

**THE EFFECT OF PROLONGED DOSAGE WITH
OESTROGENS ON THE ADULT BROWN
LEGHORN COCK**

By C. W. EMMENS

From the National Institute for Medical Research, Hampstead, London

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A GREAT deal of work has been carried out on the inhibition of the activity of the male reproductive organs in mammals by prolonged administration of oestrogens, an effect probably due to primary depression of hypophyseal activity. There is little comparable information for birds, in spite of their being specially suitable for such experiments owing to the possession of secondary sexual characters dependent on the functional activity of the gonad. Depression of testicular activity and diminution in comb size in normal cocks was shown to follow the injection of oestrone in oily solution by Morató-Manaro, Albriex & Buño [1935]. Injecting up to 50,000 I.U. (one I.U. = 0.1 μ g.) in the course of about one week, they obtained reductions in comb area amounting to 30 % of the original comb size, and found that the testes of treated birds were smaller than normal, white, and with reduced spermatogenesis and interstitial tissue. The same authors later demonstrated [Morató-Manaro *et al.* 1938] that inunction of 18,750 I.U. per day of oestrogen on the comb produced, after 10 days, a 39 % reduction in the comb area of 3-month-old White Leghorn cockerels. The combs had less elastic tissue and relatively more connective tissue, and were white and flaccid in appearance. The testes showed atrophy of the tubules and interstitial tissue and a sharp drop in spermatogenesis. Inunction of androsterone affected the testes in the same way, or to an even greater extent, but, of course, produced comb growth instead of atrophy.

Zondek [1937] recorded the production of "pituitary dwarf" fowls following the injection of 50,000-100,000 I.B.U. (one I.B.U. = 0.1 μ g.) of oestradiol benzoate twice weekly from the age of 6 weeks. The dwarf cocks were just over half the weight of normal controls, and possessed

shrivelled combs and atrophic testes, which were only 3-4 % of the normal weight, and contained only Sertoli cells and spermatogonia in the tubules. These cocks also exhibited bone changes, with sponginess and open epiphyses. In a preliminary communication [Emmens, 1938] I reported comb atrophy and testicular degeneration following the administration of oestrogens to normal Brown Leghorn cocks, accompanied by an apparent depression of the activity of the thyroids. In the present paper these findings are discussed in greater detail.

MATERIAL AND METHODS

The birds used in the investigation were adult Brown Leghorn cocks from the Institute's farm stock. Two methods were used for the administration of oestrogen. Oestradiol benzoate was given by intramuscular injection in arachis oil, at a concentration of 5 mg./ml. Oestrone was given by the implantation of tablets of compressed crystalline substance, which were inserted into the breast muscles under ether by means of a trochar and held in position by one or more stitches. Such implanted tablets become surrounded by connective tissue soon after implantation, and can easily be removed at death or at a second minor operation under anaesthesia, and cleaned and weighed in order to determine the amount of hormone absorbed.

Comb measurements were taken with dividers, and an index of the comb area obtained by multiplying the length, from the anterior insertion to a definite point at the back of the comb, by the height, from a point on the base of the comb to the tip of a particular high spike. The combs were measured at frequent intervals, but only those points which are needed to outline the curves of Figs. 1-6 have been included. Testes, thyroids and adrenals were fixed for 24 hr. in aqueous Bouin's solution and weighed after a similar period in 70 % alcohol. The pituitary glands were fixed in Susa's solution, and weighed after fixation. All sections were cut at 7μ . and stained in Meyer's haematoxylin and eosin.

EXPERIMENTAL RESULTS

A summary of the results obtained is given in the table, in which the birds are arranged in approximate order of the degree of effect produced on the comb and testes. By this arrangement they are seen to fall into two contrasting groups. The first group includes OTC 7, 8 and 9, which showed no decrease in comb size at death, and a variable amount of testicular atrophy. These birds received implantations of tablets of

oestrone. OTC 1, which was allowed to recover from the effects of oestradiol injections before being killed, also belongs to this group. The second group comprises OTC 3, 5, 4 and 2, in which considerable comb atrophy and extreme testicular atrophy occurred. These received injections of oestradiol benzoate, with or without other treatment. Apart from OTC 1, it is most convenient to consider the birds in the order in which they appear in the table.

TABLE. Data relating to adult Brown Leghorn cocks receiving prolonged dosage with oestrogens

OTC no.	Treatment	Comb area at death (initial size = 100)	Wt. of testes g.	Wt. of thyroids mg.	Wt. of adrenals mg.	Wt. of pituitary mg.
Controls (5 birds)						
—	—	—	12-30	120-296	—	ca. 10
1	(1) 65 mg. of oestradiol benzoate during 100 days in fortnightly injections. (2) A rest period of 70 days. (3) A second course of oestradiol benzoate, 40 mg. in 42 days. (4) A rest period of 80 days before killing	135	4.65	243	400	14
7	Two successive tablets of oestrone implanted intramuscularly 75 and 35 days before death, 89 mg. of which were absorbed	119	4.06	160	200	9
9	Oestrone tablet implanted 34 days prior to death, 5 mg. of which were absorbed	94	1.73	123	—	11
8	As OTC 9, with 8 mg. of the tablet absorbed, plus a daily injection of 25 mg. of a horse pituitary extract	102	1.60	125	198	9
3	(1) 65 mg. of oestradiol benzoate during 100 days, as OTC 1. (2) A rest period of 70 days. (3) A second course of oestradiol benzoate, 40 mg. in 42 days, followed by killing	53	0.92	355	—	—
5	(1) and (2) at OTC 1. (3) A second course of oestradiol benzoate, 75 mg. in 90 days, followed by implantation of an oestrone tablet for 37 days prior to killing, 17 mg. of which were absorbed.	72	0.81	172	235	9
4	(1) and (2) as OTC 1. (3) A second course of oestradiol benzoate injections, 105 mg. in 110 days, followed by killing	64	0.69	117	277	—
2	(1) and (2) as OTC 1. (3) A second course of oestradiol benzoate, 150 mg. in 130 days, during which 6 mg. of testosterone propionate were also injected, the course being followed by killing	41	0.41	260	290	11

Effect of oestrogens on testes, comb and thyroids

OTC 7 (Fig. 1) received an implantation of a 65 mg. tablet of oestrone, which in 40 days was effective in decreasing the comb size by about 50%. Subsequently, the comb began to grow again. Three days after this renewed growth a second tablet of 40 mg. of oestrone was implanted, but was ineffective in checking the growth of the comb, which continued until the bird was killed, 75 days from the beginning of the experiment. At this time the comb was larger than at the beginning of the experiment. Photographs of the comb of OTC 7 before the experiments, and when showing a maximum decrease, are given in Pl. I, figs. 8, 9.

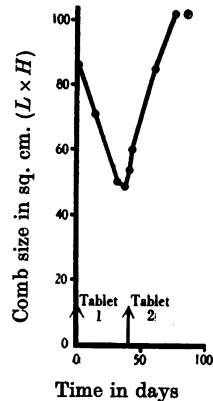


Fig. 1. Comb size of OTC 7 during treatment with oestrone. Tablet 1, implantation of a 65 mg. tablet; tablet 2, implantation of a 40 mg. tablet.

The thyroids of five normal adult Brown Leghorn cocks weighed between 120 and 296 mg. a pair; those of OTC 7 weighed 160 mg. and were therefore well within the normal weight range. Histologically, they show slightly less activity of the epithelium than falls within the normal range and an increase in vesicle size and colloid deposition (Plate III, figs. 16, 17).

OTC 9 received an implantation of a 103 mg. tablet of oestrone, and was killed 34 days later, having shown a slight increase in comb size during the experiment. The testes weighed 1.73 g. and were slightly more atrophic than those of OTC 7. There was almost complete absence of spermatogenesis, but no obvious disturbance of the intertubular tissue. The tablet recovered at autopsy weighed 98 mg., so that only 5 mg. of oestrone had been absorbed. The thyroids weighed 123 mg. and did not show any gross histological abnormalities, though they were perhaps slightly less active looking than is usual.

OTC 8 received an oestrone tablet of 102 mg., at the same time as OTC 9, together with a daily injection of 25 mg. of a gonadotrophic extract made from horse pituitaries (AP 61 B). 2.5 mg. of this extract injected in five equal daily doses into immature female rats produced a sixfold increase in ovary weight. The extract therefore possessed a high degree of gonadotrophic activity, and might have been expected to prevent testicular atrophy if this is due to depression of the gonadotrophic function of the pituitary by oestrone. The bird was killed on the 34th day

of the experiment. The testes, weighing 1.60 g., were slightly more atrophic than those of OTC 9, with tubules with only 3-4 layers of cells, showing no spermatogenesis. The comb had decreased a little in size, by about 6 % of its area at the beginning. The thyroids weighed 125 mg. and were similar to those of OTC 9 in histological appearance, again being slightly less active than normal, with somewhat large vesicles. It was found at autopsy that 8 mg. of the tablet had been absorbed.

OCT 3 (Fig. 2) was injected fortnightly for 100 days with 5 mg., and later 10 mg., of oestradiol benzoate in oil, receiving a total of 65 mg. At the end of 110 days its comb had regressed to 40 % of its former size. On

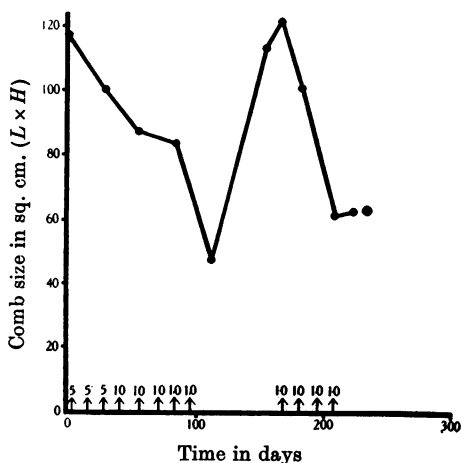


Fig. 2. Comb size of OTC 3 during and between courses of oestradiol benzoate. Each injection is indicated by a small arrow, the number above which shows the amount given in mg.

stopping the injections, the comb grew rapidly and after a further 55 days was of normal size again. Subsequently the bird received four injections, each of 10 mg., at fortnightly intervals, and once more the comb regressed, this time to 53 % of its former size. The bird was then killed. The testes weighed 0.92 g. and were much atrophied histologically. Only a few secondary spermatocytes were present in the small tubules, and the intertubular tissue appeared denser and stained more deeply than normal. The thyroids weighed 355 mg. and were large but not grossly abnormal histologically. They had again a less active appearance than is usual, with increased deposition of colloid in the large vesicles.

OCT 5 (Fig. 3) received the same treatment during its early history as OTC 3, but proved more refractory in that the comb did not regress so

much. After the end of the first course of fortnightly injections the comb rapidly grew to above its former size. It regressed again during the second course of injections, but once more exhibited refractoriness toward the end, and actually increased in size after the administration of one of the injections, of 15 mg. of the benzoate. Shortly after this a 97 mg. tablet of oestrone was implanted, the comb regressed by about 30 % in area during the next 40 days, and the bird was killed. The tablet had lost 17 mg. of its weight, and the total dose of oestradiol benzoate given was 140 mg.

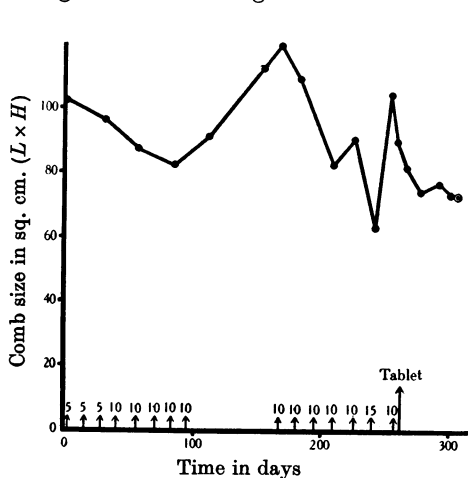


Fig. 3.

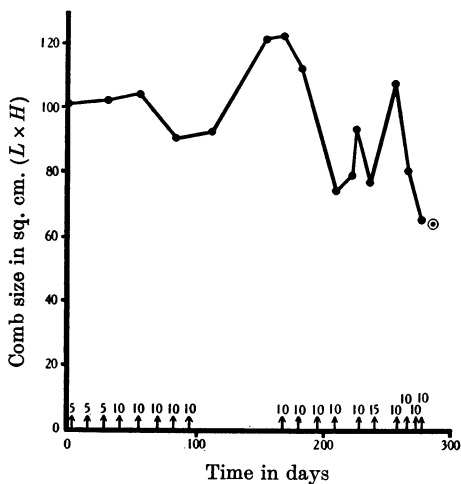


Fig. 4.

Fig. 3. Comb size of OTC 5 during and between treatments with oestradiol benzoate and oestrone. Each injection of oestradiol benzoate is indicated by a small arrow, the number above which shows the amount given in mg. "Tablet" = implantation of a 97 mg. tablet of oestrone.

Fig. 4. Comb size of OTC 4 during and between courses of oestradiol benzoate. Each injection is indicated by a small arrow, the number above which shows the amount given in mg.

The testes (Pl. II, fig. 12) weighed 0.81 g. and were rather less atrophied histologically than those of OTC 3, but otherwise they presented the same appearance. The thyroids weighed 172 mg., contained some very large colloid deposits, and the epithelium was definitely less active than normal.

OTC 4 (Fig. 4) also received the two courses of injections, the second being concluded with three weekly injections of 10 mg. of oestradiol benzoate. The same phenomenon of refractoriness was observed as in OTC 5, but the last intensive course of injections brought the comb down

to 64 % of its initial area. The testes weighed 0.69 g. and were more atrophic than any of those previously described (Pl. II, fig. 13). The very small tubules showed only Sertoli cells and spermatogonia and had a minute lumen. The intertubular tissue was dense and deeply stained. The thyroids, weighing 117 mg., showed many areas of large inactive follicles distended with colloid, but other areas had an active, normal appearance. The total dose of oestradiol benzoate received by this bird was 170 mg.

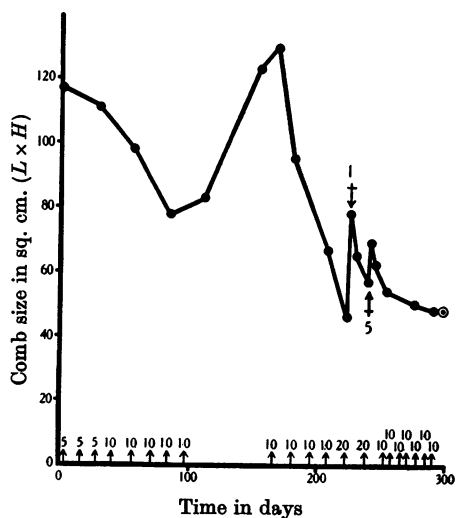


Fig. 5.

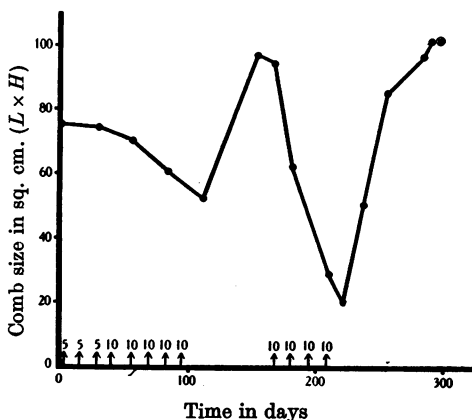


Fig. 6.

Fig. 5. Comb size of OTC 2 during and between courses of oestradiol benzoate. Each injection is indicated by a small arrow, the number above which shows the amount given in mg. † injection of testosterone propionate, amount in mg.

Fig. 6. Comb size of OTC 1 during and after courses of oestradiol benzoate. Each injection is indicated by a small arrow, the number above which shows the amount given in mg.

OTC 2 (Fig. 5), the fourth bird receiving the same early treatment as OTC 3, responded well to the injections, as measured by comb atrophy, but it also showed slight refractoriness at the end of the first course. During a prolonged intensive second course of injections of oestradiol benzoate it received two doses of 1 and 5 mg. respectively of testosterone propionate. Comb growth was observed after one of these doses of androgen, but much less than was expected. The significance of this will be discussed later. The second course finished with seven weekly injections of 10 mg. of oestradiol benzoate, at the end of which the comb

had reached its minimum size of 41 % of its original area. In all, 215 mg. of the benzoate were administered, and 6 mg. of testosterone propionate.

The testes, weighing 0.41 g., were extremely atrophic, with dense intertubular tissue and partially obliterated tubules (Pl. II, fig. 14). The thyroids (Pl. III, fig. 18) weighed 260 mg. and presented much the same appearance as those of OTC 4.

OTC 1 (Fig. 6) was allowed to recover for 80 days after a short, second course of oestradiol benzoate injections. The bird had reacted very well previously and had not shown refractoriness to the injections. At one period, after the second course, the comb had regressed to about 27 % of its initial area, and only 20 % of its final area (Pl. I, fig. 10). Recovery, as measured by comb growth, was rapid (Pl. I, fig. 11), but at autopsy the testes were still below normal weight at 4.65 g. Histologically, however, they were very nearly normal, with rather less spermatogenesis than is usual. The intertubular tissue stained less deeply and appeared normal in quantity (Pl. II, fig. 15). The thyroids weighed 243 mg. and presented the most abnormal appearance of any described. Many large, colloid-filled, inactive vesicles were present, with deposits of blood in the colloid. Surrounding them were areas with active epithelia and smaller vesicles (Pl. III, fig. 19). This bird had received a total of 105 mg. oestradiol benzoate.

Effects of oestrogens on pituitary and adrenal glands

The pituitaries of treated birds weighed between 9 and 14 mg., and were, therefore, not enlarged. Their histology has not yet been investigated. The adrenals were between 198 and 400 mg., and presented no obvious abnormalities.

Desensitization to oestrogens

The blood serum of two birds, OTC 5 and 7, which had acquired refractoriness to treatment with oestrogens, was investigated with a view to finding whether any antagonistic action to oestrone could be detected. Both birds had received treatment with oestrone itself, in the case of OTC 5 subsequently to oestradiol injections.

In an Allen-Doisy vaginal cornification test with ovariectomized albino mice, 0.1 μ g. of oestrone in oily solution gave 70 % of positive responses in a control group of twenty mice. A dose/response curve previously established showed that 0.2 μ g. of oestrone would produce 99 % of positive responses on the average, giving 100 % nine times out of ten in groups of ten mice. One group of ten mice was injected, at the



Fig. 8.

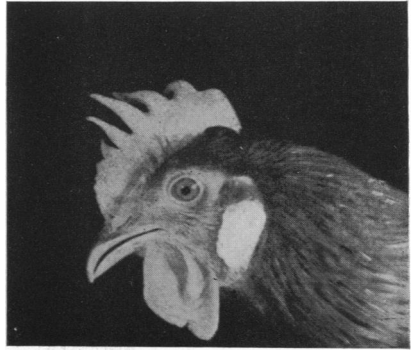


Fig. 9.



Fig. 10.

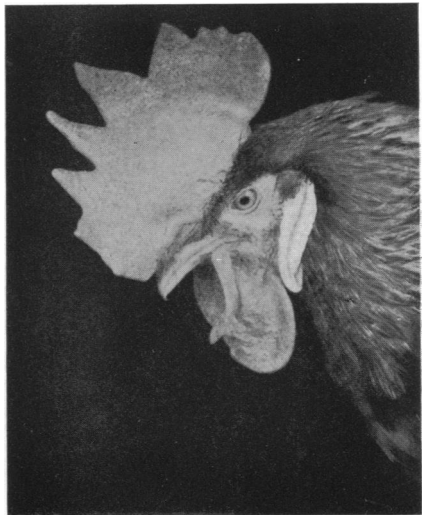


Fig. 11.

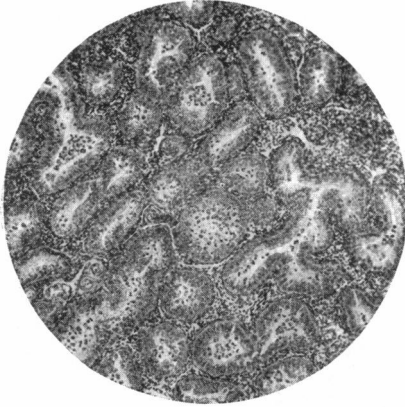


Fig. 12.

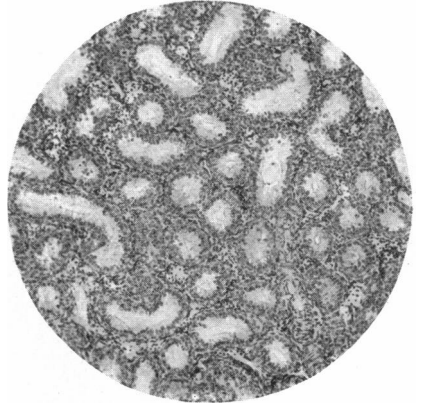


Fig. 13.

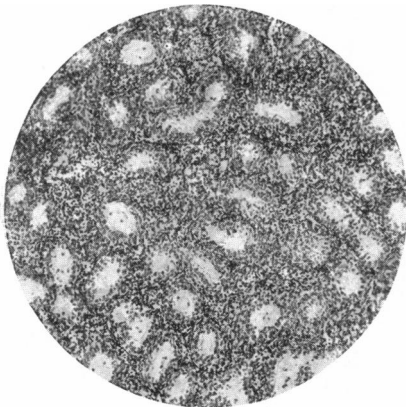


Fig. 14.



Fig. 15.

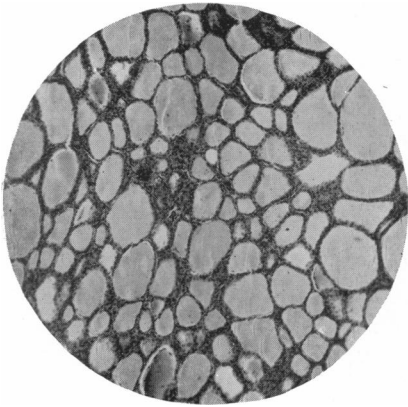


Fig. 16.

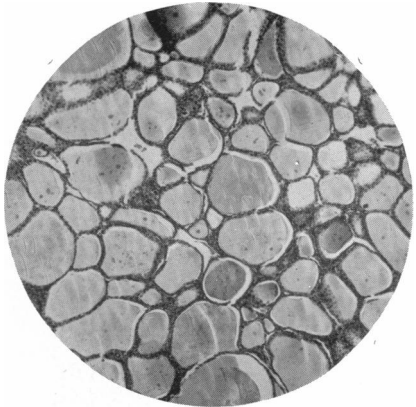


Fig. 17.

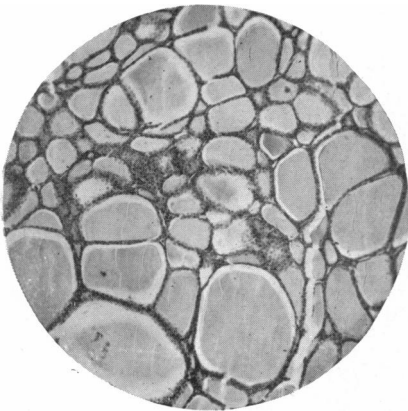


Fig. 18.

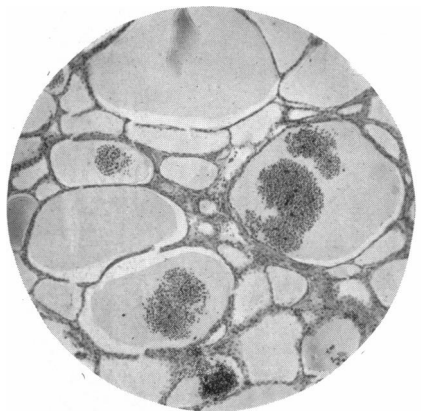


Fig. 19.

same time as the control group, with 0.2 μg . of oestrone on one side of the body, and with 1.0 ml. of serum from OTC 7 on the other. A second group of ten received 0.2 μg . of oestrone with 0.8 ml. of serum from OTC 5 similarly injected. Both groups gave 100 % of positive responses, showing that there was no inhibition of the action of the oestrone injected, and that 100 ml. of serum would therefore have no inhibitory effect on as little as 20 μg . of oestrone. It is unlikely therefore that there was any activity antagonistic to oestrone present in these sera. As oestrogen was probably present in both sera, it is still possible that a low titre of antagonist may have been present, and masked in the Allen-Doisy test by this oestrogen.

Antagonism between oestradiol and testosterone

The report of Gley & Delor [1937], that oestradiol benzoate is able to decrease the comb growth produced by testosterone, suggested that some of the comb regression observed in birds of the OTC series might be due to this action rather than to direct or indirect depression of the androgen secretion by the testis. OTC 2, when its comb was well atrophied, was given an injection of 1 mg. of testosterone propionate, preceded 3 days before by 20 mg. of oestradiol benzoate instead of the usual 10 mg. (Fig. 5). At the time of injection of the androgen the comb had already grown suddenly and rapidly, but despite the addition of male hormone it ceased growing and regressed once more, presumably as the result of the increase in the dose of oestradiol benzoate. Later, another 20 mg. of oestradiol benzoate were given simultaneously with 5 mg. of testosterone propionate, after which the comb grew a little, but much less than would have occurred in a capon after the administration of 5 mg. of testosterone propionate alone. These results indicated that the action of testosterone was being partially inhibited by the injections of female hormone, and it was thought advisable to investigate this antagonism in capons. The results obtained are shown in Fig. 7, which contains a series of curves illustrating the average growth obtained in five groups of five birds each receiving a single intramuscular injection of 150 μg . of testosterone propionate, alone or with varying amounts of oestradiol benzoate simultaneously injected into the opposite breast muscle. Some inhibition was obtained with as little as 10 μg . of oestradiol benzoate, yet the response was not entirely abolished by as much as 10 mg. Birds with the higher doses of oestradiol also showed a more rapid comb regression, after attaining their maximum growth, than did those receiving male hormone alone. Mühlbock [1938] has now shown

that a daily dose of 5 mg. of oestradiol benzoate will completely abolish the response of capons to 100 $\mu\text{g.}$ daily of androsterone, and that 500 $\mu\text{g.}$ of oestradiol benzoate, given alone, will cause regression of the already atrophic capon comb.

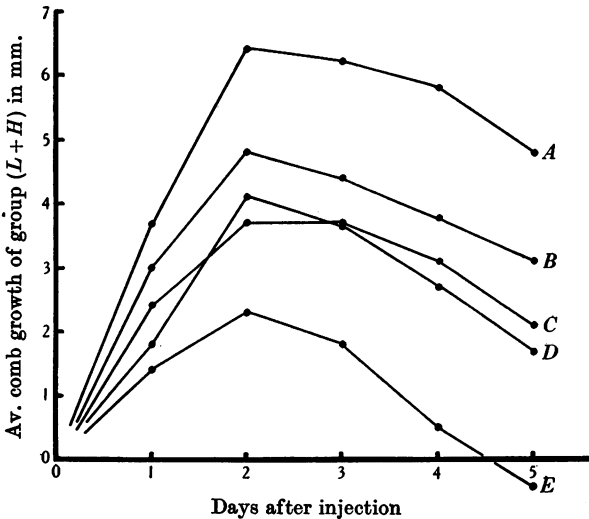


Fig. 7. Average comb growth in groups of five Brown Leghorn capons following a single injection of 150 $\mu\text{g.}$ of testosterone propionate: A, alone; B-E, together with 10, 100, 1000 and 10,000 $\mu\text{g.}$ of oestradiol benzoate respectively. The two hormones were injected at different sites.

It is clear, therefore, that very large doses of oestradiol benzoate are capable of inhibiting the action of male hormones on the capon comb, but that they are not highly effective in so doing.

DISCUSSION

There is good evidence that, in the mammal, prolonged heavy dosage with oestrogens causes depression of the pituitary activity, followed by regressive changes in the gonads and thence in accessory organs. Inhibition of growth also occurs when young animals are chronically treated with oestrogens. The pituitaries of rats respond to the treatment by an increase in size, and eventually by extreme hypertrophy [see Zondek, 1935, 1937, for review and new data].

Zondek does not record pituitary abnormalities in his dwarf fowls, and the pituitaries of my own birds were not enlarged. Since the comb regression observed may have been due to direct antagonism between

oestradiol benzoate and the naturally produced androgenic hormones of the bird, the evidence that the effects observed after prolonged dosage of the cock are solely due to depression of the pituitary body is not conclusive. There is some doubt as to the significance of the failure of a gonadotrophic extract to maintain the testis in an oestrogenized bird, since the activity on birds of the extract in question had not been controlled by tests on hypophysectomized fowls. I have been informed that a somewhat similar extract, also made from horse pituitaries, did not, in fact, show significant gonadotrophic activity in young White Leghorn and Barred Rock chicks of either sex. (Private communication to Dr A. S. Parkes from Dr S. S. Munro, Department of Agriculture, Ottawa, Ontario, Canada.)

The comb size and testis weight at death, and the total dose of oestrogen received, are correlated, and the comb was not found below normal size in any bird with testes weighing more than 1 g. Since a pair of normal testes weigh 12–30 g. (data from five control birds) it is clear that a considerable diminution in size of testis can take place without resulting in comb atrophy. In the atrophic testes there is a much higher proportion of intertubular tissue than in normal ones, becoming higher the farther regression has proceeded. No attempt to determine this proportion has been made, but inspection of Figs. 12–15 (Pl. II) shows that it is probable that very little decrease in the amount of this tissue occurs during the early stages of oestrogen treatment. The secretion of androgens by the testis might therefore be expected to continue sufficiently to maintain comb size, despite the antagonistic action of the administered oestrogen, until considerable testicular regression occurs. The fact that continued constant dosage with oestradiol benzoate eventually causes the comb to reach a minimal size, below which it will not regress without increase in dosage, indicates that the production of androgens by the testis is by no means completely inhibited by any but massive doses.

The rapidity with which comb growth takes place after injections are stopped, or even during treatment, when refractoriness occurs, seems to indicate that the greater part of the observed diminution in comb size is due to direct inhibition of the action of circulating male hormone. Nevertheless, it cannot be doubted that very severe atrophy of the testis is caused by massive dosage with oestrogen. This damage, however, appears to be reversible. The degree of testicular recovery in OTC 1 was great, no permanent damage appeared to have been caused by the two courses of oestradiol benzoate. This is perhaps an indication that the

testicular regression is due to lack of sufficient stimulation by the pituitary, and not to direct damage by the oestrogen.

Depression of functions of the pituitary gland other than the gonadotrophic action are less clear in this bird material. Spencer, D'Amour & Gustavson [1931] reported inhibition of growth in rats receiving oestrin injections over a period of 9 weeks. Zondek [1937] has shown that, both in the bird and in the rat, the growth-producing effect of the anterior pituitary is inhibited by heavy dosage with oestrogen, and Barnes, Regan & Nelson [1933] produced depression of the diabetogenic activity of the anterior pituitary in rats by the same means. The observations on the OTC series make it likely that there was some interference with the thyrotrophic function of the pituitary body. The effect was not profound, and with the dosage employed there was no production of typical thyroid-deficiency changes in the feathers. The changes observed consisted in enlargement of some of the thyroid vesicles and excess deposition of colloid, without regular enlargement of the whole gland (Pl. III, figs. 17, 18). A weight increase previously reported [Emmens, 1938] has proved to have occurred in an abnormal case, and it is not usually present. It is curious that the thyroids of OTC 1, a bird which was allowed to recover from the effects of treatment, presented these abnormalities in a more extreme degree than other birds, together with intra-vesicular haemorrhage. The condition of this bird suggests that direct damage to the thyroids may have occurred.

The dosages necessary to produce the effects described may be calculated roughly from the data available. Continuous administration by absorption from a tablet of oestrone is relatively very effective, but sufficient information is not available at present to enable dosage to be given accurately to birds by this method. Moreover, administration of high doses necessitates the implantation of several tablets. In OTC 8 and 9, 5-8 mg., absorbed over 34 days, an average of 150-250 $\mu\text{g.}/\text{day}$, was sufficient to reduce the testis weight to about 10 % of the normal, but did not affect the comb. In OTC 5, 17 mg. absorbed over 37 days, an average of 460 $\mu\text{g.}/\text{day}$, produced comb atrophy. Injection of 5 mg. of oestradiol benzoate per fortnight seems to be about the minimum dose that will cause comb regression in most birds, equivalent in total, but doubtless not in effect, to the daily injection of about 350 $\mu\text{g.}$ Since oestradiol, in the ordinary test on ovariectomized rats or mice, is several times more active than oestrone, the greater effectiveness of continuous dosage by implanting a tablet of compressed oestrogen is apparent.

SUMMARY

1. Prolonged heavy dosage of oestrone or oestradiol benzoate will cause, in the adult Brown Leghorn cock, extreme atrophy of the testes and comb, and also thyroid disturbances. No obvious abnormality of the adrenals was seen, nor were the pituitaries of treated birds enlarged.

2. Spontaneous refractiveness to treatment is common after a varying period of dosage, but has not been shown to be due to any antagonistic activity arising in the blood.

3. The comb recovers rapidly and the testes slowly after the cessation of treatment.

4. In the capon, oestradiol benzoate partially inhibits the effect of testosterone propionate. It is likely that the comb atrophy seen in treated birds is contributed to by this antagonistic action of the exogenous oestrogens towards the bird's own androgens, which continue to be produced by the testes even after atrophy of the tubules has become advanced.

My best thanks are due to Dr A. S. Parkes for the constant interest which he has taken in this work, and to Organon Laboratories for the supply of oestradiol benzoate and tablets of oestrone.

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EXPLANATION OF PLATES I-III

PLATE I. COMBS

- Fig. 8. Head of OTC 7, before treatment.
Fig. 9. Head of OTC 7, 37 days after the implantation of a 65 mg. tablet of oestrone. The comb is atrophic.
Fig. 10. Head of OTC 1, after 100 days of treatment with oestradiol benzoate by fortnightly injections (65 mg. total). The comb is atrophic.
Fig. 11. Head of OTC 1, 48 days after cessation of treatment with oestradiol benzoate. The comb has regained normal size.

PLATE II. TESTES, $\times 81$

- Fig. 12. Testis of OTC 5 (weight 0.81 g.), after prolonged treatment with oestradiol benzoate and oestrone (Fig. 3). The tubules are small and atrophic, and the intertubular tissue is dense.
- Fig. 13. Testis of OTC 4 (weight 0.69 g.), after still more intensive treatment with oestradiol benzoate (Fig. 4). The tubules show only Sertoli cells and spermatogonia.
- Fig. 14. Testis of OTC 2 (weight 0.41 g.), after very intensive treatment with oestradiol benzoate (Fig. 5). The tubules are partially obliterated.
- Fig. 15. Testis of OTC 1 (weight 4.65 g.), after 80 days in which the bird was allowed to recover from oestradiol benzoate injections (Fig. 6). Tubular regeneration is almost complete.

PLATE III. THYROIDS, $\times 66$

- Fig. 16. Thyroid of a normal cock.
- Fig. 17. Thyroid of OTC 7, after the implantation of tablets of oestrone (Fig. 1). The vesicles are partially distended with colloid and somewhat less active than normal.
- Fig. 18. Thyroid of OTC 2, after intensive treatment with oestradiol benzoate (Fig. 5). The vesicles are much enlarged in some areas, with less active epithelium than normal.
- Fig. 19. Thyroid of OTC 1, 80 days after the cessation of oestradiol benzoate injections (Fig. 6). The vesicles are nearly all much enlarged and the epithelium inactive in some regions. Deposits of blood occur in the colloid of some of the vesicles.