Biochem. J. (1971) **121**, 49–53
Printed in Great Britain

Activities of Enzymes of Ketone-Body Utilization in Brain and Other Tissues of Suckling Rats

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(*Received* 30 *July* 1970)

1. The activities of 3-hydroxybutyrate dehydrogenase and 3-oxo acid CoA-transferase in rat brain at birth were found to be about two-thirds of those of adult rat brain, expressed per g wet wt. The activities rose throughout the suckling period and at the time of weaning reached values about three times higher than those for adult brain. Later they gradually declined. 2. At birth the activity of acetoacetyl-CoA thiolase in rat brain was about 60% higher than in the adult. During the suckling period there was no significant change in activity. 3. In rat kidney the activities of the three enzymes at birth were less than one-third of those at maturity. They gradually rose and after 5 weeks approached the adult value. Similar results were obtained with rat heart. 4. The activity of glutamate dehydrogenase (a mitochondrial enzyme like 3-hydroxybutyrate dehydrogenase and 3-oxo acid CoA-transferase) also rose in brain and kidney during the suckling period, but at no stage did it exceed the adult value. 5. Throughout the suckling period the total ketone-body concentration in the blood was about six times higher than in adult fed rats, and the concentration of free fatty acids in the blood was three to four times higher. 6. It is concluded that the rate of ketone-body utilization in brains of suckling rats is determined by both the greater amounts of the key enzymes in the tissue and the high concentrations of ketone bodies in the blood. In addition, the low activities of the relevant enzymes in kidney and heart of suckling rats may make available more ketone bodies for the brain.

Experiments by Drahota, Hahn, Mourek & Trojanová (1965) and Itoh & Quastel (1970) suggest that slices of brain from infant rats utilize ketone bodies more rapidly than do slices from adult brain. This raises the question whether brain of the immature rat has higher activities of the enzymes concerned in ketone-body utilization, namely 3-hydroxybutyrate dehydrogenase (EC 1.1.1.30), 3-oxo acid CoA-transferase (EC 2.8.3.5) and acetoacetyl-CoA thiolase (EC 2.3.1.9). It has already been shown that 3-hydroxybutyrate dehydrogenase activity is higher in the brain of infant rats than in adults, and declines after weaning (Klee & Sokoloff, 1967). In adult brain the other enzymes of ketone-body utilization are also present, but like 3-hydroxybutyrate dehydrogenase do not alter in amount in situations where ketone bodies are an important fuel of respiration such as starvation and alloxan-diabetes (Williamson, Bates, Page & Krebs, 1971).

The present paper reports measurements of the activities of the three enzymes of the ketone-body utilization pathway in brain, kidney and heart of suckling and adult rats. The results indicate that the greater utilization of acetoacetate and 3-hydroxybutyrate by immature brain slices is paralleled by increased activities of 3-hydroxybutyrate dehydrogenase, 3-oxo acid CoA-transferase and acetoacetyl-CoA thiolase. Robinson & Hall (1970) have also reported briefly that the activities of these enzymes are higher in immature brain. In contrast, the activity of all three enzymes in kidney and heart of suckling rats is lower than in the adult tissues.

MATERIALS AND METHODS

Animals. Pregnant Wistar rats were kept in individual cages and fed on a commercial diet containing approx. 15% of protein, 3% of fat and 80% of carbohydrate (Oxoid breeding diet for rats and mice; Oxoid Ltd., London S.E.1, U.K.) After birth the infant rats were kept with their mother until they were weaned at 21 days.

Blood. The rats were killed by decapitation (without anaesthesia) and blood was collected in a small cup-shaped piece of Parafilm containing 5μl of heparin. A sample of blood (0.2ml) was deproteinized with 2.0ml of 6% (v/v) $HClO_4$ and neutralized as described by Williamson, Veloso, Ellington & Krebs (1969). Plasma was

obtained by centrifugation for 15min at 3000rev./min at 4°C.

Determination of metabolites. The following metabolites were determined in whole blood by standard enzymic methods: lactate and pyruvate (Hohorst, Kreutz & Bücher, 1959); acetoacetate and 3-hydroxybutyrate (Williamson, Mellanby & Krebs, 1962); glucose (Slein, 1963); glycerol (Eggstein & Kreutz, 1966). Free fatty acid concentrations were determined in plasma (Itaya & Ui, 1965).

Preparation of homogenates. Tissue homogenates of brain, liver, kidney and heart were prepared in glass homogenizers as described by Williamson *et al.* (1971). The medium of Lehninger, Sudduth & Wise (1960) was used for homogenization. Organs (two to ten) from suckling rats of the same litter were pooled to give adequate material for assay. The homogenates were treated with ultrasound for two 30s periods at 15kHz (100W model, Measuring and Scientific Equipment Ltd., London S.W.1, U.K.). The 3-hydroxybutyrate dehydrogenase determinations were carried out on the whole homogenate after sonic treatment. The supernatant obtained after centrifugation of this homogenate at 10000rev./min for 20min was used for the assay of 3-oxo acid CoA-transferase, acetoacetyl-CoA thiolase and glutamate dehydrogenase.

Determination of enzyme activities. 3-Oxo acid CoA-transferase activity was determined by measuring the rate of disappearance of acetoacetyl-CoA in the presence of succinate (Williamson *et al.* 1971). Acetoacetyl-CoA thiolase activity was determined by measuring the decrease in E_{303} due to the cleavage of acetoacetyl-CoA (Stern, 1956; Williamson *et al.* 1971). 3-Hydroxybutyrate dehydrogenase activity was determined by measuring the acetoacetate formed after incubation of the enzyme sample with DL-3-hydroxybutyrate in the presence of NAD^+ (Williamson *et al.* 1971). Glutamate dehydrogenase activity was measured as described by Williamson, Lund & Krebs (1967).

Units of enzyme activity. All measurements of enzyme activity were carried out at 25°C. A unit of enzyme activity is defined as the amount of enzyme that transforms 1 μmol of substrate/min at 25°C; specific activity is defined as units/g fresh wt. of tissue.

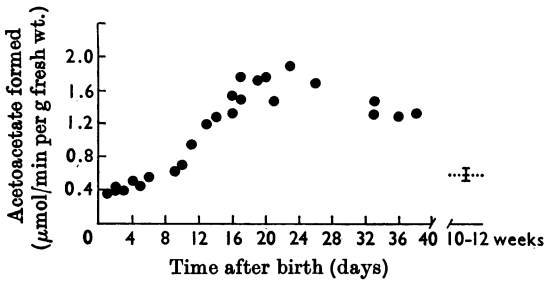

Fig. 1. Activity of 3-hydroxybutyrate dehydrogenase in brain during postnatal development of the rat. The enzyme activity was determined as described in the Materials and Methods section. Each point represents the mean value for two to ten rats taken from the same litter. The broken line with vertical bar is the mean ± S.D. of 26 adult rats.

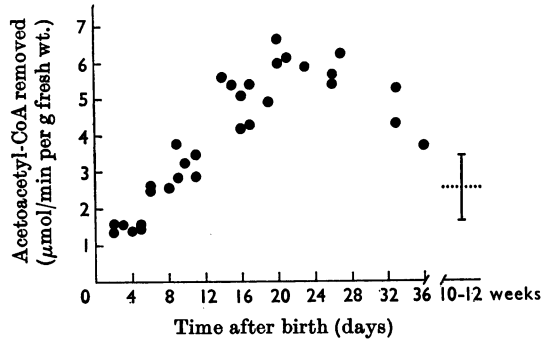

Fig. 2. Activity of 3-oxo acid CoA-transferase in brain during postnatal development of the rat. The enzyme activity was determined as described in the Materials and Methods section. Each point represents the mean value for two to ten rats taken from the same litter. The broken line with vertical bar is the mean ± S.D. of 38 adult rats.

RESULTS

Activity of the ketone-body-utilization pathway in immature brain. The activity of 3-hydroxybutyrate dehydrogenase in brain rose throughout the neonatal period and reached a maximum at the time of weaning (21 days). At birth the 3-hydroxybutyrate dehydrogenase activity was about 0.4 unit/g, which is somewhat lower than the adult value of 0.58 unit/g (Fig. 1), whereas by 21 days the activity had increased about fourfold to 1.7 units/g. Thereafter the activity slowly fell towards the adult value (Fig. 1). This pattern of change of 3-hydroxybutyrate dehydrogenase activity in immature brain is in agreement with that reported by Klee & Sokoloff (1967), although the activity observed here is higher. The changes in brain 3-oxo acid CoA-transferase activity paralleled those of 3-hydroxybutyrate dehydrogenase (Fig. 2). The transferase activity rose from 1.0 unit/g to about 6 units/g at 21 days, and then fell slowly towards the adult value of 2.5 units/g. In contrast, brain acetoacetyl-CoA thiolase remained virtually constant at approx. 4.0 units/g for 30 days after birth (Fig. 3), which is appreciably higher than that of the adult at 2.5 units/g. A similar age pattern for brain thiolase activity was reported by Lynen (1957).

Measurements of glutamate dehydrogenase activity were used as an indicator of the behaviour of other mitochondrial enzymes. Rat brain glutamate dehydrogenase activity was low at birth and increased during the next 4 weeks of development, but at no stage did the values exceed those of the adult (Table 1). A similar pattern for glutamate de-

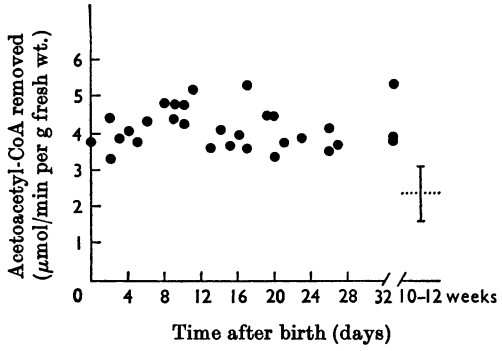

Fig. 3. Activity of acetoacetyl-CoA thiolase in brain during postnatal development of the rat. The enzyme activity was determined as described in the Materials and Methods section. Each point represents the mean value for two to ten rats taken from the same litter. The broken line with vertical bar is the mean±S.D. of 42 adult rats.

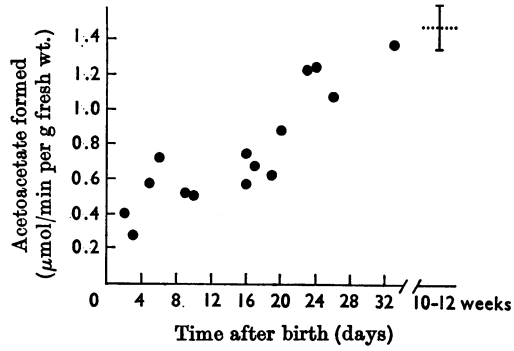

Fig. 4. Activity of 3-hydroxybutyrate dehydrogenase in kidney during postnatal development of the rat. The enzyme activity was determined as described in the Materials and Methods section. Each point represents the mean value for two to ten rats taken from the same litter. The broken line is the mean value for four adult rats.

Table 1. *Activities of glutamate dehydrogenase in brain, kidney and heart during postnatal development of the rat*

The results are expressed in μmol of oxoglutarate reduced/min per g fresh wt. of tissue and are the means of three to five observations in each group. For other experimental details see the Materials and Methods section. The adult rats weighed 150–200g.

Age of rats (days)	Tissue		
	Brain	Kidney	Heart
1–10	5.2	16.9	—
11–20	13.3	19.1	4.0
21–30	13.6	19.6	3.7
Adult	16.5	28.1	4.7

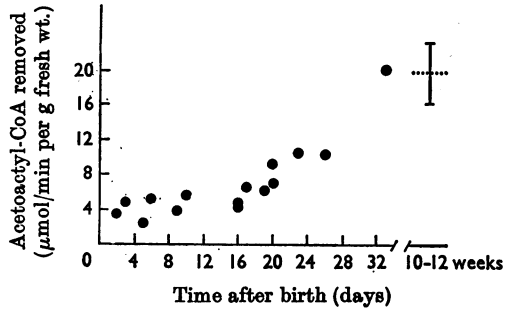

Fig. 5. Activity of 3-oxo acid CoA-transferase in kidney during postnatal development of the rat. The activity was determined as described in the Materials and Methods section. Each point represents the mean value for two to ten rats taken from the same litter. The broken line with vertical bar is the mean value±S.D. for 26 adult rats.

hydrogenase in the neonatal rat liver was found by Hommes & Richters (1969).

Activity of ketone-body-utilization pathway in immature kidney and heart. The pattern of enzyme activities in the developing rat kidney and heart was different from that in brain. In kidney the activities of 3-hydroxybutyrate dehydrogenase, 3-oxo acid CoA-transferase and acetoacetyl-CoA thiolase rose steadily from the low neonatal values to reach the adult values after 3–4 weeks (Figs. 4–6). At no time did the activities of these enzymes exceed adult values. Kidney 3-hydroxybutyrate dehydrogenase activity increased fivefold from the newborn value of 0.3 unit/g to the adult value of 1.5 units/g (Fig. 4). Similarly, kidney 3-oxo acid CoA-transferase increased from 3.0 units/g to 20 units/g, a sevenfold increase in activity in 4 weeks (Fig. 5), and acetoacetyl-CoA thiolase activity increased threefold from 6.0 units/g to 17.0 units/g (Fig. 6). Comparable, though less pronounced, increases in the activities of these enzymes were observed in heart tissue, but at no time did the activities exceed adult values during the first 3 weeks of life. Kidney and heart glutamate dehydrogenase activity behaved in a similar way to brain glutamate dehydrogenase, increasing slightly throughout the postnatal development of the rat but never exceeding the adult values (Table 1).

Blood metabolites during postnatal development. At birth the ketone-body concentration in the blood of the immature rats was similar to that of fed adult rats. After 1 day the ketone-body concentration was almost as high as the concentrations

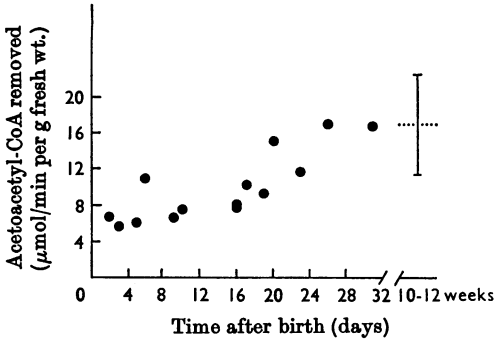

Fig. 6. Activity of acetoacetyl-CoA thiolase in kidney during postnatal development of the rat. The enzyme activity was determined as described in the Materials and Methods section. Each point represents the mean value for two to ten rats taken from the same litter. The broken line with vertical bar is the mean value ± S.D. of 21 adult rats.

for 48h-starved adult rats and this high concentration was maintained until the time of weaning, when the ketone-body concentration decreased towards the adult fed value (Table 2; see also Drahota, Hahn, Kleinzeller & Kostolánská, 1964).

The [3-hydroxybutyrate]/[acetoacetate] ratio increased throughout the neonatal period (1.4–2.6) and then decreased again on weaning.

The free fatty acid and glycerol concentrations were approx. 0.85 and 0.25mM respectively in the infant rat, compared with the fed adult concentration of 0.21 and 0.07mM respectively (Table 2). After weaning the concentration of free fatty acid and glycerol decreased towards the adult fed values. A similar pattern of change in glycerol concentration has been reported by Vernon & Walker (1970).

Glucose concentration rose steadily from 4.3mM after birth to 7.0mM at weaning (21 days). There was then a slow fall in concentration until the normal fed value of about 6mM was reached.

DISCUSSION

In the adult rat, as shown in the preceding paper (Williamson *et al.* 1971), the activities of the enzymes responsible for ketone-body utilization do not alter when the rates of ketone-body utilization change (e.g. in starvation and in alloxan-diabetes). In contrast, there are major differences between adult and suckling rats in the activities of the relevant enzymes in various tissues. In brain the activities rise during the suckling period, and at the time of weaning they are three times higher than those of the adult tissue. In kidney and heart they also rise during the early stages of development, but

Table 2. *Concentrations of blood metabolites during postnatal development of the rat*

The results are mean values (±S.D.) with at least five observations in each group except where indicated. For other experimental details see the Materials and Methods section. The adult rats weighed 150–200g.

Age of rats (days)	Glucose (mM)	3-Hydroxybutyrate (mM)	Acetoacetate (mM)	[3-Hydroxybutyrate]/[Acetoacetate]	Sum of ketone bodies (mM)	Free fatty acids (mM)	Glycerol (mM)
At birth	4.33	0.15	0.04	3.75	0.19	—	—
1–5	5.29 ± 0.62	0.94 ± 0.26	0.69 ± 0.16	1.36	1.63	0.77	0.31 ± 0.10
6–10	6.36 ± 0.62	0.67 ± 0.25	0.43 ± 0.09	1.56	10.9	0.84 ± 0.23	0.22 ± 0.04
11–15	6.70 ± 0.46	1.00 ± 0.31	0.45 ± 0.11	2.22	1.45	0.77 ± 0.19	0.29 ± 0.12
16–20	6.98 ± 0.47	0.98 ± 0.09	0.38 ± 0.06	2.58	1.36	0.90 ± 0.38	0.20 ± 0.03
21–25	6.42	0.30	0.17	1.76	0.47	0.62	—
Adult (fed)	6.15 ± 0.49	0.18 ± 0.07	0.07 ± 0.07	2.57	0.25	0.21 ± 0.05	0.07 ± 0.01
Adult (starved 48h)	4.06 ± 0.78	1.56 ± 0.04	0.55 ± 0.16	2.84	2.11	0.51 ± 0.06	0.22 ± 0.05

Table 3. *Total activities of enzymes of the ketone-body-utilization pathway in brain, heart and kidney of suckling and adult rats*

The total activity of the organ was calculated by multiplication of the mean activity/g fresh wt. of tissue by the average weight of the organ. The suckling rats refer to 20–24-day-old animals. The adult rats weighed 150–200g.

	Tissue					
	Suckling rats			Adult rats		
Enzyme	Brain	Heart	Kidney	Brain	Heart	Kidney
3-Hydroxybutyrate dehydrogenase	2.0	0.03	0.4	0.82	0.63	2.4
3-Oxo acid CoA-transferase	7.2	0.9	3.0	3.7	11.5	32
Acetoacetyl-CoA thiolase	5.4	0.5	3.6	3.7	3.4	27

the maximum adult value is not reached until after about 5 weeks. A comparison of the total activities of the enzymes of ketone-body utilization taking into consideration the weight of the tissues (Table 3) indicates that the brain is potentially a major site of ketone-body utilization in infancy. Rat milk is known to be relatively high in fat (12.3% fat, 9.2% protein and 3.0% carbohydrate) (Dymsza, Czajka & Miller, 1964) and high concentrations of free fatty acids and ketone bodies are maintained in the blood throughout the suckling period (Table 2). Unlike the hyperketonaemia of starvation, this hyperketonaemia is accompanied by a normal or slightly raised blood glucose concentration, and this raises the question of whether the ketone bodies have another metabolic role in the brain apart from serving as a fuel of respiration and sparing glucose. Klee & Sokoloff (1967) have suggested that acetoacetate could supply the acetyl-CoA required for the synthesis of the lipids involved in the myelinization process. The rate of cholesterol synthesis is high during the suckling period and very low in mature brain (Srere, Chaikoff, Treitman & Burstein, 1950), and the rate of fatty acid synthesis is known to reach a peak 15–20 days after birth (Aeberhard, Grippa & Menkes, 1969). This coincides with the time when the enzymes of ketone-body utilization show maximum activity. The question of why plasma fatty acids are not used directly by the brain as a source of acetyl-CoA cannot yet be satisfactorily answered; however, acetoacetate is a more direct source of acetyl-CoA, since only two enzymic steps are involved.

This work was supported by grants from the Medical Research Council and U.S. Public Health Service (Grant no. AM11748). D.H.W. is a member of the external Staff of the Medical Research Council.

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