An Investigation of Lipogenic and Glycolytic Enzyme Activity in the Liver of Sexually Immature and Mature Domestic Fowl

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In the non-laying pullet and the cockerel it was observed that there was no significant variation in the activities of ATP citrate lyase and 'malic' enzyme whereas in the laying hen there was a significantly greater activity of both these enzymes. Parallel increases in liver lipid content in the laying hen were also observed. Three glycolytic enzymes, phosphofructokinase, fructose diphosphate aldolase and pyruvate kinase, did not exhibit any significant variation in enzyme activity with the onset of egg laying. These results are discussed in relation to the hormonal status of the birds and also the demands of egg production for lipid.

Although changes in the activity of hepatic enzymes involved in lipid and carbohydrate metabolism have been studied in the chick during incubation and the early post-hatching period (Wallace & Newsholme, 1967; Felicioli & Gabrielli, 1967; Felicioli, Gabrielli & Rossi, 1967; Goodridge, 1968*a,b,c*; Ryder, 1970), less attention has been given to the changes in metabolism which occur in older birds in relation to the onset of sexual maturity.

The work reported in this paper was carried out to investigate the changes in lipogenic and glycolytic enzyme activity which occur in the liver of the pullet and cockerel with the onset of sexual maturity.

EXPERIMENTAL

Birds and diets. The pullets and cockerels were from the White Leghorn flock at the Agricultural Research Institute, Hillsborough. The chicks were raised from 1 day old on conventional cereal-based rations and at 18 weeks of age were placed in individual cages. Throughout the experiment the birds received food and water *ad libitum*.

Preparation of liver extracts. Birds were killed at different stages of sexual maturity and liver extracts prepared as described previously (Pearce, 1968).

Enzyme assays. ATP citrate lyase (EC 4.1.3.8) and 'malic' enzyme (EC 1.1.1.40) were assayed as previously outlined (Pearce, 1968, 1970). Phosphofructokinase (EC 2.7.1.11), fructose diphosphate aldolase (EC 4.1.2.13) and pyruvate kinase (EC 2.7.1.40) were measured as described by Pearce & Carr (1969). These assays were carried out in a Unicam SP.800 recording spectrophotometer at 40°C and in all cases the recorded activity was linear with respect to both time and protein concentration in the extract. The protein content of the cell-free extracts, the liver total lipid content and statistical analyses were performed as described by Pearce (1970).

RESULTS

The liver total lipid contents were determined in birds of different physiological states. It was observed that although the liver lipid content was very similar in both the immature non-laying pullet and the cockerel, the contents being $6.31 \pm 0.17\%$ (mean $\pm$ S.E.M., four observations) and $6.40 \pm 0.29\%$ respectively, in the laying hen the liver lipid content was considerably higher ($14.20 \pm 4.09\%$). The liver fresh weight was also similar in the non-laying pullet and cockerel but increased significantly ($P < 0.01$) in the laying hen (Table 1).

The activities of two enzymes of hepatic lipogenesis, ATP citrate lyase and the 'malic' enzyme, were not significantly different in the non-laying pullet and cockerel but in the laying hen there was a significant increase ($P < 0.05$) in the activities of both of these enzymes, expressed either in units/mg of protein in the extract or units/g of fresh liver, which coincided with the development of the ovary and oviduct and the onset of lay (Table 1). In the cockerel there was no significant variation in the specific activity of either enzyme between immature and mature birds ($P > 0.05$).

During the experiment some birds became broody and went out of lay. In this case the specific activities of ATP citrate lyase and the 'malic' enzyme decreased towards the enzyme activities observed in the cockerel and the immature pullet (Table 1). The oviduct and ovaries of these birds had regressed slightly suggesting a decrease in oestrogen secretion.

The activities of the glycolytic enzymes, phosphofructokinase, fructose diphosphate aldolase and pyruvate kinase, were determined and it was found

Table 1. *Changes in liver weight and hepatic lipogenic and glycolytic enzyme activity in immature and mature male and female domestic fowl*

	Liver weight (g)	ATP citrate lyase		'Malic' enzyme		Phosphofructokinase		Fructose diphosphate aldolase		Pyruvate kinase	
		(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)
Non-laying pullets (aged 20 weeks)	22.9 ± 1.7	3.77 ± 0.39	0.58 ± 0.02	42.46 ± 5.08	5.46 ± 0.85	43.45 ± 14.36	6.52 ± 1.98	92.04 ± 4.62	8.76 ± 0.60	103.60 ± 14.36	10.92 ± 1.72
Cockerels (aged 20 weeks)	29.9 ± 3.4	4.48 ± 0.95	0.51 ± 0.08	29.76 ± 4.79	4.82 ± 0.72	48.04 ± 16.24	7.00 ± 9.36	66.89 ± 3.98	7.53 ± 0.29	109.90 ± 8.09	11.88 ± 1.03
Laying hens (aged 28 weeks)	39.8 ± 2.7	8.17 ± 0.50	0.77 ± 0.04	99.11 ± 13.98	9.16 ± 0.85	83.48 ± 10.31	8.45 ± 1.18	91.67 ± 11.98	8.52 ± 4.08	101.55 ± 12.45	9.69 ± 1.42
Cockerels (aged 28 weeks)	24.7 ± 1.7	3.00 ± 0.45	0.37 ± 0.05	44.45 ± 8.01	5.87 ± 0.77	89.57 ± 8.30	10.83 ± 1.48	60.09 ± 4.59	7.42 ± 0.21	97.08 ± 14.33	11.87 ± 1.33
Broody hens	—	4.21 ± 0.49	0.40 ± 0.07	52.98 ± 9.13	5.77 ± 1.00	—	—	—	—	—	—

The enzyme assays were performed as given in the Experimental section. Enzyme activities are expressed as either (a) nmol of substrate metabolized/min per mg of protein in the extract, or (b) μ mol of substrate metabolized/min per g of fresh liver. Each result is the mean $\pm$ S.E.M. for four birds.

(Table 1) that there was no significant variation ($P > 0.05$) in enzyme specific activity with the onset of sexual maturity in either pullets or cockerels.

DISCUSSION

In the domestic fowl the liver is the major site of fatty acid synthesis (Goodridge, 1968*d*; O'Hea & Leveille, 1969) and also the liver has been shown to be the site of synthesis of lipids which cause oestrogen-induced lipaemia (Ranney & Chaikoff, 1951). Because of the importance of the liver as a site of lipogenesis in the domestic fowl, changes in hepatic enzyme activity, in relation to the onset of egg laying, were investigated.

Lipogenesis and the activities of ATP citrate lyase and 'malic' enzyme have been shown to be closely correlated in both rat liver (Tepperman & Tepperman, 1964; Kornacker & Lowenstein, 1965; Kornacker & Ball, 1965; Ballard & Hanson, 1967; Taylor, Bailey & Bartley, 1967; Bailey, Taylor & Bartley, 1968) and chick liver (Goodridge, 1968*a,b,c*; Balnave & Pearce, 1969; Yeh, Leveille & Wiley, 1970). In the present work the increased liver lipid content in laying hens, as compared with non-laying pullets and cockerels, was reflected in the activities of these lipogenic enzymes. There were significantly greater activities of both ATP citrate lyase and the 'malic' enzyme, whether expressed in nmol/min per mg of protein in the liver extract or μ mol/min per g of fresh liver, and this together with the increased liver size of the laying hen clearly shows a greatly increased lipogenic capacity in hen liver. These results support the observation of Husbands & Brown (1965) that hepatic lipogenesis is greater in the laying hen than in the cockerel.

The glycolytic enzymes, pyruvate kinase, phosphofructokinase and aldolase, were measured to investigate whether the increases in lipogenic enzyme activity were a result of a general increase in metabolism in the laying hen or were due to a specific stimulation of lipogenesis. The results show that there was little alteration in enzyme activity either between sexes or with physiological state, suggesting that the increases in ATP citrate lyase and 'malic' enzyme activities are the result of a specific stimulation of lipogenesis.

Previous work in this department (Pearce & Brown, 1971) with immature pullets has shown that, although gonadal hormone administration had a profound effect on the gross physiology of the birds, there were no significant variations in hepatic enzyme specific activities with hormone treatment. It is noteworthy that the situation is not the same in the mature hen and the oestrogenized pullet. In the laying hen lipogenic enzyme activity may be under the control of the hormonal

state of the bird but it clearly reflects the demand for lipogenesis in the liver. The laying hen had a great requirement for lipid for egg laying whereas there is not this demand in the cockerel or the non-laying pullet. In addition, in the non-laying broody hen the lipogenic enzyme activities were similar to those recorded for the immature non-laying hen or the cockerel.

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