The Presence of Acetyl-Coenzyme A Carboxylase Apoenzyme in the Liver of Newly Hatched Chicks

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Previous studies have shown that the increase of liver lipogenesis observed in chicks after hatching is accompanied by an increase in the activity of acetyl-CoA carboxylase (Ryder, 1970; Arinze & Mistry, 1970) and citrate ATP-lyase (Felicoli & Gabrielli, 1967; Goodridge, 1968), enzymes that are associated with this pathway.

Goodridge (1970) has suggested that the stimulation by dietary fructose of fatty acid biosynthesis in the livers of newly hatched chicks indicates that fatty acid-synthesizing enzymes must have been synthesized during embryonic life and were present in a latent form.

The present study was undertaken to investigate the presence of hepatic acetyl-CoA carboxylase apoenzyme in the embryonic period of the chick. Fertile eggs and chicks were obtained from local farms. The reaction used to assay the apoenzyme was the ATP-dependent incorporation of [^{14}C]biotin into the protein fraction precipitated by 25%-saturated $(\text{NH}_4)_2\text{SO}_4$ from the 13000g clear supernatant (Gregolin *et al.*, 1968), as described by Kosow *et al.* (1962) for propionyl-CoA carboxylase. These authors demonstrated that the rate of holoenzyme synthesis and the rate of [^{14}C]biotin binding to the protein followed the same pattern.

The reaction mixture contained 60mM-tris-HCl buffer, pH 7.3, 3mM-GSH, 5mM-MgCl₂, 2mM-ATP, 0.25 μCi of [^{14}C]biotin (specific radioactivity 58 Ci/mol; Amersham/Searle Co., Des Plaines, Ill., U.S.A.) and the enzyme preparation (dialysed overnight in 10mM-potassium phosphate buffer, pH 7.1). The mixture was incubated at 37°C for 1 h in a final volume of 1 ml. The reaction was stopped by adding 5 ml of ice-cold 6% (w/v) trichloroacetic acid. The precipitated protein was washed as described by Kosow *et al.* (1962) and finally completely dissolved in 1 ml of Soluene TM100 (Packard Instrument Co., Downers Grove, Ill., U.S.A.). The ^{14}C radioactivity bound to protein was measured in a Packard Tri-Carb liquid-scintillation spectrometer, with toluene-based scintillation mixture, with an efficiency of 82%. Protein was determined by the method of Lowry *et al.* (1951), with crystalline bovine plasma albumin (Calbiochem, Los Angeles, Calif., U.S.A.) as standard.

The amount of biotin bound was proportional to protein concentration. In 60 min the incorporation

reached a plateau, and this time was chosen for routine experiments. Addition of citrate (5–10 mM) to the reaction mixture did not affect the rate of incorporation of [^{14}C]biotin into the protein; omission of Mg^{2+} gave higher values, as reported by Kosow *et al.* (1962). The reaction was strictly dependent on ATP. A zero-time blank, in which the reaction was stopped at the start of the incubation period, was also included in some experiments.

Table 1 shows that in embryonic tissue, until the very last moment, as determined by the breaking of the shell, the incorporation of [^{14}C]biotin was very low (less than 20 c.p.m./mg of protein). In these experiments the period of incubation was extended to 120 min, without any increase in incorporation being obtained.

During the first 24 h after hatching, without any food intake, the rate of incorporation rose to about 300 c.p.m./mg of protein. This incorporation of [^{14}C]biotin represents 3.04 pmol of biotin/mg of protein. After 5 days on a balanced diet (Pollarina 2A; Protinal, Maracaibo, Venezuela) the radioactivity incorporated was more than 3 times that in the newly hatched chicks.

The results suggest that the apoenzyme is synthesized, independent of feeding, in response to hatching. In other experiments (Ryder & Teruel, 1971) on the incorporation *in vivo* of [^{14}C]biotin into liver carboxylase protein of chicks the incorporation of radioactivity in 1-day-old chicks did not correspond to the low amount of enzyme activity. This could be due to biotin fixation into apoenzyme, formed during the first day of the post-hatching period, but still in an inactive form.

The active holoenzyme might result from a stimulation of this inactive form, provoked by feeding. The presence of carboxylase holoenzyme synthetase and biotin is likely because Arinze & Mistry (1970) have demonstrated high activities for propionyl-CoA carboxylase and pyruvate carboxylase, both biotin enzymes, during the embryonic period of the chick, and it is assumed (Ochoa & Kaziro, 1965) that the same holoenzyme synthetase is active for all biotin enzymes.

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