Biochemical Pharmacology, Vol. 23, pp. 519–522. Pergamon Press, 1974. Printed in Great Britain.

DEVELOPMENTAL CHANGES IN MOUSE LIVER ALCOHOL DEHYDROGENASE*

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(*Received* 10 *March* 1973; *accepted* 27 *July* 1973)

Abstract—Alcohol dehydrogenase activity in the supernatant fraction of liver homogenates was studied in adult and newborn mice. Newborn mice were found to have a lower specific activity than adult animals. K_m values were higher in the new born animal. Electrophoresis showed two bands for the adult and only one for the newborn, with a greater intensity seen in adult.

THE PRINCIPAL enzyme responsible for degrading ethanol in mammals is believed to be alcohol dehydrogenase (ADH, EC 1.1.1.1.) which is found in the supernatant fraction of liver cell homogenate and other tissues. Like many other liver enzymes, alcohol dehydrogenase changes in activity during the fetal and postnatal period in both experimental animals and man.[1,2]

In addition to the changes in the actual activities, a specific structural form of the enzyme has been shown to dominate at a particular age period. The infantile form of the enzyme gradually disappears and is replaced concomitantly by another enzyme protein (adult form) with a similar function.[3] Human ADH has been shown to have a different electrophoretic pattern in the fetal, childhood and adult age period.[4,5] This study was undertaken to investigate further the kinetic parameters and electrophoretic properties of adult and newborn mouse alcohol dehydrogenase. Study of the developmental pattern in another species was carried out not only for comparative purposes but also to elucidate the nature of the developmental changes.

MATERIALS AND METHODS

Newborn mice were bred from our own colony of Cobbs Swiss–Webster mice (Charles River). The liver homogenates of each adult mouse were prepared and used individually. However, the amount of homogenate obtained from a single newborn mouse was sufficient for only one assay at one ethanol concentration. Therefore, the siblings in seven separate litters were pooled and seven determinations, with five different substrate concentrations, were performed for a total of 35 individual assays. A 10 per cent (w/v) homogenate was prepared in 1·15% (w/v) KCL and centrifuged for 10 min at 9000 *g* in a Sorvall refrigerated centrifuge. ADH activity was estimated according to the method of Bonnichsen and Brink,[6] recording the generation of NADH at 340 nm in a Cary model 15 recording spectrophotometer. The reaction mixture contained varying concentrations of ethanol, 1mg NAD and 0·1ml liver

* This research was supported in part by Public Health, Service Grant HD-04287.

homogenate in 0·1 M glycine–sodium hydroxide buffer, pH 9·6, to make 3·3 ml total volume. The protein content was measured from the 280 nm O.D. and activity expressed as nmoles NADH produced by 1 mg protein in 1 min. The homogenate was electrophoresed on Cellogel support (Reeve Angel) in 0·05 M Tris-PO_4, pH 8·5 buffers, from 1 to 2 hr at 250 V. Cellogel membranes are shipped and stored in methanol, which must be washed out before placing in buffer solution for electrophoresis. Ethanol, 0·045 ml, 18 mg NAD, 10 mg *p*–nitro tetrazolium blue and 0·6 mg phenazine methosulfate were added to the support membrane and incubated in a covered glass container at 37° until bands, due to reduced *p*–nitro tetrazolium blue, appeared. Visualization was also possible by observing NADH fluorescence activated under longwave ultraviolet light.

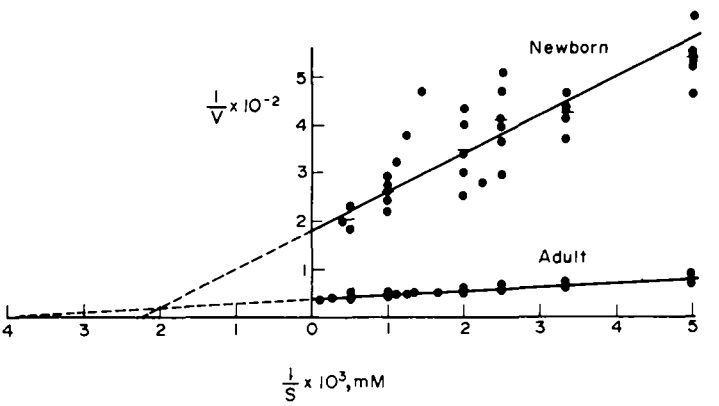

FIG. 1. Lineweaver–Burk plot for newborn and adult activities. Small horizontal bars among newborn data are the average values at those substrate concentrations. Line drawn through data points was extrapolated from computer-determined K_m and V_{max} values, although every point was treated individually.

RESULTS

The ADH activity was lower in the newborn than in the adult mouse. The result of three adult and seven newborn (pooled sibs) mice assayed at 15 different ethanol concentrations is presented in a Lineweaver–Burk plot (Fig. 1). The K_m and V_{max} values were determined by using Marquardt's techniques for the least squared estimation of nonlinear parameters. This method utilizes a computer program designed to estimate K_m and V_{max} from the observed activity at various substrate concentrations (nonlinear relationship) with a 95 per cent confidence level.[7] A K_m value of $2{\cdot}52 \pm 0{\cdot}28 \times 10^{-4}$ M was found for the adult and $4{\cdot}47 \pm 0{\cdot}82 \times 10^{-4}$ M for the newborn mouse. A straight line was drawn from the computer-determined values of $1/K_m$ to $1/V_{max}$ and extended through the observed values. The electrophoretic pattern of adult and newborn mouse alcohol dehydrogenase is shown in Fig. 2. Both migrated toward the cathode. One weak band in the adult indicates less activity present than in the other darker stained and highly fluorescent band. Both adult and newborn bands migrated toward the cathode. This electrophoretic pattern is only seen when freshly prepared homogenate is used; when the homogenate is stored frozen overnight, this electrophoretic pattern is not seen. A control without added

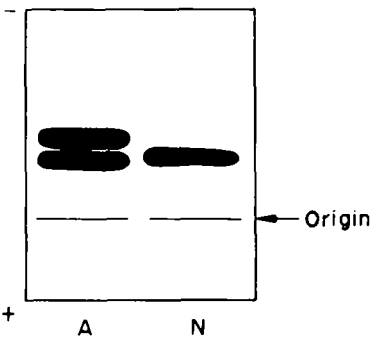

FIG. 2. Diagram of electrophoreses obtained with newborn (N) and adult (A) homogenates on Cellogel membranes. The reaction with tetrazolium blue makes visualization of the pattern possible. This same pattern also is observed by fluorescence with longwave ultraviolet illumination.

alcohol showed very little activity even after a prolonged incubation. Residual alcohol in the Cellogel membrane may have been responsible for the appearance of this slight activity.

DISCUSSION

Variation in ethanol tolerance is known to exist between different individuals and at different stages during development. Racial differences have been attributed to differing elimination rates.[8] In growing rats, an increase in the elimination rate could also be found with age.[9] A prolonged half-life was demonstrated in newborn infants who were exposed to alcohol while *in utero*.[10] These results correlate well with the lower activity of ADH found in rat and human fetal liver.[1,2] The lower activity of ADH activity generally found in newborns has now been shown to be true in mice as well. The K_m value, as well as that found for rats and man, is higher in the newborn than in the adult mouse. This indicates a higher enzyme affinity for the ethanol substrate in the newborn mouse. However, the significance of this is not understood in relation to the activity. In any case, a possible change in enzyme structure between newborn and adult is suggested.

Alcohol dehydrogenase is known to exist in more than one isoenzyme form.[11] In man, electrophoresis shows the changes in development by the appearance of one band in the newborn to four bands in the adult.[4] Moreover, band three, which is the first one to appear, seems to be more active in the fetus than in the adult. In the mouse, two bands with different intensities can be seen in the adult. It may be possible that the differences in K_m values are due to the fact that in the newborn mouse one isoenzyme dominates, while in the adult animal, the other is more developed. As previously mentioned, this phenomenon, has been observed for other enzyme systems during mammalian development.[3]

Unfortunately, it has not always been possible to correlate the overall elimination rate and alcohol response *in vivo* to the measurement of ADH activity present in liver biopsies *in vitro*.[12,13] Other explanations have also been offered, such as genetically determined differences in sensitivity.[14] Recently, the presence of a microsomal

ethanol–oxidizing enzyme system, enhanced by ethanol feeding, has been discussed.[15] In view of the new usage for ethanol in preventing premature labor, more basic information about ethanol metabolism seems to be indicated.[16]

Acknowledgements—We wish to thank Judy McConnell and Marvin Standingwolf for their technical assistance.

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