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Controversy

LETTERS TO THE EDITOR

Comments on a recent Publication by Kini and Cooper which purports to show that alcohol dehydrogenase is responsible for the physiological oxidation of methanol

Dear Sir:

In a recent publication in this journal Kini and Cooper[1] showed that, contrary to the findings of earlier workers, crystalline horse liver alcohol dehydrogenase (ADH) is capable of mediating the oxidation of methanol. From kinetic studies performed on the monkey liver ADH and from observations made on the disappearance of methanol from the blood of monkeys they concluded that "the dehydrogenase mechanism, and not the catalase-peroxide system, is responsible for the physiological oxidation of methanol." There has been a great deal of controversy regarding the enzymatic pathway involved in the physiological oxidation of methanol and since at first glance the observations of Kini and Cooper would seem to resolve the question, we feel it necessary to point out a gross error committed by these investigators in reaching their conclusion. In fact, when the error is corrected, it is possible to show, using the authors' own data, that not only has the assigned role of ADH as the primary mechanism of methanol oxidation not been proved, but, indeed, that such a role is exceedingly improbable.

From the average blood methanol disappearance rate determined during the first 22 hr after administration of 18 g (560,000 μmoles) of methanol, Kini and Cooper calculated that a monkey weighing 3 kg oxidized a maximum of 10·45 μmoles of methanol per min. At this extremely low rate 37·4 days would be required to oxidize the 18 g of methanol. This is certainly not in keeping with what is known about methanol disappearance in man or lower animals. Since this calculation was made with the assumption that oxidation was proceeding at maximum velocity, and since the V_{max} rate would not be maintained throughout the entire period of metabolism, considerably more than 37 days would be required for complete oxidation. Obviously some technical error had been made in estimating methanol disappearance from the blood or some error in the calculations had been committed. Examination of the data shows the latter possibility to be the case.

Let us consider the following statement: "With an administered oral dose of 6 g/kg of body weight, a mean value of 0·00013 per cent per min was obtained for the disappearance of methanol from the blood over a period of 22 hr. Since our monkeys had a body weight of approximately 3 kg and a presumed blood volume of about 235 ml, it can be calculated that the monkey oxidized a maximum of 10·45 μmoles methanol per min, assuming that significant pulmonary and urinary losses did not occur." While recognizing that methanol disappearance from the blood is proportional to the over-all disappearance of methanol from the whole monkey, one immediately asks why the blood *volume* should bear any relationship to total methanol oxidation. The error is simply that, while it has long been known that methanol distributes evenly throughout the total body water,[2] these investigators have made their calculations as though *all* of the administered methanol was concentrated in the blood! Since the volume which should have been considered is that of the total body water, which is represented by about 60 per cent of the body weight, or 1800 ml, it is apparent that these authors have erred by a factor of about eight-fold.

If the data provided by Kini and Cooper are properly utilized, a corrected value for the disappearance rate of methanol from the monkey can be obtained. Widmark's r is equal to the average concentration of alcohol in the entire animal divided by the concentration of alcohol in the blood.[3, 4] It follows that r also equals the change in average concentration of alcohol in the entire animal divided by the change in the concentration of alcohol in the blood. If we assume the average r value of 0·68 obtained on humans to apply also to the monkey, then 0·68 = (change in average concentration of methanol in the entire monkey)/0·00013; and 0·0000884 = change in average concentration of methanol in the entire monkey (g/100 g of monkey/min).

Thus, the actual rate of methanol disappearance in the 3-kg monkey would be $30 \times 0{\cdot}0000884 = 0{\cdot}00265$ g or 83 μmoles per min.

From their kinetic data on the oxidation of methanol by monkey ADH, Kini and Cooper estimated that 166 mg of the enzyme is necessary for the oxidation of 10·45 μmoles of methanol per min. The oxidation of 83 μmoles of methanol per min would then require about 1320 mg of ADH. These investigators find the average 3-kg monkey to possess 48 mg of liver ADH, a concentration which is in reasonable agreement with that estimated by Theorell and Bonnichsen[5] to occur in human liver. Thus, the measured monkey liver ADH can account for only 3·6 per cent of the observed methanol metabolism. If we assume that the 70-g liver reported by Kini and Cooper for the 3-kg monkey has a protein concentration of 18 per cent on the wet weight basis, the ADH necessary to metabolize the 83 μmoles of methanol per min (1320 mg) would constitute about 10 per cent of the total liver protein.

The estimation of the amount of enzyme required was made by Kini and Cooper with kinetic data obtained at 23° rather than 37°. Also, renal and pulmonary excretion of methanol were ignored, factors which would play a considerable role at the high blood methanol concentrations encountered in their studies. However, even if these considerations are taken into account, many times more enzyme would be needed to accomplish the observed rate of physiological oxidation than has been shown to be present in monkey liver. Thus it must be concluded that alcohol dehydrogenase does not play a major role in the physiological oxidation of methanol.

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Dear Sir:

The calculations that Drs. Mannering, Parks and Tephley make are based on the assumption that the 18 g of methanol administered to the monkey must be accounted for only in terms of oxidation. Gilger *et el.*[1] determined methanol levels in the plasma of ten monkeys which had received methanol at the same concentration we used in our studies (6 g/kg). When the mean methanol concentration is the plasma is extrapolated to zero time it can be calculated that 44 per cent of the alcohol was lost by pulmonary and renal routes. In our experiments the elimination of unchanged methanol by these routes amounted to 68 per cent.

In our rate figure for the disappearance of methanol from the body we used the total blood volume as a measure of this loss and, as the Wisconsin group points out, a more accurate measure necessitates the inclusion of the total body water. When our data are recalculated with this figure, and corrected for pulmonary and renal losses of methanol, we arrive at a value of 23 μmoles of methanol metabolized in the body per min. The same calculations applied to the data of Gilger *et al.* yield a figure of 28 μmoles. A reasonable agreement is apparent in these two studies, with the rate of methanol oxidation in the body averaging approximately 26 μmoles per min.* It is therefore necessary to determine whether sufficient alcohol dehydrogenase (ADH) is present in the body to account for this rate.

From Table 1 of our paper it can be calculated that monkey liver contains 48 mg of ADH and that to oxidize methanol at a rate of 26 μmoles per minute would require roughly 412 mg of the enzyme. This eight- to nine-fold discrepancy may be explained to a large extent by the following considerations: (1) The estimate of the total ADH activity in the supernatant extract of the liver mince is a minimal approximation because of the presence of DPNH oxidase in the preparation. (2) As stated in our paper, the enzyme determinations were performed at 23°, so that with the usual temperature effect on enzyme velocity prevailing it is not improbable to assume a threefold increase in ADH activity to

obtain at body temperature. (3) Finally, of course, one must take into account extrahepatic sources of of ADH such as kidney, spleen, etc.

It is obvious that the calculations made by us and by the Wisconsin group are really crude approximations and that neither group can make an unequivocal statement about the situation *in vivo* with respect to methanol oxidation. On the basis of studies with an inhibitor of catalase, the Wisconsin group feels that the catalase-peroxide system is involved in methanol oxidation. Arguments that this system does not operate *in vivo* are stated in our paper and also in a publication by Bartlett.[2] On the other hand, we have demonstrated that ADH can catalyse the oxidation of methanol, but the quantitative aspects of this activity are still open to question. The ultimate answer to the problem may be that both systems play a part.

* The *r* value of Widmark, which is quoted by the Wisconsin group, is not strictly applicable here, since it was obtained on human subjects.

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