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metabolism fetus
alcohol dehydrogenase isozymes

Development of Alcohol Dehydrogenase Activity in the Human Liver

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Extract

In the present study, alcohol dehydrogenase activity (ADH) has been measured in human liver tissue during development, and a comparison made between certain kinetic properties of crude enzyme preparations from fetal and adult liver.

The fetal livers were obtained from legal abortions. The liver tissue from children and adults was acquired during surgery of the abdomen in cases where no macroscopic abnormality of the livers was observable. The liver tissue was frozen immediately after excision. Studies indicated that the enzyme activity was stable at –20° for up to 6 days. All the determinations were made within 24 hours of the liver being sampled.

A 10 % liver homogenate was prepared in ice-cold 0.25 M sucrose containing 1 % Triton X-100. After centrifugation of the homogenate for 10 minutes at 5000 × g at 3° C, samples were taken from the supernatant for protein determination and for enzyme assays. Table I presents the enzyme activity levels in human liver during the development from a fetus to an adult organism. The results are expressed as milliunits per g liver wet weight and per 100 mg soluble liver protein. ADH activity is detectable in 2-month-old fetuses, although it amounts to no more than 3–4 % of adult activity. Activities of adult range are found after 5 years of age. Considerable varation exists in the activity of adult livers.

The relationship between pH and the rate of reaction appears in figure 1. The final pH figures of the reaction mixtures are given; these were determined with a Radiometer pH meter. Adult human liver ADH has a pH optimum of about 10.4, and the pH optimum for the fetal enzyme preparations is 10.0.

Lineweaver-Burk analyses which illustrate the relationship between the concentration of ethanol and NAD, and the ADH activity in adult and fetal liver are presented in figures 2 and 3. The results presented are typical of three cases studied in each group. Close agreement was found for all constants in the different enzyme preparations examined. The apparent K_m values for ethanol were 3400 μM and 1100 μM, and for NAD 70 μM and 150 μM in fetal and adult enzyme preparations respectively.

Speculation

These results do not constitute a foundation for a conclusion whether the fetal and the adult enzyme preparations are composed of a similar isoenzyme pattern. Studies which make use of purified enzyme and isoenzyme preparations are needed. Nevertheless, the results presented here suggest that variants may be found in alcohol dehydrogenase during development.

Introduction

Ethanol is primarily oxidized in the liver by the enzyme alcohol dehydrogenase (ADH; Enzyme Commission Number 1.1.1.1 [13]). In other tissues, there is relatively little enzyme activity, or no activity at all. ADH from human liver has been purified by VON WARTBURG *et al.* [11] and by BLAIR and VALLEE [1], who found three chromatographically distinct active forms of the enzyme. Many of the catalytic properties of the human enzyme resemble those of the enzyme from animal liver, although differences in the Michaelis constants for substrate and coenzyme and substrate specificity have been found (VON WARTBURG *et al.* [11].

The oxidation of ethanol produces marked changes in the intermediary metabolism of the liver (FORSANDER *et al.* [4]. Since ethanol passes easily through biological membranes, and thus through the placenta, the developing fetus can come into contact with this substance during pregnancy. If the fetal liver contains ADH, ethanol is oxidized and might produce alterations in the metabolism of the fetus.

The development of ADH activity has been studied in experimental animals by RÄIHÄ *et al.* [7], but the literature does not contain any reports on the developmental aspects of human liver ADH.

In the present study, ADH activity has been measured in human liver tissue during development, and a comparison made between certain kinetic properties of crude enzyme preparations from fetal and adult liver.

Material and Methods

Human liver tissue. Fetal livers were obtained from legal abortions. The liver was removed immediately, and the tissue frozen. The age of the fetuses was estimated from weight and crown-rump length, in accordance with the tables of SCAMMON and CALKINS [9] and STREETER [10].

Liver from children and adults was acquired during surgery of the abdomen in cases where no macroscopic abnormality of the livers was observable. The liver was frozen immediately after excision. Studies indicated that the enzyme activity was stable at –20° for up to 6 days. All the determinations were made within 24 hours of sampling.

Enzyme preparation and assay for ADH activity. A 10 % liver homogenate was prepared in ice-cold 0.25 M sucrose containing 1 % Triton X-100 (obtained from Rohm & Haas Co., Philadelphia). It has previously been shown (RÄIHÄ and KOSKINEN [8] that this procedure gives maximal ADH activity in liver homogenates. After centrifugation of the homogenate for 10 minutes at

Table I. Development of alcohol dehydrogenase activity in fetal and postnatal human liver. Experimental details given in the text

	Age in months	Crown-rump length cm	Weight gm	ADH activity mU/g liver wet weight	ADH activity mU/100 mg soluble protein
Fetal	2–3	5.3	22	111	97
	3–4	8.0	35	145	135
	3–4	8.0	35	155	147
	4	11.0	90	163	155
	4	11.0	100	211	201
	4–5	13.5	160	246	236
	4–5	14.0	150	239	228
	4–5	14.0	150	411	318
	5–6	21.5	500	328	321
Postnatal	0.3			495	550
	2			444	555
	7			797	1025
	years				
	2			620	1030
	5			3170	2830
	10			945	2360
	15			1940	3880
Adult	20			1625	2030
	50			2040	2550
	50			6530	5430

5000 × g at 3° C, samples were taken from the supernatant for protein determination (LOWRY *et al.* [5] and for enzyme assays. ADH activity was estimated in accordance with the method of BONNICHSEN and BRINK [2] using a Beckman DK 1A Recording Spectrophotometer. The reaction mixture contained ethanol (100 mM), NAD (1.47 mM), 0.2 ml liver supernatant and 3 ml sodium pyrophosphate—semicarbazide—glycine —NaOH buffer at pH 8.7. The total volume was 3.4 ml. The ethanol and NAD concentration used in the assay gave maximum activity with both fetal and adult enzyme preparations. Incubations were performed at 25° C. The enzyme activity has been expressed in the units recommended by the Report of the Commission of Enzymes of I.U.B. [6].

Results

Development of ADH Activity in Human Liver

Table I presents the enzyme activity levels in human liver during development from the fetus to the adult organism. The results are expressed as milliunits per g liver wet weight and per 100 mg soluble liver protein. ADH activity is detectable in 2-month-old fetuses, although it amounts to no more than 3–4 % of that of the adult. Activities in the adult range are found after 5 years of age. Considerable variation exists in the activity of adult livers.

Properties of Fetal and Adult Liver ADH

pH-Optimum. The relation between pH and the rate of reaction appears in figure 1. The final pH values of the reaction mixtures are given; these were determined with a Radiometer pH meter. Adult human liver ADH has a pH optimum of about 10.4, and the pH optimum for the fetal enzyme preparations is 10.0.

Effects of substrate concentration. Lineweaver-Burk analyses which illustrate the relation between the concentration of ethanol and NAD, and the ADH activity in adult and fetal liver are presented in figures 2 and 3. The results are typical of three cases studied in each group. Close agreement was found for all constants in the different enzyme preparations examined. The apparent K_m values for ethanol were 3400 μM and 1100 μM, and for NAD 70 μM and 150 μM in fetal and adult enzyme preparations respectively. Similar differences in K_m values were found using enzyme preparations partly purified by ammonium sulphate precipitation and dialysis.

Discussion

In this study, wide variations in the ADH activity per gram of liver of adult individuals have been found, and

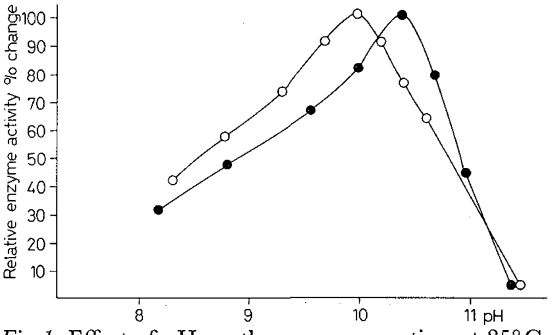

Fig. 1. Effect of pH on the enzyme reaction at 25° C. Each reaction mixture contained: ethanol (100 mM), NAD (1.47 mM), 0.2 ml adult (●) and fetal (○) human liver supernatant and 3 ml sodium pyrophosphate-semicarbazide-glycine buffer adjusted to the different pH's with NaOH. Further experimental details given in the text.

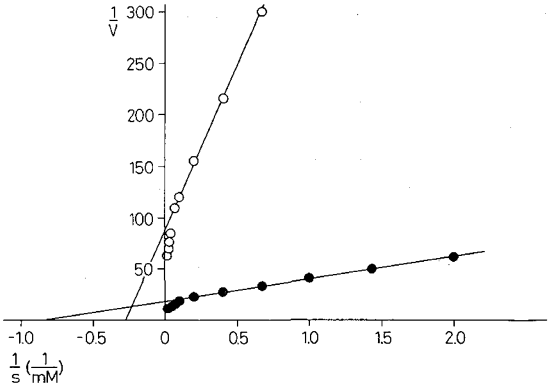

Fig. 2. Lineweaver-Burk plot of the effect of ethanol concentration on adult (●) and fetal (○) human liver ADH-activity at 1.47 mM NAD. Experimental details given in the text.

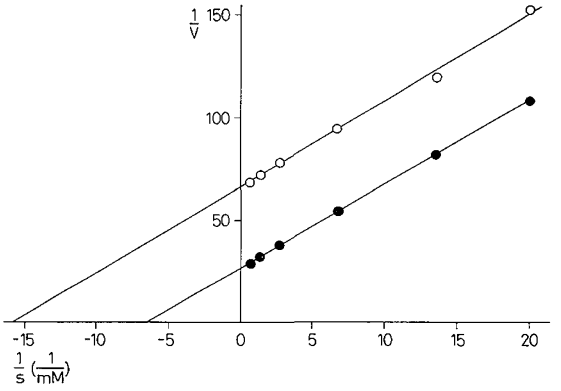

Fig. 3. Lineweaver-Burk plot of the effect of NAD concentration on adult (●) and fetal (○) human liver ADH activity at 100 mM ethanol. Experimental details given in the text.

this observation is in close agreement with the data presented by VON WARTBURG *et al.* [11] and BLAIR and VALLEE [1]. If ADH activity in liver is the only rate-limiting factor for ethanol oxidation, then the wide variation in enzyme activity between different individuals raises interesting aspects regarding variations in ethanol tolerance.

In a study of the development of liver ADH in the rat, it has been shown (RÄIHÄ *et al.* [7]) that up to a certain range the liver ADH activity is in close correspondence with the ethanol-oxidizing capacity of liver slices. It accordingly appears evident, on the basis of the present results that the human fetus and the infant have greatly reduced capacity to oxidize ethanol per gram of liver. Whether this is the case when calculated as ethanol oxidation in proportion to body weight *in vivo*, obviously depends on the size of the liver, and whether ADH is distributed evenly in the whole organ.

Only a slight difference exists in the pH optimum for ethanol oxidation between the fetal and the adult enzyme preparation, and moreover the reaction curves assume similar shapes. The apparent K_m for ethanol and for NAD differ in the fetal and adult enzyme preparations, but the divergence still falls within the same logarithmic unit. The Lineweaver-Burk plot for ethanol (fig. 2) shows a substrate activation at high ethanol concentrations. This phenomenon has recently been reported for horse liver ADH, using cyclohexanol as substrate with a large fixed NAD concentration (DALZIEL and DICKINSON [3].

These results do not constitute a foundation for determining whether the fetal and the adult enzyme preparations are composed of similar isoenzymes. Studies which make use of purified enzyme and isoenzyme preparations are needed. Nevertheless, the results presented here suggest that variants may be found in alcohol dehydrogenase during development.

Summary

The developmental pattern of human liver ADH activity has been studied, and a comparison made of some kinetic properties such as pH optimum and the effect of substrate and coenzyme concentrations on enzyme preparations from adult and fetal livers.

ADH activity is present in 2-month-old fetal livers, but amounts to no more than about 3–4 % of adult activity. The activity increases linearly, and activity comparable to that found in adult liver appears in 5-year-old subjects.

The enzyme preparation from fetal liver had a pH optimum of 10.0, and that from adult liver 10.4. The apparent K_m values for ethanol were 3400 μM and 1100 μM for fetal and adult ADH respectively, and for NAD, 70 μM and 150 μM respectively.

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