The Effect of Dietary Fat on Lipogenic Enzymes in the Liver of the Domestic Fowl

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It is well established that lipid synthesis in the liver is dependent on the dietary regime of the animal. Hepatic lipogenesis is increased when a high-carbohydrate diet is given (Lyon, Masri & Chaikoff, 1952) and is decreased when the animal is starved (Lyon *et al.* 1952; Butterworth *et al.* 1966) or fed on fat-supplemented diets (Hill, Webster, Linazasoro & Chaikoff, 1960; Bortz, Abraham & Chaikoff, 1963). The rate-limiting step in lipogenesis is acetyl-CoA carboxylase (Numa, Matsuhashi & Lynen, 1961; Ganguly, 1960) and it is at this point that control has been shown to be exerted to decrease lipogenesis in fat-fed rats (Bortz *et al.* 1963). There is a considerable volume of information on the effects of dietary manipulation on enzyme activity in mammalian liver systems, but in contrast little work has been carried out on avian systems, particularly the domestic fowl, where the situation is more complex owing to the demands of egg production. In the present work the effect of fat-feeding on the lipogenic enzymes acetyl-CoA carboxylase and citrate-cleavage enzyme were investigated in two breeds of domestic fowl after various time-intervals on a fat-supplemented diet. Isocitrate dehydrogenase was also investigated, since this enzyme is implicated in the further metabolism of extramitochondrial citrate and in the production of NADPH, which may be utilized in fatty acid biosynthesis.

Methods. Two breeds of light hybrid birds, Hyline and Hybrid 4, were maintained on a low-fat control diet (Balnave, 1968) from 1 day old. Hyline birds were maintained on this diet for 48 weeks and the Hybrid 4 birds for 45 weeks before dietary supplementation with 2% maize oil. The maize oil was substituted isocalorically with respect to the metabolizable energy of the diet by alteration of the cornflour and oathull content. Birds were killed by decapitation and bled from the jugular vein. The livers were rapidly removed, chilled and portions homogenized with 4 vol. of ice-cold $0\cdot1$ M-potassium phosphate buffer, pH 7·0, containing 7·0 mM-2-mercaptoethanol. The homogenates were centrifuged at $100000g$ for 1 hr. at 0° (MSE 40 centrifuge) and the resulting clear supernatants assayed for the following enzyme activities. NADP-

specific isocitrate dehydrogenase (EC 1.1.1.42) was assayed essentially by the method of Ochoa (1948) except that 200 μmoles of potassium phosphate buffer, pH 7·0, were used in the assay system. Citrate-cleavage enzyme (EC 4.1.3.8) was measured by the rate of reaction of oxaloacetate with NADH in the presence of malate dehydrogenase as described by Srere (1959) except that 200 μmoles of potassium phosphate buffer, pH 7·0, in a final volume of 3·0 ml. were used. Both these assays were carried out at 40° in a Unicam SP.800 recording spectrophotometer. Acetyl-CoA carboxylase (EC 6.4.1.2) was assayed as described by Levy (1963) except that potassium phosphate buffer, pH 7·0, was used. After 5 min. incubation at 40° the reaction was terminated by the addition of 0·2 ml. of 30% (w/v) $HClO_4$. The reaction mixtures were gassed with air $+ CO_2$ (95:5) for 2 min. to release trapped $^{14}CO_2$ and after spinning off the precipitated protein the incorporated radioactivity in 0·1 ml. samples was determined in a liquid-scintillation assembly as described by Brown & Badman (1961). In the above assays, enzyme activity was linear with respect to both time and protein concentration. The protein content of cell-free extracts was determined by the biuret reaction (Layne, 1957).

Results. The effects of feeding with fat-supplemented diets on hepatic enzyme activity of two breeds of laying hen are shown in Table 1. There was a marked decrease in the activities of citrate-cleavage enzyme and acetyl-CoA carboxylase after only 2 hr. on the maize-oil-supplemented diet. Changes in hepatic lipogenesis in rats over similar short time-intervals on fat-supplemented diets have been reported by Hill *et al.* (1960) and Bortz *et al.* (1963). Citrate-cleavage enzyme remained at this level of activity throughout the rest of the experiment. However, the activity of acetyl-CoA carboxylase continued to decrease during the experimental period. Although the activity of isocitrate dehydrogenase initially increased to a maximum after a few days on the maize-oil-supplemented diet, over longer time-periods the activity of this enzyme decreased to less than that of control birds.

The results with both breeds of birds were

Table 1. *Effect of length of time of fat-feeding on hepatic enzyme activity*

Enzyme activities are expressed as $m\mu$moles of substrate metabolized/min./mg. of protein in the extract, and are given as means $\pm$ S.E.M. with the numbers of birds used in parentheses. (a) Hybrid 4 birds; (b) Hyline birds. n.t., Not tested; n.d., Not detectable.

Time on fat-supplemented diet	Acetyl-CoA carboxylase		Citrate-cleavage enzyme		Isocitrate dehydrogenase	
	(a)	(b)	(a)	(b)	(a)	(b)
0 (controls)	$2\cdot68\pm0\cdot16$ (3)	$1\cdot59\pm0\cdot33$ (5)	$18\cdot31\pm1\cdot58$ (3)	$15\cdot39\pm1\cdot55$ (5)	474 ± 29 (3)	376 ± 31 (5)
2 hr.	$1\cdot61\pm0\cdot31$ (3)	n.t.	$6\cdot26\pm0\cdot72$ (3)	$7\cdot48\pm0\cdot68$ (4)	471 ± 52 (3)	352 ± 13 (4)
12 hr.	$1\cdot02\pm0\cdot11$ (4)	n.t.	$8\cdot09\pm0\cdot91$ (4)	n.t.	507 ± 24 (4)	n.t.
24 hr.	$0\cdot57\pm0\cdot17$ (4)	$1\cdot21\pm0\cdot07$ (4)	$6\cdot84\pm0\cdot53$ (4)	$7\cdot83\pm0\cdot58$ (4)	509 ± 15 (4)	427 ± 35 (4)
48 hr.	$0\cdot06\pm0\cdot01$ (4)	n.t.	$7\cdot93\pm1\cdot25$ (4)	n.t.	366 ± 12 (4)	n.t.
4 days	n.t.	$0\cdot53\pm0\cdot04$ (3)	n.t.	$5\cdot21\pm0\cdot95$ (3)	n.t.	458 ± 55 (3)
8 days	n.t.	$0\cdot20\pm0\cdot04$ (4)	n.t.	$7\cdot95\pm0\cdot43$ (4)	n.t.	465 ± 22 (4)
10 days	n.d.	n.t.	$5\cdot97\pm0\cdot37$ (3)	n.t.	262 ± 29 (3)	n.t.
28 days	n.t.	n.d.	n.t.	$8\cdot05\pm0\cdot60$ (4)	n.t.	300 ± 13 (4)

essentially similar although there was a discrepancy in the time-course of some of the observed changes, i.e. the changes in activity of acetyl-CoA carboxylase and isocitrate dehydrogenase occurred more rapidly in Hybrid 4 birds than in Hyline birds. It was concluded that this was due to strain difference.

Discussion. It has been proposed that the feedback inhibition of fat production due to dietary fat is a homoeostatic control mechanism to prevent the over-production of fat (Masoro, 1962; Bortz *et al.* 1963). The repression of acetyl-CoA carboxylase and citrate-cleavage enzyme reported here are consistent with this proposal. The decreases in activity of these enzymes are also in agreement with the results of Weiss, Naber & Johnson (1967), who showed that laying birds on fat-supplemented diets incorporated less [^{14}C]acetate into liver lipids than control birds. The fact that acetyl-CoA carboxylase decreased in activity more than citrate-cleavage enzyme clearly shows that a greater control of lipid synthesis is exercised by acetyl-CoA carboxylase; this would be expected from the function of this enzyme as the rate-limiting step in fatty acid biosynthesis.

Isocitrate dehydrogenase showed a slight increase in activity soon after the birds were placed on the fat-supplemented diet. Bortz *et al.* (1963) also showed a slight increase (10%) in isocitrate dehydrogenase activity in liver extracts from rats given fat-supplemented diets for 4 hr. This is possibly due to an adaptive stimulation of the enzyme by citrate, which would increase in concentration as a result of the repression of citrate-cleavage enzyme; citrate has been shown to activate isocitrate dehydrogenase allosterically in rat liver (Sanwal, Zink & Stachow, 1963).

The production of NADPH is a integral part of

fatty acid biosynthesis. Duncan & Common (1967), investigating the $^{14}CO_2$ production from [1-^{14}C]- and [6-^{14}C]-glucose by chicken liver slices, concluded that the pentose phosphate pathway is not operative to any great extent in immature and laying domestic fowl and that this pathway is not an important source of NADPH for fatty acid formation. In the present study isocitrate dehydrogenase was investigated as a source of NADPH. After a small initial increase in activity the rate of NADPH production decreased to less than that recorded for control birds. This is probably a reflection of the diminished requirement for NADPH under conditions of repressed fatty acid synthesis. Isocitrate dehydrogenase activity has also been observed to decrease in starvation (Numa *et al.* 1961) and alloxan-diabetes (Wieland, Neufeldt, Numa & Lynen, 1963), two other conditions that result in decreased hepatic lipogenesis, and Weber, Convery, Lea & Stamm (1966) found that the activity of isocitrate dehydrogenase *in vitro* was decreased by octanoate.

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