CLXXIII. GROWTH AND MAINTENANCE OF THE FOWL'S COMB BY ADMINISTRATION OF ANDROSTERONE.

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I. Introduction.

GREENWOOD *et al.* [1935] have discussed certain quantitative aspects of the capon comb response to androsterone. In view of the need for economy in both time and material a comparatively small degree of response is most suitable for assay work. Thus the criteria commonly used, a 20 % increase in area or an increase of 5 mm. in length + height, take the capon comb only a fraction of the way towards the size of the normal cock's comb. Owing to lack of material and preoccupation with assay work, comparatively little effort has hitherto been made to produce and maintain full-sized combs in capons.

Early in the work on the preparation of male hormone from testis, McGee *et al.* [1928] obtained comb growth in a Leghorn capon to a size ($L \times H$) of 48 cm.² in 5 weeks, about half the area (thus calculated) for a really good Brown Leghorn cock comb. Schoeller and Gehrke [1931] obtained an increase to 40.3 cm.² in 6 weeks with urine concentrates.

Ruzicka *et al.* [1934] obtained a surface area of 20 cm.² (from 6.5 cm.²) in 20 days during early work with their "synthetic" androsterone and Ruzicka and Tschopp [1935] record an increase from 4 to 27 cm.² in 16 days. The record comb of the literature, however, appears to have been produced by Freud *et al.* [1932]. As a result of nearly 4 months' injection of male hormone concentrate from bull testis, ultimately in a dose corresponding to 6 kg. of testis daily, they obtained a comb of 83 cm.² (planimeter reading), which may be considered as being full size. This problem is of importance because there is some reason to suppose that androsterone may not represent the full endocrine activity of the mammalian testis, and an examination of the requirements for the complete replacement of testicular activity in birds should be instructive.

We have also taken the opportunity of recording some observations on the effect of androsterone on the atrophic vas deferens.

II. Methods and material.

Birds. Two Brown Leghorn capons (EC 5 and 8) were obtained from the stock maintained by Dr A. W. Greenwood, having been operated on as chicks. One Brown Leghorn (EC 1) and two Plymouth Rocks (EC 2 and 3) were successfully caponised as adults and used for maintenance experiments. One Plymouth Rock (EC 7) and the Sebright Bantam were operated on as adults, and kept for more than a year before use, by which time the combs were indistinguishable from those of birds caponised as chicks. The difficulties of caponising adult cocks are considerable, and several birds were lost in obtaining reasonably clean operations. Two normal hens, one Rhode Island Red and one Brown Leghorn were also used.

Accommodation. The groups of capons discussed in Section VII were kept in an outside run open only to the north-east. The other birds were kept loose in an inside room, well lighted by two large windows, but receiving no direct sunlight.

Comb measurements. In the long period experiments combs were measured twice a week or, during periods of rapid growth, every day. In the comparative experiments described in Sections V and VIII the method of testing described by Greenwood *et al.* [1935] was used.

In order to give an adequate impression of the changes in comb size it is necessary to obtain either the surface area or the volume of the comb or an index of one of these. Length+height ($L+H$), whilst a convenient basis for assay work, does not indicate the actual changes in the appearance of the comb. We have therefore taken $L \times H$ as an index of comb size. The measurements (length $\times$ breadth in the case of the rose comb of the Sebright Bantam) were made as described by Hill and Parkes [1934].

Source of androsterone. The androsterone used in these experiments was part of the stock referred to by Callow and Deanesly [1935]. Whilst lower concentrations were used at the beginning, the bulk of the work was carried out with solutions of 10 mg./ml. arachis oil. A solution of this strength can readily be made by heating the oil, and the material afterwards remains in solution at room temperature. Stronger solutions in arachis oil can be made but they tend to crystallise out on cooling. A concentration of 10 mg./ml. represents about 100 capon units/ml. and makes it possible to keep the amount of oil injected down to a comparatively small quantity.

III. Induction of full comb growth.

Sebright Bantam capon. A Sebright Bantam cock was castrated in December 1933, when about 6 months old. The comb, 5.7×2.6 cm. at operation, atrophied rapidly, and 2 months later was 4.0×1.5 cm.; 3 months later it was 3.1×0.8 cm.,

Fig. 1. Growth of comb in a Sebright Bantam capon.

at which it remained constant for 8 months. During this time the henney plumage was superseded by typically cocky plumage and spurs developed. 13 months after castration, injections of androsterone were begun, starting at

0.5 mg., rising to 2.5 mg. and dropping again to 1.0 mg. daily. At the end of treatment, the comb was larger than at the time of castration and was in fact far larger than that of the normal cock. The treatment had no effect on the capon plumage [see Callow and Parkes, 1935]. The details of the experiment are shown in Fig. 1. It may be concluded that 0.5 mg. will cause appreciable growth of the comb of the Sebright capon, but will not take it to full size. 1.0 mg. daily, on the other hand, will grow the comb to abnormal size and will maintain it there. It may thus be supposed that the testes of the normal Sebright cock have a comb growth-promoting activity equivalent to that of rather more than 0.5 mg. androsterone administered as above.

Brown Leghorn capon. This bird was from Dr Greenwood's stock. The comb was flaccid and though rather large (5.7×2.7 cm.) when stretched, was brown and shrivelled and evidently that of a complete capon (Plate IV, fig. 7). It was thus ideal for the experiment in view, viz. the production of a full-sized comb in the minimum time. 5 mg. androsterone daily were given. Really remarkable

Fig. 2. Growth of comb in a Brown Leghorn capon.

growth was produced (Fig. 2), and after 22 days' treatment the comb measured 11.8×7.2 cm. and would readily have passed as that of a normal cock, except possibly that the papillae were not very marked (Plate IV, fig. 8). Wattles and

ear lobes were also prominent. The dose was then dropped to 1 mg. daily, which was thought to be about the probable maintenance requirement. After a set-back, due probably to the sudden drop in dosage, growth was resumed, though at a much slower rate. This second growth phase gradually died away and in the end the comb was being comfortably maintained by administration of 1 mg. daily at a size which was above rather than below the usual standard for the breed. 10 days after the first injection this bird was observed to be making vigorous efforts to "tread" a hen, and a few days later it began to crow sporadically, a condition which was maintained till it was killed. This induction of "treading" and crowing is of interest as an effect of the administration of a pure crystalline substance. According to Appel [1929] the syrinx is morphologically similar in hens, capons and cocks, so that the power to crow presumably depends merely on the "instinct" to do so.

Comparison of Brown Leghorn and Plymouth Rock capons. The combs of Brown Leghorn cocks are usually $12-14 \times 7-8$ cm. Abnormally large ones run up to 16×9 cm., or even more. The cocks of heavy breeds of fowl in general have smaller combs. The combs of Plymouth Rock cocks maintained at the Institute's Field Laboratories, for instance, are usually only $9-10 \times 4-5$ cm., and they never attain such an appearance of luxuriant growth as those of

Fig. 3. Comparison of comb growth in a Brown Leghorn and a Plymouth Rock capon.

Leghorns. It seemed of interest therefore to determine whether this difference is due to a difference in the amount of male hormone produced by the testes in the Plymouth Rocks, or to a greater sensitivity of the combs of the Leghorns. For this purpose, Leghorn and Plymouth Rock capons with combs similar

in size and shape were chosen. The Leghorn, again one of Dr Greenwood's stock, had a comb 4.7×2.2 cm. The Plymouth Rock, which had been castrated 14 months previously as an adult, had a comb which had been fairly constant at about 4.7×1.4 cm. for many months. These birds were each given 2.5 mg. daily for 8 days, then 0.5 mg. daily for 25 days and finally 2.5 mg. again (see Fig. 3). During the first 8 days the comb of the Leghorn more than tripled in $L \times H$; that of the Plymouth Rock just doubled. During the administration of 0.5 mg. daily, the Leghorn comb nearly doubled, that of the Plymouth Rock hardly grew at all. During the second period of administration of 2.5 mg. daily the Leghorn comb increased in $L \times H$ by 50 % in 21 days. The comb of the Plymouth Rock rather more than doubled in 35 days, but the actual increment was much less than in the case of the Leghorn. When injections were stopped the comb of the Plymouth Rock was rather larger than at the time of castration 16 months previously. As shown in Fig. 3 the differential rate of growth in these two birds was paralleled by the differential rate of atrophy after the last injection. This experiment suggests strongly that the difference in comb size in Leghorns and Plymouth Rocks is due to genetic difference in the sensitivity of the combs to the comb-growth-promoting hormone. A similar conclusion is arrived at from the maintenance experiments described below.

One rather curious point of difference in behaviour between the Leghorn and Plymouth Rock combs may be mentioned here. When the latter was almost stationary at quite a small size on 0.5 mg. androsterone daily, it was very noticeable that the papillae became well developed, more so than those of the fast-growing Leghorn comb. Further, the papillae of the comb of the Leghorn described on p. 1416 underwent comparatively little development during the phase of very rapid comb growth, but grew later to the normal size. This difference during induced growth is obviously correlated with the difference shown during atrophy. The comb of the Leghorn after adult castration very soon loses its papillae, and at 10 days after operation it is usually quite smooth, as well as being very blanched. The comb of the Plymouth Rock, on the other hand, retains papillae and a red colour for much longer after adult castration.

IV. *Maintenance of full comb size.*

Brown Leghorn. An adult Brown Leghorn cock, comb 12.9×7.8 cm., was castrated. 10 days later, when the comb was showing easily appreciable shrinkage and loss of colour, the injection of 1 mg. of androsterone daily was started and was continued for 12 days. This treatment caused the comb to regain colour, and the papillae began to reappear. The effect on the course of regression is shown in Fig. 4. After the first 3 days the size of the comb increased steadily, and had the injections been continued the comb would presumably have been maintained at a greater size than when injections were begun, even if not at the size observed at the time of operation. This experiment therefore confirms the supposition that the full-sized Leghorn comb can be maintained by about 1 mg. androsterone daily.

Plymouth Rock. Two Plymouth Rocks were castrated when adult. 11 days later, when both combs had shrunk considerably but were about equal in size, injection of 1 mg. androsterone daily into one bird was started, whilst the other was kept as a control. Subsequently the dosage to the first bird was raised to 2.5 mg. daily. Details of the size changes in the combs are shown in Fig. 5. The dose of 1 mg. daily restored the comb to just about its previous size and would, no doubt, have maintained it at this level. Raising the dose at this

point increased the size of comb slightly, but at the time of killing there were signs that the comb size was becoming stabilised at a point not far above the normal.

Fig. 4.

Fig. 4. Maintenance of comb size after adult castration of Brown Leghorn cock.

Fig. 5.

Fig. 5. Maintenance of comb size after adult castration of Plymouth Rock cock.

V. Comparative sensitivity of combs of Leghorn and Plymouth Rock capons.

The experiments recorded above show that the testes of the Plymouth Rock cock produce as much comb-growth factor as those of the Leghorn or even more, and that the lesser size of the Plymouth Rock comb is due to its lesser sensitivity. This conclusion has a bearing on a matter of practical importance, *viz.* choice of breed for capon comb assay work. White and Brown Leghorns have been used almost exclusively up to date, but Plymouth Rocks have the advantage that they can be easily caponised at a much later stage than can Leghorns. As a further check therefore on the difference in sensitivity, a group of 5 Leghorn capons and one of 5 Plymouth Rock capons were tested. The Leghorns were given 1 mg., and the Plymouth Rocks 5 mg. over 5 days, the final comb measurements being made 1 day after the last injection (Table I). This dosage was arranged in the hope of getting a good measurable response in both groups.

Greenwood *et al.* [1935] have shown that in Leghorns over this range of growth the size increase has an almost linear relation with the dosage, and it is

Table I.

	Average $L+H$ at start mm.	Average $L+H$ increase mm.
5 Leghorn capons each receiving 1 mg. over 5 days	45+20	11
5 Plymouth Rock capons each receiving 5 mg. over 5 days	42+16	10.7

unlikely that the Plymouth Rock combs exceeded the range over which the relation is linear. One must therefore conclude that to obtain a similar response in the Plymouth Rock capon demands about 5 times the dose required for the Leghorn capon. Breed thus appears to be an important variable in assay by the comb-growth method.

VI. *Histological observations on the vas deferens.*

The paper by Callow and Deanesly [1935] shows that pure androsterone is relatively inefficient in causing growth of the accessory organs of castrated mammals. It is thus important to know whether the internal accessory organs of capons show a response comparable with that of the comb or whether, like the mammalian accessory organs, they only respond to relatively high doses. In practice one is reduced to examination of the effect on the rudimentary or atrophic vas deferens of the capon. In the present series the vasa deferentia of the Leghorn recorded on p. 1416 and in Fig. 2, of the 2 Plymouth Rocks (p. 1418 and Fig. 5) and of an uninjected Leghorn capon are available for histological examination. The vas deferens of the uninjected capon, as might be expected, was greatly atrophied; in places the lumen, lined by low cuboidal cells, was scarcely patent (Plate IV, fig. 9). That of the uninjected Plymouth Rock, 65 days after adult castration, was less atrophic, but had obviously undergone extensive involution (Plate IV, fig. 11). In the injected Plymouth Rock the vas deferens was fully maintained as regards the appearance of the epithelium, and the general size, though subnormal, was much greater than in the uninjected control (Plate IV, fig. 12). A similar condition (Plate IV, fig. 10) was shown by the vas deferens of the Leghorn (EC 5). In view of the fact that the large size of the normal vas deferens must be partly due to distention with testicular products, it is likely that the vasa deferentia of these two capons were almost completely maintained and developed respectively by the administration of androsterone. In comparing these results with those on rodents, it should be remembered that very large amounts of androsterone were given over prolonged periods.

VII. *Effect of androsterone on the comb of the hen.*

It seems to be established that oestrin has only the smallest effect, if any, on the comb of the hen. The question thus arises as to what induces comb growth in the hen and in particular why does the comb increase in size and colour when the bird is in lay? The obvious reply is to suggest that in the same way as the fowl testis produces oestrin in addition to male hormone, so the ovary produces male hormone in addition to oestrin. We are not however aware of any experiments on the effect of male hormone on the comb of the hen.

Two hens were injected with androsterone. The first was a Rhode Island Red with a small, erect comb, and the second was a Brown Leghorn with a large, drooping comb. 2.5 mg. daily were given: the results are shown in Fig. 6. The enlargement produced was of quite a different order from that in the capon comb. In 3 weeks the comb of the Rhode Island Red barely doubled in $L \times H$, while the similar comb of the very refractory Plymouth Rock capon (p. 1418, and fig. 3)

more than doubled in $L \times H$ in 8 days on the same dosage. The Leghorn hen's comb increased in $L \times H$ from 22.0 cm.² to 45.1 cm.² in 33 days. This may be contrasted with the Leghorn capon EC 8 which, on a dose of only 0.5 mg. daily, increased in $L \times H$ from 34 cm.² to 63 cm.² in 26 days.

Fig. 6. Effect of androsterone on hens' combs.

VIII. Discussion.

It is clear from the results described above that the administration of large amounts of androsterone to capons will induce comb growth at a rate well above normal, and that crowing and "treading" occur. Further, this normal condition can be maintained by moderate daily doses. Increased efficiency is known to result from more frequent dosing [*cf.* Freud *et al.*, 1932], and moreover the comb-growth-promoting substance is probably liberated from the testis in a more effective manner than can be simulated by injection in oil. The experimental results show that daily injection of about 1 mg. of androsterone is equivalent to the comb-growth-promoting activity of the normal testis, and there is little difficulty in supposing that the testes could produce sufficient androsterone to account for the total endocrine activity as far as comb size and behaviour are concerned. It must be remembered however that there is no evidence that the testes actually do produce the particular chemical compound androsterone, which may be merely the excreted form of the testicular hormone.

The few results on the behaviour of the vasa deferentia do not allow of such definite conclusions as to the sufficiency of androsterone, and it must remain an open question whether, as in the case of rodents [Callow and Deanesly, 1935], some other substance may be involved. It should be mentioned that Callow and Parkes [1935] have shown that the plumage peculiarities of breeds such as the Sebright Bantam can only be accounted for by the assumption that the avian testis produces oestrogenic material.

A large difference was observed in the response of two different breeds of fowl to doses of androsterone. Doses of the same order are required for restoration of the comb to normal size in either Brown Leghorns or Plymouth Rocks, and the same applies to the doses necessary for maintenance. The relative sizes of the normal combs of the two breeds are however so different that the absolute increase of size of comb is several times as much for a given dose in the Leghorn as it is in the Plymouth Rock. Stated rather differently, the Leghorn comb is several times as sensitive as the Plymouth Rock comb.

It is of interest to compare the rates of comb growth obtained with these large doses given once daily with the rates obtained on similar birds under the conditions of assay employed by Greenwood *et al.* [1935]. Although a linear relation between response and dose is observed for small doses over a limited range, for larger doses (up to 8 mg. given over 5 days) the response is closely proportional to the logarithm of the dose. In the case of the Leghorn capon plotted in Fig. 2 an increase in length+height of 31 mm. occurred in 5 days with a total dose of 25 mg., and this response is of the order which would be expected on the basis of the assay results. It should be emphasised that prolonged administration of a smaller dose of androsterone will produce a comb of normal size with less total material than a brief period of heavy dosing.

As regards changes in the rate of increase on a constant dosage, Gallagher and Koch [1930] showed that with a moderate daily dosage the rate of increase was fairly constant for about a week and then fell off rapidly until the comb was approaching stability. The work recorded here shows that this kind of thing happens over a wide range of dosage, the steepness of the initial rise and the time taken for the growth curve to flatten off depend on the magnitude of the dose given, and, of course, on the breed of bird. The first Leghorn (EC 5) showed an unbroken straight line for comb growth during the period when it was getting 5 mg. daily. Such a rate of growth could obviously not have been maintained much longer, but it is probable that a very large comb would have been produced if material had been available to continue the heavy dosage indefinitely.

The relatively much lower growth rate found in hens after injection of androsterone raises an interesting problem. The strain of egg-laying might have some effect in inhibiting comb development, but actually during the period of the experiment the Rhode Island Red hen laid 1 egg only, and the Leghorn hen 2 eggs only, though the ovary was fully functional at autopsy. Three other explanations suggest themselves: (a) that the hen's comb is inherently less responsive to male hormone than the cock's, (b) that some substance in the hen, possibly oestrin, inhibits the action of androsterone on the comb, and (c) that androsterone is transformed in some way by the ovary. The first suggestion is difficult to reconcile with the known effects of testicular grafts on ovariectomised hens. As regards the second suggestion, the simultaneous injection of oestrin and male hormone concentrate according to Schoeller and Gehrke [1933] may actually increase the effect of male hormone on the comb. In an experiment carried out on capons we found that 1 mg. of androsterone *plus* 1 mg. of oestrone administered over 5 days gave a slightly lower response than the administration of 1 mg. of androsterone alone. This isolated result is not in accordance with the results of Schoeller and Gehrke, nor, on the other hand, was the difference sufficient to explain the results with intact hens. The third suggestion is at the moment speculative, and further investigation of the phenomenon is obviously necessary.

SUMMARY.

1. Very rapid growth of the combs of capons, with complete restoration to normal size in the course of a few weeks, may be induced by injection of crystalline androsterone in doses of the order of 2.5–5 mg. daily.

2. The comb can be maintained at the normal level by daily doses of the order of 1 mg. of androsterone. This requirement is not inconsistent with the view that secretion of androsterone accounts for the whole androkinetic activity of the testis of the cock. Observations on the restoration of the vas deferens have also been made.

Fig. 7.

Fig. 8.

Fig. 9.

Fig. 10.

Fig. 11.

Fig. 12.

3. The comb of the Leghorn capon is several times more sensitive to androsterone than that of the Plymouth Rock capon.
4. The comb of the hen is much less responsive than that of the capon.

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DESCRIPTION OF FIGURES IN PLATE IV.

- Fig. 7. Leghorn capon EC 5 before injection.
Fig. 8. The same bird after 22 daily injections of 5 mg. androsterone.
Fig. 9. Vas deferens of uninjected Leghorn capon, showing total atrophy. $\times 48$.
Fig. 10. Vas deferens of EC 5. Complete development of epithelium and increase in size. $\times 48$.
Fig. 11. Vas deferens of post-pubertally castrated Plymouth Rock (EC 3), 65 days after operation. Uninjected. $\times 48$.
Fig. 12. Vas deferens of post-pubertally castrated Plymouth Rock (EC 2), 63 days after operation. Injected as stated in Fig. 5, showing complete maintenance of epithelium, though the duct is not quite full size. $\times 48$.