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The Biochemistry of Methanol Poisoning

II. Metabolic Acidosis in the Monkey

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Recent investigations conducted in this laboratory (Cooper and Marchesi, 1959; Kini and Cooper, 1960) have sought to clarify the possible biochemical lesion in blindness due to methanol poisoning. Another striking manifestation of methanol poisoning, which is characteristically produced only in man, is severe metabolic acidosis. Harrop and Benedict (1920) reported a large increase in the urinary organic acids of methanol-poisoned patients. Van Slyke and Palmer (1920) titrated the organic acids in the urine of a patient who survived methanol ingestion. These workers could account for approximately 25% of the acids as lactic, formic, and acetoacetic, but the remaining 75% were unidentified.

The problem of identifying these acids appeared more amenable to solution with the report by Gilger and Potts (1955) that in monkeys the signs of methanol toxicity are identical with those seen in human subjects. Thus far, however, there has been only one report of a single experiment (Potts, 1955) in which an increased urinary excretion of titratable organic acids has been demonstrated in the primate test object.

The purpose of the present study was an attempt to identify the organic acid or acids purported to appear in increased amounts in the urine of monkeys and humans intoxicated with a dose of methanol. However, the general picture of methanol toxicity demonstrated by our animals was far different from that seen in humans.

No human material was available during the course of these studies.

[1] Taken from a thesis to be submitted by Philip Felig for the degree of Doctor of Medicine.

METHODS

Young (2–4 years of age) male and female *Rhesus macaca* monkeys in apparent good health were employed. The animals were fed their usual diet (dog biscuits, oranges, water) until the evening before the experiment, after which only water was given. A total of 12 monkeys were employed, 8 of which were re-used one to five times.

Methanol, 99.9% (Fisher Scientific Company), diluted to 20 or 30% with distilled water, was used throughout. The methanol was administered by means of a soft rubber catheter introduced orally while the jaws were held apart with a metal clamp.

Urine was collected in 24 hour specimens beginning one or more days before administration of methanol; urinary organic acids were determined by the method of Van Slyke and Palmer (1920) as modified by Potts (1955).

Serum bicarbonate was determined by the titrimetric method (Scribner and Caillouette, 1954). Blood was drawn from a femoral vein before administration of methanol and at suitable intervals thereafter and immediately centrifuged; the separated serum was kept frozen until analyses were performed.

The monkeys were observed at frequent intervals, particular note was taken of their responsiveness, spontaneous activity, equilibrium, resistance to handling, and gross response to visual stimuli. Monkeys reported below as survivors were carefully watched for 7–10 days before being removed from their individual cages.

RESULTS

Lethal Doses

Table 1 indicates that the minimal lethal dose is about 7 g/kg, while the LD_{50} lies within the range of 7–9 g/kg. This is well above the minimal

TABLE 1
SURVIVAL RATE WITH SINGLE ORAL DOSES OF METHANOL

Dosage (g/kg)	Number of experiments	Survival	
		Total	%
0.5–4	7	7	100
5	1	1	100
6	8	8	100
7	6	3	50
8	4	3	75
9	2	1	50

lethal dose of 3.0 g/kg for monkeys reported by previous investigators (Potts, 1955; Gilger *et al.*, 1956). It is also appreciably greater than the usual fatal dose in humans, among whom survival after a dose of 2.9 g/kg was considered a remarkable feat (Aronson, 1912). Moreover, our finding of no fatalities in monkeys given a dose of 6 g/kg is in sharp contrast to those of Gilger *et al.* (1959), who found 78% mortality at this dose level.

Symptomatology

The types of general clinical response may be divided into five categories:

1. Normal appearance. No symptomatic changes were observed with particular regard to those criteria outlined under Methods. This lack of obvious effects occurred in all monkeys receiving less than 5 g/kg and in a significant number receiving higher dosages.

2. Ataxia, weakness, and lethargy. These signs usually appeared about 1 or 2 hours after methanol administration and disappeared within 24 hours.

3. Transient coma. Usually about 30 minutes after administration of methanol, intoxication become evident. Progressive ataxia and weakness followed, resulting in coma at 1–4 hours. The animals were considered comatose when they became unresponsive to tactile or painful stimuli and showed no spontaneous activity. This state usually lasted from 5 to 12 hours; all animals were alert by 24 hours after poisoning.

4. Death. When methanol poisoning was fatal the picture was one of narcosis followed by death. In three cases the same response was observed as that seen in the animals of group 3, but the coma continued unabated, with death occurring at 20–30 hours. In one case, the monkey became somewhat responsive at 24–48 hours, only to die at 72 hours. This monkey, however, had received 100 ml of 4½% $NaHCO_3$ by stomach tube at 18 hours after poisoning. All the monkeys included in group 4 suffered from terminal oliguria or anuria.

5. Blindness. Blindness was considered present in an animal which did not respond to gross visual stimuli (hand moving) or had no ability to avoid obstacles placed before him in his cage. This occurred in one monkey 4 days after receiving 9 g/kg. However, the response was only transient since there appeared to be normal reaction to visual stimuli by the afternoon of the following day.

In all the monkeys with observable responses, there occurred neither deep, rapid, or irregular respiration, nor vomiting or any other manifestation of gastrointestinal disturbance. Only in one monkey was a latent period noticed between the initial signs of intoxication and those typical of imminent death. In summary, the "clinical" picture was almost always one of intoxication progressing to a narcotic state which in fatal cases was irreversible.

Acidosis

Serum bicarbonate levels with concurrent determination of urinary organic acids are reported in Table 2. Only one monkey, (monkey A) appeared to have a definite metabolic acidosis. Although the low levels

TABLE 2

EFFECT OF METHANOL ON SERUM BICARBONATE AND ON URINARY ORGANIC ACIDS

Monkey	Dose (g/kg)	Time after administration (hr)	Serum $NaHCO_3$ (mEq/liter)	Urinary organic acids (mEq/kg)
A	6.0	0	16.0	—
		18	11.3	—
		24	6.6	3.23
		42	7.3	—
		48	4.9	3.12
		66	10.7	—
B	6.0	0	9.9	—
		18	7.2	—
		24	11.9	4.85
		48	4.6	4.24
C	6.0	0	13.8	—
		24	8.8	8.70
		48	8.6	3.79

of serum bicarbonate in this animal would indicate a rather severe acidosis, characteristic Kussmaul respiration was not observed. In addition, there was no indication of an increased excretion of urinary organic acids. In the other two animals in which serum bicarbonate levels were determined, the results were equivocal.

Urinary Organic Acids

The 24-hour urinary output of organic acids (uncorrected for creatinine) was measured on fourteen occasions in normal monkeys and varied

from 0.49 to 3.94 mEq/kg, with an average value of 1.64. This is about twice the average output determined among 13 healthy young men (Van Slyke and Palmer, 1920), who were found to excrete 0.57–0.98 mEq/kg, with an average of 0.82 mEq/kg.

TABLE 3

MILLIEQUIVALENTS OF ORGANIC ACIDS IN URINE OF MONKEYS BEFORE AND AFTER METHANOL ADMINISTRATION

G/kg	Before methanol administration (mEq/kg/24 hr)	After methanol administration (mEq/kg) 10–24 Hr	24–48 Hr	48–72 Hr
0.48	1.91	0.91	—	—
0.72	1.91	0.53	—	—
1.6	1.91	0.27	—	—
2.0	0.68	1.79	—	—
3.0	0.68	2.68	—	—
3.2	1.91	2.98	—	—
4.0	1.20	1.56	—	—
5.0	1.91	2.34	—	—
6.0	1.91	3.52	—	—
6.0	1.75	4.46	—	—
6.0	1.85	2.52	3.14	1.85
6.0	0.91	2.38	2.08	—
6.0	—	2.14	—	—
6.0	—	3.23	3.12	—
6.0	—	4.85	4.24	—
6.0	—	8.70	3.79	—
7.0	1.20	1.89	—	—
7.0	1.75	0.83	2.89	—
7.0	1.85	1.27	—	—
7.0	0.91	1.19	—	—
7.0	—	3.96	5.92	—
7.0	—	3.01	—	—
8.0	1.75	4.63	4.56	4.51
8.0	—	3.62	3.82	—
8.0	—	2.06	—	—
8.0	—	2.69	—	—
9.0	1.75	8.81	5.97	0.42
9.0	—	2.26	2.78	2.88

Tables 2 and 3 illustrate the findings in treated monkeys. Regardless of the effects observed, no significant increase in excretion of organic acids occurred within 24–72 hours after the ingestion of methanol; this was true even in that monkey in which blood studies revealed a concomitant acidosis (Table 2).

The 5-fold increase in the excretion of organic acids observed in the animal which excreted 8.81 mEq, 1 day after poisoning, was insignificant when compared with the 25- to 36-fold increase found in the patient reported by Van Slyke and Palmer (1920) or with the 20- to 39-fold increase reported by Harrop and Benedict (1920). In the one report in which data on monkeys are presented, Potts (1955) described an excretion of 24.4 mEq/kg 24 hours after the administration of methanol, or almost three times as much as the highest output found in our series.

DISCUSSION

Our findings reveal that any attempt to identify the organic acids excreted in normal amounts by treated monkeys would bring us no closer to understanding which acids are responsible for the acidosis seen in humans. At first glance the output of a normal or only slightly increased amount of organic acids in the one animal shown to have a concomitant acidosis would appear paradoxical. However, this situation can be explained on the same ground as the following: in the case of citric acid, excretion varies directly with urinary pH (Ostberg, 1932). Thus, under conditions of hyperventilation or ingestion of sodium bicarbonate, citrate excretion is increased (Kuyper and Mattill, 1933). While we have no evidence to indicate that citric acid is responsible for the acidosis, those organic acids produced in our monkeys could conceivably be handled by the kidney in a manner similar to citrate, and therefore would not be excreted in increased amounts while the acidosis persisted.

With regard to the lethal dosage levels and the "clinical" response, the effects observed were highly reminiscent of those seen in lower experimental animals. Our minimal lethal dose of 7 g/kg does not differ very much from those reported in the literature for dogs and rabbits (Joffroy and Serveau, 1896; Haskell *et al.*, 1921; Gilger and Potts, 1955), and the manifestations in all the symptomatic cases were those of intoxication progressing to coma. The characteristic features of human poisoning, such as a latent period, rapid and deep breathing, vomiting, and blindness, were almost never to be found. These results would indicate that in rhesus monkeys methanol causes a general narcosis, as seen in lower animals, and this effect appears to be similar to those produced by other aliphatic alcohols.

However, since an acidosis was present and since temporary blindness and a latent period were demonstrable in isolated instances, we are probably more correct in placing the monkey in an intermediate position between lower animals and man with regard to the response to methanol. Whereas the former show only the nonspecific anesthetic effects of organic solvent narcosis, and the latter the specific ocular and acidotic effects, attributable only to methanol, the monkey may show a complex interaction of both forms of toxicity in which the resemblance to those seen in lower animals predominates.

SUMMARY

1. The urinary excretion of titratable organic acids was not increased in rhesus monkeys receiving methanol in amounts which caused observable manifestations of toxicity, including death.

2. The dose levels which cause signs of toxicity and that which could be considered as approximating the minimal lethal dose were comparable to those seen in lower animals. However, in rare instances the picture was not unlike that found in human beings. Thus, with respect to methanol poisoning, the monkey demonstrates features characteristic of both lower animals and humans.

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