CHANGES IN ACTIVITIES OF SOME ENZYMES ASSOCIATED WITH HEPATIC LIPOGENESIS IN THE RAT FROM WEANING TO OLD AGE AND THE EFFECT OF SUCROSE FEEDING

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Abstract—1. Hepatic pyruvate kinase, aldolase and ATP citrate lyase exhibit similar developmental patterns with peaks of activity at 25-35 and 55-65 days of age whereas cytosolic aconitate hydratase and isocitrate dehydrogenase have troughs of activity at these stages.

2. Glucose 6-phosphate dehydrogenase and "malic enzyme" have peaks of activity at 35-45 days of age and in mature rats the activities are higher in females than males.

3. The activity of phosphofructokinase changes little during development.

4. At all ages sucrose feeding increased the activities of each of the enzymes other than phosphofructokinase which was unaffected by diet and cytosolic aconitate hydratase and isocitrate dehydrogenase which decreased in activity.

INTRODUCTION

It is well established that the activities of many enzymes associated with hepatic lipogenesis are suppressed immediately after birth and rise after weaning (Ballard & Hanson, 1967; Taylor et al., 1967; Vernon & Walker, 1968; Lockwood et al., 1970) and that such changes can be correlated with dietary changes occurring at birth and weaning. It has also recently been shown (Webb & Bailey, 1975) that significant changes in enzyme activity also occur between birth and weaning, during which time a constant composition milk diet is consumed.

However little information is available for the time course of changes occurring after weaning, although during such time, changes such as sexual maturation and ageing processes take place. Since the results of Taylor et al. (1967) and Lockwood et al. (1970) indicate that significant changes in the activities of enzymes associated with lipogenesis do take place between weaning and maturity, we have determined the activities of enzymes of glycolysis and lipogenesis in the livers of both male and female rats between weaning and old age. Because sucrose feeding to adult rats is known to affect the activities
of many of the enzymes studied we have investigated the effect of sucrose feeding on the activities of the enzymes at various ages.

**MATERIALS AND METHODS**

*Animals and diets*

Male and female rats from the Sheffield University Animal House Colony were used throughout. The rats were killed at about the same time each day (9-10 a.m.) by a blow to the head. The animals were weaned at 21 days of age on to a rat cube diet (Oxoid diet 86, obtained from Herbert C. Styles (Bewdley) Ltd., Bewdley, Wores., U.K.). Sucrose feeding was carried out as described by Hauser & Bailey (1974). All experiments were carried out on both male and female animals but for the sake of clarity and brevity only the results for females are described, unless a sex difference is apparent.

*Measurement of enzyme activities*

Preparation of homogenates and assays of enzyme activities were carried out as described in the previous paper (Webb & Bailey, 1975). All enzyme activities

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**Fig. 2.** Developmental changes in the liver weight of male (○) and female (●) rats and the liver/body wt × 10^2 for male (△) and female (▲) rats. The results are the means of at least six determinations ± S.E.M.

**Fig. 3.** Developmental changes in the activity of phosphofructokinase and aldolase in female rat liver. The enzyme activities are expressed as units/g wet wt of liver at 25°C and are the means of at least six determinations ± S.E.M.

- ○ Phosphofructokinase in control rats.
- ● Phosphofructokinase in sucrose-fed rats.
- ▲ Aldolase in control rats.
- △ Aldolase in sucrose fed rats.
Hepatic lipogenesis from weaning to old age

Fig. 4. Developmental changes in the activity of pyruvate kinase in female rat liver. The enzyme activities are expressed as units/g wet wt of liver at 25°C and are the means of at least six determinations ± S.E.M.

- Control rats.
- Sucrose-fed rats.

are expressed as units/g wet wt of liver, 1 unit being defined as 1 μmole of substrate transformed per min at 25°C.

RESULTS

The changes in body weight and liver weight with age of the rats used are shown in Figs. 1 and 2. As is well documented the males grow more rapidly than the females after sexual maturation takes place, but little difference in growth rate is noted before 60 days of age. The liver weight/body weight ratio changes little until 70 days of age after which time it declines to about 1 year of age and then rises slightly in the old rats.

Developmental changes in the activities of the glycolytic enzymes phosphofructokinase and aldolase in the livers of female rats are shown in Fig. 3. Very similar results are obtained if male

Fig. 5. Developmental changes in the activity of glucose 6-phosphate dehydrogenase in male and female rat liver. The enzyme activities are expressed as units/g wet wt of liver at 25°C and are the means of at least six determinations ± S.E.M.

- Control male rats.
- Sucrose-fed male rats.
- Control female rats.
- Sucrose-fed female rats.
rats are used. The activity of aldolase increased after weaning, giving a slight peak of activity at about 25–35 days of age followed by a second peak at 50–60 days of age after which time the activity falls to 70 days of age before rising slowly with age. Sucrose feeding caused a slight increase in enzyme activity at all ages. The activity of phosphofructokinase varied little during development and was unaffected by the provision of a sucrose containing diet.

The results for the other glycolytic enzyme studied, pyruvate kinase, are shown in Fig. 4. The enzyme exhibited a similar developmental pattern to that of aldolase with pronounced peaks of activity at about 35 and 60 days of age and a steady rise in activity after 180 days of age. Sucrose

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**Fig. 6.** Developmental changes in the activity of "malic enzyme" in male and female rat liver. The enzyme activities are expressed as units/g wet wt of liver at 25°C and are the means of at least six determinations ± S.E.M.

- ▲ Control male rats.
- △ Sucrose-fed male rats.
- ● Control female rats.
- ○ Sucrose-fed female rats.

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**Fig. 7.** Developmental changes in the activity of cytosolic NADP-dependent isocitrate dehydrogenase in female rat liver. The enzyme activities are expressed as units/g wet wt of liver at 25°C and are the means of at least six determinations ± S.E.M.

- ● Control rats.
- ○ Sucrose-fed rats.
feeding caused a marked increase in enzyme activity at all ages studied. The results are for female rats but similar results are obtained for both sexes.

Figures 5, 6 and 7 show the results for the three enzymes possibly involved in NADPH generation for lipogenesis, i.e. glucose 6-phosphate dehydrogenase, "malic enzyme" and cytosolic isocitrate dehydrogenase. The activity of glucose 6-phosphate dehydrogenase (Fig. 5) in male rat livers increases rapidly after weaning to a peak of activity at about 40 days of age before declining to 180 days of age, after which time the activity changes little. In the case of female rats the enzyme activity again increases after weaning but does not decline until about 70 days of age so that during sexual maturation the enzyme activity becomes much higher in females than males. The enzyme activity although falling after 70 days of age, remains higher in the females than males. Sucrose feeding increased the activity of the enzyme in both sexes at all ages studied.

Developmental changes in the activity of "malic enzyme" are shown in Fig. 6. The activity of the enzyme in the livers of both male and female rats increases rapidly after weaning to a peak of activity at 35–45 days of age before falling steadily to 180 days of age after which time activity changes little. After 70 days of age a sex differences is apparent with activities being significantly higher in females than males. Sucrose feeding increased the activity of the enzyme in both sexes at all ages studied.

Hepatic lipogenesis from weaning to old age

Fig. 8. Developmental changes in the activity of ATP-citrate lyase in female rat liver. The enzyme activities are expressed as units/g wet wt of liver at 25°C and are the means of at least six determinations ± S.E.M.

○ Control rats.
○ Sucrose-fed rats.

Fig. 9. Developmental changes in the activity of cytosolic aconitate hydratase in female rat liver. The enzymes are expressed as units/g wet wt of liver at 25°C and are the means of at least six determinations ± S.E.M.

○ Control rats.
○ Sucrose-fed rats.
The results for cytosolic NADP-dependent isocitrate dehydrogenase in female rats are shown in Fig. 7. The activity of the enzyme decreased after weaning to 30 days of age and then rose to a peak at about 45 days before falling to 60 days of age, rising to 180 days of age and then falling slightly to old age. At all ages studied sucrose feeding brings about a decrease in enzyme activity. Similar results are obtained using male rats.

The developmental patterns for the citrate metabolizing enzymes ATP citrate lyase and cytosolic aconitate hydratase are shown in Figs. 8 and 9. In both cases the results are for female rats, but similar changes are observed with male animals. The activity of ATP citrate lyase (Fig. 8) exhibits similar developmental changes to aldolase and pyruvate kinase with peaks of activity at about 35 and 60 days of age and a slight increase in activity after 180 days of age. As with the glycolytic enzymes, sucrose feeding caused an increase in enzyme activity at all ages studied. The developmental pattern for aconitate hydratase (Fig. 9) is somewhat similar to that for isocitrate dehydrogenase and in many ways the reciprocal of that for ATP citrate lyase in that the activity of the enzyme decreases in the immediate post weaning period and has a peak of activity at about 45 days of age. In contrast ATP citrate lyase increases in activity after weaning and has a trough of activity at 45 days of age. In common with isocitrate dehydrogenase and in contrast to ATP citrate lyase, sucrose feeding decreases the activity of aconitate hydratase at all ages studied.

**DISCUSSION**

It is quite clear that significant changes occur in the activities of many enzymes involved in carbohydrate conversion to lipid between weaning and old age. The rapid increase in activities of aldolase, pyruvate kinase, ATP Citrate lyase, glucose 6-phosphate dehydrogenase and "malic enzyme" immediately after weaning can be correlated with the change from a high fat content milk diet to a high carbohydrate content laboratory diet and the increase in hepatic lipid synthesis which ensues (Ballard & Hanson, 1967; Taylor et al., 1967). However since the diet is constant from weaning to old age a change in nutrient cannot be invoked to explain the changes in enzyme activity occurring after about 30 days of age. Further since with the exception of glucose 6-phosphate dehydrogenase and "malic enzyme" similar developmental patterns are obtained for male and female rats it is unlikely that sex hormones are involved. Irrespective of the cause of the changes, it is important to note that, in the normal laboratory rat given a commonly used diet, some hepatic enzymes never reach a steady activity although in most cases there is very little change in activity between 180 and 300 days.

It is of interest that the activity of phosphofructokinase which is probably the key regulatory enzyme in glycolysis (Newsholme & Start, 1973) exhibits few developmental changes in activity and is unaffected by sucrose feeding whereas the other glycolytic enzymes studied, pyruvate kinase and aldolase, show marked fluctuations in activity during development and are induced by sucrose feeding. Presumably phosphofructokinase can increase glycolytic flow considerably by marked changes in enzyme activity brought about by allosteric effectors whereas the other enzymes are much more dependent on induction of enzyme synthesis.

"Malic enzyme" and glucose 6-phosphate dehydrogenase appeared to be the only enzymes studied which showed a sex difference after sexual maturation. Tepperman & Tepperman (1964) reported that treatment of adult rats with oestrogen caused an increase in activity of glucose 6-phosphate dehydrogenase and to a lesser extent "malic enzyme" in the liver. Lockwood et al. (1970) showed that the activities of the enzymes were reduced in female rats which had been ovariectomized and elevated in male rats which had been castrated and these results suggest that both male and female sex hormones are involved in controlling the levels of the enzymes.

It is noteworthy that developmental changes in the activities of cytosolic aconitate hydratase and NADP-dependent isocitrate dehydrogenase appear in many respects to be the opposite to those for ATP citrate lyase. A similar comment applies with regard to the effect of sucrose feeding on the three enzymes of cytosolic citrate metabolism. In the liver, considerable amounts of citrate are probably always being transported from the mitochondria to the cytosol (Robinson, 1973) and such citrate can either be metabolised by ATP citrate lyase to provide acetyl-CoA for lipogenesis or by aconitate hydratase and isocitrate dehydrogenase to provide NADPH for biosynthesis (Chappell, 1968; Walker & Bailey, 1969; Lockwood et al., 1970). It appears that in lipogenic situations, e.g. sucrose feeding, when ATP citrate lyase is induced then less citrate is metabolized via aconitate hydratase and isocitrate dehydrogenase and the activities of the enzymes decrease.

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**REFERENCES**


**Key Word Index**—Rat liver; glycolysis; lipogenesis; development; sucrose.