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THE EFFECTS OF VARYING DOSES OF OESTRADIOL ON LIVER LIPID METABOLISM IN THE IMMATURE PULLET

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Abstract—An experiment was carried out to examine the time-effects of various intramuscularly administered doses of oestradiol (0, 0·5, 1, 2 and 4 mg) on liver lipid metabolism in immature pullets. After 2 days of hormone treatment there was a close-related response in liver weight, liver lipid content and lipogenic enzyme specific activities up to maximal values with a dose of 2 mg hormone. After 8 days of hormone treatment there were similar dose related increases in liver weight and liver lipid content; again the maximal values were observed with a dose of 2 mg hormone. However, the lipogenic enzyme specific activities were reduced in oestradiol-treated birds compared with non-hormone treated birds; these results are discussed in relation to the situation in the laying hen. The fatty acid composition of the liver lipid from the oestrogenized pullets showed a pattern intermediate between that found in the immature bird and that in the mature laying hen. These results indicate the role of oestrogens in liver lipid metabolism in the mature bird and show that some of the changes in metabolism can be simulated by the administration of oestradiol to immature pullets. The results also suggest that in studies with immature birds metabolic effects within 2 days of hormone treatment are more indicative of the physiological role of oestradiol in the mature bird than longer periods of hormone administration.

It is well known that changes in the physiology of birds, similar to those encountered when the pullet comes into lay, can be induced by the administration of oestrogen to immature pullets [1]. One such oestrogen-induced effect is an increase in the blood and liver lipid content of oestrogen-treated birds. In the domestic fowl the liver is the major site of lipogenesis [2] and also of the oestrogen-induced lipaemia in immature pullets [3].

It has recently been shown that this oestrogen-induced increase in the liver lipid content of immature pullets is accompanied by an increase in liver lipogenic enzyme activity [4, 5]. These workers showed that increased lipogenic enzyme activities were observed 2 days following the intramuscular administration of 2 mg oestradiol. After longer time intervals (8 and 9 days of hormone administration) the lipogenic enzyme activities were similar or slightly lower in oestradiol-treated pullets compared with non-hormone treated birds. In this work intramuscular injections of 2 mg oestradiol were given on alternate days throughout the experiment. This procedure of administering hormones on alternate days has been standard practice in experiments investigating the effects of gonadal hormones on the metabolism of immature domestic fowl in this and other centres [6–14]. Balnave [15] suggested that although the administration of oestradiol to immature pullets induces changes in blood and liver fatty acids which are qualitatively similar to those obtained at sexual maturity, quantitative differences may nevertheless exist depending on the amount of hormone administered.

The present work was undertaken to study the effects of different doses of oestradiol on the physiology and liver lipid metabolism of immature pullets after 2 and 8 days of hormone treatment.

MATERIALS AND METHODS

The pullets used were a strain-cross White Leghorn (H and N Nick Chicks) and were obtained at 1-day-old from a local hatchery. The birds were reared to 4 weeks of age on a standard chick mash and then were randomly distributed to five treatment groups. There were 10 pullets in each treatment group and they received the following hormone treatments:

Treatment 1: 0·2 ml corn oil (control group),
Treatment 2: 0·5 mg oestradiol dipropionate in 0·2 ml corn oil,
Treatment 3: 1·0 mg oestradiol dipropionate in 0·2 ml corn oil,
Treatment 4: 2·0 mg oestradiol dipropionate in 0·2 ml corn oil,
Treatment 5: 4·0 mg oestradiol dipropionate in 0·2 ml corn oil.

These hormone treatments were administered as intramuscular injections on altermate days. Five birds from each group were killed 2 days after the commencement of hormone treatment and the remaining five birds were killed 8 days after the commencement of hormone treatment. The birds killed after 2 days had therefore received only a single hormone injection.

After the intervals of gonadal hormone treatment stated the birds were killed by decapitation and exsanguination. The livers were rapidly removed and

cell-free extracts were prepared and assayed for ATP-citrate lyase (EC 4.1.3.8) and NADP-malate dehydrogenase (EC 1.1.1.40) activities as previously described [5] using a Unicam SP 8000 spectrophotometer. In all cases the recorded activity was linear with respect to both time and the protein content of the extract.

The liver total lipid content and the protein contents of cell-free extracts were determined as previously described by Balnave and Pearce [5]. The fatty acid composition of the liver lipids was determined by the method described by Balnave and Pearce [16] using a Hewlett–Packard 5750 research chromatograph. The physiological responses of liver weight and oviduct weight were expressed as a percentage of body weight and liver lipid content as a percentage of liver fresh weight. These results and the observed fatty acid percentages were transformed by the angular transformation method and analysis of variance was carried out; the back transformed data are given in the tables along with the S.E.M. of the transformed data. The results of the lipogenic enzyme assays were examined by analysis of variance. All statistical significances referred to are at the 5% level ($P < 0.05$).

RESULTS

The effects of different doses of oestradiol on various physiological parameters are shown in Table 1. Analysis of variance indicated that both the duration and level of oestradiol administration significantly influenced the liver and oviduct weight. Liver weight, expressed as a percentage of body weight, showed a significant step-wise increase with increasing dose level to 2 mg of oestradiol; this occurred for birds which were killed after both 2 days and 8 days of hormone administration. At both time intervals the liver weights of birds receiving 4 mg oestradiol were significantly less than in those receiving 2 mg hormone. No significant differences in the liver weights of the control birds were observed after 2 and 8 days of administration, but at all dose levels of oestradiol the liver weights were significantly greater after 8 days compared with 2 days of hormone administration. Two days following a single injection of oestradiol a significant dose-related response was observed in oviduct weight up to 1 mg of hormone but the responses to 1, 2 and 4 mg oestradiol were not significantly different. The responses in oviduct weight were much larger after 8 days, the heaviest oviducts being found in the birds receiving the largest dose of oestradiol although the responses to 2 and 4 mg of hormone were not significantly different. Analysis of variance indicated that the level but not the duration of hormone administration had a significant effect on liver total lipid content. Liver total lipid content increased with increasing level of hormone administered up to a maximal value in birds receiving 2 mg oestradiol and this increase relative to controls attained significance at the 1, 2 and 4 mg hormone levels. Again there was a decrease in response when the dose level increased from 2 to 4 mg oestradiol.

Analysis of variance indicated that the duration of hormone treatment exerted a significant effect on the specific activities of hepatic lipogenic enzymes. Although the level of hormone administered had no overall significant effect on the specific activities of ATP-citrate lyase and NADP-malate dehydrogenase at 2 days, a dose-related response in both enzymes was observed up to 2 mg oestradiol (Table 2). At this time reductions in the specific activities of these lipogenic enzymes were observed on increasing the oestradiol level from 2 to 4 mg; these changes paralleled the observed reductions in liver size and liver lipid content on increasing the dose from 2 to 4 mg oestradiol. No dose-related responses in hepatic enzyme activities were observed on day 8; at this time maximal enzyme specific activities were observed with 0·5 mg oestradiol and at higher dose levels the enzyme activities were less than in control birds.

Analysis of variance indicated that oestradiol treatment had a significant influence on the liver fatty acid composition, this effect being observed with all the individual fatty acids. In addition the duration of hormone treatment significantly influenced the amounts of palmitoleic, stearic and oleic acids. On day 2 there was a significant stepwise variation in the individual liver non-essential fatty acid concentrations up to 1 mg oestradiol, but no significant differences between the 1, 2 and 4 mg dose levels were observed (Table 3). No obvious variations were observed with the essential fatty acids; the linoleic acid content at all dose levels of oestradiol was significantly reduced compared with controls on day 2 and no significant differences were observed between oestradiol levels. In addition no significant differences in the arachidonic acid levels were observed between any treatment

Table 1. The effects of dose of oestradiol on oviduct weight, liver weight and liver lipid content

	Time (days)	Oestradiol administered (mg) 0	0·5	1·0	2·0	4·0	S.E.M. (40 *d.f.*—transformed data)
Bodyweight (g)	2	245*	250	249	248	257	
	8	280	288	292	267	264	
Oviduct weight	2	0·022	0·074	0·107	0·101	0·097	
(% of body weight)	8	0·036	0·747	0·769	1·004	1·095	0·11
Liver weight	2	2·89	3·71	4·49	5·01	4·62	
(% of body weight)	8	2·98	4·62	5·24	5·48	5·38	0·25
Liver lipid	2	4·69	7·84	14·73	15·81	11·39	
(% of liver weight)	8	6·06	9·12	10·23	14·02	10·19	1·23

* Each result is the mean of 5 observations.

Table 2. The effects of dose of oestradiol on the specific activities of hepatic lipogenic enzymes

Enzyme	Time (days)	Oestradiol administered (mg) 0	0·5	1·0	2·0	4·0	S.E.M. (40 *d.f.*)
ATP-citrate lyase	2	9·83*	10·95	12·08	14·47	10·43	1·04
	8	7·82	8·43	6·74	6·55	5·93	
NADP-malate dehydrogenase	2	214·24	218·94	236·57	233·26	169·39	18·05
	8	200·19	202·15	152.15	178·95	168·71	

* The results are expressed in nmoles substrate metabolised/min per mg protein and each result is the mean of 5 observations.

groups. Similar patterns of response in the individual fatty acids were found after 8 days. Equivalent changes in the percentages of palmitic, stearic and oleic acids occurred at smaller dose levels and the extent of the responses continued beyond the range observed on day 2. The palmitoleic, linoleic and arachidonic acid percentages were similar on both days.

DISCUSSION

The experiment described, although pharmacological in concept, was carried out to investigate the effects of varying the dose of administered hormone in simulating some of the physiological changes encountered in the point-of-lay pullet.

The physiological responses to oestrogen were similar to those reported previously by the present and other workers [5–8, 11, 15, 17]. However in none of these investigations where a dose-related response to oestrogen was investigated, was a reduced response to oestrogen observed at higher dose levels. This may be related to the fact that birds of equivalent bodyweight to those used in the present work were given relatively smaller doses of hormone [8] or else birds of much larger body weight were used, thereby reducing the dose per unit of body weight [6, 7, 17]. In the present work increasing the dose of administered hormone progressively increased liver weight and liver total lipid content up to a maximum with the 2 mg dosage level. The larger dose of 4 mg oestradiol, given either as a single dose or as four injections on alternate days over a period of 8 days, reduced these changes although the values obtained were still significantly greater than for control birds.

In confirmation of earlier results [4, 5] oestradiol increased hepatic lipogenic enzyme activities 2 days after the initial hormone administration. There was a step-wise increase in the enzyme specific activities with increasing oestradiol dosage to 2 mg which paralleled the changes in liver total lipid content. The effect of 4 mg oestradiol was to reduce both liver lipid content and the specific activities of the lipogenic enzymes. In general the liver data indicate that, at the higher dose levels, oestradiol exerted a pronounced pharmacological effect, perhaps through feed-back effects on hormone balance, and that such levels of hormone should not be administered when attempting to simulate natural changes in liver metabolism. It is therefore noteworthy that in preliminary studies, the present authors have found that the circulating plasma oestradiol concentration 2 days following the single intramuscular administration of 0·5 mg hormone, was similar to that observed in the point-of-lay pullet. Furthermore, in the present study the enzyme specific activities were significantly reduced after 8 days of hormone administration. Similar results after long periods (8–10 days) of hormone treatment have been observed by Pearce and Brown [12] and Balnave and Pearce [5]. This may be due to feedback repression by the synthesized lipid which, in contrast

Table 3. The effects of dose of oestradiol on the fatty acid composition of liver lipid

Fatty acid	Time (days	Oestradiol administered (mg) 0	0·5	1·0	2·0	4·0	S.E.M. (40 *d.f.*-transformed data)
C16:0	2	21·1*	22·6	23·6	24·5	25·4	0·52
	8	20·7	23·6	23·9	26·6	26·8	
C16:1	2	2·1	3·0	3·5	3·5	2·9	0·54
	8	1·9	2·9	2·5	3·4	2·0	
C18:0	2	26·4	22·3	19·1	18·3	19·4	0·52
	8	24·7	20·4	18·0	14·7	16·1	
C18:1	2	18·3	21·5	26·6	24·3	24·4	0·68
	8	13·4	25·2	28·0	30·7	28·9	
C18:2	2	18·5	14·9	15·7	14·7	14·8	0·74
	8	19·3	14·6	15·3	14·7	15·0	
C20:4	2	9·2	9·5	6·8	6·9	8·3	0·75
	8	13·5	9·4	8·7	6·7	7·9	

* Each result is expressed as a percentage of the total fatty acids and is the mean of 5 observations.

to the mature fowl, is not removed from the body through the egg production process. These results suggest that changes in liver lipid metabolism following long-term hormone administration may be pharmacological rather than induced physiological effects. The present and previous results [5] indicate that in relation to liver lipid metabolism short-term investigations within 2 days of oestradiol administration should be more indicative of the role of oestrogens in the mature bird.

The patterns of response to oestradiol in liver fatty acid composition are similar to those reported previously [11, 15, 16]. Although higher dose levels of oestradiol administered for extended periods appear to exert a pharmacological effect on liver metabolism (see above), the patterns of response observed in the fatty acids were similar to those obtained 2 days after a single injection of 0·5 mg oestradiol. When the liver fatty acid compositions of oestrogenized pullets are compared with those of mature laying hens [15, 18] it is evident that the oestradiol-induced changes are intermediate between the immature and mature physiological patterns.

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