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Observations on the Vitamin Metabolism of the Common Fowl

2. The Effects of Oestrogen and Progesterone Injections in Immature Pullets on the Riboflavin Content of the Magnum

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Riboflavin exists in the body of the fowl as the free vitamin, as flavin-mononucleotide and as flavin-adenine-dinucleotide. When a bird is about to ovulate, the amount of free riboflavin in the magnum rises to about 40% of the total vitamin present; after the egg has passed through the magnum and received its layers of albumen, the amount of free riboflavin in the magnum falls to about 30% of the total vitamin present. This decrease in free riboflavin content is approximately equal to the amount in the albumen of the eggs laid (Bolton, 1952).

The fluctuations in the quantity of free riboflavin in the magnum are most likely controlled by the endocrine system, probably by ovarian hormones. Fraps, Hooker & Forbes (1948) found progesterone in the blood plasma of laying hens and suggested that this hormone was of ovarian, and most likely follicular, origin. Hertz, Dhyse & Tullner (1949) showed that, while the biotin content of the blood serum of chicks was increased about fivefold by stilboestrol treatment, no avidin was formed in the oviduct. The simultaneous injection of stilboestrol and progesterone evoked both an increase of biotin in the blood serum and formation of avidin in the oviduct, thus suggesting that progesterone is involved in the secretion of egg albumen.

It is known (Common & Bolton, 1946; Common, Rutledge & Bolton, 1947–8; Common, Bolton & Rutledge, 1947–8) that injections of oestradiol dipropionate will increase the serum-riboflavin concentration of immature pullets about fifteenfold and (Bolton, 1950) that this increase is almost entirely due to an increase in free riboflavin.

It is also known (Bolton, 1951) that the higher level of riboflavin in the serums of laying birds, compared with non-laying birds, is due almost entirely to free riboflavin. Thus the changes in serum riboflavin content that occur as the fowl comes into lay can also be evoked by the administration of oestrogen.

The experiments described in the present paper were undertaken to ascertain the effects of oestradiol dipropionate and progesterone on the free riboflavin content of the magnum.

EXPERIMENTAL

Animals and diets. Brown Leghorn pullets were bred at this Centre and came from a single strain. All groups were reared under the same conditions. During the experimental period the birds were housed separately in all-metal laying cages and received the same daily ration of an all-mash laying diet, made up as follows: ground wheat 26, ground yellow maize 26, ground oats 25, wheatfeed 10, fish meal 4, earthnut-cake meal 6, cod-liver oil 1, precipitated chalk 1·75 and common salt 0·25 parts. Hydrated manganese sulphate (20 g) was added to each 100 lb. mash. This diet contained 1·7 μg riboflavin/g food and 1·0% calcium.

Pullets at this Centre are recorded as having laid when 18 weeks old (Greenwood & Blyth, 1946). The deposition of yolk and the hypertrophy of the oviduct would have begun when these birds were about 16 weeks old. Therefore, to ensure that the effects evoked by the hormone treatments are not affected by normal changes, birds should not be older than 16 weeks at the conclusion of the experiment and should preferably be slightly younger. For this reason the experiments were begun when the birds were 11 weeks old.

Hormones. The hormones used were oestradiol dipropionate and progesterone. The progesterone was dissolved in the same oily base (arachis oil) as that used for the oestradiol dipropionate and the oil administered was always brought to the same volume by adding a suitable amount of diluent. The need to do this has additional importance because of the observations of Crafts (1942) and Bruce & Tobin (1940) that injections of sesame oil caused a reduction of testis weight and so acted like an oestrogen. The choice of arachis oil as the solvent for the hormones was fortunate, for Russell (1952) has shown that injecting ethyl oleate into rabbits leads to the formation of 'cheesy' deposits at the site of injection. This finding has cast doubt on the efficacy of hormone absorption from this medium.

The hormone solutions were injected into the pectoralis muscles, the route used for injections of oestrogen and androgen by Common, Bolton & Rutledge (1947–8) and Common, Rutledge & Bolton (1947–8).

Analytical methods. The riboflavin content of the diet was computed from the amounts in the ingredients, which were assayed by the method of Kodicek & Wang (1949). Blood-serum riboflavin was determined by the method for total riboflavin of Burch, Bessey & Lowry (1948), and the partition of riboflavin into free vitamin, flavin-mononucleotide and flavin-adenine-dinucleotide in the magnum and liver by that of Bessey, Lowry & Love (1949). Macromodifications of the last two methods were adopted, as the microfluorimeter necessary for the original methods was not

available. Serum-calcium determinations were made by the modified method of Halverson & Bergeim as described by Peters & Van Slyke (1932). It is necessary to remove protein from the serums of birds treated with oestrogens before attempting to precipitate calcium oxalate. Calcium in the food and faeces was estimated by Godden's (1937) method. The dry-matter content of the liver was ascertained by drying overnight at 100° and the fatty-acid content of this tissue by the method of McCance & Shipp (1933). Comb size is expressed as length plus height and the distance between the pelvic bones was directly measured during dissection.

Design of experiment. Groups of six birds were used in each of three experiments. The first bird of each group received oil alone and acted as a control, the second received a single dose of oestrogen, and the third a double dose. The remaining three birds all received progesterone, and of them one received no oestrogen, one a single and one a double dose. The birds were injected on alternate days until a total of six injections had been given to each: they were killed before the morning feed on the day following the last injection.

RESULTS

The results set out in Table 1 are the means of those obtained for birds in all three groups. They show that oestrogen administration evoked the usual hypertrophy of the oviduct and that the oviducts from the birds receiving the higher level of oestrogen alone were comparable in length and weight with those found by Common, Bolton & Rutledge (1947–8) and Common, Rutledge & Bolton (1947–8) after the same dose of oestradiol dipropionate. On the other hand, these workers found that the simultaneous injection of androgen had a synergistic effect on the growth of the oviduct, whereas in these experiments simultaneous injections of progesterone had an antagonistic effect. The riboflavin contents of the magnum, liver and blood serum are set out in Table 2.

DISCUSSION

Initial and final live weights. The results show that there was no significant difference between groups in either initial or final live weight, indicating that the hormone treatments had not had any effect.

Ovaries. Accurate dissection of the ovaries was not attempted, but the appearance and weights confirmed that they were quiescent in all birds. The other changes observed can therefore be taken as due to the experimental treatments.

Comb development. Progesterone alone did not have any effect on comb growth; oestrogen alone or in conjunction with progesterone caused a regression in comb size.

Spleen. The spleens from the birds receiving oestrogen, alone or in conjunction with progesterone, were paler in colour than those of the control birds or of birds receiving progesterone alone; the differences in weight were not significant.

Pelvic distances. The distance between the pelvic bones increased with administration of oestrogen, an increase that was not affected by progesterone treatment.

Liver. The amount of dry matter in the livers was not affected by either oestrogen or progesterone. The livers of the birds receiving oestrogen were heavier than those of

Table 1. *Effect of injections of oestradiol dipropionate and progesterone on pullets*

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Least significant difference ($P=0.05$)
Dosage of oestradiol dipropionate (mg)	6×0	6×1	6×2	6×0	6×1	6×2	—
Dosage of progesterone (mg)	6×0	6×0	6×0	6×1	6×1	6×1	—
Live weight:							
Initial (kg)	0·67	0·67	0·67	0·66	0·71	0·69	0·13
Final (kg)	0·75	0·75	0·74	0·73	0·75	0·75	0·14
Comb size:							
Initial (mm)	32	29	35	37	35	29	—
Change (mm)	+5	−1	−3	+6	−2	−1	2·7
Ovary:							
State	Quiescent	Quiescent	Quiescent	Quiescent	Quiescent	Quiescent	—
Weight (g)	0·20	0·17	0·15	0·20	0·14	0·17	0·04
Oviduct:							
State	Undeveloped	Developed magnum with white corded appearance	Developed magnum with white corded appearance	Undeveloped	Developed magnum with white corded appearance	Developing magnum with white corded appearance	—
Weight (g)	0·13	7·62	10·45	0·37	7·24	6·76	2·21
Length (mm)	65	275	360	93	250	232	39
Spleen weight (g)	1·19	1·13	0·93	1·28	1·04	0·82	0·70
Pelvic distance (mm)	11	15	28	11	17	31	7
Liver:							
Weight (g)	16·4	20·2	23·7	15·6	19·8	25·1	3·0
Dry-matter content (%)	29·3	30·4	30·5	29·0	30·3	30·8	3·2
Fatty-acids content (percentage of fresh tissue)	3·69	5·14	5·71	3·62	5·02	6·50	0·55
Serum calcium (mg/100 ml.)	12·6	45·3	73·1	13·0	40·3	69·7	24·8
Calcium retention (g)	1·66	1·82	1·87	2·03	1·77	2·23	0·94

Table 2. *Riboflavin contents of magnum, liver and blood serum*

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Least significant difference ($P=0.05$)
Dosage of oestradiol dipropionate (mg)	6 × 0	6 × 1	6 × 2	6 × 0	6 × 1	6 × 2	—
Dosage of progesterone (mg)	6 × 0	6 × 0	6 × 0	6 × 1	6 × 1	6 × 1	—
Magnum:							
Weight (g)	—	2·61	3·66	—	2·27	2·02	1·19
Length (mm)	—	132	153	—	112	101	26
Riboflavin content of magnum:							
Free riboflavin (μg/g)	—	0·97	0·94	—	1·60	1·29	0·36
Flavin-mononucleotide (μg/g)	—	0·28	0·38	—	0·24	0·33	0·38
Flavin-adenine-dinucleotide (μg/g)	—	2·50	2·45	—	2·59	1·74	1·19
Free riboflavin (percentage of total)	—	25·2	25·1	—	37·6	39·5	11·4
Riboflavin content of liver:							
Free riboflavin (percentage of total)	8·9	11·3	10·8	12·5	8·2	8·8	4·8
Total riboflavin (μg/g)	20·6	19·3	17·6	22·4	19·6	18·2	4·2
Total riboflavin in tissue (μg)	341	379	418	346	407	458	85
Serum riboflavin (μg/ml.)	0·05	0·28	1·12	0·06	0·71	1·29	0·39

either the controls or the birds receiving progesterone alone. The fatty-acid contents of the livers showed an increase with oestrogen treatment, but progesterone was without effect. Common, Bolton & Rutledge (1947–8) also found that the livers of birds dosed with oestrogen were heavier and had a higher content of fatty acids than those of control birds, and György & Rose (1949) that oestrogen administration increased the fat content of rat liver, but that progesterone was without effect.

Oviduct. The usual hypertrophy of the oviduct was observed after oestrogen treatment. Statistical analysis also showed a significant interaction between the two hormones, suggesting that they acted antagonistically on the growth of the oviduct. A similar examination of oviduct weights quoted by Common, Rutledge & Hale (1948) showed that androgen and oestrogen acted synergistically. An experiment is in progress to ascertain what happens when all three hormones are administered together.

Serum calcium and calcium retention. The usual rise in serum calcium was observed after oestrogen treatment, but progesterone was without effect. Calcium retention was not affected by either hormone or by their simultaneous administration.

Serum and liver riboflavin. Oestrogen evoked the usual increase in serum-riboflavin content. The increase in serum riboflavin after simultaneous administration of oestrogen and progesterone was significantly greater than after oestrogen alone when a single dose of oestrogen was given, but not after a double dose of oestrogen. These experiments therefore leave it undecided whether or not oestrogen and progesterone act synergistically on the concentration of serum riboflavin.

The total riboflavin content of the liver was unchanged with increasing oestrogen dosage. It will be noted that the percentage of free vitamin in the liver did not show any appreciable change, thereby supporting the suggestion put forward (Bolton, 1952) that this organ does not play any part in meeting the demand for free riboflavin as the egg passes down the magnum.

The total content of riboflavin in the liver showed an increase with oestrogen dosage. It is therefore considered highly unlikely that the extra riboflavin in the blood serum and in the magnum could be due to the mobilization of body reserves, for it is most probable that the liver store would then be depleted rather than augmented. It is therefore concluded that the increased content of riboflavin in liver, blood serum and oviduct is a symptom of increased absorption or retention from the alimentary tract. This view is in accord with that of Common, Rutledge & Bolton (1947–8).

Magnum riboflavin. The degree of hypertrophy of the magnum was greater when oestrogen was administered alone rather than in conjunction with progesterone. The interaction between oestrogen and progesterone almost reached the 5% level of significance, suggesting that the antagonistic effects of progesterone on oestrogen seen in the whole oviduct may also apply to the magnum.

The hypertrophy of the magnum in birds receiving oestrogen was sufficiently great to permit the riboflavin present to be partitioned into free vitamin, flavin-mononucleotide and flavin-adenine-dinucleotide. The results show that the contents of flavin-mononucleotide and flavin-adenine-dinucleotide were of the same order, but that the content of free riboflavin was increased by the simultaneous administration of progesterone and oestrogen to a level above that produced by oestrogen alone.

To compare the results of this experiment with those obtained previously, the content of free riboflavin in the magnum was expressed as a percentage of the total vitamin present. When the birds were dosed with oestrogen alone, about 25% of the riboflavin was present as the free vitamin. The simultaneous administration of progesterone increased the percentage to about 40. These figures are in close agreement with the values of 40% of free riboflavin in the magnum just before ovulation and 30% after the egg has reached the shell gland (Bolton, 1952). As already mentioned, progesterone has been detected in the blood plasma of laying birds (Fraps *et al.* 1948).

The hypothesis is therefore put forward that the increased content of free riboflavin in the magnum occurring just before ovulation results from the actions of oestrogen and progesterone.

SUMMARY

1. The effects of oestrogen and progesterone injections in immature female fowls were studied.

2. Oestrogen alone evoked hypertrophy of the oviduct, a decrease in comb size, increases in liver weight and total liver riboflavin, increases in serum calcium and serum riboflavin and an increase in liver fatty-acid content.

3. Progesterone antagonized the oestrogen effects on the growth of the oviduct, and increased the percentage of free riboflavin in the hypertrophied magnum from 25 to 40.

4. It is suggested that the increase in the percentage of free riboflavin in the magnum of a bird about to ovulate is due to the simultaneous action of oestrogen and progesterone.

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