Developmental Changes in Alcohol-Dehydrogenase Activity in Rat and Guinea-Pig Liver

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1. Alcohol-dehydrogenase activity is first detectable in the rat foetus on about the eighteenth day of gestation, after which time it increases to about 25% of the adult activity at birth. Adult activity is reached at about 18 days after birth. The ethanol-oxidizing capacity of liver slices from rats correlates well with the increase of the enzyme activity *in vitro*. 2. In the guinea pig there is a steady linear increase from about 17 days before term to 5 days after birth. Adult activity is reached between the sixth and eighth postnatal day. 3. Some kinetic properties of liver alcohol dehydrogenase are very similar in newborn and adult rats. 4. Administration of ethanol to pregnant rats during the latter half of gestation had no effect on alcohol-dehydrogenase activity in the liver of the newborn offspring. Intraperitoneal injections of ethanol to newborn and young rats had no effect on the alcohol-dehydrogenase activity of the livers. 5. Intraperitoneal injections of hydrocortisone and triamcinolone to newborn and adult non-adrenalectomized rats had no significant effect on the increase of the alcohol-dehydrogenase activity as studied up to 4 days after the injection.

The activities of many enzymes in the liver are known to change during the foetal and postnatal development of the mammal and such changes reflect important alterations in the physiological functions of the liver and of the whole organism (Kretchmer, Greenberg & Sereni, 1963; Sereni & Principi, 1965).

The enzyme ADH* (EC 1.1.1.1) occurs in the hepatic parenchymal cells of most mammals studied and plays a vital role in the intermediary metabolism of ethanol (Lieber & Davidson, 1962). This enzyme has been extensively studied with respect to its kinetics and mechanism of action (Sund & Theorell, 1963). Some workers (Greenberger, Cohen & Isselbacher, 1965) studying the adaptation of ADH in adult mammalian liver to administration of ethanol have found that its activity is little affected. Others report a significant increase in ADH activity in rats after chronic ethanol administration (Hawkins, Kalant & Khanna, 1966).

The authors are not aware of any studies on developmental changes in ADH activity in mammalian liver. Since oxidation of ethanol in the liver has been shown to produce marked effects on the overall intermediary metabolism of the liver (Forsander, Räihä, Salaspuro & Mäenpää, 1965), it would be desirable to know whether at any stage of its development the foetal or newborn animal is capable of oxidizing ethanol.

In the present paper the course of development of ADH activity, as assayed by experiments *in vitro* and the ethanol-oxidizing capacity of liver slices, have been studied during perinatal development in rats and guinea pigs. The effect of administration of ethanol to pregnant and newborn animals was also investigated and it was shown that the development of ADH activity could not be affected by ethanol administration.

* Abbreviation: ADH, alcohol dehydrogenase.

MATERIALS AND METHODS

Animals. The rats used were from our own Wistar albino strain fed on a standard laboratory diet. Birth dates of the litters studied were carefully recorded after frequent inspection. The gestational age of the rats was estimated from their weight according to Donaldson's (1924) charts. The adult rats used were males aged 2–4 months. The age of the foetal guinea pigs was estimated from Draper's Tables (Needham, 1931). The animals were killed by decapitation and the liver samples blotted and weighed when cold.

Assay for alcohol-dehydrogenase activity. For the estimation of ADH activity a 10% liver homogenate was prepared in ice-cold 0·25M-sucrose containing 1% Triton X-100 (obtained from Rohm and Haas Co., Philadelphia, Pa., U.S.A.), which gives maximal ADH activity in liver homogenates (Räihä & Koskinen, 1964). After centrifugation of the homogenate for 10min. at 5000*g*, samples were taken from the supernatant for protein determination

(Lowry, Rosebrough, Farr & Randall, 1951) and for enzyme assay. ADH activity was estimated by the method of Bonnichsen & Brink (1955) at pH 8·7 and 25°, with a Beckman DK 1A recording spectrophotometer. Activity is expressed in the units recommended by the Commission of Enzymes of I.U.B. (1961) both per g. wet wt. of liver and per mg. of soluble protein. Usually the assays for ADH activity were performed immediately after excision of the livers, but in some cases the livers were stored overnight. The stability of the ADH activity in stored liver samples was examined and found to be stable for 5 days when the liver was stored at −18°. At a temperature of 1°, ADH activity of rat liver was unchanged up to 4 hr.

Ethanol oxidation by liver slices. The capacity of the liver to oxidize ethanol was measured by using conditions similar to those described by Forsander (1966). The assay medium consisted of ethanol (6·8 mM) and glucose (11·0 mM) in 10 ml. of Krebs–Ringer phosphate buffer, pH 7·4. Approx. 0·5 g. of liver slices prepared with a Stadie–Riggs tissue slicer were used. The gas phase was O_2+CO_2 (95:5) and the final volume of the reaction mixture was 10·8 ml. The tissue slices were incubated at 37° for 1 hr. in stoppered flasks and the reaction was ended by addition of perchloric acid. Two flasks to estimate evaporation of ethanol during incubation were run in each experiment. After deproteinization the ethanol in the supernatant was determined enzymically by the method described by Bücher & Redetzki (1951) with Biochemica Boehringer (Mannheim, W. Germany) test kits.

Effect of ethanol. The effect of ethanol on liver ADH activity was determined by administration of 10% ethanol to pregnant rats as the sole fluid available from the twelfth day of pregnancy onward. In addition, the pregnant rats received 375 mg. of ethanol (30%, v/v)/100 g. body wt. daily by stomach tube during 4 days before delivery. The livers of the newborn rats were assayed for ADH activity at birth. Other experiments were performed in which 200 mg. of ethanol/100 g. body wt. was administered intraperitoneally daily to newborn rats of different ages, controls receiving the same volume of 0·9% NaCl. The animals were killed 24 or 72 hr. later by decapitation and their livers analysed for ADH activity.

Effects of steroid hormones. A newborn litter of rats was divided into two parts, one part being injected intraperitoneally with 20 mg. of hydrocortisone–sodium succinate/100 g. body wt. (The Upjohn Co., Kalamazoo, Mich., U.S.A.). The rest of the litter, serving as a control, was given an equal volume of 0·9% NaCl. The ADH activity of the liver was studied 24 and 72 hr. later. In experiments on adult rats (2 months old) 10 mg. of hydrocortisone/100 g. body wt. was injected intraperitoneally and ADH activity was studied in the liver 24 hr. later. In other experiments 6 mg. of triamcinolone/100 g. body wt. (Lederle Laboratories, Pearl River, N.Y., U.S.A.) was injected intraperitoneally into newborn rats and the development of ADH activity was studied for 4 days after the injection. Controls received an equal volume of 0·9% NaCl.

RESULTS

Development of alcohol-dehydrogenase activity and ethanol-oxidizing capacity in the foetal, postnatal and adult rat and guinea-pig liver. The developmental pattern of ADH activity in rat liver is shown in Fig. 1. ADH activity is first detectable in measurable quantities in the foetal liver on about the eighteenth day of gestation. From this age on, the activity increases fairly linearly, reaching adult levels at approx. 18 days after birth. At birth the activity is one-fourth of the adult activity. When the ADH activity is expressed in milliunits/mg. of soluble protein the increase is similar. Fig. 2 shows that the ethanol-oxidizing capacity of liver slices correlates well with the developmental pattern of the ADH activity measured *in vitro*.

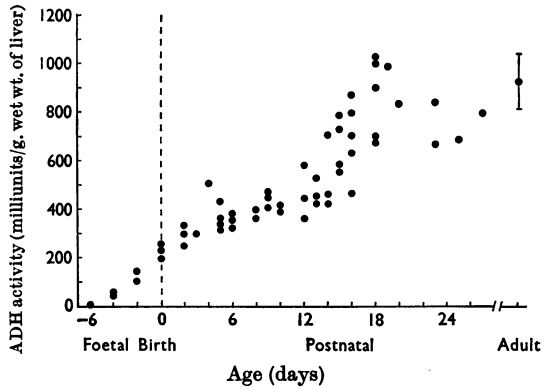

Fig. 1. Development of ADH activity in the foetal and postnatal rat liver.

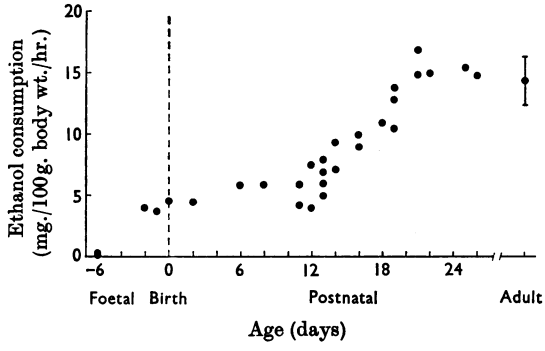

Fig. 2. Development of ethanol-oxidizing capacity in the foetal and postnatal rat liver.

The first measurable ADH activity in guinea-pig liver during development was detected 17 days before birth. From this age on the increase in activity is slow and linear until the fifth postnatal day. The activity at birth is 20% of adult activity. Adult activity is reached between the sixth and the eighth day postnatally.

Properties of alcohol-dehydrogenase activity during development. The properties of ADH of adult and newborn rat liver were studied with respect to dependency on pH, NAD and ethanol concentra-

Table 1. *Effect of ethanol administration on alcohol-dehydrogenase activity in newborn and postnatal rat liver.*

Pregnant rats received 10% ethanol as the only fluid intake during the latter half of gestation, and in addition, 375mg. of ethanol (30%, v/v)/100g. body wt. by stomach tube daily during the 4 last days of pregnancy. Control rats received water *ad lib.* and 0·9% NaCl by stomach tube during the 4 last days of gestation. The offspring were studied at birth. Postnatally the litters, two in each experiment, were divided so that one half received intraperitoneal injections of 200mg. of ethanol (20%, v/v)/100g. body wt. daily, the other half receiving 0·9% NaCl. Newborn rats were studied 24hr. and older offspring 72hr. after the first injection. The results are expressed as means ± S.D. Each determination represents five pooled livers.

Expt. no.		Age of rats when killed for assay (days)	No. of observations	ADH activity (milliunits/g. wet wt. of liver)
1	Ethanol	0	8	416 ± 85
	Control	0	5	438 ± 91
2	Ethanol	1	4	589 ± 74
	Control	1	3	594 ± 44
3	Ethanol	8	2	757
	Control	8	2	754
4	Ethanol	12	4	967 ± 47
	Control	12	2	975

tion. It was found that the pH optimum was about 8·7 in both cases. The effect of change in NAD or ethanol concentration was very similar in newborn and adult liver. The apparent K_m values, as determined by the method of Lineweaver & Burk (1934), were 280 μM and 270 μM for NAD and 240 μM and 330 μM for ethanol in newborn and adult liver respectively.

Effect of ethanol administration on alcohol-dehydrogenase activity in newborn and postnatal rat liver. Administration of ethanol to pregnant mothers had no effect on the liver ADH activity at birth (Table 1). In experiments in which ethanol was administered as intraperitoneal injections to young rats of various ages and for periods up to 3 days no significant effect on the postnatal increase of ADH activity could be detected (Table 1).

Effect of steroid hormones on the development of alcohol-dehydrogenase activity. A single intraperitoneal injection of triamcinolone (6mg./100g. body wt.) to newborn rats had no effect on the postnatal increase of the ADH activity as measured up to 4 days after the injection. Two experiments were performed and three livers from different animals were pooled in each experiment. The control animals received an equal volume of 0·9% sodium chloride. No change was observed in the amount of soluble supernatant protein in the two groups during this time. When adult animals were given intraperitoneal injections of 12mg. of triamcinolone/100g. body wt. no increase in ADH activity was observed as compared with controls receiving 0·9% sodium chloride up to 6 days after the injection. Hydrocortisone injections at birth had no effect on the postnatal increase of ADH activity as studied up to 3 days after the injection. Hydrocortisone was without effect on the ADH activity in adult liver studied 24hr. after the injection.

In preliminary experiments on four animals it was shown that adrenalectomy performed at birth had no effect on the postnatal increase of ADH activity as studied 48hr. after the operation.

DISCUSSION

The developmental pattern of liver ADH in the rat and the guinea pig shows a linear increase of activity throughout development and no post-parturition peak is observed, as in many other enzymes such as tryptophan peroxidase (Nemeth, 1959), tyrosine transaminase (Sereni, Kenney & Kretchmer, 1959), some of the gluconeogenic enzymes (Ballard & Oliver, 1962) and the urea-synthesizing enzymes (Kennan & Cohen, 1959).

In the rat the development of ADH activity, assayed in experiments *in vitro*, is positively correlated with the developmental pattern of the ethanol-oxidizing capacity of liver slices, indicating that at least during development, when ADH activity is lower than in adult animals, the activity of the enzyme present is a factor regulating the rate of ethanol oxidation.

During mammalian development one well-known phenomenon is the appearance of one form of protein enzyme at a specific period, after which it disappears and is replaced by another with a similar function. The change in the pattern of isoenzymes of lactate dehydrogenase during development has been demonstrated by Cahn, Kaplan, Levine & Zwilling (1962). A similar pattern has been observed in liver glucokinase (Walker, 1962; Ballard & Oliver, 1964).

In the light of these observations the kinetic properties of the ADH present in liver of newborn animals were compared with those of liver of adult animals. O. A. Forsander & P. Saarinen (personal communication) have demonstrated the presence of at least four different ADH isoenzymes in adult rat liver. The absence of any change in the studied properties of ADH during development does not necessarily exclude the possibility that the isoenzyme pattern changes. This will depend on whether the studied properties of the isoenzyme forms are the same.

In an attempt to influence the linear increase of ADH activity during development by substrate induction, ethanol was administered at different stages of development and for various periods. As is observed from the results, ethanol had no effect on the linear development of ADH activity. In long-term experiments with adult rats a slight increase in liver ADH activity has been reported (Hawkins *et al.* 1966), although contradictory results have also been found (Greenberger *et al.* 1965). Thus it seems unlikely that ADH activity can be induced in mammalian liver by ethanol, although some other enzymes, especially some concerned with nitrogen metabolism, can be induced in liver by a substrate-type mechanism (Knox & Greengard, 1965). Whether some other substrates of liver ADH can induce the enzyme activity in the liver remains to be studied.

The activity of many liver enzymes can be increased by administration of steroid hormones, but whether this reflects a general increase in liver protein synthesis or a selective induction of certain enzyme proteins is still uncertain (Kenney, Greenman, Wicks & Albritton, 1965).

It has been suggested that the magnitude of response of an enzyme to an agent causing an increase in the rate of synthesis will depend on the rate of turnover of the enzyme (Berlin, 1965). The present experiments show that ADH is not affected by steroid hormone administration during the time-period studied.

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