with increasing doses but rates of formate metabolism to CO_2 in monkeys were only about half those observed in rats, findings which may explain why formate accumulates in the methanol-poisoned monkey but not in the rat. Compared with results obtained with control monkeys, the rate of [^{14}C]formate metabolism to $^{14}CO_2$ and the rate of elimination of formate from the blood were markedly reduced in monkeys which were in a state of folate deficiency. Also, an increased accumulation of formate in the blood of folate-deficient monkeys was seen after the administration of methanol. Folate pretreatment of control monkeys led to an increased rate of formate metabolism to CO_2. Administration of the catalase inhibitor, 3-amino-1,2,4-triazole had no effect on formate metabolism. These results suggest that a folate-dependent pathway is the major route of formate metabolism in the monkey. These studies show that a quantitative difference exists in the utilization of folate-dependent system for formate metabolism between rats and monkeys and that this difference leads to differences in formate accumulation and sensitivity to methanol.

At present, many new uses of methanol are being proposed, generally for the production of energy (Posner, 1975). Methanol toxicity in man is well known and is distinguished by a 12 to 24 hour latent period, followed by metabolic acidosis, ocular toxicity and death (Röe, 1955). Recent studies in our laboratory (McMartin *et al.*, 1975; Martin-Amat *et al.*, 1977; Hayreh *et al.*, 1977; Baumbach *et al.*, 1977) and other laboratories (Clay *et al.*, 1975) have demonstrated that the rhesus, as well as the pigtail monkey, may serve as an appropriate model for the study of methanol poisoning in man. The course of development of metabolic acidosis in the methanol-poisoned monkey parallels the accumulation of formic acid and decreases of bicarbonate in the blood. These recent studies have established that the accumulation of

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formic acid plays the predominant role in the production of metabolic acidosis in methanol poisoning in the monkey. The increase in blood formate concentrations accounted for the decrease in blood bicarbonate levels.

In contrast, methanol administration to the rat does not produce signs of toxicity similar to those observed in the monkey and in man; no metabolic acidosis nor significant formate accumulation occurs (McMartin *et al.*, 1975). Since the accumulation of formate in the animal appears to be intimately involved in the generation of metabolic acidosis and, at least, part of the etiology of methanol toxicity, information on the metabolic disposition of formate in sensitive and insensitive species is needed.

Two pathways have been proposed for the conversion of formate to CO_2. Many studies have shown that formate can be metabolized by the catalase-peroxidative system in *in vitro* preparations. Chance (1950) showed that the catalase-peroxide complex 1 reacts with formate, and Aebi *et al.* (1957) reported that formate oxidation by guinea-pig liver was inhibited *in vitro* when animals were pretreated with 3-amino-1,2,4-triazole (AT), an inhibitor of catalase. A second possible pathway is metabolism *via* a folate-dependent one-carbon system. Formate enters this pool by combining with tetrahydrofolate (THF) to form [^{10}N]formyl-THF, a reaction catalyzed by 10-formyl-THF synthetase, a ubiquitous enzyme in mammalian tissues (Whiteley, 1960). Various enzymatic reactions can direct the [^{10}N]formyl-THF to other pathways, such as the Krebs' cycle, or to [^{10}N]formyl-THF: NADP oxidoreductase. With respect to the latter enzyme, Kutzbach and Stokstad (1968) have reported that it catalyzes the oxidation of the formyl group on [^{10}N]formyl-THF directly to CO_2.

Recently, investigations in the rat *in vivo* by Palese and Tephly (1975) have shown that formate is metabolized to CO_2 by a folate-dependent pathway rather than by the catalase-peroxidative system. The catalase inhibitor, AT, and the alternate substrate, ethanol, had no effect on formate oxidation to CO_2 *in vivo* in the rat, but the state of folate deficiency induced by feeding rats a folate-deficient diet allowed for a marked decrease in the rate of formate conversion to CO_2 in the rat *in vivo* (Palese and Tephly, 1975). Only in folate-deficient rats can a role for catalase in formate oxidation be demonstrated (Palese and Tephly, 1975). The present study evaluates these metabolic pathways for formate metabolism in the monkey.

Materials and Methods

Sodium [^{14}C]formate (1–5 mCi/mmol) and [^{14}C]methanol (2–5 mCi/mmol) were purchased from New England Nuclear, Boston, Mass. Preblend 3a70B scintillation cocktail was obtained from Research Products International Corp., Elk Grove, Ill. 3-Amino-1,2,4-triazole and folic acid were purchased from Sigma Chemical Company, St. Louis, Mo. All other reagents employed in these investigations were of the highest available purity. Metabolism chambers and small primate restraining chairs were purchased from Plas-Labs, Lansing, Mich. The folic acid-deficient monkey diet and the control diet were prepared by Bio-Serv Inc., Frenchtown, N.J. Folate assay kits were purchased from Diagnostic Biochemistry, Inc., San Diego, Calif. Formimino-L-glutamic acid (FIGlu) assay kits were obtained from Sigma Chemical Company. Soft-tissue biopsy needle syringes were purchased from Kormed Inc., Minneapolis, Minn.

Animal preparation. Young male and female cynomolgus (*Macaca fascicularis*) and pigtail (*Macaca nemestrina*) monkeys (2.3–3.5 kg) were employed. The monkeys were prepared for metabolic experiments as previously described (McMartin *et al.*, 1975). For formate metabolism experiments, the femoral or axillary vein was cannulated; for exprimments in which methanol was administered, the femoral artery was cannulated. Blood samples were obtained and prepared as previously described (McMartin *et al.*, 1975).

Assays. Methanol blood levels were determined using the gas chromatographic assay described by Baker *et al.* (1969), and formate levels were measured by employing the specific assay described by Makar *et al.* (1975). Blood gases and blood pH were measured using a blood gas analyzer (Instrumentation Laboratories, model 713). Bicarbonate values were calculated from pH and pCO_2 values.

Formate metabolism studies. Sodium [^{14}C]formate (2.5 M, specific activity 90,000 dpm/mg of formate) was administered i.v. at doses of 1 to 10 mmol/kg b.wt. over 1 to 2 minutes and the monkey was immediately placed in the metabolic chamber. At timed intervals, NaOH solutions were removed from the scrubbers and rates of $^{14}CO_2$ formation were determined as previously described (McMartin *et al.*, 1975) except that 3a70B was used in the scintillation cocktail. Preliminary experiments indicated that the metabolism of formate to CO_2 was the same whether administered as the sodium salt or as the free acid. Similar rates of metabolism were seen whether injections were made by i.p. or i.v. routes of administration. Since the sodium salt was less toxic than the free acid and, in general, did not affect the

acid-base balance, sodium formate was used for all studies reported here. Blood samples were obtained from the venous cannula periodically and analyzed for formate levels.

Methanol experiments. A 20% (w/v) solution of methanol was administered *via* a nasogastric tube to the monkey. Blood samples were obtained from an arterial cannula for methanol, formate, pCO_2 and pH analysis.

Folate deficiency in the monkey. Male cynomolgus monkeys were divided into two groups and fed either a folic acid-deficient diet or the corresponding control diet which contained approximately the equivalent of 2.5 mg of folic acid per kg diet. Liver samples were obtained from each group of monkeys by percutaneous needle biopsy and prepared for folate analysis by a method based on that of Chanarin *et al.* (1966). Liver samples were weighed and homogenates (1:1000, w/v) were prepared with ice-cold 0.1 M potassium phosphate, pH 6.0, containing 1% sodium ascorbate. The homogenates were heated for 15 minutes in a boiling water bath, cooled, centrifuged, and the supernatant was withdrawn and stored at −80°C until analyzed. Blood samples were withdrawn from a superficial leg vein into heparin-treated syringes. They were immediately centrifuged to obtain the plasma, which was diluted 1:1 with 0.1 M potassium phosphate, pH 6.0, containing 1% sodium ascorbate and stored at −80°C until analyzed. Folate levels in liver and plasma samples were determined by a radioassay, similar to the method described by Longo and Herbert (1976), using the kits supplied by Diagnostic Biochemistry.

FIGlu excretion. Twenty-four hour urine samples were collected in order to determine the excretion of FIGlu in monkeys. Monkeys were not administered a loading dose of histidine prior to urine collection. Samples were centrifuged to remove debris, and 10-ml aliquots of the supernatant were removed. After 0.1 ml of concentrated HCl was added, the samples were stored at −80°C until analyzed. The urinary excretion of FIGlu was determined by the method of Tabor and Wyngarden (1958) using kits obtained from Sigma Chemical Company.

Statistical evaluation. Significance levels for statistically different values were determined using the Student's *t* test. Linear regression analyses were performed using the least squares-fit method.

Results

Toxicity of methanol in cynomolgus monkeys. Our studies on the toxicity of methanol (McMartin *et al.*, 1975) were performed using rhesus and pigtail monkeys. However, since the cynomolgus monkey is a more readily available and less expensive animal than the other two species, experiments were performed to determine the susceptibility of the cynomolgus monkey to methanol poisoning. The oral administration of methanol (3 g/kg) produced a syndrome similar to that observed in the rhesus or pigtail monkeys, with a latent period of 8 to 12 hours, followed by metabolic acidosis, coma and death. The data in figure 1 show that formic acid accumulates in the blood to levels as high as those in the other species (12 mEq/l) and that decreases in arterial blood bicarbonate values correlate with increases of formate ions. Other data, such as the methanol blood levels and the rate of metabolism of methanol to CO_2, were also similar to those reported for the rhesus and pigtail monkeys (McMartin *et al.*, 1975). Therefore, the cynomolgus monkey is an appropriate model for studies on methanol toxicity and was used for most of the studies reported.

Metabolism of formate to CO_2 in the monkey. The data in figure 2 show the pattern of [^{14}C]formate metabolism to $^{14}CO_2$ in the monkey. The rate of metabolism to CO_2 increases with increasing dose of formate. Figure 3 shows the dose dependence of the rate of formate oxidation to CO_2. Rates of metabolism were calculated from data in figure 2 during periods of time when the rate of $^{14}CO_2$ formation approximated linearity. The maximal rate occurs at about 10 mmol/kg of formate but higher doses were not employed as the dose of 10 mmol/kg of formate induced severe vomiting in a number of animals. Also included in figure 3 for comparison are data for the rat, derived from results presented by Palese and Tephly (1975). At the higher dose levels of formate, the rate of formate metabolism to CO_2 in the monkey is

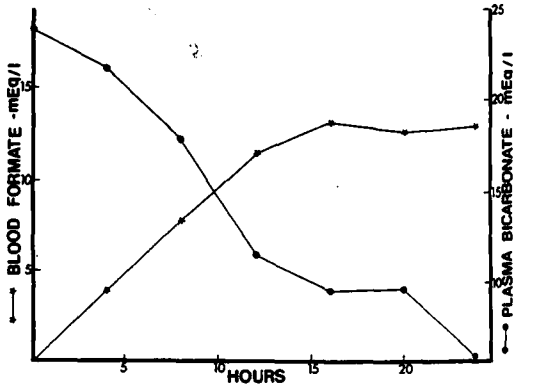

FIG. 1. Toxicity of methanol in cynomolgus monkey. [^{14}C]methanol (3 g/kg) was administered p.o. as a 20% (w/v) solution. Bicarbonate values were calculated from the arterial blood pH and pCO_2 values.

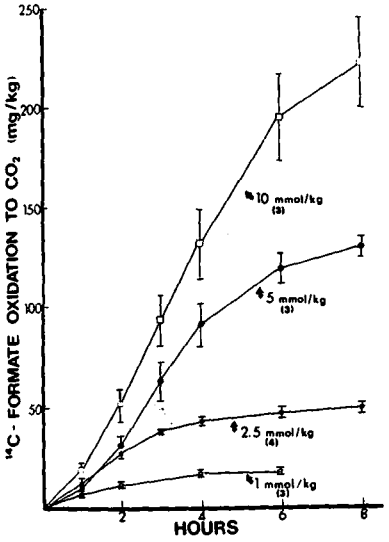

FIG. 2. Metabolism of formate to CO_2 in the monkey. Sodium [^{14}C]formate was administered i.v. at zero time in the dose indicated for each curve. Each point represents the mean ± S.E.M. Numerals within parentheses indicate the number of animals.

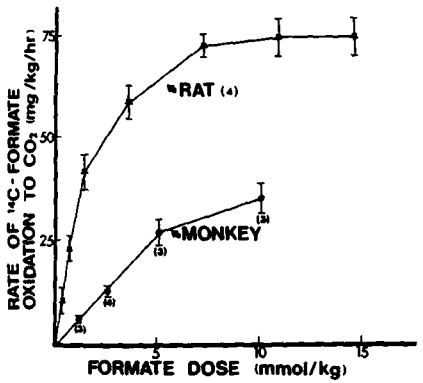

FIG. 3. Species differences in the rate of formate metabolism. Sodium [^{14}C]formate was administered i.v. to the monkey. Rates of formate oxidation to $^{14}CO_2$ were determined from data in figure 2 as described in the text. Values for the rat are obtained from a paper by Palese and Tephly (1975). Each point represents the mean ± S.E.M. Numerals in parentheses indicate the number of animals.

less than half that observed in the rat. At lower doses the rate of oxidation to CO_2 in the monkey is about 25% of that in the rat. The lower rate of formate metabolism to CO_2 in the monkey must be considered as an important factor in explaining why formate accumulates and produces acidosis in the methanol-poisoned monkey but not in the rat.

Role of the catalase-peroxidative pathway in formate metabolism in the monkey. Because of the difference in the rate of formate metabolism to CO_2 in the monkey and in the rat, studies were designed to define the route by which formate is converted to CO_2 in the monkey. The dose of 2.5 mmol/kg of formate was chosen for these and subsequent studies. This dose produces rates less than the maximal rate of formate metabolism. At this dose, inhibitions are most apt to be revealed.

To test the role of the catalase-peroxidative system in formate oxidation in the monkey *in vivo*, AT, in a dose of 1 g/kg was administered i.p., 1 hour prior to [^{14}C]formate. This dose of AT has been shown previously (Makar *et al.*, 1968) to decrease monkey hepatic catalase activity to 10% of control values. The results in figure 4 show the lack of effect of AT on the metabolism of formate to CO_2 in the monkey. Furthermore, AT pretreatment does not affect the rate of formate disappearance from the blood of the monkey. The half-life for formate elimination from the blood of monkeys treated with AT was 38 ± 5 minutes, whereas the value in control monkeys was 37 ± 5 minutes. These results were not unexpected because previous studies (Makar *et al.*, 1968) have shown that the catalase-peroxidative system does not function in methanol metabolism to CO_2 in the monkey. Thus, the results suggest that the catalase-peroxidative system is not important for the metabolism of formate in the monkey *in*

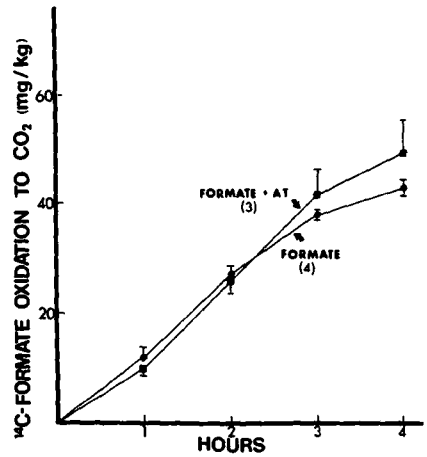

FIG. 4. Effect of AT on formate metabolism in the monkey. Sodium [^{14}C]formate (2.5 mmol/kg) was administered i.v. and AT (1 g/kg) was injected i.p. 1 hour before [^{14}C]formate. Each point represents the mean ± S.E.M. Numerals in parentheses indicate the number of animals. The values for AT-treated animals are not significantly different from the values obtained for animals treated with formate alone.

vivo. A similar conclusion was reported by Palese and Tephly (1975) in studies on formate metabolism in the rat.

Role of a folate-dependent system in formate metabolism in the rat. In order to evaluate the role of the folate-dependent one-carbon pool in the metabolism of formate, monkeys were divided into two groups, one group receiving a folate-deficient diet, the other receiving a control diet. The level of folate in the liver was used as an index of tissue folate concentration. After 14 weeks on the diet, liver biopsies were performed and folate measurements were made on these samples. The data in figure 5 show that hepatic folate concentrations for monkeys on a folate-deficient diet are markedly depressed compared with those of control monkeys. Also, the urinary excretion of FIGlu in the monkeys fed the folate-deficient diet was significantly increased above that of control animals. The excretion of FIGlu has been used as an indirect measure of tissue folate (Siddons, 1974) and allows for an alternate means of assessing this status.

Along with the striking decrease in hepatic folate levels, the results in figure 6 show that the rate of formate metabolism to CO_2 in folate-deficient monkeys (9.6 ± 0.4 mg/kg/hr) is about half that observed in control monkeys (17.3 ± 2.8mg/kg/hr). After 20 weeks on the diet, the rate is further reduced to 7.3 ± 1.3 along with a decrease in hepatic folate level to 0.7 ± 0.4 μg/g of liver, a value which is less than that seen in animals on the diet for 14 weeks (fig. 5). Furthermore, the data in figure 7 demonstrate that the rate of elimination of formate from the blood of folate-deficient monkeys was about half that of control monkeys. The half-life of formate disappearance from the blood of folate-deficient monkeys is 74 minutes, a value almost double the value of 39 minutes which was observed in control monkeys. Thus, a folate-dependent one-carbon pathway is primarily responsible for formate oxidation in the monkey *in vivo*.

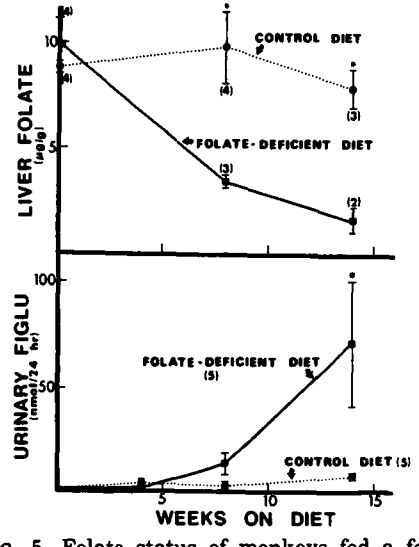

FIG. 5. Folate status of monkeys fed a folate-deficient diet. Monkeys were divided into two groups; one group was fed the folate-deficient diet and the others were fed the control diet. Hepatic folate concentrations were determined as described under "Methods" and are expressed on the basis of wet weight of liver. Each point represents the mean ± S.E.M. Numerals in parentheses indicate the number of animals. * Indicates significant difference from the control value ($P < .05$).

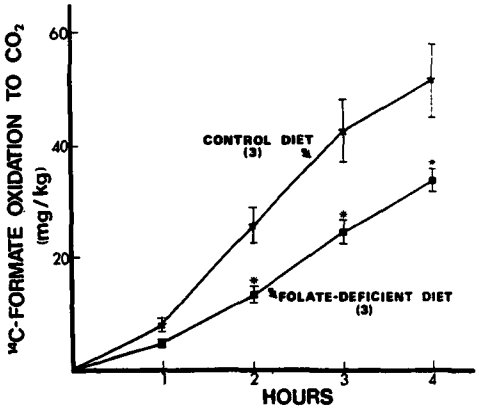

FIG. 6. Effect of folate deficiency on formate metabolism in the monkey. Sodium [^{14}C]formate (2.5 mmol/kg) was administered i.v. to monkeys maintained on the control diet or on the folate-deficient diet. Each point represents the mean ± S.E.M. Numerals in parentheses indicate the number of animals. * Indicates significant difference from the control value ($P < .05$).

Administration of AT to folate-deficient monkeys in a dose of 1 g/kg i.p. 1 hour prior to formate, produced no further decrease in the rate of metabolism of formate to CO_2. These data indicate that the catalase-peroxidative system does not function in the metabolism of formate to CO_2 even in monkeys whose rate of formate oxidation has been decreased by the induction of folate deficiency.

Effect of exogenous folate on formate metabolism in the monkey. Whereas folate has been shown to play a major role in formate oxidation through studies with folate-deficient monkeys, the question was raised as to whether

folate administered exogenously could have an effect on the rate of formate metabolism to CO_2 in the control monkey. Thus, sodium folate, in doses of 50 mg/kg b.wt. was administered i.p. at 48, 24 and 1 hour prior to [^{14}C]formate administration. This treatment led to plasma folate levels of about 2000-fold times control values, *i.e.*, from 11.1 ± 1.3 to 25,300 ± 4,500 ng/ml. Liver folate measurements are not available from these animals. The data in figure 8 show that folate pretreatment increased the rate of formate oxidation to about 50% above the rate observed in control animals. This result provides further evidence for a role of the one-carbon pool in the metabolism of formate in the monkey *in vivo*.

Effect of the rate of formate metabolism on formate accumulation after methanol administration to the monkey. Since folate-deficient monkeys display a slower rate of formate oxidation than control monkeys, folate-deficient monkeys should be more susceptible to the effects of methanol than the control monkey. The data in figure 9 demonstrate that, after the

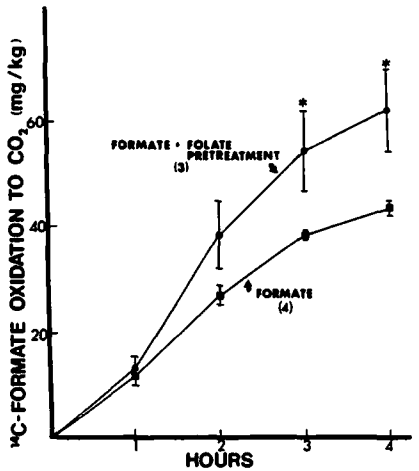

FIG. 8. Effect of exogenous folate administration on formate metabolism in the monkey. Sodium [^{14}C]formate (2.5 mmol/kg) was administered i.v., and sodium folate [folic acid, 50 mg/kg in a 7.5% (w/v) solution of sodium bicarbonate] was injected i.p. 48, 24 and 1 hour before [^{14}C]formate. Each point represents the mean ± S.E.M. Numerals in parentheses indicate the number of animals. * Indicates significant difference from the value obtained for animals treated with formate alone ($P < .05$).

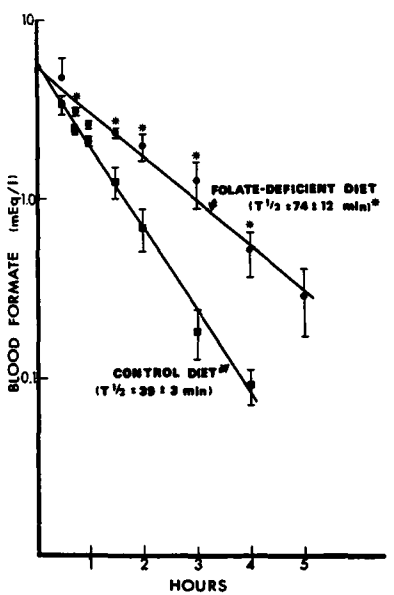

FIG. 7. Formate elimination from the blood of folate-deficient monkeys. Sodium [^{14}C]formate (2.5 mmol/kg) was administered i.v. to monkeys maintained on the folate-deficient diet or on the control diet. Each point represents the mean ± S.E.M. for three animals. ——, regression analyses on the linear phase of the disappearance curves. The half-life represents the mean value ± S.E.M. for the values obtained from regression analysis of the disappearance curve for each animal * Indicates significant difference from the control value ($P < .05$)

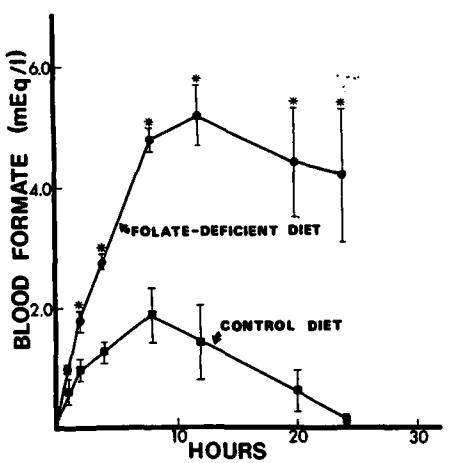

FIG. 9. Effect of folate deficiency on methanol toxicity in the monkey. Methanol (0.5 g/kg) was administered p.o., as a 20% (w/v) solution, to monkeys maintained on the control diet or on the folate-deficient diet. Each value represents the mean ± S.E.M. for three animals. * Indicates significant difference from the control value ($P < .05$).

administration of a low dose of methanol (0.5 g/kg p.o.), there is a striking increase in the level of formic acid in the blood of the folate-deficient monkey as compared with monkeys fed the control diet. These results suggest a major role for the folate-dependent pathway in the metabolism of formate and its role in regulating the

susceptibility of the animal to methanol toxicity.

Discussion

The use of the monkey as a model of methanol poisoning in man was first proposed by Gilger and Potts (1955) and whereas other workers were unable to confirm their results (Cooper and Felig, 1961), recent studies (McMartin *et al.*, 1975; Martin-Amat *et al.*, 1977; Hayreh *et al.*, 1977; Baumbach *et al.*, 1977; Clay *et al.*, 1975) have described a methanol toxicity syndrome in the monkey which has similarities to that described for man. Our laboratory has studied the rhesus, pigtail and cynomolgus species of monkeys and has found that each is equally sensitive to methanol and in each a similar pattern of events occurs.

An outstanding feature of methanol toxicity in man as well as in the monkey is metabolic acidosis. Studies by McMartin *et al.* (1975) and Clay *et al.* (1975) have shown that formic acid is the major determinant of metabolic acidosis in methanol-poisoned monkeys. Clay *et al.* (1975) showed that the increase in blood formate concentrations fully accounted for the decrease in blood bicarbonate levels and that there was only a slight elevation in the concentrations of various organic acids which were measurable by gas chromatographic-mass spectrometric methods. McMartin *et al.* (1975) demonstrated that the anion gap increases in methanol-poisoned monkeys and that formate was the major determinant of the increase in the anion gap. Other studies have shown (fig. 10) that one can correlate decreased blood bicarbonate concentrations with increases in formate ions. Herken *et al.* (1969) have shown a correlation between formate blood levels and a base deficit observed in methanol-intoxicated amethopterin-treated dogs. Our laboratory has shown that when methanol metabolism in the monkey was inhibited by administration of 4-methylpyrazole, an inhibitor of hepatic alcohol dehydrogenase, no formic acid accumulated in the blood and no acidosis developed (McMartin *et al.*, 1975). Presumably, formate also plays a major role in the acidosis observed in man. We have observed initial blood formate levels of about 11 mEq/l in one patient recently admitted to University Hospital in Iowa City with methanol poisoning. This value is consistent with that observed in monkeys (fig. 1).

In contrast to results in monkeys and in man, administration of methanol to the rat, in a dose (6 g/kg) twice that given the monkey, produced no formate accumulation in the blood and no acidosis (McMartin *et al.*, 1975). The accumulation of formate in the blood of monkeys, but not rats, after methanol administration may be explained by a difference in the rate of formate metabolism to CO_2 in this species. In the monkey, formate probably accumulates in the blood because the rate of formate production from methanol is greater than the rate of formate elimination. Differences in the urinary excretion of formate do not account for the differences in formate elimination in the two species (Clay *et al.*, 1975) and it is unlikely that different or unique distribution or storage mechanisms occur in these species. The present results show that the rat metabolizes formate to CO_2 at about twice the rate observed in the monkey. When the rate of formate metabolism to CO_2 in the rat is decreased about 50%, or to values normally seen in the monkey, methanol treatment produces an accumulation of formate in the blood which is accompanied by a severe

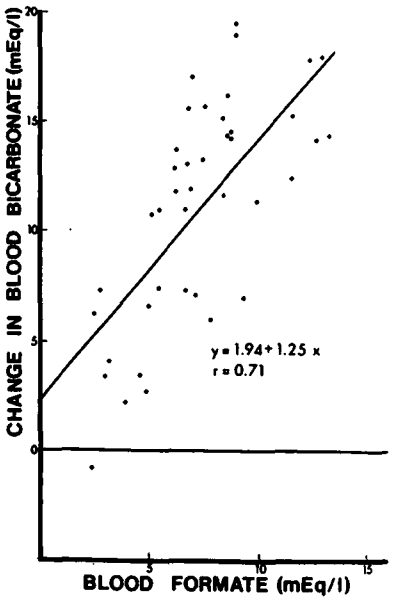

FIG. 10. Correlation between arterial blood bicarbonate and formate values in methanol-poisoned monkeys. Methanol (3 g/kg) was administered p.o. as a 20% (w/v) solution to nine monkeys. Each point represents the values for arterial blood samples which were obtained at timed intervals and analyzed for formate concentrations and blood gases. Bicarbonate values were calculated from the arterial blood pH and pCO_2 values and are expressed as the change from the zero-time value, which was used as the control value for each animal.

acidosis (Makar and Tephly, 1976). In the current studies, when the rate of formate metabolism is decreased, the monkey becomes more susceptible to methanol poisoning. Rietbrock *et al.* (1966) induced folate deficiency in the dog by pretreatment with amethopterin and demonstrated a slight increase in blood formate with a probable acidosis.

The results in this study as well as those in the report by Palese and Tephly (1975) suggest that the rat and monkey metabolize formate by the same pathway, a folate-dependent one-carbon system rather by a catalase-peroxidative system. The lack of activity of the catalase-peroxidative system with methanol or formate in the monkey *in vivo* is probably due to a mixture of factors including the low level of peroxidative capacity of hepatic particulate catalase activity and the low activity of peroxide-generating oxidases in monkey liver (Mannering *et al.*, 1969; Tephly *et al.*, 1974). The difference in the rate of formate metabolism between monkeys and rats and, therefore, the ability to eliminate formate from the blood in the two species are presumably due to a greater efficiency or capacity of the folate pathway in the rat as compared with the monkey. The enzymatic steps and the regulation of the concentration of substrates and cofactors important in the functioning of this system for formate metabolism are not known; this is the subject of future investigations which will attempt to explain the differences in capacity of the one-carbon pool in various species that are sensitive and insensitive to methanol poisoning.

The data showing an increase in the rate of formate metabolism in monkeys pretreated with folate indicate a possible use of folate therapy in the treatment of methanol poisoning in man. Since formate plays a major role in the production of metabolic acidosis from methanol and since the rate of formate metabolism seems to be the factor controlling the amount of formate accumulation, any procedure which decreases the rate of methanol oxidation or increases the rate of formate metabolism would be useful in the treatment of methanol poisoning. The use of ethanol or 4-methylpyrazole would accomplish the former procedure; means to stimulate the one-carbon pool system would provide the latter. The use of folate administration in methanol toxicity has also been suggested by Rietbrock *et al.* (1966) who showed an increase in methanol elimination and a decrease in blood formate in dogs treated with folate and then with methanol. Studies designed to evaluate the efficacy of folate treatment in methanol poisoning in the monkey are currently underway.

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