Biochemical Pharmacology, Vol. 22, pp. 163–173. Pergamon Press, 1973. Printed in Great Britain.

CHANGES IN BLOOD METHANOL CONCENTRATIONS IN CHIMPANZEES DURING PERIODS OF CHRONIC ETHANOL INGESTION*

W. A. PIEPER and MARIANNE J. SKEEN

Georgia State University and Yerkes Regional Primate Research Center, Emory University, Atlanta, Ga. 30322, U.S.A.

(*Received* 29 *April* 1972; *accepted* 21 *July* 1972)

Abstract—Accumulation of blood methanol was observed in young chimpanzees (*Pan troglodytes*) during 6–14 week periods of chronic ethanol ingestion when blood ethanol levels were continually above 10–20 mg/100 ml blood. This occurred when the daily dose of ethanol was greater than that amount which could be eliminated during the 24-hr period. If these conditions were maintained, blood methanol concentrations increased for 4–5 days, then reached a plateau and remained elevated at fluctuating levels until blood ethanol concentrations decreased to less than 60–15 mg/100 ml. If ethanol concentrations continued to decline below these levels, methanol concentrations then decreased linearly at a rate which was positively correlated with the rate of elimination of blood ethanol. Accumulation of blood methanol is probably the result of competitive inhibition of methanol oxidation by ethanol. The relationship between the observed increases in elimination rates of both ethanol and methanol and the possibility of ethanol-stimulated enzymatic alterations are discussed.

ACCUMULATION of endogenously produced methanol has recently been reported to occur in human alcoholic volunteers during a 10–15 day period of chronic ethanol intake by Majchrowicz *et al.*[1,2] Blood and urine methanol levels were less than 0·1–0·2 mg/100 ml prior to drinking as compared with maximum concentrations of 2–4 mg/100 ml during periods when blood and urine ethanol levels fluctuated between 100–500 mg/100 ml. Majchrowicz *et al.*[3] have also reported the accumulation of blood methanol in four rhesus monkeys that consumed ethanol to avoid the onset of an electric shock. In each of these investigations, the authors concluded that methanol from endogenous sources accumulated as a result of competitive inhibition of alcohol dehydrogenase (alcohol:NAD oxidoreductase, EC 1.1.1.1) by ethanol. Additional evidence for the presence of endogenous methanol has also been provided by Eriksen and Kulkarni.[4] Using gas chromatography and samples of expired air from normal, non-drinking human subjects, they were able to detect and measure methanol in concentrations ranging from 0·06 to 0·49 μg/l. Although the source of this endogenous methanol is uncertain, an enzyme system capable of producing methanol from *S*-adenosylmethionine has been isolated from pituitary glands of several mammalian species by Axelrod and Daly.[5] Von Wartburg *et al.*[6] and Kini and Cooper[7] have demonstrated that alcohol dehydrogenase (ADH) isolated from

* Supported by PHS Grants MH-12090 and RR-00165 from N.I.H. In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care" as set forth by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences, National Research Council. Animals were maintained in animal care facilities fully accredited by the American Association for Accreditation of Laboratory Animal Care.

man and rhesus monkeys, respectively, catalyzes the oxidation of methanol as well as ethanol and that ethanol competitively inhibits the oxidation of methanol. Thus during periods of chronic ethanol intake, the relatively high concentrations of blood ethanol might inhibit the oxidation of methanol which is being produced either by the pituitary enzyme described by Axelrod and Daly[5] or by some other as yet unidentified enzyme system. This inhibition would result in the observed accumulation of blood methanol and would be consistent with the observation of Majchrowicz and Mendelson[1] that methanol which had accumulated in the blood of human subjects in the presence of high levels of ethanol disappeared from the blood only after ethanol levels decreased to 70–20 mg/100 ml blood.

Although it is generally thought that the physiological oxidation of ethanol is mediated primarily by ADH,[8] it has also been demonstrated[9] that catalase (H_2O_2: H_2O_2 oxidoreductase EC 1.11.1.6) is able to convert both ethanol and methanol to their corresponding aldehydes via a peroxidative pathway if H_2O_2 is available. Furthermore, Tephly *et al.*[10] have shown that ethanol competitively inhibits methanol oxidation in a purified catalase–glucose oxidase system. The relative importance of ADH and catalase in the metabolism of methanol *in vivo* has been a matter of controversy, although species differences demonstrated by Makar *et al.*[11] have provided an explanation for much of the disagreement. These authors showed that ADH is primarily responsible for methanol metabolism in the rhesus monkey, whereas catalase is quantitatively more important in the rat.[12] In addition to ADH and catalase, a hepatic microsomal-oxidizing system has been described[13,14] which is capable of catalyzing the conversion of both methanol and ethanol to their corresponding aldehydes. Orme-Johnson and Ziegler[13] also found that ethanol inhibited methanol oxidation by microsomal preparations isolated from liver homogenates obtained from rats, rabbits and pigs. Some evidence suggests, however, that the observed microsomal oxidation is due primarily to catalase activity.[15–17] Additionally, recent reports indicate that the microsomal-oxidizing system is not quantitatively important in the oxidation of ethanol *in vivo*.[18,19] Nevertheless, it is possible that enzyme systems other than ADH, such as catalase and the hepatic microsomal-oxidizing system, may be involved in the observed accumulation of blood methanol during periods of chronic ethanol ingestion, even though these enzymes may quantitatively play only a minor role in the oxidation of ethanol in the non-adapted animal.

During periods of chronic ethanol ingestion, increases in ethanol elimination rates have been reported for both man[20,21] and experimental animals.[22,24] Although increases in ADH activity have been demonstrated,[24,25] it has been suggested that increases in enzyme systems other than ADH might be responsible for these changes. Trémolières and Carré[26] found that ethanol was catabolized by blood plasma obtained from chronic alcoholics but not by plasma obtained from either normal subjects or abstinent alcoholics. They postulated that this difference was due to an ethanol-stimulated increase in the peroxidative activity of catalase coupled with xanthine oxidase. Additionally, Lieber and DeCarli[14] found adaptive increases in the microsomal-oxidizing system of the rat during periods of chronic ethanol ingestion, although Mezey[27] recently showed that increased ethanol disappearance rates returned to normal after withdrawal more quickly than did the microsomal-oxidizing activity and concluded that the two events were not functionally related. Thus changes may occur

in one or more of the enzyme systems which are capable of oxidizing methanol as well as ethanol during periods when methanol has been observed to accumulate in the blood. The effects of these potential changes upon the elimination rate of blood methanol have not previously been investigated.

If, as suggested, common biochemical mechanisms are responsible for the metabolic fate of both ethanol and methanol, then (1) the amount of methanol which accumulates should be related to the degree of inhibition and thus to the concentrations of blood ethanol which are present, (2) the rate of accumulation of blood methanol should be related to the general rate of increase in daily blood ethanol levels during the period when blood methanol is accumulating and (3) if blood ethanol disappearance rates increase during periods of chronic ethanol ingestion, then similar increases in blood methanol elimination rates might be predicted. The purpose of this study was to investigate these relationships in a group of seven young chimpanzees in which blood methanol accumulation was observed during 6–14 week periods of chronic ethanol ingestion.

METHODS

The subjects were three male and four female chimpanzees (*Pan troglodytes*) ranging in age from 1 to 30 months. Ethanol mixed with a liquid diet was offered to these animals at regularly scheduled feeding times (4–5 times daily). Details concerning the nature of the liquid diet and the procedure used to induce oral acceptance of pharmacologically significant ethanol doses have been reported elsewhere.[23,28] Briefly, ethanol in initial concentrations of 1–2% (w/v) was mixed with a high protein (19 per cent of total calories) liquid diet and offered to the animals 4–5 times daily. Over a period of 3–6 weeks ethanol concentrations were gradually increased to dose levels of 6–8 g/kg/day (approx. 10% w/v).

Blood ethanol and methanol levels were analyzed using 50-μl capillary blood samples obtained from the heels of the subjects. These samples were mixed with 50 μl of 5% $ZnSO_4$, 50 μl of 0·3 N $Ba(OH)_2$ and 50 μl of 0·4% isopropanol, and then centrifuged for 2 min in a Beckman microfuge. One-μl samples of supernatant were analyzed using the gas chromatographic procedure described by Roach and Creaven.[29] Their procedure was modified slightly in that samples were injected onto a 6 ft $\frac{1}{8}$ in. o.d. stainless steel column packed with Porapak Q 80–100 mesh which was housed in a Varian Aerograph model 1740 gas chromatograph with a hydrogen flame ionization detector. Nitrogen was used as the carrier gas. Operating temperatures were as follows: column 145°, injector 160° and detector 165°. Peak height of methanol was obtained directly, while peak areas of ethanol and isopropanol were evaluated with a Disc integrator. Blood ethanol and methanol levels were quantified using a ratio technique with isopropanol serving as the internal standard. Since this procedure required only 50-μl samples of blood, serial determinations were accomplished with minimal stress to the animals.

Methanol was identified under these analytical conditions by determining that aqueous solutions of reagent grade methanol had a retention time identical to the retention time of the peak in the biological samples. This identification was independently verified in another laboratory* in which the specificity of the gas chromatographic analysis of methanol had been confirmed chemically using a chromotropic

* We thank Dr. E. Majchrowicz for his assistance and cooperation.

acid procedure.[1] Gas chromatographic identification of methanol has previously been reported by Eriksen and Kulkarni,[4] Baker *et al.*[30] and Majchrowicz and Mendelson.[1] A standard reference curve for the quantification of methanol was developed by sampling seven different concentrations of methanol ranging from 0·2 to 1·4 mg/100 ml of water. Ratios of methanol peak height to isopropanol peak areas were calculated and the relationship between these ratios and the corresponding concentrations of methanol was calculated by the method of least squares to obtain a standard equation. The linearity of the analysis and the degree of precision with which blood methanol levels were determined using this procedure are indicated by the coefficients of determination[31] obtained for the initial equation ($r^2 = 0{\cdot}987$) and for two subsequent replications ($r^2 = 0{\cdot}990$, $r^2 = 0{\cdot}982$). Values were accurate to within ± 6 per cent and recovery of methanol added to blood was approximately 95 per cent. The U.S.P. reagent grade absolute ethanol used in this study was analyzed under the gas chromatographic conditions described above. No methanol contamination was detectable under these conditions, indicating that no more than 0·05 mg methanol/ml was present. Majchrowicz and Mendelson[1] report methanol contamination of approximately 0·001 mg/ml in 50% U.S.P. grain ethanol.

RESULTS

Methanol began to accumulate in the blood of the chimpanzees when the amount of ethanol accepted by these animals had increased to a level at which the total daily dose could not be eliminated from the blood during a 24-hr period. This condition was determined by obtaining blood samples for ethanol and methanol analysis between 8:00 and 8:30 a.m. each morning (before the first morning feeding). At this time blood ethanol concentrations were at their daily minimums, since the longest interval between regularly scheduled feeding times occurred between the night and morning feedings (9–10 hr). Ethanol concentrations greater than 10–20 mg/100 ml blood were present in the morning sample when ethanol dose levels in excess of 5–7 g/kg/day were maintained. Ethanol disappearance rates were determined frequently for each animal and provided further evidence that the administered ethanol doses could not be totally eliminated in a 24-hr period. During these periods when blood ethanol concentrations were continually in excess of 10–20 mg/100 ml, methanol began to accumulate in the blood and increasing concentrations of methanol were found in morning blood samples on successive days (e.g. see Fig. 1). Once it accumulated, methanol remained elevated at fluctuating levels until blood ethanol concentrations fell below values which ranged from 60 to 15 mg/100 ml in the different animals. If ethanol concentrations continued to decline below these levels, however, then methanol decreased as a linear function of time.

Accumulation of blood methanol. By obtaining blood samples from the chimpanzees each morning, the daily changes in blood ethanol and methanol levels were monitored and the relationship between the concentrations of these two alcohols was examined. Blood ethanol levels fluctuated considerably during a 24-hr period, increasing after the administration of each dose and decreasing as ethanol was metabolized. When the amount of ethanol ingested exceeded the amount which could be eliminated in a 24-hr period, the excess was present in the morning blood sample. Since the dose for the following day was then added to this excess ethanol remaining

from the previous day's dose, morning blood ethanol levels generally tended to increase on subsequent days if the dose level was either maintained or increased.

Morning blood methanol levels tended to increase gradually as a linear function for periods of 4–5 days before reaching a plateau if: (1) blood ethanol levels were generally increasing during this period and (2) blood methanol levels were relatively low because methanol had not yet accumulated or because methanol levels had declined from a previously established plateau. A wide range of methanol build-up rates was observed in these chimpanzees (0·10–0·27 mg/100 ml/day, with r^2 values ranging from 0·90 to 0·99). It appeared that blood methanol tended to accumulate at a greater rate when morning blood ethanol levels were rapidly increasing, suggesting a relationship between methanol accumulation rate and the rate of increase in morning blood ethanol levels. In order to evaluate the relationship between these two rates, the slope of the straight line which best fitted the blood ethanol concentrations obtained on successive mornings during the 4–5 days of linear methanol accumulation was considered the most reasonable estimate of the rate of change in ethanol levels, even though these increases in morning blood ethanol levels were not always significant linear functions (r^2 values ranged from 0·049 to 0·914). Using these values, the correlation between methanol accumulation rate and the corresponding rate of increase in morning blood ethanol concentration was statistically reliable ($r = +0{\cdot}875$, $P < 0{\cdot}001$). These data as well as additional information concerning changes in blood ethanol and methanol levels during periods of linear methanol accumulation are presented in Table 1. Although the relationship between rates of increase in morning blood ethanol and methanol levels was statistically reliable, the relationship between mean blood ethanol levels during this period and methanol accumulation rates was not statistically reliable ($r = +0{\cdot}495$). Thus it appears that the rate at which blood methanol accumulated was primarily related to the rate of change in morning blood ethanol level rather than to the average amount of ethanol present in the blood. However, the relationship between the concentration of methanol which accumulated

TABLE 1. CHANGES IN BLOOD ETHANOL AND METHANOL LEVELS DURING PERIODS OF LINEAR METHANOL ACCUMULATION

Methanol accumulation rate (mg/100 ml/day)	Increase in morning blood ethanol level (mg/100 ml/day)*	Mean morning blood ethanol level (mg/100 ml)†	Maximum morning blood methanol level (mg/100 ml)‡	Minimum and maximum morning blood ethanol levels (mg/100 ml)
0·100	4·1	177	1·00	106–235
0·119	40·6	104	0·83	4–170
0·124	8·8	41	0·75	9–76
0·130	19·3	44	0·60	5–99
0·174	44·2	176	0·70	0–230
0·210	80·5	137	0·73	4–296
0·229	51·6	267	1·46	192–429
0·271	79·7	148	0·86	0–250

* Correlation between this variable and methanol accumulation rate: $r = +0{\cdot}875$, $P < 0{\cdot}001$.
† Correlation between this variable and methanol accumulation rate: $r = +0{\cdot}495$, $P > 0{\cdot}05$.
‡ Correlation between this variable and mean blood ethanol level: $r = +0{\cdot}808$, $P < 0{\cdot}01$.

during this period of build-up and the concentration of blood ethanol did prove to be statistically significant ($r = +0{\cdot}808$, $P < 0{\cdot}01$). This comparison was made by correlating the maximum morning blood methanol levels obtained during linear methanol accumulation periods with the mean morning blood ethanol levels during corresponding periods (see Table 1). Additionally, the relationship between maximum blood methanol levels and maximum blood ethanol levels during periods of methanol accumulation was statistically significant ($r = +0{\cdot}777$, $P < 0{\cdot}01$.) Therefore, even though the rate of methanol accumulation was not significantly related to blood ethanol concentration, the amount of methanol which accumulated did appear to covary with blood ethanol concentration. These findings are consistent with the hypothesis that blood methanol accumulates as a result of competitive inhibition of methanol oxidation by ethanol.

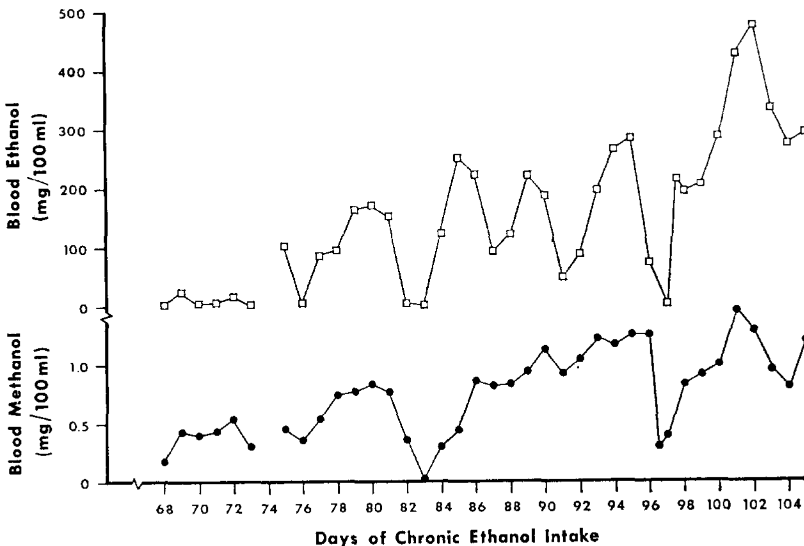

FIG. 1. Relationship between blood ethanol level and corresponding blood methanol level. Each point represents the concentration of ethanol or methanol in blood samples obtained between 8:00 and 8:30 a.m. each morning from one chimpanzee, male no. 353, who was maintained on a chronic ethanol ingestion schedule for 105 days.

Maintenance of elevated blood ethanol levels. After this 4–5 day period during which methanol accumulated as a linear function, blood methanol concentrations tended to plateau and remain relatively stable unless blood ethanol levels decreased to below approximately 50 mg/100 ml. Figure 1 illustrates the relationship between morning blood ethanol and blood methanol levels in one animal, male no. 353. These data were selected as representative for two reasons: (1) morning blood ethanol levels in this animal varied considerably, thus demonstrating this relationship over a wide range of corresponding ethanol levels and (2) more data were obtained for this animal than for others because of the length of time during which he was maintained at high ethanol doses. As can be seen in Fig. 1, the general shape of the methanol curve approximated that of the ethanol curve. However, sharp decreases in blood methanol concentrations occurred only when corresponding blood ethanol levels decreased to zero. In contrast, although ethanol levels varied widely between days 86 and 96, no

dramatic alterations in morning blood methanol concentrations were apparent during this period. Methanol levels tended to increase and decrease slightly as corresponding ethanol concentrations increased and decreased, but no rapid declines in methanol levels occurred until ethanol levels approached zero on day 96. This decline was reversed as ethanol levels subsequently increased.

Ethanol was abruptly withdrawn from the diet of this animal on day 105, the final dose of ethanol being administered in the 11:00 p.m. feeding on day 104. During the 4–5 days preceding withdrawal, morning blood ethanol levels were extremely high, reaching a maximum of 477 mg/100 ml. Blood methanol levels also reached their maximum (1·46 mg/100 ml) during this time and in general fluctuated in the same direction that blood ethanol concentrations fluctuated. Feeding times were adjusted to meet the needs of the animal and to provide more equivalent intervals between ethanol doses during these 4–5 days; therefore, the 11:00 p.m. feeding was frequently offered later during the night. Even under these conditions, however, the morning blood ethanol levels still approximated the diurnal minimum levels. The finding that maximum methanol levels tend to be attained during periods when ethanol levels are also at their highest is consistent with the previously noted positive correlation between methanol and ethanol concentrations during periods when methanol is accumulating as a linear function.

Elimination of accumulated blood methanol. The linear decline of accumulated blood methanol levels was examined in six chimpanzees that had continually maintained blood ethanol levels greater than 10–20 mg/100 ml for periods of 1–5 weeks. Ethanol was abruptly withdrawn from the diet of these animals to permit the elimination of ethanol from the blood. As blood ethanol concentrations decreased to less than 60–15 mg/100 ml, blood methanol levels began to decrease rapidly. During these withdrawal periods, blood samples were analyzed at 30–90 min intervals as ethanol and methanol levels declined, and elimination rates were calculated from these values using the method of least squares. Although the individual functions were highly reliable (r^2 = 0·87–0·98), methanol disappearance rates varied considerably, ranging from 0·13 to 0·35 mg/100 ml/hr. Ethanol disappearance rates were also variable, however, and the relationship between the methanol disappearance rate and the

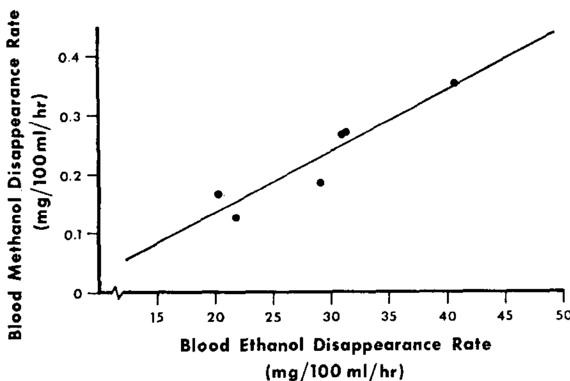

Fig. 2. Relationship between ethanol and methanol disappearance rates. Each point represents the ethanol disappearance rate and corresponding methanol disappearance rate calculated for each animal on the day after abrupt withdrawal of ethanol from the diet. The correlation coefficient for this relationship is $r = +0{\cdot}937$.

corresponding ethanol disappearance rate was statistically significant ($r = +0.937$, $P < 0.001$). The positive correlation between the disappearance rates for ethanol and methanol in this group of chimpanzees is illustrated in Fig. 2. The amounts of ethanol received by these animals during the periods of chronic ethanol intake prior to withdrawal had differed from animal to animal. In conjunction with these varying dose schedules, differences in the elimination rate of ethanol were observed at the time of withdrawal. The significant positive correlation between ethanol dose level and disappearance rate of blood ethanol has been reported elsewhere.[23] Thus the correlation between methanol and ethanol disappearance rates reported here indicates that when the ethanol elimination rate was enhanced during a period of chronic ethanol ingestion, a similar degree of enhancement of methanol disappearance rate occurred concomitantly. Other factors which were considered potentially related to the observed variability in the methanol disappearance rate were examined and no statistically significant correlation was found between the methanol disappearance rate and (1) the animal's age ($r = +0.216$), (2) the degree of elevation of blood methanol at the time of ethanol withdrawal ($r = +0.558$) or (3) the blood ethanol level on the morning of withdrawal ($r = +0.500$).

DISCUSSION

The accumulation of blood methanol observed in these chimpanzees during periods of chronic ethanol ingestion is probably due to the inhibition of methanol oxidation by the relatively large concentrations of blood ethanol which result from chronic ingestion. Whether ethanol inhibits methanol oxidation by ADH, or by an alternative enzyme system such as a catalase peroxidative system or the hepatic microsomal-oxidizing system, or by some combination of these systems is not known. From the kinetic data for primate ADH which are summarized in Table 2, it can be seen that ethanol remains competitive for ADH at relatively low physiological concentrations. Assuming comparable K_m values for chimpanzee ADH, competitive amounts of ethanol are present virtually all of the time during periods when morning blood ethanol levels exceed 10–20 mg/100 ml. Additionally, since only small amounts (1–2 mg/100 ml) of blood methanol accumulate during these periods, the ratio of ethanol to methanol remains greater than 10:1 and may be as high as 400:1. With such unfavorable concentration ratios and with the difference in Michaelis constants for ethanol and methanol, oxidation of methanol by ADH should essentially be completely inhibited by ethanol under these conditions. Similarly these concentration ratios should

TABLE 2. MICHAELIS CONSTANTS

Enzyme system	Species	Substrate	K_m
ADH	Man[32]	Ethanol	1.0×10^{-3} M (5·5 mg/100 ml)
		Methanol	3.0×10^{-2} M (96·0 mg/100 ml)
	Rhesus monkey[7]	Ethanol	2.7×10^{-3} M (12·4 mg/100 ml)
		Methanol	1.7×10^{-2} M (54·4 mg/100 ml)
Catalase	Rat[10]	Ethanol	1.5×10^{-3} M (6·9 mg/100 ml)
		Methanol	1.5×10^{-3} M (4·8 mg/100 ml)

result in nearly complete inhibition of any methanol oxidation which is mediated by catalase, even though ethanol and methanol have approximately equal affinities for this enzyme (see Table 2). Although K_m values for the hepatic microsomal-oxidizing systems are not available, Orme–Johnson and Ziegler[13] reported inhibition of methanol metabolism by equimolar amounts of ethanol, indicating that ratios of ethanol of 10–250:1 would result in significant inhibition.

If inhibition of methanol oxidation were essentially complete, however, it might be predicted that blood methanol levels would continue to increase as long as blood ethanol levels exceeded 10–20 mg/100 ml, assuming that endogenous production of methanol continued at a relatively constant rate. Instead blood methanol levels increased for 4–5 days at a rate which was significantly related to the rate of increase in blood ethanol levels on those days and then reached a plateau. Although the reason for the stabilization of blood methanol levels after 4–5 days cannot be determined from the data available, several possible explanations are suggested. First, accumulation of blood methanol may result in feedback inhibition of the methanol-producing system resulting in decreased production of endogenous methanol. Second, a portion of the methanol may be eliminated by pulmonary and renal excretion. Makar *et al.*[11] showed that in the monkey approximately 50 per cent of a large dose (6 g/kg) of methanol was eliminated in this manner. Although the percentage of methanol which is excreted via the lungs and kidneys is dose related,[12] Eriksen and Kulkarni[4] found trace amounts of methanol in the breath of normal, non-drinking human subjects. Thus small amounts of methanol may be eliminated via these routes and prevent further increases in blood methanol levels. Third, ethanol may not bind efficiently to catalase in the intact organism resulting in incomplete inhibition of methanol oxidation by catalase even in the presence of large ethanol to methanol concentration ratios. Although studies *in vitro* indicate that ethanol has approximately equal affinity for ADH and catalase (see Table 2), ethanol oxidation *in vivo* is mediated primarily by ADH, with 90 per cent inhibition of catalase having no effect on the rate of ethanol oxidation.[33] One reason for this might be that ethanol and catalase do not bind effectively *in vivo*. Fourth, an alternative enzyme system might exist for which methanol has a greater affinity than ethanol. This would allow for some oxidation of methanol even in the presence of unfavorable concentration ratios. The correlations between blood ethanol concentrations and blood methanol accumulation found in this study suggest that a mechanism which allows for partial inhibition of methanol oxidation (such as the latter two possibilities discussed above) is responsible for the accumulation of blood methanol and its maintenance at relatively stable levels.

With respect to the parallel increases in ethanol and methanol disappearance rates, it is not known if these changes are due to adaptive increases in ADH, catalase, the microsomal-oxidizing system or some other system. However, ADH is saturated only at relatively high concentrations of blood methanol (see Table 2), and the observed methanol disappearance rates are linear. Therefore, catalase, which has a much lower K_m for methanol than ADH, might be responsible for the oxidation of the low concentrations of accumulated blood methanol when ethanol concentrations approach zero. Further investigation of the mechanisms underlying the accumulation and oxidation of methanol in ethanol-adapted organisms may aid in determining the enzymatic mechanism underlying the development of metabolic tolerance.

Accumulation of similar quantities of blood methanol has been reported in human alcoholic volunteers who were chronically ingesting intoxicating amounts of ethanol under experimental conditions.[1] In these subjects the blood ethanol elimination rate was 27·2 ± 3 mg/100 ml/hr and the blood methanol elimination rate was 0·29 ± 0·4 mg/100 ml/hr. If these values are compared with the ranges of elimination rates illustrated in Fig. 2, it may be seen that chimpanzees with ethanol disappearance rates comparable to those reported for the human subject group (24–30 mg/100 ml/hr) had methanol disappearance rates similar to those reported for the humans. In addition, Majchrowicz and Mendelson[1] report that methanol is not eliminated from the blood in humans until ethanol levels decrease to approximately 70–20 mg/100 ml, which is in close agreement with the range found for chimpanzees. Majchrowicz and Mendelson suggest that methanol may play some role in the development of the alcohol withdrawal syndrome, although they emphasize that evidence of methanol accumulation does not necessarily imply such a relationship. While it is recognized that the amount of methanol found in both human and chimpanzee subjects is far below the levels (50–275 mg/100 ml) associated with methanol intoxication and toxicity,[34] the chronic presence of even small quantities of blood methanol may affect the organism, especially the central nervous system. Cohen and Collins[35] have shown that both acetaldehyde and formaldehyde condense with catecholamines to form 1, 2, 3, 4-tetrahydroisoquinoline alkaloids which are similar in structure to pharmacologically active naturally occurring alkaloids. Additionally, formaldehyde, which is the primary oxidation product of methanol, condenses with catecholamines 25–50 times faster than does acetaldehyde. Synthesis of tetrahydroisoquinolines *in vivo* in rats pretreated for 3 days with i.p. injections of methanol (1–4 g/kg) was demonstrated by Cohen and Barrett[36] using fluorescence microscopy.

The similarity of the relationships between blood ethanol and blood methanol concentrations in man and in the chimpanzee, as well as the observed accumulation of blood methanol in the rhesus monkey,[3] suggests that the chronic effects of low concentrations of blood methanol may be appropriately investigated in a non-human primate. Furthermore, it has been established that although non-primates develop a different pattern of symptomatology to toxic levels of methanol, the rhesus monkey develops acute acidosis and visual impairment as does man.[37] Thus the non-human primate may serve as a particularly useful experimental model for evaluating the significance of the role that methanol may play in the development of alcoholism.

REFERENCES

1. E. MAJCHROWICZ and J. H. MENDELSON, *J. Pharmac. exp. Ther.* **179**, 293 (1971).
2. E. MAJCHROWICZ and V. C. SUTHERLAND, *Pharmacologist* **13**, 219 (1971).
3. E. MAJCHROWICZ, J. H. MENDELSON and N. K. MELLO, 160th National Meeting Am. Chem. Soc., Abstr. no. 127, Chicago (1970).
4. S. P. ERIKSEN and A. B. KULKARNI, *Science, N.Y.* **141**, 639 (1963).
5. J. AXELROD and J. DALY, *Science, N.Y.* **150**, 892 (1965).
6. J.-P. VON WARTBURG, J. L. BETHUNE and B. L. VALLEE, *Biochemistry, N.Y.* **3**, 1775 (1964).
7. M. M. KINI and J. R. COOPER, *Biochem. Pharmac.* **8**, 207 (1961).
8. H. THEORELL and R. BONNICHSEN, *Acta chem. Scand.* **5**, 1105 (1951).
9. D. KEILIN and E. F. HARTREE, *Biochem. J.* **39**, 293 (1945).
10. T. R. TEPHLY, R. E. PARKS and G. J. MANNERING, *J. Pharmac. exp. Ther.* **131**, 147 (1961).
11. A. B. MAKAR, T. R. TEPHLY and G. J. MANNERING, *Molec. Pharmac.* **4**, 471 (1968).
12. T. R. TEPHLY, R. E. PARKS and G. J. MANNERING, *J. Pharmac. exp. Ther.* **143**, 292 (1964).
13. W. H. ORME-JOHNSON and D. M. ZIEGLER, *Biochem. biophys. Res. Commun.* **21**, 78 (1965).

14. C. S. Lieber and L. M. DeCarli, *Science, N.Y.* **162,** 917 (1968).
15. M. K. Roach, W. N. Reese and P. J. Creaven, *Biochem. biophys. Res. Commun.* **36,** 596 (1969).
16. K. J. Isselbacher and E. A. Carter, *Biochem. biophys. Res. Commun.* **39,** 530 (1970).
17. R. G. Thurman, H. G. Ley and R. Scholz, *Eur. J. Biochem.* **25,** 420 (1972).
18. J. M. Khanna, H. Kalant, G. Lin and G. O. Bustos, *Biochem. Pharmac.* **20,** 3269 (1971).
19. M. K. Roach, M. Khan, M. Knapp and W. M. Reese, *Q. Jl Stud. Alcohol,* **33,** 751 (1972).
20. H. Isbell, H. F. Fraser, A. Wikler, R. E. Belleville and A. J. Eisenman, *Q. Jl Stud. Alcohol* **16,** 1 (1955).
21. R. M. Kater, N. Carulli and F. L. Iber, *Am. J. clin. Nutr.* **22,** 1608 (1969).
22. R. D. Hawkins, H. Kalant and J. M. Khanna, *Can. J. Physiol. Pharmac.* **44,** 241 (1966).
23. W. A. Pieper, M. J. Skeen, H. M. McClure and P. G. Bourne, *Science, N.Y.* **176,** 71 (1972).
24. J. M. Khanna, H. Kalant and G. Bustos, *Can. J. Physiol. Pharmac.* **45,** 777 (1967).
25. K. Schlesinger, E. L. Bennett, M. Hebert and G. E. McClearn, *Nature, Lond.* **209,** 488 (1966).
26. J. Trémolières and L. Carré, *C. r. Seanc. Soc. Biol.* **155,** 1022 (1961).
27. E. Mezey, *Biochem. Pharmac.* **21,** 137 (1972).
28. W. A. Pieper and M. J. Skeen, *Lab. Anim. Sci.,* in press.
29. M. K. Roach and P. J. Creaven, *Clinica chim. Acta* **21,** 275 (1968).
30. R. N. Baker, A. L. Alenty and J. F. Zack, Jr., *J. Chromat.* **7,** 312 (1969).
31. B. Ostle, *Statistics in Research,* p. 225. Iowa State University Press, Ames, Iowa (1963).
32. A. H. Blair and B. L. Valle, *Biochemistry, N.Y.* **5,** 2026 (1966).
33. M. E. Smith, *J. Pharmac. exp. Ther.* **134,** 233 (1961).
34. O. Roe, *Acta med. Scand.* **113,** 558 (1943).
35. G. Cohen and M. Collins, *Science, N.Y.* **167,** 1749 (1970).
36. G. Cohen and R. Barrett, *Fedn Proc.* **28,** 288 (1969).
37. A. P. Gilger and A. M. Potts, *Am. J. Ophthal.* **39,** 63 (1955).