Adaptive Behaviour of some Enzymes Involved in Glucose Utilization and Formation in Rat Liver during the Weaning Period

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1. The effects of premature weaning on to normal and high-glucose diets and of normal weaning on to high-protein and high-fat diets on the activities of hepatic enzymes involved in both the utilization of glucose and the formation of glucose by gluconeogenesis in rats during the weaning period were investigated. 2. The effects of weaning and high-glucose diets were, in general, to bring about increases in the activities of enzymes involved in glucose utilization and a more rapid decrease of the activities of the gluconeogenic enzymes towards normal adult values. 3. The effects of weaning on to high-fat and high-protein diets were, in general, to lower the rate of increase in activities of the enzymes involved in glucose utilization, and to prevent the decrease in activity of the gluconeogenic enzymes that occurs during normal weaning. 4. The changes in the activity of cytoplasmic phosphopyruvate carboxylase appear to be of special significance; this and other results are in keeping with current theories about the pathways and control of gluconeogenesis. 5. The activity of ATP citrate lyase appears to be particularly important in lipid formation. 6. The results are interpreted in terms of the known adaptive behaviour of these enzymes in the adult rat. The roles of enzyme development and of enzyme adaptation in the elaboration of metabolic homeostasis in the weanling rat are discussed.

The development of a mammalian tissue towards maturation involves many changes, including the appearance of enzymes not previously detectable and adaptation of the activity of others (Herrmann & Tootle, 1964; Moog, 1966; Walker, 1967). Many such changes have been recorded for enzymes involved in glucose utilization and formation in rat liver (Dawkins, 1966; Walker, 1967), and the preceding paper (Vernon & Walker, 1968) presented an integrated picture of such changes during the neonatal and weaning periods in the rat. Many of the changes noted could be explained in terms of the known adaptive behaviour of the same enzymes in adult rat liver when they were assessed against the known changes in the dietary supplies to the neonatal and weanling rat.

In the present paper we report the effects of various dietary modifications on several enzymic activities in rat liver at the commencement of and during the weaning period. The observations support and extend the conclusions reached in the preceding paper (Vernon & Walker, 1968). A preliminary report has been given (Walker & Vernon, 1967).

EXPERIMENTAL

Materials and assay methods. The procedure for the assay of all the enzymes and of protein and the source of the materials used therein have been described by Vernon & Walker (1968). Glucose (AnalaR grade), dextrin and casein (Hammarsten quality) were obtained from British Drug Houses Ltd. (Poole, Dorset) and the sources of the other ingredients of the diets were as follows: Solka Floc (cellulose), Johnson, Jorgensen and Wettre Ltd. (London, E.C. 4); liver (desiccated), Oxoid Ltd. (London, E.C. 4); casein (low-vitamin content), Genatosan Division, Fisons Pharmaceutical Ltd. (Loughborough, Leics.); corn oil, B.P., Boots Pure Drug Co. (Nottingham).

All results are expressed as before (Vernon & Walker, 1968). Statistical significances between means were calculated by a *t*-test and expressed as follows: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Animals and diets. The type of animal, normal diet and the normal weaning practice have been described by Vernon & Walker (1968). Though the weanling rats were normally removed from their mothers when 30–32 days old, weaning at 21 days is a common practice and we found that it was possible to wean as early as 17 days, provided that the animals were relatively large for their age. Three types of experiments involving premature weaning have been used in the present study, as follows.

(1) For the experiments on pyruvate kinase (EC 2.7.1.40) (results in Fig. 1) involving rats aged 12–15 days and 15–18 days, the animals were removed from their mothers during the daytime and given, by intubation into the oesophagus, an artificial liquid diet; full details of the procedures have been given by Walker & Eaton (1967). The normal liquid diet contained, in a total volume of 100 ml.: 7 g. of casein (Hammarsten quality), 15 g. of corn oil, 1.5 g. of dextrin, 0.5 g. of glucose, 0.5 g. of salt mixture (U.S.P. XIV), 0.08 g. of choline and 1 g. of a vitamin mixture described by Walker & Eaton (1967). The high-glucose liquid diet was similar except that it contained 10 g. of glucose and 10 g. of corn oil. These two diets are approximately isocaloric. The problems and limitations of these procedures have been discussed by Walker & Eaton (1967).

(2) For all the experiments in which animals were weaned at 17–18 days or 21 days after birth, one of two procedures was adopted. (a) The effect simply of weaning was assessed by taking a litter of ten rats and removing five from the mother. These five were given normal manufactured rat diet except that this was finely crumbled in a pestle and mortar and then set in 0.5% (w/v) non-nutrient agar (Kornacker & Lowenstein, 1965) and again crumbled by hand; the weaned rats appeared to ingest this moistened form of diet more readily and thrived much better. Five young rats (marked by ear tattoo) from another litter of the same age were added to the five remaining with the mother to keep the number of animals feeding off the mother the same, but these marked animals were not otherwise included in the experiments. (b) To examine the effect of weaning on to a normal versus a high-glucose diet on the change in enzymic activity, a litter of ten was removed completely from the mother and divided at random into two groups of approximately equal total weight. One group was fed on the normal crumbled diet, as in (a) above, and the other group was fed on a diet consisting of 30% (w/v) glucose and 70% (w/v) powdered rat diet suspended in agar and crumbled as above. The success of these regimens was judged on the basis of continued weight increase after weaning; the gain in weight was negligible on the first day after weaning but thereafter was approximately normal.

(3) The effects of weaning in the presence of a lactating mother on to either a high-fat or a high-protein diet were examined by using two litters each of ten young animals. Each of the two halves of one of these litters, taken at random when the animals were 16 days old, were pooled with half of the other litter, selected similarly. Each set of ten young animals were then placed in cages containing either a normal synthetic diet or the other diet (details given below) as indicated in the Results section. The two mothers of the two litters were interchanged between the two cages at 9 a.m. each day during the experimental period. The neonatal animals thus were weaned on to a fixed diet while the two mothers had the two diets on alternate days. Five animals, selected at random, from each cage were then killed for assay when 30 days old. The high-fat diet was similar to diet C described by Walker & Eaton (1967), but contained 30% (w/w) corn oil and 30% (w/w) Solka Floc, and the high-protein diet was diet D, also described by Walker & Eaton (1967). The normal diet in these experiments was the pelleted rat cake, some of which was crumbled and placed in a dish for the benefit of the weanling animals.

In view of the results in the preceding paper (Vernon &

Walker, 1968), male and female weanling rats were taken at random with no attention to their sex.

RESULTS

Enzymes of carbohydrate-lipid conversion. It was shown in an earlier study (Walker & Eaton, 1967) that, though hepatic glucokinase (EC 2.7.1.2) activity normally first appears in rat liver about 16 days after birth, and reaches adult values some 12 days later (Walker & Holland, 1965), a high-glucose diet will induce high glucokinase activities very rapidly once the competence to synthesize the enzyme develops. A similar situation exists with respect to the increase in pyruvate kinase activity that occurs during normal weaning (Vernon & Walker, 1968). Fig. 1 shows that when neonatal rats in the age periods 12–15 and 15–18 days are given a limited amount of glucose, in the form of milk containing 10% of glucose, there is a significant ($P < 0.01$) increase in the enzymic activities in the livers of these animals with respect to their control litter mates, although the absolute activities are still

Fig. 1. Effect of a high-glucose diet and weaning on the activity of pyruvate kinase in livers of weanling rats. Four separate experiments over the age periods 12–15, 15–18, 18–21 and 21–24 days are shown. In the first two experiments, neonatal rats were removed from the mother during the daytime and fed on either a normal milk or a high-glucose milk diet, and returned to the mother overnight. In the latter two experiments, animals were completely weaned at either 18 or 21 days respectively on to either a normal solid diet or a high-glucose solid diet. Further details of the diets and regimen are given in the text. Activities were determined as is also described in the Experimental section. Results are given as means $\pm$ S.E.M. (where the latter is sufficiently large to record) for five animals in each group. ○, Activities at the time of the start of each experiment for litter mates of the experimental animals; □, animals fed on normal milk; △, animals fed on high-glucose milk; ■, animals weaned on to normal solid diet; ▲, animals weaned on to high-glucose diet; A, mean normal adult activity.

Fig. 2. Effect of weaning at 17 days of age on to a normal or a high-glucose diet on the activities in the livers of rats at 20 days of age of ATP citrate lyase and NADP-specific malate dehydrogenase. The animals weaned on to the two diets gained weight over the period in magnitude statistically insignificantly different from those of normal litter mates left with the mothers. Activities were determined as described in the text. Results are means $\pm$ S.E.M. for five animals in each group. Open symbols (○, △, □) represent activities of ATP citrate lyase and closed symbols (●, ▲, ■) represent activities of NADP-specific malate dehydrogenase; circles (○, ●) represent initial activities; triangles (△, ▲) represent activities on a high-glucose diet; squares (□, ■) represent activities on a normal solid diet; A, mean normal adult NADP-specific malate dehydrogenase activity; B, mean normal adult ATP citrate lyase activity.

comparatively low. When rats are weaned on to solid diets at 18 or 21 days, their hepatic pyruvate kinase activities increase significantly ($P < 0.05$ or better) above the non-weaned value and the animals receiving the high-glucose diet have still higher activities ($P < 0.001$ for the 18–21-day experiment and $P < 0.05$ for the 21–24-day experiment) than those weaned on to the normal laboratory diet. The much larger increase in activity for the latter animals in the 21–24-day experiment (Fig. 1) probably reflects the increase in activity of the digestive hydrolases.

Fig. 2 shows the effects of weaning on to solid diets on the activities of ATP citrate lyase (EC 4.1.3.8) and NADP-specific malate dehydrogenase (EC 1.1.1.40). In unweaned animals the activities of both enzymes are less than $0.1 \mu\text{mole/min.}/100\text{mg. of protein}$ at 20 days of age (Vernon & Walker, 1968), so that weaning significantly ($P < 0.001$) increases both activities and the high-glucose diet has a greater effect ($P < 0.01$) than the normal diet.

The ratio of the activities malate dehydrogenase/ATP citrate lyase are 2.2 and 2.3 for the animals weaned on to the normal and the high-glucose diets respectively.

If animals are allowed to wean naturally, while left with their mothers, on to a high-protein diet, the normal developmental increase (cf. Vernon & Walker, 1968) in activity of pyruvate kinase is lowered significantly (Table 1); the increase is inhibited completely with a high-fat diet. Under the same dietary regimens, increases in ATP citrate lyase and NADP-specific malate dehydrogenase are not prevented, but the increase in ATP citrate lyase is significantly lowered by the high-fat diet (Table 1) and the increase in the malate dehydrogenase is significantly lowered by the high-protein diet. Some data on the growth characteristics of the animals used in these experiments are given in Table 2. Animals weaned on to the high-fat diet increase in body weight much as do the normal controls; the weight gain in the animals weaned on to the high-protein diet is lower, but the liver weight/body weight ratio is higher in such animals. On an activity/100g. body wt. basis therefore the lowering of the NADP-specific malate dehydrogenase activity on the high-protein diet is less marked but still significant ($P < 0.01$), and the significance of the effect of the high-fat diet on ATP citrate lyase activity remains the same ($P < 0.05$).

Dietary effects on NADP-specific dehydrogenases. The activities of glucose 6-phosphate dehydrogenase (EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (EC 1.1.1.44) normally show some increase during weaning (Vernon & Walker, 1968). Premature weaning on to a normal diet significantly ($P < 0.05$ for the first and $P < 0.01$ for the second enzyme) increases the activity of both enzymes 3 days later. A typical result is shown in Fig. 3(a); in other similar experiments the absolute activities varied considerably (i.e. between various litters), but within one litter the effects were very similar. The variations between litters are apparent from a comparison of the results of weaning the two different groups on to normal diet in the experiments in Figs. 3(a) and 3(b). Nevertheless, though the effects both of weaning and of the high-glucose versus the normal diet on 6-phosphogluconate dehydrogenase activity are variable, the greatly increased glucose 6-phosphate dehydrogenase activity as a result of weaning on to a high-glucose diet (Fig. 3b) is highly significant ($P < 0.01$). This was confirmed in other experiments.

Soluble NADP-specific isocitrate dehydrogenase (EC 1.1.1.42) activities were also measured in all these experiments and no significant change in activity was recorded. This applied also to experiments in which the animals were allowed to wean when 16 days old on to high-fat and high-protein

Table 1. *Effect of high-fat and high-protein diets on the activities of pyruvate kinase, ATP citrate lyase and soluble malate dehydrogenase (NADP) (decarboxylating) in the livers of weanling rats*

Animals were selected at random from two litters for each experiment when 16 days old, as described in the text. They were then left with lactating females and weaned on to a normal, a high-fat or a high-protein diet and killed for enzyme assays when 30 days old. The pre-weaning activities refer to a few litter mates killed when 16 days old. Activities were determined as described in the text, and the results are expressed as means $\pm$ s.e.m. with the numbers of observations in parentheses. Significance of results: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Expt. no.	Diet	Pyruvate kinase activity (μ moles/min.)		ATP citrate lyase activity (μ moles/min.)		NADP-specific malate dehydrogenase activity (μ moles/min.)	
		(per g. wet wt.)	(per 100 mg. of protein)	(per g. wet wt.)	(per 100 mg. of protein)	(per g. wet wt.)	(per 100 mg. of protein)
1A	Normal	44.6 $\pm$ 4.0 (7)	53.4 $\pm$ 5.3 (7)				
	High-fat	16.9 $\pm$ 0.7 (8)***	17.5 $\pm$ 0.7 (8)***				
7	Normal	42.2 $\pm$ 2.4 (5)	46.4 $\pm$ 1.6 (5)				
	High-protein	28.4 $\pm$ 2.0 (5)**	28.2 $\pm$ 2.0 (5)***				
6	Pre-weaning (16 days)	0.01 (4)	0.02 (4)	0.05 $\pm$ 0.02 (4)	0.07 $\pm$ 0.03 (4)		
	Normal	0.37 $\pm$ 0.02 (5)	0.40 $\pm$ 0.02 (5)	0.85 $\pm$ 0.02 (5)	0.95 $\pm$ 0.02 (5)		
	High-fat	0.28 $\pm$ 0.02 (4)*	0.30 $\pm$ 0.02 (4)**	1.00 $\pm$ 0.07 (5)	1.07 $\pm$ 0.06 (5)		
7	Pre-weaning (16 days)	0.03 (4)	0.04 (4)	0.05 $\pm$ 0.02 (4)	0.06 $\pm$ 0.02 (4)		
	Normal	0.31 $\pm$ 0.02 (5)	0.34 $\pm$ 0.02 (5)	1.11 $\pm$ 0.06 (5)	1.22 $\pm$ 0.05 (5)		
	High-protein	0.28 $\pm$ 0.05 (5)	0.28 $\pm$ 0.04 (5)	0.64 $\pm$ 0.10 (5)**	0.63 $\pm$ 0.10 (5)***		

Table 2. *Summary of the data for animals weaned on to either a high-fat or a high-protein diet*

In each experiment the groups of animals fed on the normal and the special diets were selected at random, as described in the Experimental section. Results in the last two columns are means $\pm$ s.e.m.

Expt. no.	Diet	No. of animals	Mean			Mean liver wt. (g.)	Mean ratio, 100 $\times$ liver wt./body wt.	Supernatant protein (mg./g. wet wt. of liver)
			initial body wt. at 16 days (g.)	final body wt. at 30 days (g.)	increase in body wt., 16-30 days (g.)			
1A	Normal	7	32.0	67.2	35.2	3.08	4.59 $\pm$ 0.09	83.1 $\pm$ 1.9
	High-fat	8	31.3	59.0	27.7	2.52	4.27 $\pm$ 0.10	97.0 $\pm$ 2.3
6	Normal	5	28.5	69.0	40.5	3.35	4.72 $\pm$ 0.08	90.9 $\pm$ 1.5
	High-fat	5	28.4	73.0	44.6	3.32	4.51 $\pm$ 0.13	92.7 $\pm$ 0.5
1	Normal	5	33.5	89.9	56.4	4.23	4.74 $\pm$ 0.12	92.3 $\pm$ 2.5
	High-fat	5	33.5	88.0	54.5	3.82	4.51 $\pm$ 0.08	107.5 $\pm$ 3.9
5	Normal	5	27.0	79.0	52.0	3.70	4.79 $\pm$ 0.13	93.0 $\pm$ 2.2
	High-fat	5	28.0	79.0	51.0	3.66	4.72 $\pm$ 0.32	109.0 $\pm$ 3.7
7	Normal	5	28.0	70.0	42.0	3.16	4.51 $\pm$ 0.10	91.0 $\pm$ 3.4
	High-protein	5	27.0	62.0	35.0	3.17	5.11 $\pm$ 0.10	100.6 $\pm$ 0.3
2	Normal	5	28.4	83.5	55.1	3.86	4.68 $\pm$ 0.09	92.3 $\pm$ 1.1
	High-protein	5	27.5	63.9	36.4	3.16	4.86 $\pm$ 0.19	106.3 $\pm$ 2.1
4	Normal	5	31.6	83.3	51.7	3.95	4.68 $\pm$ 0.05	93.6 $\pm$ 1.6
	High-protein	5	30.8	64.1	33.3	3.51	5.32 $\pm$ 0.19	103.2 $\pm$ 1.6

diets and killed for assay when 30 days old. In these same experiments the normal increase in activity of glucose 6-phosphate dehydrogenase is accelerated in some but not all animals weaned on

to the high-protein diet, whereas weaning on to the high-fat diet retards significantly ($P < 0.001$) the increase in 6-phosphogluconate dehydrogenase activity (Table 3).

Fig. 3. Effect of (a) weaning and (b) weaning on to normal and high-glucose diets on the activities of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. The animals were weaned on to the diets at 18 days of age and killed for assay when 21 days old; weight gains were statistically insignificantly different from those of normal litter mates left with the mothers. Activities were determined as described in the text. Results are means $\pm$ S.E.M. for five animals in each group. Open symbols ($\circ$, $\triangle$, $\square$) represent activities of glucose 6-phosphate dehydrogenase and closed symbols ($\bullet$, $\blacktriangle$, $\blacksquare$) activities of 6-phosphogluconate dehydrogenase; circles ($\circ$, $\bullet$) represent initial activities; triangles ($\triangle$, $\blacktriangle$) represent activities on a high-glucose diet; squares ($\square$, $\blacksquare$) represent activities on a normal solid diet.

first-named enzymes the high-glucose diet is more effective than the normal diet. The latter diet results in the lowering of the phosphopyruvate carboxylase activity to the normal adult value so that the high-glucose diet has no further effect in lowering the activity of this enzyme. On the other hand, the activities of all three enzymes remain high and do not decrease towards adult values at 30 days of age, as they do in normally weaned animals (Vernon & Walker, 1968), if the animals are offered a high-fat or a high-protein diet (Table 5).

Of the several different effects tried, only weaning on to the high-protein diet has any effect different from normal weaning on the activities of soluble NAD-specific malate dehydrogenase (EC 1.1.1.37) and soluble fumarase (EC 4.2.1.2) (Table 6). Both activities are significantly higher on this diet.

DISCUSSION

The weaning period involves a change from the low-carbohydrate but high-fat and high-protein diet of milk (Dymysz, Czajka & Miller, 1964) to a solid diet. Most commercially available rat diets contain 60–70% of carbohydrate, but a 'normal' diet can cover a wide range of percentage compositions and the animals can continue to grow and thrive, albeit at different growth rates, on the wide variety of diets. Many differences in metabolic functions result from varying the carbohydrate/fat ratio (Carroll, 1963, 1964; Carroll & Bright, 1965; Baldwin, Ronning, Radanovics & Plange, 1966) and these can be explained in terms of adaptations of a wide range of enzymes involved in glycogen metabolism, glycolysis, gluconeogenesis and lipogenesis.

Hepatic glucokinase (EC 2.7.1.2) first appears about 16 days after birth in the rat (Walker & Holland, 1965). The competence to synthesize a normal complement of this enzyme develops within about 2 days and the normal slower developmental curve during normal weaning can be interpreted in terms of the gradual switch from milk to solid diet (Walker & Eaton, 1967). The effects of premature weaning and of weaning on to various diets on the activities of pyruvate kinase, ATP citrate lyase and NADP-specific malate dehydrogenase (Figs. 1 and 2, and Table 1) follow a not dissimilar pattern to glucokinase. Weaning on to the normal diet requires that the pancreatic and intestinal carboxylases are available if glucose is to be formed for absorption, and these enzymes also increase in activity during the weaning period (for references see Walker & Eaton, 1967). Weaning on to a high-glucose diet by-passes the need for hydrolysis of polysaccharides and results in a large increase in glucokinase (Walker & Eaton, 1967), pyruvate kinase, ATP citrate lyase and NADP-specific malate dehydrogenase. The normal increases in

Enzymes of gluconeogenesis. Weaning neonatal rats on to both normal and high-glucose diets lowers the activities of glucose 6-phosphatase (EC 3.1.3.9), fructose 1,6-diphosphatase (EC 3.1.3.11) and phosphopyruvate carboxylase (EC 4.1.1.32) in every case (Table 4). For the two

Table 3. *Effect of high-fat and high-protein diets on the activities of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in the livers of weanling rats*

Animals were selected and weaned as described in Table 1. The pre-weaning activities refer to a few littermates killed when 16 days old; all the other animals were weaned when 16 days old and killed for assay when 30 days old. Activities were determined as described in the text, and the results are expressed as means $\pm$ s.e.m. with the numbers of observations in parentheses. Significance of results: ***, $P < 0.001$.

Expt. no.	Diet	Glucose 6-phosphate dehydrogenase activity (μ moles/min.)		6-Phosphogluconate dehydrogenase activity (μ moles/min.)	
		(per g. wet wt.)	(per 100 mg. of protein)	(per g. wet wt.)	(per 100 mg. of protein)
6	Pre-weaning (16 days)	1.14 $\pm$ 0.07 (4)	1.38 $\pm$ 0.14 (4)	1.61 $\pm$ 0.11 (4)	1.91 $\pm$ 0.07 (4)
	Normal	1.41 $\pm$ 0.08 (5)	1.55 $\pm$ 0.09 (5)	4.32 $\pm$ 0.08 (5)	4.76 $\pm$ 0.10 (5)
	High-fat	1.48 $\pm$ 0.14 (5)	1.59 $\pm$ 0.15 (5)	3.51 $\pm$ 0.12 (5)***	3.78 $\pm$ 0.14 (5)***
7	Pre-weaning (16 days)	1.58 $\pm$ 0.04 (4)	1.83 $\pm$ 0.07 (4)	1.97 $\pm$ 0.06 (4)	2.38 $\pm$ 0.04 (4)
	Normal	1.53 $\pm$ 0.07 (5)	1.68 $\pm$ 0.02 (5)	4.10 $\pm$ 0.12 (5)	4.51 $\pm$ 0.14 (5)
	High-protein	2.23 $\pm$ 0.32 (5)	2.22 $\pm$ 0.31 (5)	4.09 $\pm$ 0.17 (5)	4.06 $\pm$ 0.15 (5)

Table 4. *Effect of weaning on to normal and high-glucose diets on the activities of glucose 6-phosphatase, fructose 1,6-diphosphatase and phosphopyruvate carboxylase in the livers of weanling rats*

Animals were weaned when 18 days old on to either a normal or a high-glucose diet and killed for assay 3 days later, when 21 days old. Activities were determined as described in the text and the results are expressed as means $\pm$ s.e.m. for five animals in each group. Significance of results: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Expt. no.	Studies and diet	Activity (μ moles/min.)				
		Glucose 6-phosphatase (per g. wet wt.)	Fructose 1,6-diphosphatase		Phosphopyruvate carboxylase	
			(per g. wet wt.)	(per 100 mg. of protein)	(per g. wet wt.)	(per 100 mg. of protein)
11	Normal, unweaned	23.0 $\pm$ 1.6	2.14 $\pm$ 0.08	2.40 $\pm$ 0.04	2.88 $\pm$ 0.23	3.22 $\pm$ 0.18
	Weaned on to normal diet	14.3 $\pm$ 0.3**	1.75 $\pm$ 0.07**	2.21 $\pm$ 0.07*	1.31 $\pm$ 0.06***	1.66 $\pm$ 0.07***
10	Weaned on to normal diet	17.5 $\pm$ 0.9	2.10 $\pm$ 0.13	2.44 $\pm$ 0.10	1.64 $\pm$ 0.19	1.91 $\pm$ 0.19
	Weaned on to high-glucose diet	14.4 $\pm$ 0.5*	1.88 $\pm$ 0.05	2.15 $\pm$ 0.04*	1.74 $\pm$ 0.11	2.01 $\pm$ 0.09

Table 5. *Effect of high-fat and high-protein diets on the activities of glucose 6-phosphatase, fructose 1,6-diphosphatase and phosphopyruvate carboxylase in the livers of weanling rats*

Animals were selected and weaned when 16 days old as described in Table 2. They were killed for assay when 30 days old. Activities were determined as described in the text and the results are expressed as means $\pm$ s.e.m. for five animals in each group. Significance of results: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Expt. no.	Diet	Glucose 6-phosphatase activity (μ moles/min./g. wet wt.)	Phosphopyruvate carboxylase activity (μ moles/min.)	
			(per g. wet wt.)	(per 100 mg. of protein)
5	Normal	13.9 $\pm$ 0.5	1.89 $\pm$ 0.27	2.03 $\pm$ 0.35
	High-fat	25.7 $\pm$ 0.8***	4.05 $\pm$ 0.20***	3.75 $\pm$ 0.23**
4	Normal	12.6 $\pm$ 0.5	2.40 $\pm$ 0.25	2.58 $\pm$ 0.30
	High-protein	20.2 $\pm$ 0.5***	5.55 $\pm$ 0.45***	5.38 $\pm$ 0.39***
Fructose 1,6-diphosphatase activity (μ moles/min.)				
		(per g. wet wt.)	(per 100 mg. of protein)	
6	Normal	1.87 $\pm$ 0.05	2.06 $\pm$ 0.06	
	High-fat	2.07 $\pm$ 0.01**	2.23 $\pm$ 0.02*	
7	Normal	1.91 $\pm$ 0.10	2.09 $\pm$ 0.09	
	High-protein	2.83 $\pm$ 0.08***	2.80 $\pm$ 0.08***	

Table 6. *Effects of weaning and high-glucose, high-fat and high-protein diets on activities of soluble NAD-specific malate dehydrogenase and soluble fumarase activities in livers of weanling rats*

Animals were selected and, in Expts. 5 and 4, allowed to wean while remaining with mothers, as in Table 2. Activities were determined in the supernatant fraction of liver homogenates as described in the text. Results are given as means $\pm$ S.E.M. for five animals in each group. Significance of results: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Expt. no.	Diet and time period	Age when killed for assay (days)	Activities (μ moles/min.)			
			NAD-specific malate dehydrogenase		Fumarase	
			(per g. wet wt.)	(per 100 mg. of protein)	(per g. wet wt.)	(per 100 mg. of protein)
11	Unweaned, normal	21	279 $\pm$ 9	314 $\pm$ 4	18.4 $\pm$ 0.7	20.7 $\pm$ 0.3
	Weaned at 18 days on to normal diet	21	266 $\pm$ 13	336 $\pm$ 14	16.3 $\pm$ 1.5	20.6 $\pm$ 2.1
10	Weaned at 18 days on to normal diet	21	342 $\pm$ 9	400 $\pm$ 12	15.8 $\pm$ 1.6	20.2 $\pm$ 1.1
	Weaned at 18 days on to high-glucose diet	21	340 $\pm$ 8	389 $\pm$ 6	18.4 $\pm$ 1.5	23.1 $\pm$ 1.1
5	Normal, 16-30 days	30	322 $\pm$ 8	347 $\pm$ 13	20.6 $\pm$ 0.9	22.4 $\pm$ 0.7
	High-fat, 16-30 days	30	319 $\pm$ 10	294 $\pm$ 15*	27.7 $\pm$ 2.1*	25.9 $\pm$ 2.7
4	Normal, 16-30 days	30	299 $\pm$ 8	320 $\pm$ 13	22.9 $\pm$ 0.9	24.5 $\pm$ 0.7
	High-protein, 16-30 days	30	450 $\pm$ 21***	439 $\pm$ 26**	57.0 $\pm$ 2.4***	55.2 $\pm$ 1.7***

glucokinase and pyruvate kinase during weaning are retarded by weaning on to a high-fat or a high-protein diet (Table 1). The inhibition by a high-fat diet of the normal increase in ATP citrate lyase activity is reasonable, for in this situation fatty acid oxidation provides the source of energy, and the conversion of glucose into lipid is low (Ballard & Hanson, 1967). Glucose 6-phosphate dehydrogenase activity is greatly increased by the high-glucose diet (Fig. 3), as is to be expected from the known influence of diet on this enzyme in the adult rat (Tepperman & Tepperman, 1964).

For reasons of immaturity discussed previously by Walker & Eaton (1967), it is not possible to introduce much glucose to the rat in the first 2 weeks of extrauterine life. It has therefore not proved feasible to examine what effects a substantial amount of carbohydrate might have on the enzymic activities before the time of normal weaning. An increased flux of glucose through the glycolytic sequence and thence to lipogenesis is unlikely before glucokinase development, because glucose phosphorylation is of rate-limiting significance in the overall utilization of glucose (Walker, 1966). The initiation of glucokinase development is not significantly advanced by glucose therapy (Walker & Holland, 1965; Walker & Eaton, 1967).

Weaning rats on to high-fat and high-protein diets results in the activities of the three gluconeogenic enzymes remaining higher than normal (Table 5); this is to be expected. It also suggests that the rapid development of glucose 6-phos-

phatase, fructose 1,6-diphosphatase and phosphopyruvate carboxylase activities well above normal adult values, such as are found within a few days of birth, can be regarded as the rapid adaptation to the milk diet rather than an overshoot in synthesis of a normal amount of the three enzymes. Other factors including the rate of enzyme degradation may also change and therefore influence the recorded activities. Early weaning (Table 5) on to normal or high-glucose diets results in a lowering of the activities of all three enzymes. The activity of phosphopyruvate carboxylase falls to normal adult values within the 3-day experimental period not only with the high-glucose diet but also with the normal diet. At the same time the activities of glucose 6-phosphatase and fructose 1,6-diphosphatase are lowered significantly, but still remain above the normal adult value. This difference may simply relate to a higher rate of protein turnover of phosphopyruvate carboxylase compared with the other two enzymes. It emphasizes the view (Vernon & Walker, 1968) that the activity of phosphopyruvate carboxylase, the first cytoplasmic enzyme involved in glucose formation from gluconeogenic precursors by rat liver (Lardy, 1966), is of rate-controlling significance in the regulation of gluconeogenesis in the neonatal rat. The mechanisms regulating phosphopyruvate carboxylase activity in the liver include dietary, hormonal and extrahepatic factors that operate in a complex, unknown way (Foster, Ray & Lardy, 1966). Lardy, Foster, Young, Shrago & Ray (1965) have suggested

that gluconeogenic amino acids may stabilize the enzyme in some manner; this could be of special importance in the neonatal period.

A role for the NAD-specific malate dehydrogenase and fumarase in the extramitochondrial compartment of the cell has been proposed (Lardy *et al.* 1965; Krebs, Gascoyne & Notton, 1967). Oxidation of malate, supplied from the mitochondria, is a means of producing the NADH in the cytoplasm that is required in gluconeogenesis. Large quantities of amino acids will supply the carbon chains required for gluconeogenesis in the neonatal animal with the production, among other substances, of aspartate. This will also pass from the mitochondria into the cytoplasm, where it is used for urea synthesis. Urea formation increases markedly at weaning (Illnerová, 1966) and the fumarate formed by the cleavage of argininosuccinate can be converted into malate in the presence of fumarase. It is therefore noteworthy that cytoplasmic fumarase activity increases during the neonatal period from a low value at birth (Vernon & Walker, 1968) and that weaning on to a high-protein diet (Table 6) results in a very significant increase in fumarase activity; this is the only situation in the present experiments in which fumarase activity is different from normal.

It is thus possible to interpret many of the enzymic adaptations that occur during the neonatal and weaning periods of the rat in terms of adaptations known to occur in the adult animal in response to dietary changes. Two enzymes, glucokinase (Walker & Holland, 1965) and NADP-specific malate dehydrogenase (Ballard & Hanson, 1967; Bailey, Taylor & Bartley, 1967; Vernon & Walker, 1968), develop at the time of weaning in response to the increased dietary supply of carbohydrate, but the triggering mechanism is unknown. The increases in activity of pyruvate kinase (Fig. 1) and of ATP citrate lyase and NADP-specific malate dehydrogenase (Fig. 2) may be dependent on the development of glucokinase, which results in increased flux through the glycolytic intermediates. No decision can be made on this possibility in the absence of knowledge about the role, if any, of the intermediates in the control, either directly or via other regulatory compounds, of synthesis or degradation of enzymes. The changing diet may also influence the release and effectiveness of hormones such as insulin, glucagon and the anti-insulin hormones of the adrenal cortex and anterior pituitary glands; the significance of any such changes to the neonatal animal are unknown.

A major limitation in the interpretation of the results is that the quantitative role of the changing

activities of the digestive enzymes cannot be assessed. The different effects of weaning on to normal and high-glucose diets on certain enzyme activities emphasize that it is the nature of the absorbed components rather than the distribution of diet between the classical divisions of carbohydrate, fat and protein that influences the observed activities of the adaptive enzymes. The balance between the nature and amount of the supply of all the various nutrients that reach the liver cells, plus the effects of changing concentrations of various hormones that act directly on the liver, together with other factors, all determine the observed enzymic activity at any time.

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