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# LACK OF A ROLE FOR FORMALDEHYDE IN METHANOL POISONING IN THE MONKEY

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**Abstract**—Methanol was administered either to untreated cynomolgus monkeys or to a folate-deficient cynomolgus monkey which exhibits exceptional sensitivity to the toxic effects of methanol. Marked formic acid accumulation in the blood and in body fluids and tissues was observed. No formaldehyde accumulation was observed in the blood and no formaldehyde was detected in the urine, cerebrospinal fluid, vitreous humor, liver, kidney, optic nerve, and brain in these monkeys at a time when marked metabolic acidosis and other characteristics of methanol poisoning were observed. Following intravenous infusion into the monkey, formaldehyde was rapidly eliminated from the blood with a half-life of about 1.5 min and formic acid levels promptly increased in the blood. Since formic acid accumulation accounted for the metabolic acidosis and since ocular toxicity essentially identical to that produced in methanol poisoning has been described after formate treatment, the predominant role of formic acid as the major metabolic agent for methanol toxicity is certified. Also, results suggest that formaldehyde is not a major factor in the toxic syndrome produced by methanol in the monkey.

Methanol poisoning in humans is characterized by a latent period of 12 to 24 hr followed by metabolic acidosis, ocular toxicity, coma and death. It is now generally accepted that the toxicity of methanol is due to the formation of toxic metabolites [1, 2], either formaldehyde or formic acid. Since both formaldehyde and formic acid are more toxic to animals than is methanol [3], each has been championed as the toxic metabolite in methanol poisoning [4–7]. However, only recently has it been possible to identify the toxic agent responsible for certain of the effects observed of methanol poisoning in a species susceptible to the agent. Much of the problem was due to a species difference in susceptibility towards methanol and the use of inappropriate animal models, a feature that has not always been considered in toxicological studies. The nonprimate species have often been used for the elucidation of the mechanism of toxicity [8], with no evidence of methanol poisoning and no accumulation of toxic metabolites in these species [9].

Although initial studies of methanol toxicity in the monkey produced conflicting results [5, 10], recent work carried out by our laboratory [11–15] and by Clay *et al.* [16] has established that various strains and species of monkeys could be used as an experimental model of methanol intoxication in the human. After administration of sufficient doses of methanol to the monkey, a syndrome was produced characterized by a latent period of 8 to 12 hr, followed by the development of metabolic acidosis, ocular toxicity, coma and death [11–14].

Since the monkey appears to be the most suitable model for methanol intoxication the present studies were designed to assess the role of formaldehyde in methanol poisoning in the monkey. Formaldehyde accumulation after methanol administration was also studied in the folate-deficient monkey, a model that appears to be extremely sensitive to methanol [15]. The respective and quantitative roles of formic acid and formaldehyde in the pathogenesis of methanol toxicity are also discussed in light of previous work which has shown the key role of formic acid accumulation in the production of metabolic acidosis [11, 12] and ocular toxicity [17].

## MATERIALS AND METHODS

### *Materials*

Metabolism chambers and small primate restraining chairs were purchased from Plas-Labs, Lansing, MI. 4,5-Dihydroxy-2,7-naphthalenedisulfonic acid disodium salt (chromatropic acid) and 5,5-dimethyl-1,3-cyclohexanedione (dimedon) were acquired from Eastman Organic Chemicals. [$^{14}$C]methanol (2–5 mCi/mmol) and [$^{14}$C]formaldehyde (10 mCi/mmol) were purchased from New England Nuclear. Preblend 3a70B scintillation cocktail was obtained from Research Products International. All other reagents employed in these investigations were of the highest available purity.

### *Methods*

*Formaldehyde accumulation in methanol poisoned monkeys.* Young male and female cynomologus (*Macaca fascicularis*) monkeys (2–3.5 kg) maintained on either a control or a folate-deficient diet [15] were employed. Folate deficiency was ascertained by measuring formiminoglutamate concentrations in the urine and by hepatic folate levels as described by McMartin *et al.* [15]. The monkeys were prepared for experimen-

tation as previously described [11]. Methanol was administered via a nasogastric tube as a 20% (w/v) solution. When the dimedon assay for formaldehyde was to be employed, a 20% solution of [$^{14}$C]methanol (1300 dpm/$\mu$mol of methanol) was administered.

During the development of the toxic syndrome, arterial blood samples and urine samples were obtained and prepared for formate analysis as previously described [11]. Analysis of body fluid and tissue samples for formate concentrations were performed by the method of Maker *et al.* [18]. Blood samples were immediately analyzed for blood gases and blood pH using a digital blood gas analyzer (IL Model 713). Plasma bicarbonate values were calculated from the arterial pH and $pCO_2$ values. Blood samples (2 ml) for formaldehyde analysis via the chromatropic acid method were drawn directly into syringes containing 0.1 ml of a solution of semicarbazide (167 mg/ml). Aliquots of urine (2 ml) were mixed with 0.1 ml of the semicarbazide solution. This procedure produced a final concentration of 75 $\mu$mol/ml of semicarbazide in body fluid samples. Blood samples (0.5 ml) were taken for formaldehyde analysis by the dimedon method by aspiration into heparinized syringes and immediately transferring this mixture to tubes containing 25 $\mu$l of 1.5 M semicarbazide solution in order to obtain a final concentration of 75 $\mu$mol/ml. After mixing, 15 $\mu$l of a 37% solution of formaldehyde were added as a carrier, and samples were again mixed. Aliquots (0.5 ml) of urine were prepared in a similar manner. Blood and urine samples were deproteinized by the $ZnSO_4$–NaOH method described previously [11]. Recovery of added [$^{14}$C]formaldehyde by this procedure was 73 per cent and 80 per cent for blood and urine samples, respectively.

At a time when the signs and symptoms of methanol poisoning were apparent, the monkeys were anesthetized with Sernylan and pentobarbital [12, 13]. Cerebrospinal fluid samples were collected by suboccipital puncture directly into syringes and prepared for formate or formaldehyde analysis by methods described above. The abdomen was exposed and samples of liver were removed either by freeze-clamping at liquid nitrogen temperatures or by surgical excision. Freeze-clamped samples were kept at −80° until analyzed. Surgically excised samples were immediately placed either in a 0.2 M potassium phosphate buffer, pH 6.0, for formate analysis or in a 1.15% solution of KCl containing 11.15 mg/ml of semicarbazide for formaldehyde analysis and then stored at 4° until homogenized. Following sacrifice of the animal with intravenous administration of KCl, the vitreous humor was immediately removed from the eye by syringe and prepared for formate and formaldehyde analysis by methods described for blood. The remainder of the eye and the posterior portion of the optic nerve were rapidly removed; the anterior optic nerve and optic disc were removed, combined with the posterior optic nerve, and saved in either phosphate buffer or KCl-semicarbazide at 4° until homogenized. The kidneys and the brain were rapidly removed, the brain was divided into cerebrum and midbrain and samples were stored in phosphate buffer or KCl-semicarbazide at 4° until homogenized. Homogenates (25%, w/v) were prepared from the tissues stored in phosphate buffer and aliquots (0.5 ml) were deproteinized for formate analysis in a similar manner as described for blood samples. Homogenates (25%, w/v) were prepared from the tissues stored in KCl-semicarbazide. Homogenates were used directly in the chromatropic acid method. Aliquots (0.5 ml) of homogenates were added to tubes containing 15 $\mu$l of 37 per cent formaldehyde carrier when the dimedon method was employed. The samples were mixed and then deproteinized by the $ZnSO_4$–NaOH method [11]. Recovery of added [$^{14}$C]formaldehyde by this procedure was approximately 85 per cent.

Homogenates (25%, w/v) were also prepared from the freeze-clamped liver samples in either potassium phosphate (for formate analysis) or in KCl-semicarbazide (for formaldehyde analysis). All operations were carried out in a cold room at 4°. Freeze-clamped samples were crushed with a mortar and pestle precooled with liquid nitrogen. Samples were then rapidly weighed, with balance pan precooled with liquid nitrogen. Samples were added to the homogenizer and homogenates immediately prepared for either formaldehyde or formate measurement as described above.

*Formaldehyde metabolism in the monkey.* For these experiments, the monkeys were prepared as described previously [11] except that both the femoral vein and femoral artery in the same leg were cannulated. A 0.2 M solution of formaldehyde was freshly prepared by dissolving 0.6 g of paraformaldehyde in 100 ml of 0.9% saline. This was used to prepare [$^{14}$C]formaldehyde solutions of specific activity 1500 dpm/$\mu$mol and 115,000 dpm/$\mu$mol. At zero time, [$^{14}$C]formaldehyde was slowly infused intravenously in a dose of 1 mmol/kg over a 3–4 min period. During the infusion and at various times afterwards, arterial blood samples were withdrawn and prepared for pH and $pCO_2$, for formate analysis [18] and for formaldehyde evaluation by the dimedon method.

*Formaldehyde assay by the chromatropic acid method.* The method of MacFadyen [19] was used to measure formaldehyde levels in certain tissue samples. Two ml of blood or other body fluid, tissue homogenate, or standard were added to 4 ml of a 30% solution of trichloroacetic acid and then distilled. To 4 ml of the distillate or to 4 ml of a standard solution, 0.2 ml of a 0.5% solution of chromatropic acid were added. Then, while the solution was kept at 4° in ice, 4 ml of concentrated sulfuric acid were slowly added from a burette. After mixing, the solution was heated in a boiling water bath for 30 min, cooled to room temperature and diluted to 10 ml with water. The absorbance was read against a reagent blank. The formaldehyde recovery by this procedure was about 50 per cent.

*Formaldehyde assay by the dimedon method.* Dimedon has been used to quantitatively isolate formaldehyde by gravimetric [20] or by radiometric methods [21]; a modification of these methods was used to measure formaldehyde levels in various samples. Aliquots (0.5 ml) of a formaldehyde solution or of deproteinized supernatant of blood or other body fluid, tissue homogenate, or standard were added to one ml of 1 M sodium acetate, pH 4.5. Then, 10 ml of a 0.5% dimedon solution were added, the solution was heated in a boiling water bath for 5 min and then cooled in an ice bath for about an hour. The solution was filtered using a millipore apparatus with Whatman No. 2 paper and the filter paper washed twice with ice-cold water. The filter paper was placed in a scintillation vial and the millipore filter chimney was rinsed with acetone into the vial.

After evaporation of the acetone, 10 ml of Preblend 3a80B scintillation cocktail was added and the samples were analysed for radioactivity using a Packard Tri-Carb liquid scintillation spectrometer. Recovery of [$^{14}$C]formaldehyde standard was about 55 per cent by this procedure.

## RESULTS

Previous studies [2, 22] on the accumulation of formaldehyde following methanol treatment were conducted using rabbits, a species which is not sensitive to methanol poisoning [5]. No appreciable concentration of formaldehyde was found. The cynomolgus monkey is sensitive to methanol poisoning and the folate-deficient cynomolgus monkey is markedly susceptible to this agent [15]. Results in Fig. 1 show that no formaldehyde accumulated in the blood of either a methanol treated control monkey (3 g/kg methanol) or a methanol treated, folate-deficient monkey (2 g/kg methanol) during the time when a marked acidosis was observed. At 12 hr after methanol administration in the folate-deficient monkey (A) and at 18 hr in the control monkey (B) the arterial bicarbonate levels were 8.8 mequiv/l and 8.4 mequiv/l respectively. The formate levels in the blood were elevated but the formate levels in the folate-deficient monkey were about twice those seen in the control monkey.

When the marked metabolic acidosis was present and the monkeys were showing characteristics of methanol poisoning (optic disc edema, vomiting) blood fluid samples were collected. Then, the animals were killed and body tissue samples were taken as described in Methods. No formaldehyde was detected in the cerebrospinal fluid, vitreous humor and urine of these monkeys (Table 1), where the detectability limits for formaldehyde levels as determined by the chromatropic acid method and dimedon method were about 25 and 200 $\mu$M, respectively. The formate level in the urine is about 10-fold higher than that in the blood, indicating a concentration of formate by the kidneys. In the vitreous humor, the formate concentrations were markedly elevated, although they were somewhat less than the blood. The formate level in the cerebrospinal fluid was measured in one monkey and found to be about one half the blood level. Other studies in methanol-poisoned

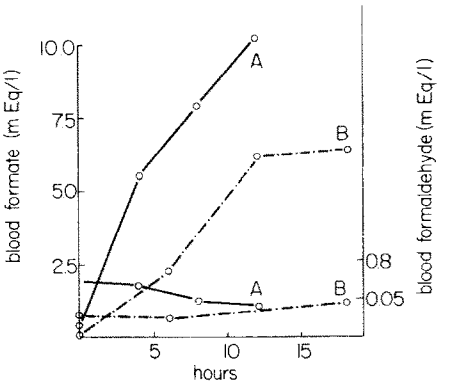


Fig. 1. Formaldehyde and formate blood levels in methanol-poisoned monkeys. Formaldehyde blood level was determined by the chromatropic acid assay described in Methods. Methanol was administered orally as a 20% (w/v) solution in a dose of 2 g/kg to monkey A (folate-deficient) and in a dose of 3 g/kg to monkey B (control). The upper lines refer to the blood formate concentrations and the lower lines represent formaldehyde blood levels.

Table 1. Levels of formaldehyde and formate in the body fluids and tissues of methanol poisoned monkeys

| Sample** | Monkey A* | | Monkey B | | Monkey C | |
|---|---|---|---|---|---|---|
| | Formate | Formaldehyde | Formate | Formaldehyde | Formate | Formaldehyde |
| Zero-time Blood | 0.18 | 0.068 | 0 | 0.027 | 0.27 | ND† |
| Blood—18 hour | 10.20 | 0.038 | 6.40 | 0.045 | 10.48 | ND† |
| Vitreous Humor | 7.90 | ND† | 3.12 | ND† | 4.01 | ND† |
| Cerebrospinal fluid | — | ND† | — | ND† | 4.96 | ND† |
| Urine | 115.80 | ND† | 87.80 | ND† | 163.10 | ND† |
| Liver (surgical specimen) | — | ND† | 6.10 | ND† | 1.80 | ND† |
| Liver (freeze-clamped)‡ | — | ND† | 11.86 | ND† | 6.45 | ND† |
| Kidney | — | ND† | 6.33 | ND† | 0.44 | ND† |
| Optic Nerve | — | — | 3.14 | ND† | — | ND† |
| Cerebrum | — | ND† | 4.01 | ND† | 2.04 | ND† |
| Midbrain | — | 0.14 | 2.16 | ND† | 1.02 | ND† |

* Methanol (2 g/kg) was administered orally as a 20% (w/v) solution at zero time to monkey A (folate-deficient). At 12 hr, samples were collected as described in Methods. Methanol (3 g/kg) was administered orally as a 20% (w/v) solution at zero time to monkeys B and C (controls). At 18 hr, samples were obtained as described in Methods. Formaldehyde levels in body tissues were determined by chromatropic acid method for samples from monkeys A and B and by dimedon method for samples from monkey C.

** Values represent mEq/l for formate concentrations in body fluids, mmol/l for formaldehyde in body fluids and $\mu$mol/g of wet weight of tissue for formate or formaldehyde where body tissues are indicated.

† Detectability limit for formaldehyde concentrations by chromatropic acid method was 0.025 mmol/l. Detectability limit for formaldehyde concentrations by dimedon method was 0.200 mmol/l for body fluids and 0.80 $\mu$mol/g of wet weight tissue.

‡ Liver samples were obtained by freeze-clamping as described in Methods.

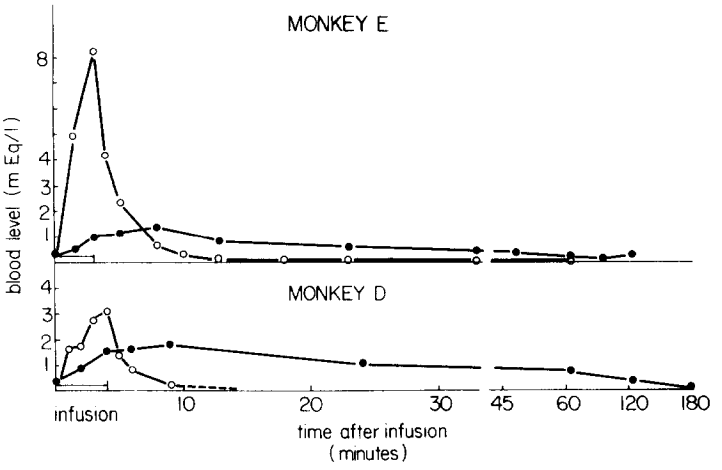


Fig. 2. Formate and formaldehyde blood levels in the monkey following formaldehyde infusion. ○---○ Formaldehyde blood level. ●——● formate blood level. Formaldehyde blood levels were determined by the dimedon assay as described in Methods. [$^{14}$C]-formaldehyde (1 mmol/kg) was infused intravenously in untreated monkeys as a 0.2 M solution in 0.9% saline over the time period indicated by the open bars, specific activity used for monkey D was 1500 dpm/μmol and for monkey E was 115,000 dpm/μmol.

monkeys showed that formate levels in the cerebrospinal fluid were the same or somewhat higher than those detected in the blood [12].

Essentially no formaldehyde was detectable (Table 1) in the following tissues of methanol-poisoned monkeys: liver, kidney, optic nerve, cerebrum, and midbrain, where the detectable limits for formaldehyde concentration as determined by the chromatropic acid and dimedon methods were 0.09 and 0.80 μmol/g of wet weight tissue, respectively. Furthermore, in samples of liver obtained from the control monkeys (B and C) by freeze-clamping techniques, no formaldehyde could be detected. The formate levels in the freeze-clamped liver samples were at least twice the level measured in the samples which were taken by surgical excision. These results indicate that considerable disappearance of formate may have occurred in the latter samples before they were deproteinized and prepared for assay. In the presence of high formate values and definitive evidence of toxicity in methanol-poisoned monkeys, no measurable formaldehyde was found in the body tissues that were tested. Makar and Tephly [23] also were unable to detect formaldehyde in the presence of high formate concentrations in methanol-poisoned, folate-deficient rats.

In order to verify that our methods allowed for the detection of formaldehyde when it was present in the blood of the monkey and to study the kinetics of disappearance from the blood [$^{14}$C]formaldehyde (1 μmol/kg) was infused into the femoral vein of a monkey and blood samples were obtained from the femoral artery on the same side. Results (Fig. 2) show that the dimedon method was useful for detecting formaldehyde in the blood. In monkey D, when the specific activity of [$^{14}$C]formaldehyde was about the same as that of the [$^{14}$C]methanol used in monkey C, formaldehyde was detected for about five minutes after the infusion was completed (detectability limit about 160 μM). However, when a [$^{14}$C]formaldehyde solution of a 100-fold higher specific activity was infused into monkey E, formaldehyde could be detected (over 5 μM) for at least 60 min. The half-life for formaldehyde elimination from the blood in these two experiments was approximately 1.5 min, a value similar to that reported for dogs, cats, guinea pigs and rats [24, 25]. No significant decline in arterial bicarbonate levels was observed in either monkey, probably because the blood formic acid concentrations did not reach a level which altered acid–base balance significantly. A short vomiting episode was observed in monkey E about three minutes after the infusion of formaldehyde was completed; otherwise, no toxic effects at this dose of formaldehyde were noted in either monkey.

## DISCUSSION

Formaldehyde has often been regarded as the toxic agent in methanol poisoning [6, 26, 27] even though there has been no evidence for the accumulation of formaldehyde in the intact organism after methanol administration. Keeser [22] reported, using qualitative tests, the presence of formaldehyde in the cerebrospinal fluid, vitreous humour and peritoneal fluids of rabbits which had been given methanol. However, the data were incomplete, the methods lacked sensitivity and specificity, and the rabbit does not exhibit signs of methanol poisoning. In other studies no formaldehyde could be detected in blood, urine, or tissues obtained from methanol intoxicated non-primates [2] or humans [4, 28]. In this report, the cynomolgus monkey, which is an appropriate model for human methanol intoxication [5, 11–16] was used and no formaldehyde accumulation was observed in either body fluids or tissues following methanol administration. Indeed, no formaldehyde was detected even in samples from a folate-deficient monkey which is especially susceptible to the effects of methanol [15]. Several methods were employed in the experiments and rapid collection methods and freezing were employed.

The methods of detection as employed in this work

were very sensitive and the level of sensitivity would have permitted us to detect concentrations of formaldehyde that had been shown to produce retinal toxicity *in vitro* [29].

The inability to detect formaldehyde following methanol administration was probably due to its rapid metabolism to formate in the liver [30, 31] as well as in the blood [32, 33]. Formaldehyde was rapidly eliminated from the blood of monkeys following intravenous infusion with a half-life of approximately 1.5 min. Similar results were obtained by Rietbrock [24] in dogs, cats, rabbits, guinea pigs and rats. Infusion of formaldehyde in the monkey produced rapid increases in the level of formate in the blood to a peak of about 1–2 mg/l within 5 min after the end of the infusion. 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 the formaldehyde was rapidly and almost completely metabolized to formic acid [25].

Previously, formic acid has been shown to be the major determinant of the metabolic acidosis resulting from methanol administration to the monkey [11, 16] and to the folate-deficient rat [9]. Recent studies in our laboratory [17] show conclusively that formate is capable of producing an ocular toxicity essentially identical to that produced by methanol in the monkey. No formaldehyde was detected in blood and liver samples obtained from these formate-infused monkeys [17]. Thus, whereas one can associate formate intimately with ocular toxicity in the monkey, no association of formaldehyde with ocular toxicity can be made at this time.

It is not possible to completely eliminate formaldehyde as a toxic intermediate because formaldehyde could be formed slowly within cells and interfere with normal cellular function without ever obtaining levels that were detectable in body fluids (25 $\mu$M) or tissues (90 nmol/g wet weight). However, the demonstrated role of formic acid in the metabolic acidosis of methanol poisoning [11, 16] and of the formate ion in the ocular toxicity of methanol [17] leads us to the conclusion that formate accounts for the methanol poisoning syndrome in the monkey.

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