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KINETICS OF ETHANOL METABOLISM IN THE INTACT RAT AND MONKEY

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Abstract—Because ethanol distributes evenly throughout body water and because it can be given in saturating doses, it offers an opportunity for a comparison of kinetic studies *in vivo* with those performed *in vitro*. Ethanol was given intraperitoneally to rats and monkeys in a dose (1 g/kg) which not only saturated the enzyme system responsible for its oxidation, but which permitted sufficient time for its even distribution throughout body water before metabolism reduced its concentration to a level where its oxidation proceeded in accordance with first order kinetics. Blood alcohol concentrations were determined at intervals and these data were used to calculate apparent kinetic constants *in vivo* for the oxidation of ethanol. The apparent Michaelis constants *in vivo* obtained in this manner compared quite favorably with those for the reaction of ethanol with crude alcohol dehydrogenase preparations obtained from the livers of the same species. The calculated apparent maximum velocities *in vivo* were quite similar to the observed maximum rates *in vivo* of ethanol metabolism seen in each of the species. Kinetic data were more closely correlative in the rat than in the monkey.

ETHANOL offers special advantages for the study of the kinetics of the metabolism of a foreign substance *in vivo*. Because it distributes evenly throughout body water, its concentration at the metabolic site after a given dose can be predicted and it is one of the few compounds of pharmacologic interest that can be given in doses high enough to saturate the system responsible for its metabolism. The even distribution of ethanol at any desired concentration in the body water can be achieved by giving a dose of the alcohol considerably higher than that sought and allowing the excess to be removed by metabolism. Recently in our laboratory, the Michaelis constants of crude preparations of hepatic alcohol dehydrogenase (ADH) from the rat and monkey were determined using ethanol and methanol as substrates.[1] The purpose of the current study was to determine how closely the apparent kinetic constants for the oxidation of ethanol in the intact rat and monkey compare with the kinetic constants for the oxidation of ethanol by ADH isolated from the livers of the same species. Similar studies employing humans have been described by Lundquist and Wolthers.[2]

METHODS

Treatment of animals. Four male Sprague–Dawley strain rats (250–300 g) and four male rhesus monkeys (1600–2000 g) were employed. All animals received 1 g of ethanol per kg, given intraperitoneally as a 10% solution in 0·9% NaCl solution. This dose

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permits adequate time for absorption and even distribution of ethanol throughout body water before the concentration of ethanol is reduced through metabolism to the point where the oxidation of the alcohol will proceed in accordance with first order kinetics. Blood specimens were taken at irregular time intervals until ethanol concentrations of about 0·05% were reached, after which time collections were made about every 20 min. Blood was collected in tubes in which 0·2 ml of a 1% solution of disodium ethylaminetetraacetate had been evaporated. Rat blood was obtained by clipping the tip of the tail and allowing about 0·3 ml of blood to fall directly into the tube. Monkeys were tied to an operating board in a supine position and an indwelling catheter was placed in a hindlimb vein. Using a syringe, 1 to 2 ml of blood was drawn from the catheter and transferred to collection tubes. Clotting of blood was prevented by keeping heparinized saline in the catheter between sampling. The catheter was rinsed with blood drawn from the animal just previous to sampling. The tubes were capped.

Measurement of ethanol concentration in blood. Gas–liquid chromatography provides the degree of sensitivity needed to measure the low ethanol concentrations in the blood required to reveal the dependency of the rate of ethanol oxidation on substrate concentration. A Barber–Colman 10 gas chromatograph equipped with a hydrogen flame ionization detector was used. The column was a 6 ft by 6 mm, i.d., U-shaped borosilicate glass tube with a column packing consisting of Carbowax 400 as the liquid phase and silanized diatomaceous earth, Gas-Chrom S (80–100 mesh), as the solid support. The column was conditioned overnight at 150° with the carrier gas (argon) flowing. The relative gain was 10^{-9} ampere. The gas pressures were: air, 30; hydrogen, 14; and argon, 10, psig. The temperature of the column was 80°. The flash heater was not turned on. Glass wool was placed in the top of the column to prevent contamination of the column with blood. The glass wool was changed each day analyses were performed.

Standard curves were constructed from the chromatography of duplicate 10 μl vol. of 0·005, 0·02 and 0·05% aqueous solutions of ethanol. Ethanol gave a retention time of about 6 min. Water appeared about 16 min after the injection and produced a negative response. If the initial injection is followed 4 min later with a second injection, duplicate ethanol peaks can be obtained before the appearance of water from either injection. Quantitation was achieved by cutting from the chart paper the areas representing the ethanol response and weighing them. Standard curves were obtained each day analyses were performed.

Undiluted blood was injected in volumes ranging from 1 to 10 μl. Very small and very large responses were avoided by choosing suitable blood volumes for injection and by attenuation. All analyses were performed in triplicate. Recovery studies demonstrated the validity of applying the standard curve to the analysis of ethanol in undiluted blood. Blood containing as little as 0·001% ethanol could be analyzed accurately.

Calculation of kinetic values. The nonlinear portions of the ethanol concentration–time curves (Fig. 2) were used to construct the familiar Lineweaver–Burk plot from which the apparent Michaelis constant *in vivo* (K_m)* and the apparent maximum

* The apparent Michaelis constant *in vivo* is defined as the concentration of ethanol in m-moles per l. of body water at which ethanol is oxidized at one-half the rate calculated to occur at infinite substrate concentration.

velocity *in vivo* (V_{max})* were derived. In order to give a more accurate estimate of substrate concentration during a given time interval than that represented by an arithmetic mean, advantage was taken of an integrated form of a rate equation provided by Lundquist and Wolthers.[2] Because it was convenient to use the computer program already in use in our laboratory for the calculation of kinetic data using the l/v vs. l/S plot (v = velocity, S = substrate concentration) rather than the v vs. v/S plot employed by Lundquist and Wolthers,[2] the equation developed by these investigators was used only to calculate S. Lundquist and Wolthers plotted v vs. v/S by

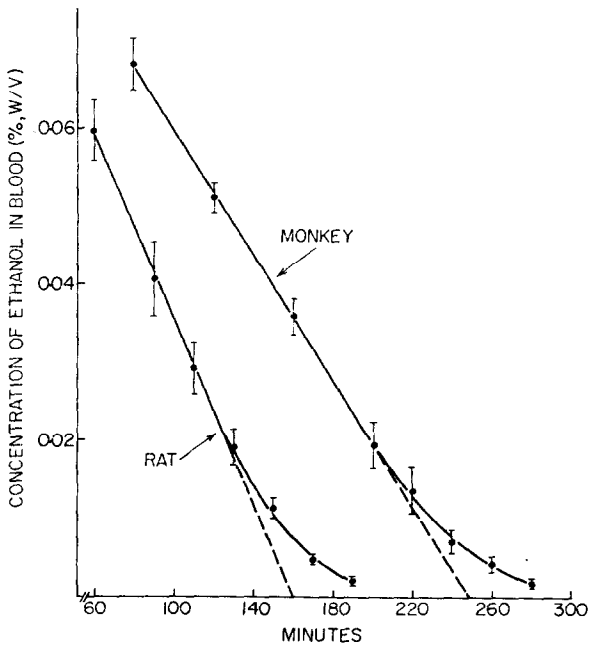

FIG. 1. Disappearance of ethanol from the blood of rats and monkeys after the intraperitoneal injection of 1 g of ethanol/kg. Each point represents the mean value of four animals (vertical bars = ± S. E.).

equating $v = l/t\,(c_0 - c)$ and $v/S = l/t \ln c_0/c$. It follows then that $S = \frac{c_0 - c}{\ln c_0/c}$ where c_0 is the blood ethanol concentration in blood water at the beginning of a given 20-min time interval and c is the ethanol concentration at the end of that time interval. To avoid bias in constructing the Lineweaver–Burk plots, the data employed in the derivation of the kinetic values were submitted to a statistical analysis provided by Wilkinson[3] with calculations performed on a digital computer using a FORTRAN program written by Cleland.[4]

* The apparent maximum velocity *in vivo* is defined as the calculated rate of ethanol oxidation at infinite concentration of the substrate in body water.

RESULTS

The time courses of the disappearance of ethanol from the blood of rats and monkeys after the administration of 1 g of ethanol/kg are shown in Fig. 1. During the linear portion of the curve, the ethanol content of the blood of the rat was found to decline at the rate of about 0·038 ± 0·0032 per cent per hr. Assuming the water content of the whole rat and of the blood to be 70 and 80 per cent, respectively, it can be calculated that ethanol was metabolized by the intact rat at the rate of about 330 mg (7·4 m-moles)/kg/hr. This compares remarkably well with the 325 mg/kg/hr rate previously observed in our laboratory when the disappearance of ethanol was obtained directly

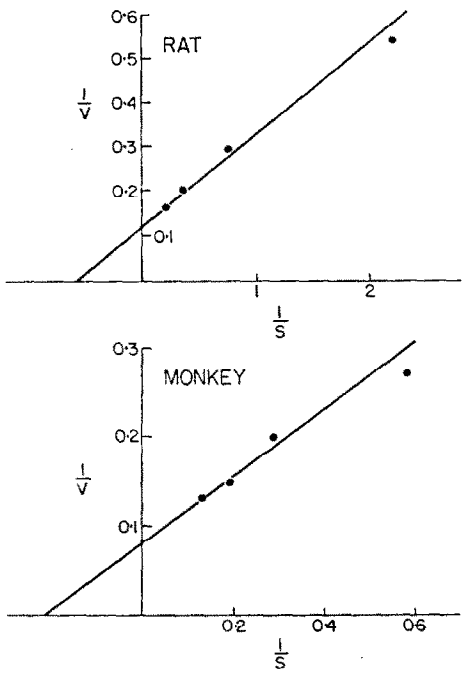

FIG. 2. Representative Lineweaver–Burk plots of the oxidation of ethanol *in vivo* by the rat and monkey derived from rates of disappearance of ethanol from the blood as described in Methods. The points are observed values, but the plot was drawn with the aid of statistical analysis. v = m-moles of ethanol oxidized per l. of body water per hr; S = m-moles of methanol per l. of blood (or body) water.

by determining the loss of ethanol from the homogenized whole carcass.[5] The rate of ethanol oxidation is seen to depart from linearity at a concentration of about 0·02 per cent in the blood or about 0·025 per cent of blood water, which is equivalent to a solution of ethanol in blood water or other body water of about 5·4 mM.

During the linear portion of the curve, the ethanol content of the blood of the monkey declined at the rate of about 0·023 ± 0·0017 per cent. Applying the same calculations as those used for the rat, it can be estimated that the monkey metabolized ethanol at the rate of about 195 mg (4·2 m-moles)/kg/hr. In a previous study[1] where the rate of ethanol oxidation in the monkey was obtained by measuring the amount of pulmonary $^{14}CO_2$ produced from 1-^{14}C-ethanol, about 34 per cent, or about 5·3 m-moles of a 15·6 m-mole dose of the labeled ethanol was oxidized between the first and second hours after administration, a period when the rate of oxidation was linear.

The rate of ethanol oxidation is seen to depart from linearity at about the same concentration of ethanol as that seen in the rat.

Data from the nonlinear portions of the curves shown in Fig. 1 were employed as described in Methods for the determination of apparent kinetic constants *in vivo*. Typical examples of Lineweaver–Burk plots obtained from the four rats and four monkeys represented in Fig. 1 are shown in Fig. 2. Summaries of the kinetic data obtained from all of the animals in this manner are given in Table 1.

TABLE 1. CALCULATED AND OBSERVED KINETICS OF ETHANOL OXIDATION IN THE RAT AND MONKEY

Species	Apparent K_m *in vivo* (mM)	K_m using liver ADH (mM)	Calculated apparent V_{max}* *in vivo*	Observed maximum rate of ethanol metabolism *in vivo** A†	B‡	C§
Rat	2·7 ± 1·3	1·9‖	11·3 ± 0·76	10·6	10·9	
Monkey	4·6 ± 2·3	1·8‖	13·6 ± 3·5	6·0		7·6

* Millimoles of ethanol oxidized per l. of body water per hr.
† Calculated from the disappearance of ethanol from the blood (current study) on the basis that the whole animal and blood contain 70 and 80 per cent water respectively.
‡ Value obtained previously[5] by direct measurement of the loss of ethanol from the homogenized whole carcass at various times after the administration of a saturating dose of ethanol (2·7 g/kg).
§ Value derived previously[1] by measuring the pulmonary excretion of $^{14}CO_2$ produced from the administration of a saturating dose of 1-^{14}C-ethanol (0·72 g/kg).
‖ Value obtained previously[1] from two crude liver ADH preparations.

DISCUSSION

Table 1 includes data from previous publications[1, 5] as well as a summary of the current data. In the rat, the apparent Michaelis constant *in vivo* for ethanol oxidation compares quite favorably with that obtained using a crude ADH preparation from rat liver. The calculated apparent maximum velocity *in vivo* and the observed maximum rate of ethanol oxidation *in vivo* are also in good agreement. The rate of metabolism obtained by direct measurement of ethanol disappearance from the homogenized carcass is almost exactly that obtained indirectly by measurement of ethanol disappearance from the blood, thus validating the use of blood determinations as an indirect means of measuring the rate of ethanol oxidation *in vivo* in this species. In the monkey, agreement between observed and calculated kinetic values was not as good as that seen in the rat; nevertheless, values were still well within the same order of magnitude.

In a previous publication from our laboratory[1] it was reported that ethanol was metabolized more rapidly in the intact rat than could be accounted for by the amount of ADH extracted from the liver. This led to the suggestion that a significant amount of ethanol may be oxidized in the rat by some mechanism not involving ADH or catalase. An alternative explanation offered in this publication was the possibility that the procedure for the extraction of ADH from the liver may have yielded a poor recovery of the enzyme. The finding that in the rat the apparent K_m *in vivo* agrees well with the K_m for the oxidation of ethanol by crude preparations of ADH from rat liver suggests that the latter explanation is more probable. On the other hand, although it would be fortuitous, an alternative mechanism for ethanol oxidation could involve an enzyme

system with a K_m for ethanol quite similar to that for ADH. It is presently not possible to distinguish between these two possibilities.

Most of our knowledge of biochemistry has been derived from the study of systems *in vitro*. Frequently there has been little interest in whether mechanisms thus derived have any meaning with respect to events occurring *in vivo*. The current studies lend some encouragement to the view that, at least in certain cases, kinetic studies *in vitro* can be applied to situations *in vivo*.

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