Hepatic Acetyl CoA Carboxylase, Propionyl CoA Carboxylase and Pyruvate Carboxylase Activities During Embryonic Development and Growth in Chickens[1,2] (35093)

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The development of gluconeogenic and lipogenic pathways in animals as well as the enzymes involved in these pathways has been investigated by various workers (1–9). Lipid synthesis is negligible in the developing chicken embryo (6) but increases after hatching to normal levels in 8–10 days. Citrate cleavage enzyme and "malic" enzyme activities parallel these changes in lipogenesis (7, 8). Since the developing avian egg is endowed with a limited supply of carbohydrate (10), there is a need for increased glucose synthesis during embryogenesis. In the chicken embryo the hepatic activities of glucose-6-phosphatase and fructose-1, 6-diphosphatase are very high, whereas the activities of hexokinase, phosphofructokinase, and pyruvate kinase are very low in comparison with the adult (5).

In the present study we have examined the activities of acetyl CoA carboxylase, propionyl CoA carboxylase, and pyruvate carboxylase in the livers of chickens during embryonic development and until adulthood.

Materials and Methods. Animals. Incubated eggs were obtained from the University of Illinois Poultry Farm through the courtesy of Dr. H. M. Scott. Newly hatched female chicks (New Hampshire males × Columbian females) were placed on the day of hatch in raised wire floor cages with adequate heating and fed *ad libitum* a corn-based chick diet[3] containing 59% carbohydrate. Water was provided at all times.

Enzyme preparations and assays. Chickens were killed by cervical dislocation and the livers were quickly removed and placed in the homogenizing medium. All enzyme preparations were done at 0–4°. Samples for pyruvate carboxylase and propionyl CoA carboxylase were prepared by homogenizing the liver and sonicating the homogenate (5%) in a 0.25 *M* sucrose medium containing 0.02 *M* Tris–HCl buffer, pH 7.4, 1 m*M* reduced glutathione (GSH) and 1 m*M* EDTA (11). Livers from eight embryos were pooled and homogenized in 5–10 ml of medium. The sonicated homogenate was centrifuged at 20,000*g* for 20 min and appropriate aliquots of the supernatant were used for enzyme assays. Samples for acetyl CoA carboxylase were prepared by homogenizing the liver (pooled for 8 embryos) for 1 min, using a Potter-Elvehjem homogenizer, in a medium containing 0.05 *M* Tris–HCl buffer, pH 7.2, 0.15 *M* KCl and 0.1 m*M* EDTA. The homogenate (5%) was centrifuged at 20,000*g* for 10 min. The supernatant was recentrifuged at 100,000*g* for 1 hr. An aliquot (0.1 ml) of the 100,000*g* supernatant was used for the assay.

Pyruvate carboxylase was assayed as described by Deodhar and Mistry (11) except that the incubation period was 3 min.

Propionyl CoA carboxylase was assayed by the $^{14}CO_2$-fixation method of Lane and Halenz (12) except that the reaction was stopped after 8 min with 0.3 ml of 20%

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[3] University of Illinois Agr. Exp. Sta. Chicken Diet No. 521.

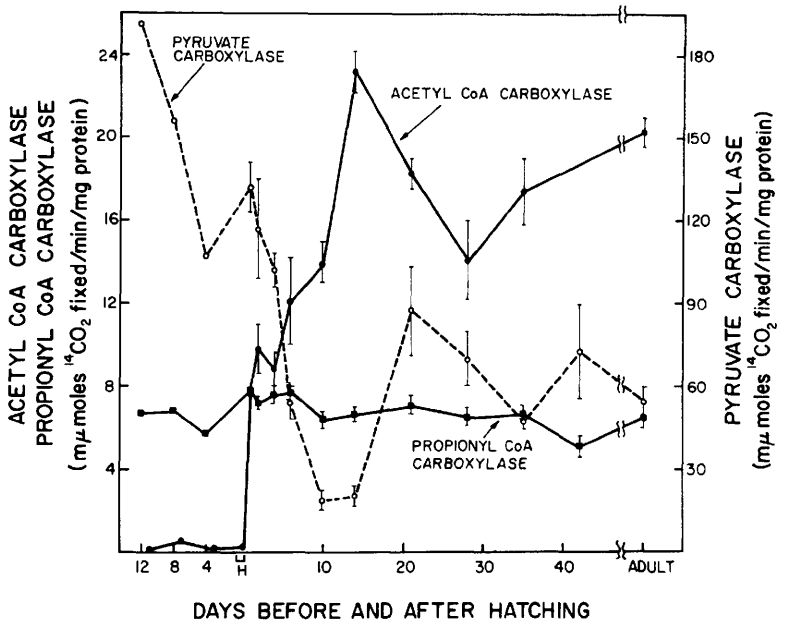

FIG. 1. Hepatic acetyl CoA carboxylase, propionyl CoA carboxylase, and pyruvate carboxylase activities during embryonic development and growth in chickens. Each point represents the mean ± standard error of 4–12 chickens; vertical bars are omitted where pooled samples were used or where the standard error is too small to be represented; H indicates the day of hatching.

trichloroacetic acid.

Acetyl CoA carboxylase was assayed by a slight modification of the method of Greenspan and Lowenstein (13). The reaction mixture in a total volume of 1 ml contained (μmoles): Tris–HCl buffer (pH 7.5), 10; $MgCl_2$, 10; sodium citrate, 10; GSH, 2; ATP, 2; acetyl CoA, 0.2; $NaH^{14}CO_3$ (0.2 μCi/μmole), 20; and BSA[4], 0.6 mg. The reaction was initiated by adding the enzyme and stopped after 5 min with 0.2 ml 6 *N* HCl. Both $MgCl_2$ and citrate were omitted from the blanks. Very little activity was observed in the absence of BSA.

In all assays the residual ^{14}C-bicarbonate was flushed out of the protein-free supernate by the repeated additions of pieces of dry ice (11). Aliquots were then counted in a liquid scintillation counter after adding 15 ml of dioxane scintillant (14). Protein was determined by the procedure of Lowry *et al.* (15).

Results and Discussion. Hepatic biotin enzyme activities in the chicken from the embryonic stage to adulthood are shown in Fig. 1. Acetyl CoA carboxylase activity was hardly detectable in embryos. With the onset of feeding after hatching, the activity increased markedly in the first 10 days, representing a 50-fold increase from the day of hatch. Values comparable to adult hepatic activities were reached in 10–14 days. This finding is in agreement with the data of Goodridge (6) regarding the development of hepatic lipogenesis in chicks. The low rates of fatty acid synthesis from glucose observed in embryonic chicken liver (6) and adipose tissue (16) increased after hatching to normal levels in 6–12 days. Goodridge (6) has attributed this increase in lipogenesis following hatching to the transition from the high-fat, low-carbohydrate environment of egg yolk to the low-fat, high-carbohydrate diet of growing chickens.

It is now generally agreed that actyl CoA carboxylase is the rate-limiting enzyme in fatty acid biosynthesis because of its allosteric properties (17–19). The activity of this enzyme in the liver (20) and adipose tissue

[4] Bovine serum albumin, fatty acid poor, from Mann Research Lab., N.Y.

(21) of the rat increases when the animals are fed a diet high in carbohydrate. On the other hand, diets rich in fat have the opposite effect. The acetyl CoA carboxylase profile shown in Fig. 1 resembles the activity profiles of "malic" enzyme and citrate cleavage enzyme which correlate well with the developmental pattern of lipogenesis in embryonic and growing chicks (6, 7). From the evolutionary standpoint, it is understandable that there was no necessity to elaborate these enzymes since the embryonic environment is rich in lipids.

In the chicken embryo, hepatic pyruvate carboxylase activity was 2–3-fold higher than in the adult liver (Fig. 1). After hatching, the enzyme activity progressively declined to minimal values in 10–14 days, only to display a pronounced increase in the third week. Thereafter, the activity profile showed no further significant changes until adulthood.

The activities of key gluconeogenic enzymes, namely, pyruvate carboxylase (see Fig. 1), glucose-6-phosphatase and fructose-1, 6-diphosphatase (5) are high in chicken embryos whereas hexokinase, pyruvate kinase, and phosphofructokinase activities are low (5) although U-^{14}C-alanine, 2-^{14}C-glutamate and ^{14}C-bicarbonate have been claimed to be incorporated into glucose to the same extent in embryos as in adult chickens (2). Our results, the enzyme data of Wallace and Newsholme (5), and the data of Goodridge (6, 8) on glucose metabolism in embryonic, newly-hatched, and growing chickens establish clearly that prior to hatching the pathway of gluconeogenesis is very active.

In the data of Nelson *et al.* (3), embryonic hepatic pyruvate carboxylase activity was reported to be only 50% of the adult value. We found the activity of this enzyme 2–3-fold higher in the liver of the embryo than in the adult. The extremely low activities for unbuffered KCl liver homogenates reported by these authors (0.30 μmoles/min/g of liver for the adult versus 10.93 μmoles/min/g of liver in the present study) was very likely the result of the method employed for the preparation of the homogenate. In a recent study on chicken liver pyruvate carboxylase, Madappally (22) has also observed 50% greater activity even in day-old chickens compared to the activity in adult birds. We interpret the marked decline in the activity of pyruvate carboxylase, from 117 in the 2-day-old chicken, to about 20 μmoles/min/mg of protein 10–14 days after hatching, as an adaptation to carbohydrate availability before and after hatching.

The activity of propionyl CoA carboxylase remained relatively constant, between 5 and 8 mμmoles/min/mg of protein, throughout the period examined. This enzyme is associated with the degradative pathway of aliphatic branched chain amino acids but can also function in lipid metabolism since propionyl CoA results from the breakdown of odd chain fatty acids. In contrast to hepatic pyruvate carboxylase and acetyl CoA carboxylase, propionyl CoA carboxylase does not appear to be adaptive to carbohydrate availability before or after hatching.

Summary. Acetyl CoA carboxylase activity was negligible in embryonic chicken liver, but increased markedly after hatching to adult levels in 2 weeks. Very high pyruvate carboxylase activity observed in embryonic livers progressively decreased, reached minimum values 10–14 days posthatching, and thereafter increased to adult levels. Propionyl CoA carboxylase, on the other hand, showed no such adaptive changes throughout the period studied.

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