BIOCHEMICAL MEDICINE 13, 319–333 (19

Methanol Poisoning I. The Role of Formic Acid in the Development of Metabolic Acidosis in the Monkey and the Reversal by 4-Methylpyrazole[1]

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Received July 7, 1975

INTRODUCTION

Methanol poisoning in man is characterized by a modest central nervous system depressant effect, an asymptomatic latent period of 12–24 hr, followed by weakness, abdominal pain, visual disturbances, severe metabolic acidosis, and hyperpnea. Coma and permanent blindness and/or death may ensue. The toxicity has been attributed to the formation of toxic metabolites of methanol (1), because of the latent period prior to acidosis and because ethanol, which presumably blocks methanol metabolism, often delays or alleviates the acidosis and toxicity in humans (2).

Methanol poisoning, while rare, may reach epidemic proportions (3). However, since it has been proposed that methanol serve as a gasoline extender (4), the importance of determining the etiology and an effective treatment of methanol poisoning becomes more immediate. However, research has been hampered due to the question of which animal should be employed as a valid model for the toxic syndrome seen in man. In a variety of nonprimates, toxic doses of methanol produce only an effect similar to that produced by ethanol (1), and the latent period, ocular toxicity, and severe acidosis are not observed (5). The rhesus monkey has been proposed as a model for methanol toxicity by Potts and his coworkers (5–7). Typical symptoms of human methanol poisoning were described, including the latent period, metabolic acidosis, signs of retinal dysfunction, coma, and death. They suggested that formaldehyde is the agent responsible for methanol poisoning in man, rather than formic acid since the formate level, as they determined it, did not account for the

[1] This work was supported in part by NIH Grant GM 19420, and in part by NIH Grant GM 12675.

organic acid concentration in the animal. In contrast, Cooper and Felig (8) were unable to confirm these results in rhesus monkeys. They used comparable doses of methanol and found no consistent features of methanol toxicity, except a central nervous system depression similar to that seen in nonprimate species. However, since in a few cases a possible acidosis was determined, the monkey's susceptibility to methanol was categorized between that of man and nonprimates.

Makar *et al.* (9) studied the role of alcohol dehydrogenase (E.C.1.1.1.1.) and catalase (E.C.1.11.1.6) in methanol oxidation in the monkey, and occasionally administered doses which were twice the minimum lethal dose as determined by Gilger and Potts (5). Though the studies were not designed to examine the characteristics of methanol toxicity, no deaths were reported in these monkeys.

The present study was designed to review the position of whether the monkey (rhesus or pigtail) could serve as a model for human methanol poisoning and to evaluate the property of 4-methylpyrazole to inhibit methanol metabolism through its ability to inhibit monkey hepatic alcohol dehydrogenase (10). In addition, a new specific and sensitive assay for formate (11) was employed to determine the relation between metabolic acidosis and blood concentration of formic acid.

MATERIALS

Methanol-^{14}C (2-5 mCi/mmole) and Aquasol were purchased from New England Nuclear. 4-Methylpyrazole was purchased from Research Plus Laboratories, Inc., Denville, N.J. Sernylan (phencyclidine hydrochloride) was obtained from Bio-Ceutic Laboratories, Inc., St. Joseph, Mo. All other reagents employed in these investigations were of the highest available purity. Metabolism chambers and small restraining chairs were purchased from Plas-Labs, Lansing, Michigan.

METHODS

Metabolic Experiments

Young male and female rhesus (*Macaca mulatta*) and pigtail (*Macaca nemestrina*) monkeys (2.2–6.5 kg) were employed. Sernylan was injected intramuscularly (1 mg/kg) in order to produce a quiescent state required for surgical procedures. The femoral artery was cannulated, and an indwelling catheter was fixed in order to obtain arterial blood samples for methanol, formate, pO_2, pCO_2, pH, and electrolyte analysis. On occasion, a superficial leg vein was cannulated as an alternative. The monkeys were restrained in a small chair that forms part of the metabolism chamber, and their limbs were tied so as to prevent them from disrupting the function of the apparatus during the experiment. The

monkeys were allowed to recover fully from the behavioral effects of Sernylan (about 1–4 hr).

A nasogastric tube was put in place, and a 20% (w/v) solution of [^{14}C]methanol (3 g/kg; specific activity 1500 dpm/mg of methanol) was administered over 5–10 min. The monkey was then immediately placed in the clear plastic metabolic chamber. Throughout the experiments, when the chamber was opened, the monkeys were allowed to drink water and to eat fruit. In some experiments, those beyond 24 hr, monkeys were administered about 3 ounces of Sego (Pet, Incorporated) via a nasogastric tube. The monkeys were observed almost constantly during the experiment for clinical signs and symptoms, which related to methanol toxicity, or possible hyperactivity which would have led to removal of the cannula.

Room air was drawn through the chamber (about 10 l/min) and passed through a column containing about 400 g calcium sulfate to trap water, and approximately 250 g magnesium perchlorate to collect expired methanol (9,12). The air was delivered to three scrubbers, each containing 200 ml of 2 N NaOH, in order to collect expired $^{14}CO_2$. At timed intervals during the experiment, the NaOH solutions were removed from the scrubbing flasks, the flasks were rinsed and refilled with fresh alkaline solutions.

The rate of $^{14}CO_2$ evolution was determined by analysis of 1 ml aliquots of the NaOH solutions. To each aliquot, in scintillation vials, 4 ml of water and 15 ml of Aquasol were added. The resulting gel was cooled and dark-adapted for 2–4 hr to avoid chemiluminescence. The samples were analyzed for radioactivity using a Packard Tri-Carb liquid scintillation spectrometer (Model 3320). The overall efficiency of this system was approximately 85%.

No [^{14}C]methanol was detected in the NaOH solutions. The $MgClO_4$-$CaSO_4$ column was triturated with distilled water, and expired methanol was determined. Addition of saturated $BaCl_2$ solutions precipitated any dissolved CO_2. The supernatant radioactivity was not altered by this procedure, confirming that no $^{14}CO_2$ was trapped in the $MgClO_4$-$CaSO_4$ column. Finally, analysis of the contents of a trap situated between the three NaOH scrubbing flasks and the vacuum pump revealed no radioactivity, showing that the scrubbing system was efficient in removing $^{14}CO_2$.

Analysis of Blood Gases and Electrolytes

At various time intervals, the metabolic chamber was opened and blood samples (usually 2 ml) were withdrawn from the arterial cannula into heparinized syringes. Then, 2 ml of 0.9% NaCl was administered to the animal followed by 0.25 ml (the cannula volume) of a heparin solu-

tion (10 units/ml). Control blood samples were obtained at the start of the experiment just prior to the administration of the methanol solution. When plasma electrolytes were determined, 0.75 ml of the blood was immediately centrifuged to obtain plasma. The plasma sample was then submitted to the Clinical Pathology Laboratories, University of Iowa Hospital, for determination of plasma Na^+, K^+, and Cl^-. Blood samples were immediately analyzed for blood gases and blood pH using a digital blood gas analyzer (Instrumentation Laboratories, Model 213). Plasma bicarbonate and anion gap measurements were estimated from arterial pH, pCO_2, and electrolyte values.

Formic Acid and Methanol Analysis

Blood (0.5 ml) placed in vacutainer tubes was kept at 4°C until used for formate and methanol analysis. Blood samples were diluted with 0.7 ml of H_2O, thoroughly mixed and allowed to stand for 5 min. Then, 0.15 ml of 7.5% $ZnSO_4 \cdot 7H_2O$ was added and samples were mixed again. Finally, 0.15 ml of 0.4 N NaOH was added dropwise with mixing and samples were centrifuged at 35,000*g* for 20 min in a RC-2B Sorvall centrifuge. Aliquots of the protein-free supernatant were used for formate and methanol measurement. Methanol blood levels were determined using the gas chromatographic assay described by Baker *et al.* (13), and formate was measured by a specific and sensitive assay recently described by us (11). This method is based on the conversion of formate to CO_2 by the bacterial enzyme, formate dehydrogenase, coupled to an NADH-dependent reaction catalyzed by diaphorase. In the process, the nonfluorescent dye, resazurin, is converted to the fluorophore, resorufin. Recovery of formate and methanol by this procedure is approximately 95–100%.

Urine samples were collected at various times and immediately stored at 4°C. Aliquots were centrifuged to remove sediment and analyzed as such for methanol and formate.

4-Methylpyrazole Experiments

In these studies, the monkeys were treated as described previously, except that, at 15 min before the administration of [^{14}C]methanol, 4-methylpyrazole (50 mg/kg) was injected intraperitoneally as a 1.0% solution in 0.9% NaCl. After [^{14}C]methanol administration, the monkeys were placed in the metabolic chamber and the experiments were conducted as described above.

Rat Experiments

Male Sprague–Dawley rats (280-310 g) were used. Methanol was injected intraperitoneally as a 30% (w/v) solution in H_2O at a dose level of 6 g/kg. Venous blood samples (0.7 ml) were obtained from the tail vein and placed in heparinized tubes. Blood samples were analyzed for blood gases, blood pH, formate, and methanol as described for the monkey.

RESULTS

Clinical Observations

The administration of methanol (3 g/kg) to both rhesus and pigtail monkeys produced a picture resembling that described for methanol poisoning in man. This included, moderate central nervous system depression lasting approximately 1–2 hr, followed by a latent period where no obvious signs were seen (8–12 hr). Then, the animal's condition progressively deteriorated with signs of anorexia, photophobia, weakness, restlessness, and profound hyperpnea. In each animal, coma ensued, followed by death, which occurred from 12–33 hr after the administration of methanol. There appeared to be no difference in this pattern produced by methanol between the rhesus monkey and the pigtail monkey. Furthermore, there were no significant differences between any of the parameters evaluated in the rhesus monkey and those in the pigtail monkey. The data presented in the Results represents a combination of data obtained from both the pigtail and rhesus monkeys.

Metabolic Acidosis

Data in Fig. 1 show that metabolic acidosis gradually develops in methanol-poisoned monkeys. During the initial latent period, the arterial blood pH remained within normal limits, but was balanced by respiratory compensatory mechanisms. On the average, at about 16 hr, the animals' blood pH was decreased to a mean value of 7.15, whereas the

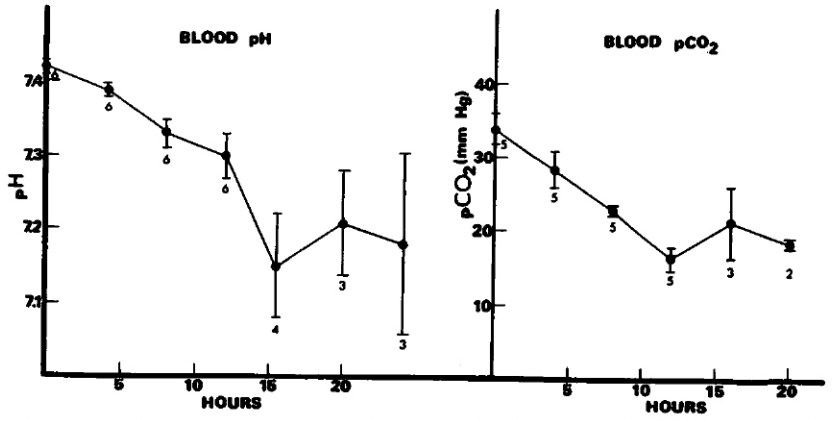

FIG. 1. Arterial blood pH and pCO_2 in methanol-poisoned monkeys. [^{14}C]methanol (3 g/kg) was administered orally as a 20% (w/v) solution. Each point represents the mean value ± SEM. Numerals beneath each point represent the number of animals.

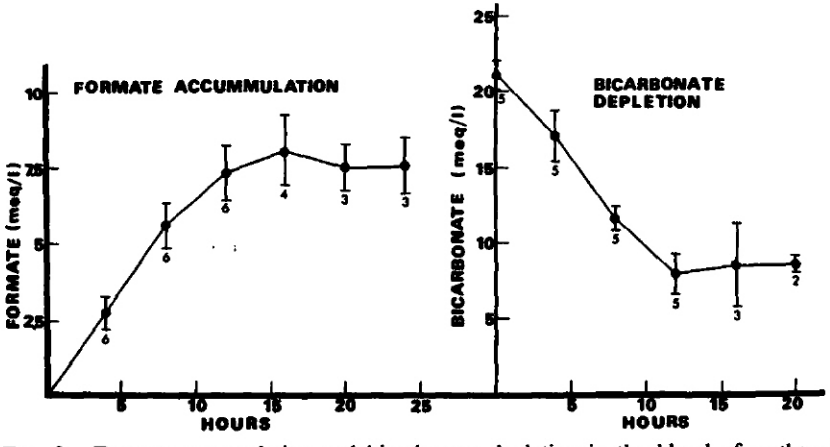

FIG. 2. Formate accumulation and bicarbonate depletion in the blood of methanol-poisoned monkeys. [^{14}C]methanol (3 g/kg) was administered orally as a 20% (w/v) solution. Bicarbonate values were calculated from the arterial blood pH and pCO_2 values presented in Fig. 1. Each point represents the mean value ± SEM. Numerals beneath each point represent the number of animals.

arterial blood pCO_2 was reduced to an average of 20 mm Hg, values that reflect metabolic acidosis. Prior to death, metabolic acidosis was usually very severe, with values of arterial blood pH and pCO_2 as low as 7.02 and 11.1 mm Hg, respectively. The blood pO_2 values did not differ significantly from the control value (89 ± 3 mm Hg) throughout the experiment.

The plasma bicarbonate values plotted in Fig. 2 were computed from the blood pH and pCO_2 levels using the Henderson–Hasselbalch relationship. Plasma bicarbonate decreased from the control value of 21 meq/l to approximately 8 meq/l within 12 hr after methanol administration. During the severe acidosis prior to death, plasma bicarbonate levels of the monkeys in these experiments were recorded as low as 3 meq/l. The level of formic acid rapidly accumulated in the blood of monkeys poisoned with methanol (Fig. 2). Approximately 12 hr after methanol administration, the blood formate concentration reached a plateau of 7.5 meq/l. The accumulation of formate coincided with the decrease in plasma bicarbonate concentration and the development of the state of metabolic acidosis. Such concurrent changes suggest that the formic acid, produced through the metabolism of methanol, leads to the loss of bicarbonate and thereby plays a role in the genesis of metabolic acidosis. The possibility that formate alone accounts for the loss of bicarbonate was investigated by calculating the sum of the concentrations of bicarbonate and formate in the blood and comparing these values with control bicarbonate values. Data in Fig. 3 show that the sum of bicarbonate and formate concentrations 8 hr or longer during the experiments

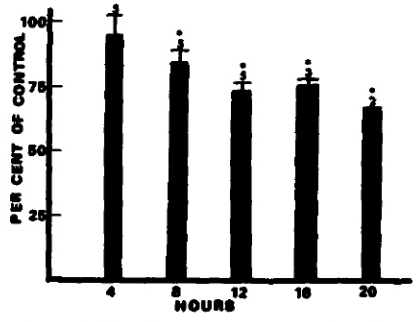

FIG. 3. Sum of formate and bicarbonate concentrations in the blood of methanol-poisoned monkeys. [^{14}C]methanol (3 g/kg) was administered orally as a 20% (w/v) solution. The plasma bicarbonate value at zero time (at which time blood formate is 0 meq/l) was defined as 100% of control. The sum of the values of blood formate and bicarbonate concentration presented in Fig. 2 at each time period represented percentage of control values. Each bar represents the mean ± SEM. Numerals above each bar indicate the number of animals. Results obtained from five experiments were used to provide the control value. * Indicates significant difference from 100% of control value ($P < 0.05$), as determined by Student's paired t-tests.

is significantly different ($P < 0.05$) from control bicarbonate levels. Thus, the loss of bicarbonate anion cannot be accounted for by the concentration of formate anion.

In order to rule out the possibility of changes in other normal electrolytes as a basis for changes in bicarbonate, plasma electrolytes, Na^+, K^+, and Cl^-, were obtained in two monkeys poisoned with methanol. These electrolytes remained within normal limits throughout the experiment (Table 1), except that plasma potassium concentration rose during the phase of severe acidosis which preceded death. The anion gap is defined as the difference between the sum of the concentration of Na^+ and K^+ in plasma, and the sum of the concentrations of Cl^- and HCO_3^- in plasma. The normal values for anion gap (Table 1) range from 15 meq/l to 20 meq/l. After methanol administration, the value of anion gap increases more than twofold. Since the plasma electrolytes remain essentially normal, such an increase in the anion gap represents a loss in bicarbonate and an elevation in organic anion concentration. Formate levels (Table 1) can account for only about 50% of the increase in the anion gap in methanol-poisoned monkeys. This indicates that other organic anions may also accumulate in monkeys which have received toxic doses of methanol.

In contrast to the results produced in both rhesus and pigtail monkeys, administration of methanol (6 g/kg) to rats does not produce any of the signs seen in the monkey, results which correspond with the findings of Gilger and Potts (5). Values of venous blood pH and pCO_2 remain normal, and formic acid does not accumulate in the blood of rats treated

TABLE I

FORMATE AND THE INCREASE IN ANION GAP IN METHANOL-POISONED MONKEYS

Species	Time after methanol[a] (hr)	Plasma electrolytes			HCO_3^- (meq/l)	Anion gap[b] (meq/l)	Blood formate[c] (meq/l)	Increase in anion gap (meq/l)	Formate contribution to anion gap increase (%)
		Na^+ (meq/l)	K^+ (meq/l)	Cl^- (meq/l)					
Pigtail	0	137	5.5	110	18.8	13.7	—	—	—
	4	139	4.1	110	14.7	18.4	3.2	4.7	68
	8	135	5.7	104	11.4	25.3	5.4	11.6	47
	12	138	7.2	108	7.0	30.2	6.9	16.5	42
Rhesus	0	142	4.2	105	20.6	20.6	—	—	—
	4	139	3.9	102	17.2	23.7	4.6	3.1	—
	8	139	4.1	97	9.0	37.1	8.3	16.5	50
	12	139	4.8	97	5.4	41.4	11.5	20.8	55
	16	141	6.8	94	2.9	50.9	12.3	30.3	41

[a] Methanol (3 g/kg) was administered orally to monkeys at zero time. At times indicated, arterial blood samples were obtained as described in Methods.

[b] Computed as difference between the sum of Na^+ and K^+ and the sum of Cl^- and HCO_3^- concentration in arterial blood.

[c] Blood formate concentrations were measured as described by Makar *et al.* (11).

TABLE 2
BLOOD pH, pCO_2, AND FORMATE LEVELS IN METHANOL-TREATED RATS

Time after methanol[a] (hr)	Blood pH	Blood pCO_2 (mm Hg)	Blood formate[b] (meq/l)	Blood methanol[c] (mg/100 ml blood)
0	7.39	53	0	0
	7.39	50	0	0
16	7.42	51	0.04	502
	7.39	46	0.14	446
24	7.42	52	0.06	372
	7.40	54	0.11	354
30	7.43	51	0.13	—
	7.38	45	0.22	—

[a] Methanol (6 g/kg) was injected intraperitoneally to rats at zero time. Venous blood samples were collected at indicated times as described in Methods.
[b] Measured as described by Makar *et al.* (11).
[c] Determined using the assay of Baker *et al.* (13).

with methanol (Table 2). Thus, these studies show one aspect of why the rhesus or pigtail monkey, and not the rat, might serve as an appropriate model of methanol poisoning.

Effect of 4-Methylpyrazole (4-MP) on Methanol Metabolism and Poisoning

Alcohol dehydrogenase is the major catalyst for the first step in the oxidation of methanol in the monkey (9). Pyrazole, a potent inhibitor of alcohol dehydrogenase has been shown to inhibit methanol metabolism in the monkey (14). 4-Methylpyrazole (4-MP) has been reported to be less toxic than pyrazole (15) and to be a more potent inhibitor of alcohol dehydrogenase (16,17). Therefore, 4-MP (50 mg/kg) was administered to both pigtail and rhesus monkeys 15 min prior to methanol (3 g/kg) in order to examine its effects on methanol toxicity. Figure 4 shows the effect of 4-MP on methanol metabolism to CO_2 in the monkey. The rate of metabolism of methanol in monkeys treated with methanol alone is not linear with time, thus, an initial rate of 62 mg/kg/hr declines to about 15 mg/kg/hr at 24 hr after methanol administration. Pretreatment of monkeys with a single dose of 4-MP decreased the rate of metabolism of methanol to CO_2 in monkeys to approximately 25% of the rates obtained in the absence of 4-MP. Furthermore, no formic acid accumulates in the blood of monkeys pretreated with 4-MP during the first 36 hr after methanol administration (Fig. 5) and no metabolic acidosis results. These data indicate that 4-MP inhibits the metabolism of methanol and the accumulation of formic acid in the blood. No overt signs of toxicity develop during the first 36 hr after 4-MP and methanol. The monkeys remain calm, alert, strong, and retain their control behavioral pattern.

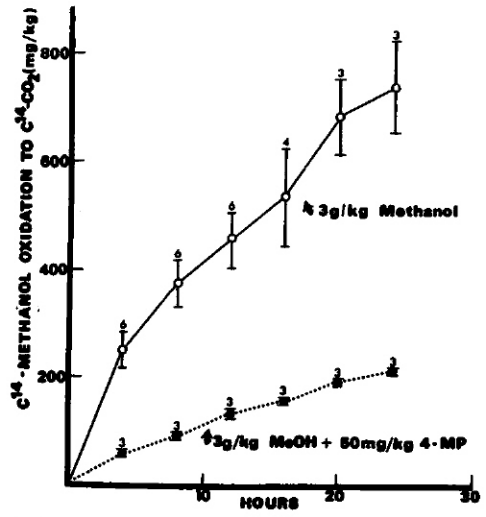

FIG. 4. Effect of 4-methylpyrazole (4-MP) on methanol metabolism to CO_2 in the monkey. o——o [^{14}C]methanol (3 g/kg) administered orally; •···• [^{14}C]methanol (3 g/kg) administered orally 15 min after 4-methylpyrazole (50 mg/kg) injected intraperitoneally. Rates of $^{14}CO_2$ production in 4-MP-treated monkeys are significantly different from rates in monkeys treated with methanol alone at each time ($P < 0.05$). Each point represents the mean value ± SEM, and numerals above each point represent the number of animals used.

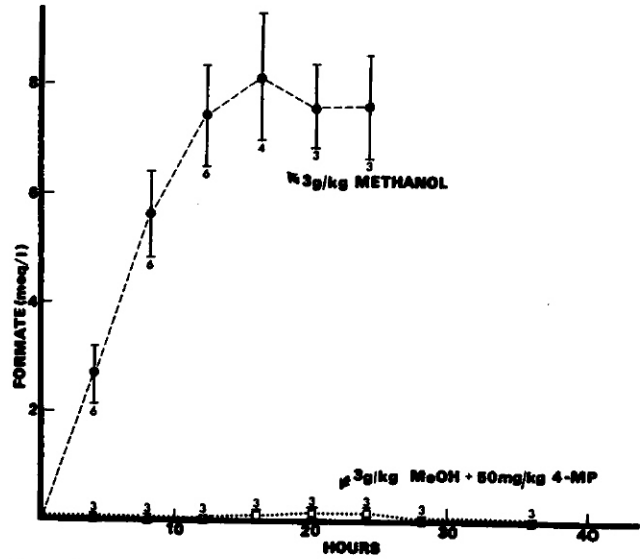

FIG. 5. Effect of 4-methylpyrazole (4-MP) on the accumulation of formate in the blood of monkeys poisoned with methanol. •---• [^{14}C]methanol (3 g/kg) administered orally; □···□ [^{14}C]methanol (3 g/kg) orally 15 min after 4-methylpyrazole (50 mg/kg) injected intraperitoneally. Blood formate concentrations in 4-MP-treated monkeys are significantly different ($P < 0.05$) from concentrations in monkeys treated with methanol alone at each time. Each point represents the mean value ± SEM. Small numerals associated with each point represent the number of animals used.

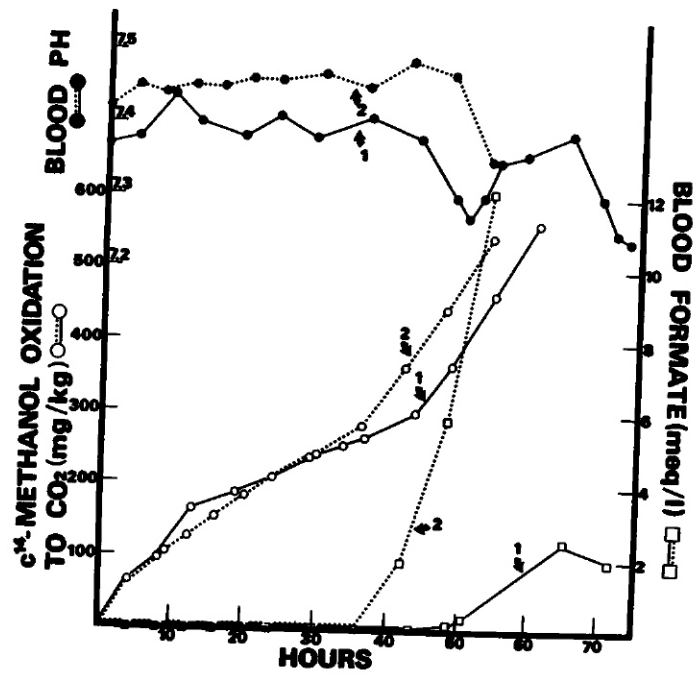

FIG. 6. Delay in methanol toxicity in the monkey produced by singular administration of 4-methylpyrazole (4-MP). 1(——), pigtail monkey; 2(...), rhesus monkey. ○, [^{14}C]methanol oxidation to $^{14}CO_2$; •, blood pH; □, blood formate concentrations. [^{14}C]methanol (3 g/kg) was administered orally 15 min after a single dose of 4-methylpyrazole (50 mg/kg) was injected intraperitoneally. Venous blood was obtained from the pigtail monkey for pH and formate analysis, whereas arterial blood was obtained from the rhesus monkey. During the first 36 hr, the arterial blood pCO_2 remained essentially normal (25–30 mm Hg) in the rhesus monkey.

After the administration of methanol orally (3 g/kg) the peak blood level (± SEM) obtained was 319 ± 10 mg of methanol per 100 ml of blood. The rate of disappearance of methanol from the blood of monkeys is slow. The mean half-life (± SEM) for methanol, calculated from individual regression lines for seven animals is 23.7 ± 3.0 hr. Treatment of monkeys with 4-MP prior to methanol administration increased the mean half-life of methanol to 48.6 ± 14.1 hr, results predictable on the basis of the inhibition of alcohol dehydrogenase and the oxidation of methanol to CO_2. Since blood levels of methanol remain high in 4-MP treated monkeys and no sign of toxicity are found, methanol itself is not responsible for the toxicity of methanol poisoning.

One dose of 4-MP (50 mg/kg) is effective in blocking the metabolism of methanol to CO_2, the accumulation of formate, and the production of toxic symptoms for at least 36 hr. After this time, if no more 4-MP is administered, the rate of methanol metabolism (Fig. 6) increases from

about 5 mg/kg/hr to 16 mg/kg/hr, a rate observed 24 hr after methanol administration where no 4-MP was given. With the return of methanol metabolism to normal rates, formic acid accumulates in the blood and metabolic acidosis along with the typical signs of methanol toxicity are seen. Thus, one dose of 4-MP (50 mg/kg) given 15 mins before an oral dose of methanol (3 g/kg) is capable of preventing methanol toxicity for at least 36 hr, but when the concentration of 4-MP decreases below that necessary for the inhibition of methanol metabolism, there remains sufficient methanol in the blood of these monkeys so as to produce the toxic syndrome.

DISCUSSION

Roe and others (1,18) have observed that methanol toxicity in lower animals corresponds to Richardson's rule, but that the toxicity in man represents an exception to that rule. In man, methanol produces a syndrome characterized by a central nervous system depression followed by a latent period where no observable signs or symptoms are seen. Then, 12–24 hr later a metabolic acidosis occurs with profound respiratory effects secondary to that acidosis. The use of an appropriate experimental model for methanol poisoning was emphasized by Potts and his coworkers (5,6,7) who employed the rhesus monkey in experiments and showed metabolic acidosis and ocular toxicity in this species. While these results seemed clear, other laboratories were unable to confirm them (8). Indeed, we have participated in experiments in the past (9) where the toxicity was not apparent under the conditions employed in that work. However, the major thrust of the latter studies dealt with the pathways involved in the conversion of methanol to formaldehyde, rather than the description of the syndrome of methanol toxicity in the monkey. Therefore, because of an increase in our knowledge of the various pathways responsible for methanol oxidation in the rat and the monkey, studies in the current report were initiated in order to determine whether the monkey would be a suitable model for methanol poisoning, and what experimental conditions were required for the production of that syndrome.

Under the conditions of these experiments, both the pigtail and the rhesus monkey displayed certain aspects of the toxicity which are seen in man. After the administration of methanol (3 g/kg) there was an initial central nervous system depression, a latent period of 8–12 hr during which time the animals displayed no obvious signs of toxicity, followed by a progressive deterioration in the animals' condition, a metabolic acidosis, hyperpnea, tachypnea, coma, and death within 12–33 hr after the initial administration. The metabolic acidosis developed gradually and was coincident with the accumulation of formic acid in the blood.

These results agree in part with those of Potts and his coworkers

(5,6,7) with respect to the description of a toxic syndrome in the monkey. However, our conclusions differ from theirs in that there appears to be a correlation between the rate of accumulation of formic acid in the blood and the onset and progression of the metabolic acidosis. Potts and his colleagues concluded that formic acid was not responsible for the toxicity of methanol and suggested that the syndrome was related to the generation of formaldehyde (7). Our studies show that formic acid production coincides with the onset and progression of metabolic acidosis and when the accumulation of formic acid is prevented, e.g., through the use of 4-methylpyrazole, the acidosis does not ensue. That formic acid may not be entirely responsible for the metabolic acidosis was shown where the anion gap determined in these experiments could not be explained completely by the generation of formic acid. Therefore, other organic anions may also be involved in the production of the metabolic acidosis due to methanol poisoning. Perhaps the reason why these studies show the correlation between formic acid accumulation and metabolic acidosis relates to the availability of a specific and sensitive analysis for formic acid in the blood which was not available at the time that Potts and his colleagues did their work. Formaldehyde measurements were performed and were found to be negative in all samples tested thus far.

Cooper and Felig (8) were unable to repeat and verify the experiments of Potts *et al.* (5,6). Although their work showed a possible increase in urinary organic acid excretion, they concluded that the monkey may lie between lower species and man in the sensitivity to methanol. We have noticed that certain possible differences exist between experiments performed in this report and those of previous reports (8,9). These may, in part, explain the development of metabolic acidosis in our monkeys. In the current work, care was taken to measure arterial blood gases, electrolytes, formic acid, and blood pH throughout the course of the experiments. Another difference was that the rate of conversion of [^{14}C]methanol to $^{14}CO_2$ was followed. In previous experiments, the animal was allowed to remain relatively unrestrained over the course of the study (8,9). In the current work, the nature of the experimental design required severe restraint of the animal in order to allow for easy access to arterial blood, urine, feces, and pulmonary expired air. It is conceivable that the prolonged restraint required by the experimental design, in part, led to the evolution of metabolic acidosis and death in these animals. That restraint alone was not responsible for the production of metabolic acidosis and death was seen in experiments where animals were carried through the course of the study without the administration of methanol, or in experiments where 4-methylpyrazole was administered 15 min before the administration of methanol. In animals that received 4-methylpyrazole, metabolic acidosis was seen only after about 36 hr, at a

time when the methylpyrazole was likely to have been eliminated through metabolic and excretory mechanisms. In another experiment, repeated injections of 4-methylpyrazole allowed for the complete recovery of the animal after the toxic syndrome had developed. Therefore, the inhibition of alcohol dehydrogenase by 4-methylpyrazole served as an internal control in that, not only did it prevent the onset of metabolic acidosis during those time periods where control animals displayed metabolic acidosis, but also these animals were susceptible to metabolic acidosis once the 4-methylpyrazole effect had dissipated.

The role of 4-methylpyrazole in blocking methanol metabolism to CO_2, preventing the accumulation of formic acid, and delaying the onset of metabolic acidosis is probably due to its effectiveness in inhibiting alcohol dehydrogenase in the monkey. Makar and Tephly (10) have shown that hepatic alcohol dehydrogenase activity in the monkey is inhibited by 4-methylpyrazole *in vitro* and that it is a more effective inhibitor than pyrazole in *in vitro* studies. It is probably a more appropriate substance to be employed *in vivo* since pyrazole has displayed toxicity in monkeys (14), and Blomstrand and Theorell have shown that 4-methylpyrazole administered to man is useful in inhibiting ethanol metabolism (19). Also, 4-methylpyrazole does not inhibit catalase activity *in vitro* or *in vivo* as does pyrazole (20). However, more work must be done before 4-methylpyrazole can be assumed safe for use in the treatment of methanol poisoning in man. Its effectiveness in the monkey is currently under study.

More studies must be done before a definitive statement can be made concerning the visual toxicity of methanol in the monkey. It must be recalled that an important characteristic of methanol poisoning in man is its effect on the eye. Amaurosis leading to eventual blindness, with or without recovery, has been observed by others (2,3); and Potts and his coworkers have reported the production of retinal edema in the monkey after the administration of methanol (5,6). At this time, it can be stated that there is no certain production of retinal edema within the first 24 hr after the administration of methanol in the animals used in the present report. However, more work must be done using more quantitative and sophisticated techniques before this can be said with certainty and before the effect of methanol on the visual system of the monkey can be discussed. These studies are also currently underway and will be the subject of another report.

SUMMARY

The administration of methanol (3 g/kg) to rhesus and pigtail monkeys produced signs and symptoms similar to those described for methanol poisoning in man. These were a mild central nervous system depression, a latent period of 8–12 hr when no signs were observed, followed by a severe metabolic acidosis leading to coma and death 12–33 hr after the initial administration. The gradual development of metabolic acidosis coincided with the accumulation of formic acid in the blood, and the decrease of bicarbonate in the plasma. There was an increase in the anion gap during the period of metabolic acidosis, and formic acid concentration accounted for about one-half of the increase observed. Therefore, formic acid was a major, but not the only, determinant of the metabolic acidosis. Administration of 4-methylpyrazole (50 mg/kg), a potent inhibitor of monkey hepatic alcohol dehydrogenase, produced a 75% inhibition of the rate of [^{14}C]methanol metabolism to $^{14}CO_2$ in the monkey. During the first 36 hr following the administration of 4-methylpyrazole and methanol, no metabolic acidosis developed, no formate accumulated in the blood, and no signs of toxicity were observed. After a single dose of 4-methylpyrazole and methanol, the toxic syndrome was delayed by about 36 hr in the monkey, after which time the onset of metabolic acidosis and the accumulation of formic in blood was noted. The use of the monkey as a model for the study of methanol poisoning is presented, and the possible use of 4-methylpyrazole in the treatment of methanol poisoning is implicit.

REFERENCES

1. Röe, O., *Pharmacol. Rev.* **7**, 399 (1959).
2. Röe, O., *Acta Med. Scand.* **126**, (*Suppl. 182*) (1946).
3. Bennett, I. L., Cary, F. H., Mitchell, G. L., and Cooper, M. N., *Medicine* **32**, 431 (1953).
4. Reed, T. B., and Lerner, R. M., *Science* **182**, 1299 (1973).
5. Gilger, A. P. and Potts, A. M., *Amer. J. Ophthalmol.* **39**, 63 (1955).
6. Potts, A. M., Praglin, J., Farkas, I., Orbison, L., and Chickering, D., *Amer. J. Ophthalmol.* **40**, 76 (1955).
7. Gilger, A. P., Potts, A. M., and Farkas, I. S., *Amer. J. Ophthalmol.* **42**, 244 (1956).
8. Cooper, J. R. and Felig, F., *Toxicol. Appl. Pharmacol.* **3**, 202 (1961).
9. Makar, A. B., Tephly, T. R., and Mannering, G. J., *Molec. Pharmacol.* **4**, 471 (1968).
10. Makar, A. B., and Tephly, T. R., *Biochem. Med.* **13**, 334 (1975).
11. Makar, A. B., McMartin, K. E., Palese, M., and Tephly, T. R., *Biochem. Med.* (1975). (In press.)
12. Tephly, T. R., Parks, R. E., Jr., and Manning, G. J., *J. Pharmacol. Exp. Ther.* **143**, 292 (1964).
13. Baker, R. N., Alenty, A. L., and Zack, J. R., Jr., *J. Chromatogr. Sci.* **7**, 312 (1969).
14. Watkins, W. D., Goodman, J. I., and Tephly, T. R., *Molec. Pharmacol.* **6**, 567 (1970).
15. Magnusson, G., Nyberg, J. -A., Bodin, N. -O., and Hansson, E., *Experientia* **28**, 1198 (1972).
16. Dahlbom, R., Tolf, B. R., Akeson, A., Lundquist, G., and Theorell, H., *Biochem. Biophys. Res. Commun.* **57**, 549 (1974).
17. Theorell, H., Yonetani, T., and Sjoberg, B., *Acta Chem. Scand.* **23**, 255 (1969).
18. Koivusalo, M., *in* "International Encyclopedia of Pharmacology and Therapeutics", Sec. 20, Vol. 2. Alcohols and Derivatives, 465 (1970).
19. Blomstrand, R., and Theorell, H., *Life Sci.* **9**, 631 (1970).
20. Lieber, C. S., Rubin, E., De Carli, L. M., Misra, P., and Gang, H., *Lab. Invest.* **22**, 615 (1970).