Effect of Development on Chicken Liver Acetyl-Coenzyme A Carboxylase

By ELENA RYDER

Instituto de Investigación Clínica, Facultad de Medicina, Universidad del Zulia, Apartado 1151, Maracaibo, Venezuela

(Received 10 August 1970)

Lipogenesis is exceedingly active in the liver of rats, especially during the later part of embryonic life; it decreases markedly at birth, but after weaning high activities of fatty acid synthesis, which characterize the adult, are again obtained (Ballard & Hanson, 1967; Vilee & Hagerman, 1958). In the chicken, however, lipogenesis appears only after hatching and attains very high values during the first few days (Goodridge, 1968a). In both cases a close relationship exists between the rate of lipogenesis and the diet given to the animal. The increase of lipogenesis observed in chicks after hatching is accompanied by an increase in the activity of enzymes such as ATP citrate lyase and 'malic' enzyme, which are associated with fatty acid synthesis (Felicoli & Gabrielli, 1967; Goodridge, 1968b).

The regulation of lipogenesis has been much investigated, and considerable attention has been paid to the possible importance of acetyl-CoA carboxylase as a regulatory enzyme (Numa, Bortz & Lynen, 1965). This enzyme can exist as an inactive monomer or as an active polymer (Vagelos, Alberts & Martin, 1963; Matsushashi, Matsushashi & Lynen, 1964; Numa *et al.* 1965; Gregolin, Ryder, Warner, Kleinschmidt & Lane, 1966); the conversion takes place in the presence of citrate and is the point where feedback inhibition is exerted. I have studied the activity of this regulatory enzyme in livers from chicks of different ages, from 9 days of incubation to 18 days after hatching.

Fertile eggs and chicks were obtained from local farms. The chicks were maintained with a balanced diet of Pollarina 2A (Protinal C.A., Maracaibo, Venezuela), and had water *ad libitum*. At the specified ages they were killed by decapitation and liver homogenates prepared and tested for enzymic activity by the procedure of Chang, Seidman, Teebor & Lane (1967). Units of enzymic activity are expressed as nmol of malonyl-CoA formed/min per mg of protein (mU/mg). Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951).

The results are shown in Fig. 1. During the early embryonic period the activity of acetyl-CoA carboxylase is very low (less than 1 mU/mg), and rises only very slowly to 1.6 mU/mg at birth. After

hatching the activity rises markedly and progressively, attaining a maximum on the eleventh day of about 21 mU/mg. This is 15.6 times the activity in the liver of the newly hatched chick, and is 100 times that found in the embryos.

I have observed that the enzyme activity shows a complete dependence on the presence of citrate. The optimum concentration of citrate was 5 mM, and a marked inhibition was observed at 20 mM. The enzyme was active without any preincubation with citrate, provided that the latter was present at optimum concentrations in the test system; reactions started by the addition of enzyme showed no lag period. In the absence of citrate no increase in activity with time was observed. All these properties were similar throughout the life period studied.

The possible contamination of the liver homogenate by avidin, contained in the egg albumin, was avoided by preincubation of the enzyme preparation with an excess of biotin. The presence of biotin had no effect on the enzyme activity.

Fig. 1. Acetyl-CoA carboxylase activity in chick liver. Points from the incubation period represent averages of pooled tissue samples. Points after hatching represent the means of two to five experiments. The vertical bars indicate S.D. In each assay four protein concentrations were tested to ensure proportionality of initial reaction rates to amount of enzyme.

The great amount of fat contained in egg yolk may possibly explain the absence of lipogenic activity during the embryonic period, since the carboxylase is known to be strongly inhibited by free fatty acids and their CoA derivatives (Numa *et al.* 1965). After hatching of the chick, a diet rich in carbohydrates might decrease such inhibition, although the liver still contains much fat. The increase in fatty acid synthesis is probably largely due, however, to other factors; the elevation of the activity of enzymes concerned with lipogenesis observed on re-feeding rats previously subjected to a starvation period is due to an adaptive synthesis of new enzyme proteins (Hicks, Allman & Gibson, 1965).

An apparent discrepancy exists between the pattern of appearance of lipogenesis and of carboxylase activity during the first few days after hatching. Goodridge (1968a) found very high activities of lipogenesis on the first day, when the carboxylase activity is still low. The same difference appears to exist between carboxylase activity and that of relevant enzymes as ATP citrate lyase and 'malic' enzyme. These latter enzyme activities increase rapidly after hatching and feeding (Goodridge, 1968b).

A preliminary account of this work was presented at the 19th Convención Anual de AsoVAC, Caracas, Venezuela, in May 1969.

- Ballard, F. J. & Hanson, R. W. (1967). *Biochem. J.* **102**, 952.
- Chang, H. C., Seidman, I., Teebor, G. & Lane, M. D. (1967). *Biochem. biophys. Res. Commun.* **28**, 682.
- Felicioli, R. A. & Gabrielli, F. (1967). *Experientia*, **23**, 1000.
- Goodridge, A. G. (1968a). *Biochem. J.* **108**, 655.
- Goodridge, A. G. (1968b). *Biochem. J.* **108**, 663.
- Gregolin, C., Ryder, E., Warner, R. C., Kleinschmidt, A. K. & Lane, M. D. (1966). *Proc. natn. Acad. Sci. U.S.A.* **56**, 1751.
- Hicks, S. E., Allman, D. W. & Gibson, D. M. (1965). *Biochim. biophys. Acta*, **106**, 441.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *J. biol. Chem.* **193**, 265.
- Matsuhashi, M., Matsuhashi, S. & Lynen, F. (1964). *Biochem. Z.* **340**, 263.
- Numa, S., Bortz, W. & Lynen, F. (1965). In *Advances in Enzyme Regulation*, vol. 3, p. 407. Ed. by Weber, G. Oxford: Pergamon Press Ltd.
- Vagelos, P. R., Alberts, A. W. & Martin, D. B. (1963). *J. biol. Chem.* **238**, 533.
- Villee, C. A. & Hagerman, D. D. (1958). *Am. J. Physiol.* **194**, 457.