Changes in Glutaminase Activities of Rat Liver and Kidney during Pre- and Post-Natal Development

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The activities of two phosphate-dependent glutaminase reactions characteristic of adult rat liver and kidney were determined in these organs from 2½ days before to 7 days after birth and compared with the activities in the adult tissues. In the kidney, before and after birth, only the kidney type of activity was detected, and it increased in concentration in parallel with the steady growth of that organ throughout the period examined. In the liver, however, the kidney type of activity was the only one present 2½ days before birth, and its concentration decreased to barely significant values by the end of the first week after birth. In contrast, the liver type of activity appeared only just before birth and increased to 60% of adult values over the next 4 days. There was no obvious relation between these changes in glutaminase type and changes in liver weight, protein content and total cell number that occurred during this time. But there was a very close correlation between the fall in kidney-type activity and the estimated fall in haematopoietic-cell number in liver.

In the adult rat two kinds of phosphate-dependent glutaminase activities (EC 3.5.1.2) can be distinguished, one characteristic of the liver, the other characteristic of the kidney and present also in most other tissues (Horowitz & Knox, 1968). New ways of measuring these two activities under optimum conditions were devised, and preliminary experiments indicated that foetal liver did not contain the adult liver type of enzyme but instead contained the kidney type (Horowitz & Knox, 1968). The kidney-type enzyme had also been found in neoplastic liver (Linder-Horowitz, Knox & Morris (1969). Foetal kidney contained the kidney type of glutaminase but at concentrations much lower than in the adult. The purpose of the research reported here was to document the changes in the activities of these two forms of glutaminase in liver and kidney immediately before and after birth in relation to organ size, cell number, protein content and other biochemical properties that change during development.

METHODS

Foetal and newborn rats were of the NEDH strain obtained from litters of seven to 11 animals. With foetal rats entire litters were used, whereas with newborn rats

two or four from a given litter were used. For all rats the length from crown to rump (in mm.) was measured and averaged for all rats of the same age. Results are given as means $\pm$ s.d. The age of foetal rats was estimated by using the data of Gonzalez (1932). (Such ages were used in plotting the changes, with age, of the various parameters.)

Preparation of tissues. For foetal rats, livers from a whole or half litter, as indicated, were pooled, weighed and homogenized with 4 vol. of 0.15 M-KCl. Kidneys from a whole litter were always pooled and, after being weighed, were homogenized with 9 vol. of 0.15 M-KCl. Livers or kidneys of two or four newborn rats were pooled, weighed and homogenized as for foetal organs. Portions of homogenates were diluted with 0.9% NaCl to a final concentration of 1% tissue. Nuclei were counted as described by Knox, Horowitz & Friedell (1969), and protein content (as albumin) was determined by the method of Lowry (Layne, 1957) with this diluted homogenate. Undiluted homogenates were assayed for glutaminase activities.

Assay of glutaminase activities. Assays were based on those of Horowitz & Knox (1968) for measurement of maximum phosphate-dependent glutaminase activities in adult rat liver and kidney. Assays were performed in duplicate and depended upon the amount of ammonia formed from glutamine during a 20 min. incubation with 0.2 ml. of tissue homogenate at 37° in a total vol. of 1.0 ml. Reactions were stopped by chilling the samples to 0° and adding *p*-mercuribenzoate to a final concentration of 2 mM. Ammonia was distilled from the samples by the method of Archibald (1944) by using the apparatus described by Preuss, Bise & Schreiner (1966) with a water aspirator. In this procedure, samples were brought to pH 10.8 with saturated borate buffer and immersed in a 60° water bath

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for exactly 5 min., from the addition of borate. The ammonia was trapped in 4.0 ml. of 0.04 M- H_2SO_4 and determined in samples (4.5 ml. or less) to which 0.5 ml. of Nessler's solution (Koch-McMeekin) (Fischer Scientific Co., Mass., U.S.A) was added. The extinction was measured exactly 30 min. thereafter in a Klett colorimeter with no. 42 filter.

The activities of the two different phosphate-dependent glutaminase reactions, when present together or alone, could be calculated by using simultaneous equations from the results of assays under four conditions: (a) pH 7.6, 0.10 M-L-glutamine, 2 mM-*p*-mercuribenzoate; (b) pH 7.6, 0.10 M-L-glutamine, 0.010 M-phosphate; (c) pH 8.6, 0.040 M-L-glutamine, 2 mM-*p*-mercuribenzoate; (d) pH 8.6, 0.040 M-L-glutamine, 0.20 M-phosphate. The simultaneous equations were:

Liver-type activity = $1.240(\text{I}) - 0.186(\text{II})$
(as at pH 7.6, 0.050 M-phosphate)

Kidney-type activity = $1.166(\text{II}) - 1.104(\text{I})$
(as at pH 8.6, 0.20 M-phosphate).

where (I) is the difference between values for conditions (b) and (a) and (II) is the difference between values for conditions (d) and (c). The phosphate-independent glutaminase activity present was determined directly from the assay (c). These equations were based on results obtained with liver and kidney homogenates from adult rats (Horowitz & Knox, 1968), assuming that no kidney type of activity was present in adult liver, and vice versa. From assays of mixtures of adult kidney and liver homogenates, the error of this method for determining a given activity was about 15% if the activity comprised more than 40% of the total activity in the mixture. It was about 50% when the activity comprised less than 20% of the total.

Activities calculated (or determined directly) are expressed as $\mu\text{moles of NH}_3$ formed/min. (units)/g. of tissue, or as total units/organ, a pair of kidneys being considered one organ.

RESULTS

Changes in organ weight, protein content and cellularity. Figs. 1 and 2 show the changes in organ weight, protein content and cellularity of liver and kidney with crown-rump length and age. The period covered was from 2.4 days before birth to 7 days after birth. There was a large increase in liver weight in the last days of gestation (an increase of about 0.13 g./day/rat) (Fig. 1). At birth the liver weight fell dramatically, then remained unchanged for 4 days and increased to the pre-birth value by the seventh day. My findings are similar to those of Lang (1965) before birth and of Oliver, Ballard, Shield & Bentley (1962) after birth. About a day before the fall in liver weight at birth, there was a decrease in the total number of liver cells that continued through the first day after birth. And, although liver weight remained static during the next 3 days, the cell number increased slowly and continued to increase at the same rate through to the seventh day. There were sizable changes in

Fig. 1. Changes in crown-rump length, liver weight, cellularity and protein content with development before and after birth in the rat. Values plotted are means for the numbers of rats used (given in parentheses) or for individual determinations on three different pools of livers. Individual values were within 10% of the value of the mean except for those starred (*), which were within 20%. The ages of foetal rats were estimated from the crown-rump length by using the data of Gonzalez (1932). The total number of cells/liver was estimated from the number of nuclei counted/g. of tissue, assuming one nucleus/cell. $\circ$, Liver weight (g.); $\bullet$, total no. of cells (no. of nuclei/liver); $\blacktriangle$, cellularity (no. of cell nuclei/g.); $\triangle$, protein content (mg./g.).

the number of cells counted/g. of liver during the period examined. In the last days of gestation the number fell by about 60%, suggesting a large increase in the size of at least some of the cells present. There was relatively little change thereafter up to 7 days after birth, but even then the number of cells/g. was still three times that of adult liver. Protein content, which averaged about half the adult value before birth, increased to within 15% of the adult value by the end of the first week after birth.

The growth of the kidney proceeded with less fluctuation than that of the liver (Fig. 2). Kidney weight and total number of cells increased linearly, fourfold, in the last 2½ days before birth, and increased steadily but at a lower rate during the first week after birth (threefold in 7 days). There was little change in the number of kidney cells/g. during the time examined, but the number was only about twice that of adult kidney. Protein content averaged about two-thirds that of the adult value

Fig. 2. Changes in crown-rump length, kidney weight, cellularity and protein content with development before and after birth in the rat. Values given are as in Fig. 1, but determinations of cell number and protein content were done on only two pools of tissue each. O, Kidney weight (g.); ●, total no. of cells (no. of nuclei/kidney); ▲, cellularity (no. of cell nuclei/g.); △, protein content (mg./g.).

before birth and increased nearly to adult values by the end of the first week after birth.

Changes in glutaminase activities in liver. There were dramatic changes in the concentrations of two glutaminase isoenzymes in liver during the period examined (Fig. 3 and Table 1). About 2 days before birth foetal liver contained only one form of glutaminase and this was not the type of phosphate-dependent enzyme found in the adult organ but rather the 'kidney type' of phosphate-dependent glutaminase found in most non-hepatic tissues (Horowitz & Knox, 1968). Kidney-type glutaminase activity fell sharply during the period before birth and continued to fall after birth to barely significant values at the end of the first week after birth. The fall seemed to be exponential whether plotted as units/g. (Fig. 3) or as total units/organ (Table 1), with a half-time of 3–4 days. There was no obvious correlation between the fall in kidney-type glutaminase activity and the changes in liver weight and cellularity that occurred. However, these changes are complicated by the presence of considerable amounts of haematopoietic tissue in liver, as discussed below.

The type of glutaminase characteristic of adult liver was first detected about $\frac{1}{2}$ day before birth, and rose dramatically to concentrations equal to

Fig. 3. Changes in concentrations of kidney and liver types of phosphate-dependent glutaminase activities in livers of rats before and after birth. Determinations of kidney-type (●) and liver-type (×) activity of individual pools of livers were plotted as units/g. The stippled areas encompass all the individual determinations.

those of the kidney-type enzyme directly after birth and reached about 60% of the concentration in adult liver by the fourth day after birth (Fig. 3). Again there was no obvious correlation between the rise in liver-type activity and the changes in liver weight and cellularity. Instead, the changes in activity occurred during a period in which there was little cell proliferation and in which the average cell size tended to decrease, if it changed at all.

Very small amounts of *p*-mercuribenzoate-insensitive glutaminase activity ('phosphate-independent') were detected beginning about 2 days before birth and throughout the period examined (Table 1). But in contrast with the report by Katunuma, Katsumura, Tomino & Matsuda (1968), this activity did not disappear by the end of the first week after birth and could be detected even in the liver homogenates from 66-day-old rats.

Enzyme changes in the kidney. Changes in the concentrations and type of glutaminase were smaller in the kidney than in the liver (Table 2). A considerable concentration of the phosphate-dependent glutaminase normally found in adult kidney was present more than 2 days before birth. This increased two- to three-fold during the first 4 days

Table 1. *Changes in the concentrations of kidney-type and liver-type phosphate-dependent glutaminase activities in rat liver before and after birth*

Crown-rump lengths are given as means $\pm$ s.d., with numbers of rats in parentheses. For foetal rats, livers from a whole or a half litter were pooled and assayed (see the Methods section). Ages of foetal rats were estimated from the crown-rump length by using the data of Gonzalez (1932). Values for glutaminase activity are the means of assays of three individual pools of liver. Individual values were within 10% of the mean.

Crown-rump length (mm.)	Age (days)	Glutaminase activity (total units/organ)		
		Phosphate-dependent (pH 8.6)	Phosphate-dependent	
			Kidney type	Liver type
28 $\pm$ 1 (11)	-2.4	0.00	0.61	0.01
29 $\pm$ 1 (23)	-2.2	0.02	0.76	0.01
32 $\pm$ 1 (19)	-1.7	0.09	0.96	0.03
39 $\pm$ 1 (15)	-0.5	0.11	0.73	0.30
	Birth			
39 $\pm$ 1 (6)	+0.5	0.06	0.50	0.65
43 $\pm$ 1 (10)	+3.8	0.07	0.30	1.57
47 $\pm$ 3 (6)	+6.8	0.17	0.22	2.07
	Adult			
(6)	66	0.6 $\pm$ 0.2*	0.0 $\pm$ 0.1*	7.7 $\pm$ 2.4*
(6)	86	0.1 $\pm$ 0.1*	0.0 $\pm$ 0.1*	10.0 $\pm$ 2.7*

* Activity given as units/g. rather than units/organ.

Table 2. *Changes in glutaminase activities in rat kidney before and after birth*

Crown-rump lengths are given as means $\pm$ s.d., with numbers of rats in parentheses. Ages of foetal rats were estimated from the crown-rump length by using the data of Gonzalez (1932). Values given for glutaminase activity are the means of assays of two individual pools of kidney, except those for liver-type activity, which are assays of single pools of kidney. Individual values were within 10% of the mean except for those starred (*) which were within 20% of the mean.

Crown-rump length (mm.)	Age (days)	Glutaminase activity			
		(units/g.)		(total units/organ)	
		Phosphate-dependent		Phosphate-independent (pH 8.6)	Phosphate-dependent kidney type
		Kidney type	Liver type		
28 $\pm$ 1 (11)	-2.4	6.5	—	0.0	0.06
29 $\pm$ 1 (23)	-2.2	3.5*	—	0.0	0.04*
32 $\pm$ 1 (8)	-1.7	12.6	-0.6	0.6	0.29
39 $\pm$ 1 (15)	-0.5	8.3	+0.5	0.9*	0.29
	Birth				
39 $\pm$ 1 (6)	+0.5	11.2	-0.1	1.3*	0.59
43 $\pm$ 1 (10)	+3.8	19.6	-1.7	1.3	1.69
47 $\pm$ 3 (6)	+6.8	16.8	0.9	1.1	2.41
	Adult				
(6)	66	28.0 $\pm$ 8.2	0.0	5.9 $\pm$ 1.0	
(6)	86	38.5 $\pm$ 8.4	0.0	5.0 $\pm$ 1.3	

after birth to about half the adult value. In terms of total enzyme units/organ, the activity increased linearly with age during the period examined and a good correlation (correlation coefficient = 0.98, 11 degrees of freedom, $P \leq 0.001$) between total units and organ weight could be shown. Little phosphate-independent glutaminase, which also occurs in the adult kidney (Horowitz & Knox, 1968; Katunuma *et al.* 1968), was detected until just

before birth, and its amount remained relatively low during the first week after birth (Table 2).

DISCUSSION

Others have reported the presence of phosphate-dependent glutaminase activity in foetal rat liver (Katunuma, Tomino & Nishino, 1966; Raina & Ramakrishnan, 1964) and pig kidney (Radde &

McCance, 1959) and followed the development of the activity in rat kidney after birth (Wacker, Zarkowsky & Burch, 1961). My results with new assay methods confirmed the presence of glutaminase in the foetal tissues and identified the predominant isoenzyme in both tissues as the phosphate-dependent glutaminase typical of adult kidney. In the kidney of the newborn rat, the concentration of phosphate-dependent glutaminase increased in a manner related to kidney growth during the first week after birth. In the liver, the dramatic change in isoenzyme content that occurred mainly within the 2 days before and after birth was not correlated with changes in the other parameters examined here: liver weight, protein content and total cell number. The sharp fall in liver weight at birth described here has not been commonly reported, but was a very consistent result in this laboratory. As much as one-third of this fall in weight may be accounted for by the fall in liver glycogen content occurring at this time. Just before birth glycogen comprises up to 10% of the liver weight of the rat foetus, but falls to less than 1% within 24 hr. (Ballard & Oliver, 1963). The fall in liver weight may also reflect a release of haematopoietic cells from the liver. But cell counts during this period suggest that this is not a major factor, since haematopoietic cells seem to be released at fairly constant rates throughout the end of gestation and after birth (Finck & Thiel, 1964; Oliver, Blumer & Witham (1963). Moreover, I did not find a sharp fall in total cell number coincident with birth. Thus the major reason for the fall in liver weight at birth remains to be explained as a reflection of dehydration or other occurrences.

In the foetal rat a large proportion of the cells in liver is haematopoietic. During foetal life and during the first week after birth the ratio of haematopoietic to total cells decreases nearly exponentially, falling from about 46% at 3 days before birth to about 28% at birth (Finck & Thiel, 1964) and then to about 7% by the seventh day after birth (Oliver *et al.* 1963). The number of haematopoietic cells in liver at various times during this period may be estimated from these reported results and my total cell counts. When the results are compared with the fall in total units of kidney-type phosphate-dependent glutaminase activity that occurs (Table 2, last column) a very good correlation is obtained (correlation coefficient = 0.86, 6 degrees of freedom, $P \leq 0.01$) (Fig. 4). Thus it is possible that the kidney type of activity is present in the haematopoietic cells rather than the parenchymal cells of foetal liver, and as the former are released the enzyme concentration falls. Since the work of Greenstein, Fodor & Leuthardt (1949) and Greenstein (1954) the enzyme patterns and isoenzyme contents of hepatomas and other neoplastic

Fig. 4. Correlation between total amount of kidney-type glutaminase activity in liver and number of haematopoietic cells. Total units of kidney-type glutaminase activity in livers of rats before and after birth were plotted against numbers of haematopoietic cells present at these times as reported by Finck & Thiel (1964) and Oliver *et al.* (1963).

tissues have increasingly been compared with those of foetal liver, and many similarities have been found (Rutter, Blostein, Woodfin & Weber, 1963; Schapira, Dreyfuss & Schapira, 1963; Sheid, Morris & Roth, 1965; Sugimura, Ikeda, Hirota, Hozumi & Morris, 1966). It may be that these similarities are actually to be ascribed to the haematopoietic cells present in foetal liver, and that it is the enzyme pattern of these cells rather than that of the parenchymal liver cells that is similar to that of neoplasms.

In contrast with the kidney-type phosphate-dependent glutaminase in liver, the adult liver-type enzyme is not detectable until immediately before birth and increases within 4 days to 60% of the adult concentration (about 85% of the concentration in 2-month-old rat liver). The rise in this, a mitochondrial enzyme (Horowitz & Knox, 1968), may be associated with the rise in the concentration of liver cell mitochondria that occurs at this time and is nearly complete by the fifth day after birth (Dawkins, 1959).

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