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Citrate-Cleavage Enzyme, 'Malic' Enzyme and Certain Dehydrogenases in Embryonic and Growing Chicks

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The activities of several enzymes possibly implicated in lipogenesis were measured in the soluble fraction of homogenates of liver and adipose tissue of embryonic and growing chicks. The activities of adipose-tissue enzymes showed little or no change. The activities of hepatic hexose monophosphate-shunt dehydrogenases, malate dehydrogenase, 3-phosphoglyceraldehyde dehydrogenase and NAD-linked α-glycerophosphate dehydrogenase also showed little or no change. Isocitrate dehydrogenase activity in liver rose to a peak on the day of hatching and fell to half the peak value during the next 12 days, where it remained to 26 days after hatching. The activities of 'malic' enzyme and citrate-cleavage enzyme showed very low stable values in embryonic liver and remarkable rises during the early part of the post-hatching period. An 85-fold increase in the activity of 'malic' enzyme activity was completed in 7 days and a 15-fold increase in that of citrate-cleavage enzyme in 5 days. The activities then attained were maintained up to 26 days after hatching. 2. The increases in the activities of hepatic citrate-cleavage enzyme and 'malic' enzyme occurred simultaneously with a marked increase in lipogenesis, suggesting a relationship of these enzymes to lipogenesis in chick liver. By contrast, activity of the hexose monophosphate-shunt dehydrogenases does not appear to be thus associated.

The preceding paper (Goodridge, 1968*b*) described the increase in hepatic fatty acid synthesis and glucose oxidation that occurred after newly hatched chicks were fed on a high-carbohydrate low-fat mash diet. The present paper extends this work by reporting the results of time-course studies in embryonic and growing chicks of the activities of several liver and adipose-tissue enzymes involved in the metabolism of glucose and synthesis of fatty acids.

MATERIALS AND METHODS

The care and handling of the chicks and embryos and the removal and homogenization of the liver were as described in the preceding paper (Goodridge, 1968*b*). Lateral thoracic and lateral abdominal adipose tissue was removed from the animals and placed in ice-cold 0·25 M-sucrose. Homogenates of adipose tissue (20%, w/v) were prepared in 0·25 M-sucrose with a TenBroeck homogenizer. Homogenization of liver was performed at ice-bath temperatures; for adipose tissue the homogenizer was warmed by contact with the hands to minimize congealing of the lipid. Samples of the whole homogenates were removed for determination of N by a micro-Kjeldahl procedure (Frerichs & Ball, 1962). The remainder was centrifuged at 40000*g* for 15 min. at 0–3° and the resulting supernatant fraction used for the enzyme assays (in some cases it was diluted with 0·25 M-sucrose).

'Malic' enzyme [L-malate–NADP oxidoreductase (decarboxylating), EC 1.1.1.40] was assayed by the method of Wise & Ball (1964), citrate-cleavage enzyme [ATP–citrate oxaloacetate-lyase (CoA-acetylating and ATP-dephosphorylating), EC 4.1.3.8] by the method of Srere (1959) as modified by Kornacker & Ball (1965) and malate dehydrogenase (L-malate–NAD oxidoreductase, EC 1.1.1.37) by the method of Ochoa (1955). Total HMP-shunt† dehydrogenase [D-glucose 6-phosphate–NADP oxidoreductase (EC 1.1.1.49) plus 6-phospho-D-gluconate–NADP oxidoreductase (decarboxylating) (EC 1.1.1.44)] activity was assayed by the procedure of Glock & McLean (1953) as modified by Ball & Jungas (1963). Isocitrate dehydrogenase [*threo*-D$_s$-isocitrate–NADP oxidoreductase (decarboxylating), EC 1.1.1.42] was assayed by the method of Abraham, Migliorini, Bortz & Chaikoff (1962). α-Glycerophosphate dehydrogenase (L-glycerol 3-phosphate–NAD oxidoreductase, EC 1.1.1.8) and glyceraldehyde 3-phosphate dehydrogenase [D-glyceraldehyde 3-phosphate–NAD oxidoreductase (phosphorylating), EC 1.2.1.12] were assayed by modifications of

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† Abbreviation: HMP shunt, hexose monophosphate shunt.

the procedures of Burch *et al.* (1963). In the α-glycerophosphate dehydrogenase assay the dihydroxyacetone phosphate concentration was decreased from 5 mM to 1 mM. In the glyceraldehyde 3-phosphate dehydrogenase assay the glyceraldehyde 3-phosphate concentration was decreased from 2 mM to 1 mM and a 0·1 M-potassium phosphate buffer, pH 7·4, was used instead of the dimethylglycine buffer. All enzyme assays were carried out at 40° in a Gilford recording spectrophotometer.

Glucose 6-phosphate (barium salt), 6-phosphogluconate (barium salt), sodium pyruvate, malic acid, $NADP^+$, NADH, ATP and malate dehydrogenase were obtained from Sigma Chemical Co. (St Louis, Mo., U.S.A.); nicotinic acid, trisodium citrate and oxaloacetic acid were from Calbiochem (Los Angeles, Calif., U.S.A.); dihydroxyacetone dimethylketal (dicyclohexylammonium salt) and DL-glyceraldehyde 3-phosphate diethylacetal (barium salt) were from Boehringer (Mannheim) Corp. (New York, N.Y., U.S.A.) CoA was from P-L Biochemicals Inc. (Milwaukee, Wis., U.S.A.). The barium salts of glucose 6-phosphate and 6-phosphogluconate were converted into the sodium salts with Na_2SO_4 or on AG-50W (X2) cation-exchange resin (BioRad Laboratories, Richmond, Calif., U.S.A.). The sodium salts of dihydroxyacetone phosphate and glyceraldehyde 3-phosphate were generated from the dimethylketal and diethylacetal respectively by boiling for 3 min. in the presence of the AG-50W (X2) resin.

Significance of the data was tested where appropriate by the Mann–Whitney test (Siegel, 1956). Standard errors are provided to indicate the degree of variance in the data.

RESULTS

The activity of citrate-cleavage enzyme in liver and adipose tissue from embryos and chicks of various ages is shown in Fig. 1. Activity of the adipose-tissue enzyme was very low and unchanged throughout the period studied. In embryonic liver activity decreased from 2·6 μmoles/mg. of N/hr. in 14-day embryos to 1·1 μmoles/mg. of N/hr. at 19 days of incubation. Activity increased slightly but significantly during the hatching period and the starvation period before feeding. After 24 hr. of feeding citrate-cleavage enzyme activity had increased 4·5-fold. The enzyme continued to increase in activity until the chicks were 6–7 days old (5–6 days of feeding). There were no significant changes in activity from 6–7 to 26 days of age. A similar pattern for the development of hepatic citrate-cleavage enzyme in the chick has been described by Felicioli & Gabrielli (1967).

The pattern of activity changes for liver and adipose-tissue 'malic' enzyme (Fig. 2) was similar to that observed for citrate-cleavage enzyme. The activity of 'malic' enzyme was very low in both liver and adipose tissue from embryos and day-of-hatching and 1-day-old chicks. At 1 day after the initiation of feeding, activity of the adipose-tissue enzyme was unchanged whereas that of the liver had increased almost six-fold. Activity of the liver enzyme continued to increase rapidly, reaching 84 times the prefeeding level in 8-day-old chicks. After the chicks were 8 days old there were no further significant changes in the activity of liver 'malic' enzyme.

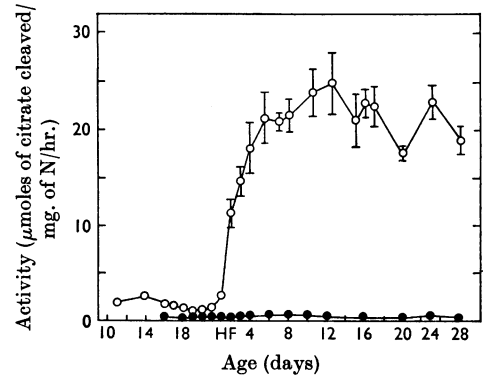

Fig. 1. Activity of citrate-cleavage enzyme in liver (○) and adipose tissue (●). Each point represents the mean of four to 12 experiments (some experiments represent averages of pooled tissue samples). The vertical bars indicate S.E.M. (the lack of a vertical bar indicates that the S.E.M. was too small to put on the graph). Numbers to the left of H on the abscissa refer to days of incubation; those to the right refer to age of chicks. H represents the day of hatching; F indicates the day of first feeding.

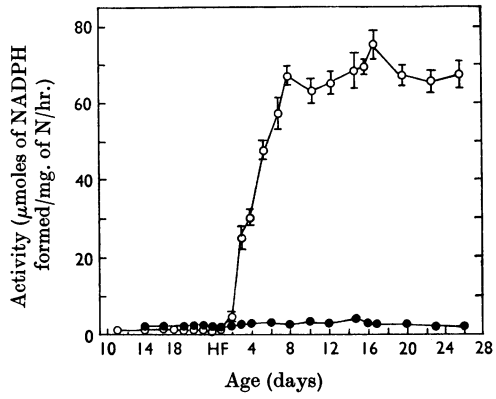

Fig. 2. Activity of 'malic' enzyme in liver (○) and adipose tissue (●). Each point represents the mean of four to 12 experiments. For further explanation see the legend to Fig. 1.

The activities of liver and adipose-tissue HMP-shunt dehydrogenase changed little during the period studied (Fig. 3). Activity in the liver increased from 5 μmoles/mg. of N/hr. at the end of the day of starvation after hatching to a peak value of 7·5 μmoles/mg. of N/hr. 2 days after feeding was initiated. As the chicks grew older the small (but statistically significant) oscillations in liver HMP-shunt dehydrogenase activity appeared to be

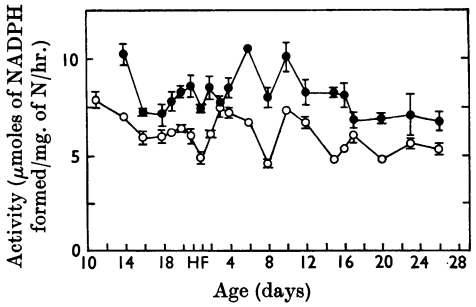

Fig. 3. Activity of HMP-shunt dehydrogenases in liver (○) and adipose tissue (●). Expression of data and numbers of experiments are the same as in Fig. 2. For further explanation see the legend to Fig. 1.

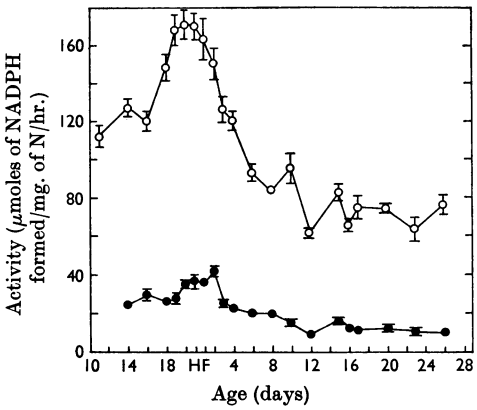

Fig. 4. Activity of isocitrate dehydrogenase in liver (○) and adipose tissue (●). Expression of data and numbers of experiments are the same as in Fig. 2. For further explanation see the legend to Fig. 1.

damped out. Changes in the activity of the adipose-tissue enzyme were not significant.

The activity of the soluble NADP-linked isocitrate dehydrogenase of liver (Fig. 4) increased from 112 μmoles/mg. of N/hr. in 11-day embryos to a peak of 170 μmoles/mg. of N/hr. at the time of hatching. Activity decreased rapidly after hatching. Some fluctuation occurred between 8 and 17 days, but thereafter activity stabilized at about 75 μmoles/mg. of N/hr. The activity pattern for the adipose-tissue enzyme was similar to that for the liver enzyme except that the magnitude of the increase was much smaller and peak activity occurred 2 days after hatching rather than at hatching.

The activities of adipose-tissue and liver soluble NAD-linked malate dehydrogenase showed no significant trends during the period studied. The liver enzyme varied in activity from 700 to 1200 μmoles/mg. of N/hr. and the adipose-tissue enzyme from 330 to 550 μmoles/mg. of N/hr. Similar patterns of activity for liver soluble malate dehydrogenase have been reported by Solomon (1959) for the embryo and chick and by Rinaudo & Giunta (1967) for the embryo.

The activities of hepatic glyceraldehyde 3-phosphate dehydrogenase and soluble NAD-linked α-glycerophosphate dehydrogenase also showed no significant changes during the period studied, except for a 44% decrease in the activity of the glyceraldehyde 3-phosphate dehydrogenase between 16 and 28–30 days of age.

Patterns of enzyme activity in liver and adipose tissue would be the same as just described if the data were expressed on a tissue-wet-weight or body-weight basis. Liver nitrogen concentration was essentially constant during the period of study, fluctuating from about 2·4 to 3·0 mg. of N/100 mg. wet wt. during the period of observation. Nitrogen content of adipose tissue was 0·9 mg. of N/100 mg. wet wt. in 14-day embryos. This value decreased to 0·2 mg. of N/100 mg. wet wt. in hatching chicks. After a slight rise in the starved chicks, it returned to 0·2 mg. of N/100 mg. wet wt. in 6-day-old chicks and remained at that level for the rest of the period studied.

DISCUSSION

Lipogenesis and the activities of 'malic' enzyme and citrate-cleavage enzyme were closely correlated in chick liver and adipose tissue. Fatty acid synthesis (Goodridge, 1968*a*,*b*), 'malic' enzyme activity and citrate-cleavage enzyme activity were very low in chick adipose tissue at all ages and in livers from embryos, but were very high in the livers of older chicks. The activities of 'malic' enzyme and citrate-cleavage enzyme increased at the same time as lipogenesis in the livers of the newly hatched chicks. The increases in lipogenesis and the activities of these two enzymes were food-dependent (citrate-cleavage enzyme activity continued to increase in the absence of food, but the change was only a fraction of that observed when the chicks were fed) (Goodridge, 1968*c*). The chick can therefore be added to the list of species in which 'malic' enzyme and citrate-cleavage enzyme appear to play an important role in hepatic lipogenesis.

Soluble malate dehydrogenase activity was high in both embryonic and chick liver and showed essentially no change as hepatic lipogenesis increased. Shrago & Lardy (1966) and Walter, Paetkau & Lardy (1966) have suggested that this enzyme may be involved in the formation of phosphoenolpyruvate from dicarboxylic acids and amino acids in rat liver. Since gluconeogenesis is a very active process in embryonic chick liver (Kilsheimer, Weber & Ashmore, 1960; Wallace & Newsholme, 1967), a high activity of malate dehydrogenase might be expected.

The dehydrogenases of the HMP shunt have also been implicated in lipogenesis in liver, adipose tissue and mammary-gland tissue of rats and mice (Masoro, 1962; Ball, 1966). In general, changes in the activities of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase parallel changes in the activities of 'malic' enzyme and citrate-cleavage enzyme and fatty acid synthesis in mammalian tissues. The fact that HMP-shunt dehydrogenase activity did not increase in chick liver as lipogenesis increased suggests that these enzymes may not play an important role in chick hepatic lipogenesis. Duncan & Common (1967) reached a similar conclusion. They found that the value of the quotient $^{14}CO_2$ liberated from [1-^{14}C]-glucose/$^{14}CO_2$ liberated from [6-^{14}C]glucose did not deviate appreciably from unity in liver slices from chickens of different ages and sex. They concluded that the HMP shunt, if active at all in chicken liver, played a minor role in the catabolism of glucose as compared with its role in rat liver. Evidence presented by Goodridge & Ball (1966, 1967) suggests that the HMP-shunt dehydrogenases also play a minor role in lipogenesis in pigeon liver.

The activity of the soluble NADP-linked isocitrate dehydrogenase was measured in these experiments because of its possible involvement in the production of extramitochondrial NADPH. Except for the increase in activity that occurred in late embryos, the activity pattern for isocitrate dehydrogenase was the opposite of that for 'malic' enzyme, citrate-cleavage enzyme and lipogenesis. The results suggest that soluble NADP-linked isocitrate dehydrogenase may be involved in a metabolic pathway other than lipogenesis (see Goodridge, 1968*c*).

Neither soluble NAD-linked α-glycerophosphate dehydrogenase nor glyceraldehyde 3-phosphate dehydrogenase showed any significant change in activity when the newly hatched chicks were fed. These findings are in keeping with the role of α-glycerophosphate dehydrogenase as a producer of α-glycerophosphate, which is used in triglyceride synthesis. Esterification of fatty acids to triglycerides may be high in livers from both embryos and chicks. The findings are also in keeping with the dual role of glyceraldehyde 3-phosphate dehydrogenase in gluconeogenesis (high in embryonic liver) and glycolysis (high in liver of growing chicks).

The increases in citrate-cleavage enzyme and 'malic' enzyme activities in chick liver after hatching strongly resemble the increases in the activities of these enzymes observed in weanling rats (Ballard & Hanson, 1967; Taylor, Bailey & Bartley, 1967; Vernon & Walker, 1968). In both cases the changes are associated with dramatic increases in lipogenesis (Goodridge, 1968*b*; Ballard & Hanson, 1967; Taylor *et al.* 1967) and with a change from a high-fat low-carbohydrate diet to a low-fat high-carbohydrate diet. The enzymic changes may have led to the increase in lipogenesis, or, more likely, they may have been secondary to an increased flow of metabolites from glucose to fatty acids (cf. Goodridge & Ball, 1967; Srere & Foster, 1967).

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