

CLXXII. QUANTITATIVE STUDIES ON THE RESPONSE OF THE CAPON'S COMB TO ANDROSTERONE.

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I. Introduction.

SINCE it was first reported by Pézard in 1911 that injection of extracts of testicular substance restored the characteristic sexual phase of growth in the comb of the castrated bird, this response has been used extensively to determine the efficacy of preparations containing the male hormone. A review of the literature to 1931 on the assay of male hormone will be found in the article by Koch [1932].

The earlier work of Gallagher and Koch and their co-workers, 1928-1930, [cf. Koch, 1932] showed that the marked individual variations in the responses of the capons to the hormone were not determined by such factors as age, weight of birds and initial size and shape of comb. This lack of influence of body weight and initial comb size on the extent of the reaction was confirmed by Blyth *et al.* [1931] but not by Freud *et al.* [1932]. It was found however that the effect of these individual variations could be minimised by previously selecting the birds and by using a number for each test.

Gallagher and his co-workers defined a bird unit as the amount of hormone which, when injected daily for 5 days, yields an average of 5 mm. increase in length *plus* height of the combs of at least 5 Brown Leghorn capons. In the studies recorded here the American procedure has been followed mainly for the reason that considerable familiarity with this method over a long period of time has led, in our opinion, to results which are as free from obvious irregularities as can be expected of any biological measurement.

Another method of estimating the amount of comb growth induced is that in use in the Laqueur laboratory where the increase in total area of the comb is determined by means of planimetric measurements of the photographic shadow. On this basis the unit of male hormone has been defined as one fourth of the smallest amount which, administered in 8 doses of 0.5 ml. in 4 successive days (morning and evening) subcutaneously or intramuscularly in oil solution, produces on the fifth day, in at least 2 of a group of 3 animals, a comb increase of at least 15 %, or if more than 3 birds are used, in the majority of the animals. Reference may also be made to the methods described by Schoeller and Gehrke [1931], Butenandt and Tscherning [1934], and Ruzicka and Tschopp [Ruzicka *et al.*, 1934].

The standardisation of preparations of male hormone by the comb growth method, like other biological assays, is unsatisfactory in that experiments carried out in different laboratories with the same technical procedure and the same hormone preparation do not necessarily give the same response. (Results of an

international experiment carried out in 1933 under the direction of Koch in the laboratories of Laqueur, Koch, Butenandt and Greenwood (unpublished.) The discovery of a method for the artificial preparation of the male hormone, androsterone, by Ruzicka *et al.* [1934; 1935] and its production in larger quantity has suggested an extensive examination of the variables involved in the reaction between the hormone and the comb.

II. *Material and methods.*

Androsterone. This material was prepared in London by the method described by Ruzicka *et al.* [1934] with certain minor modifications [*cf.* Callow and Deanesly, 1935]. Material recrystallised from ethyl acetate and having a melting-point not below 182° was used. Solutions in arachis oil for injection were prepared by warming on the water-bath.

The stability of the oil solution was tested by heating a sealed ampoule containing the solution in an oil-bath, the temperature of which was thermostatically controlled at 149–151°, for 24 hours.

Care of capons. The birds on which these observations have been made were bred and kept at the Institute of Animal Genetics. They are housed under uniform conditions in a roof-lit, brick poultry house divided into pens each capable of holding 16–20 birds; the fowls are given access daily to floored sun porches but never come in contact with the ground.

Husbandry has been standardised as far as possible; the composition of the food remains the same throughout the year and consists of grain and mash in the summer months, while in the winter-time a hot mash is added. Cod-liver oil, green stuff, minerals and grit are also provided to supply the necessary special food requirements which cannot be picked up as on free range.

From October to March lighting is switched on in the evening until 6 o'clock and in the summer the birds are confined to the building from this time until 9 a.m. No artificial heating is provided.

Routine observations of the size of comb and body weight are made regularly. For the present series of experiments altogether 63 Brown Leghorn capons have been used, varying in age from 4 months to 6 years. The age at which the birds were castrated varied considerably as shown in Table I.

In preparation for the experiments measurements of the birds' combs and body weights were made (18. ii. 35) and 40 of the large group of 2-year-old castrates divided up into lots of 5 so that the average comb size and body weight of each was similar (see Tables II and III).

Table I. *Age at castration in different age groups.*

Prefix to no. of bird	Hatched	No. of birds	Average age at castration days	Range in days
A	1929	4	25	8–54
B	1930	4	26	18–33
D	1932	5	47	29–51
E	1933	45	39	21–51
G	1935	5	42	Nil

Comb measurement. All the birds were measured at the time of first injection and the differences in the average comb size (length *plus* height) of the groups from the preliminary measurement varied from –0.2 to +0.8 mm.

Since it appears that the technique of comb measurement employed by various operators does not yield strictly comparable results, a fairly detailed

description of the procedure used in Edinburgh will be given. The measurements of greatest length and greatest height of the comb were made by means of a small pliable celluloid scale graduated in mm., and all data recorded were obtained from the measurements made by a single observer. The determination of the height of the comb usually presents no great obstacles, though very occasionally it is difficult to obtain a definite base line between the comb and the head. The length however is a different matter: as an anterior point from which to measure, the junction of the comb with the beak is not satisfactory since it fairly frequently happens that the bird damages this region with the result that the fleshy tissue retracts, and, if this occurs during an experiment a source of error is introduced into the results. While in a few of the capons the front of the comb runs down in an unbroken line to meet the beak, in most there is a slight transverse groove towards the front, and this wherever possible has been used as the anterior limit. In the normal cock the comb ends in a large blade extending posteriorly, but in the capon this part of the undeveloped comb frequently curves upwards so that its upper edge lies parallel with the last point of the comb; when growth is initiated it begins to move through an arc towards the position it would occupy in the normal cock, so that the line of greatest length from a fixed anterior point would not fall consistently on the same posterior point. In order to minimise the error in these cases, the initial measurement is made with the posterior blade of the comb pulled down to the position it would subsequently occupy. Apart from this manipulation the comb is not abnormally stretched in measuring; an attempt is merely made to get it lying as flat as possible between the points to be measured.

The injections were made at approximately the same time each day, and all groups of birds, with one exception, received the hormone preparation deep into the pectoral muscles, alternate injections being given in the left and right sides. The exceptional group received subcutaneous injections.

In all cases where small doses were given daily (0.2 ml.) the substance was administered by means of a 1 ml. tuberculin syringe graduated in 0.01 ml.

During the course of the experiments the combs were measured normally 3 and 5 days after the initial injection was given but, exceptionally, daily measurements were made to which special reference will be made later. The rate of comb regression following the cessation of injections has been determined for some groups by measurements up to 30 days from the initial injection.

III. *Examination of variables.*

Relation between dose and response. The first experiment, on the relation between the amount of hormone injected and the magnitude of the comb response, was performed on a series of 25 capons divided into groups of 5, each bird receiving daily injections of 0.2 ml. of arachis oil solution for 5 days. The amount of hormone given was doubled in each successive group, and the total dose per bird ranged from 0.5 to 8 mg. of androsterone.

The data are recorded in Table II and plotted in Fig. 1. The individual points on the graph show that the variability of the response to the different doses is very similar. Thus, for the mean responses in the five groups receiving 0.5, 1, 2, 4 and 8 mg. total dose, σ is respectively 3.0, 1.9, 2.3, 2.5 and 1.8. This means that the relative variability (coefficient of variation, or $\sigma/\text{mean} \times 100$) is much less for the larger responses than for the smaller ones, which suggests that for comparative work it is desirable to aim at a substantial response. In

Table II.

Series A. Dose/response data. (Figs. 1, 2 and 6.)

No. of bird	Body weight g.		Comb size (mm.)				Increase in		Comb size (mm.)					
	18. ii. 35	4. iii	18. ii	27. ii	2. iii	4. iii	3 days	5 days	6. iii	9. iii	14. iii	19. iii	24. iii	29. iii
Group 1. Total dose 0.5 mg.														
E. 99	2350	2400	66	66	68	74	2	8	73	72	69	69	69	69
214	2850	2900	71	70	69	71	-1	1	72	70	69	68	68	67
232	3300	3250	67	71	71	72	0	1	72	71	71	70	71	68
399	2800	2850	60	61	63	64	2	3	64	62	62	61	61	60
533	2550	2600	75	75	75	76	0	1	76	75	76	74	74	73
Average	2770	2780	67.8	68.6	69.2	71.4	0.6	2.8	71.4	70.0	69.4	68.4	68.6	67.4
Group 2. Total dose 1 mg.														
E. 153	3200	3200	73	73	75	78	2	5	77	76	76	75	74	73
182	2700	2700	61	61	63	67	2	6	67	65	64	65	64	62
275	2900	2900	70	71	73	80	2	9	79	76	76	74	74	75
330	3150	3150	80	80	82	87	2	7	88	85	84	85	81	83
653	1950	1950	55	56	57	60	1	4	61	59	58	56	56	56
Average	2780	2780	67.8	68.2	70.0	74.4	1.8	6.2	74.4	72.2	71.6	71.0	69.8	69.8
Group 3. Total dose 2 mg.														
E. 10	2200	2200	77	78	86	91	8	13	90	87	86	85	85	85
227	2650	2650	53	54	58	61	4	7	61	59	59	58	58	57
317	3400	3500	74	72	78	84	6	12	84	82	81	78	77	78
350	2650	2700	65	64	69	74	5	10	74	72	71	71	70	70
403	3050	3100	70	70	74	81	4	11	79	76	74	71	71	70
Average	2790	2830	67.8	67.6	73.0	78.2	5.4	10.6	77.6	75.2	74.2	72.6	72.2	72.0
Group 4. Total dose 4 mg.														
E. 126	2050	2100	51	53	63	70	10	17	69	65	63	63	63	63
145	3200	3250	76	76	82	90	6	14	91	88	87	84	83	83
187	3400	3400	74	75	80	89	5	14	88	87	84	81	80	81
328	2600	2600	66	63	74	82	11	19	82	76	74	76	75	74
385	2700	2700	71	70	77	83	7	13	84	81	80	76	75	74
Average	2790	2810	67.6	67.4	75.2	82.8	7.8	15.4	82.8	79.4	77.6	76.0	75.2	75.0
Group 5. Total dose 8 mg.														
E. 24	2650	2600	75	76	87	93	11	17	93	89	88	87	86	86
230	2700	2800	68	69	80	86	11	17	87	85	82	79	78	79
412	2900	2950	71	69	79	89	10	20	89	84	83	81	79	78
536	2900	2850	63	64	74	82	10	18	81	78	76	74	74	74
585	2900	2850	62	64	71	79	7	15	80	76	74	73	72	72
Average	2810	2810	67.8	68.4	78.2	85.8	9.8	17.4	86.0	82.4	80.6	78.8	77.8	77.8

(Daily dose: 0.2 ml.)

view of the constant magnitude of the variability of the response with different doses it is legitimate to consider the whole of the 25 observations together and to calculate a combined σ . The figure obtained is 2.1, which may be taken as the standard deviation of the comb response under the conditions and range of dosage used. The standard error of the response may therefore be taken as ± 1.0

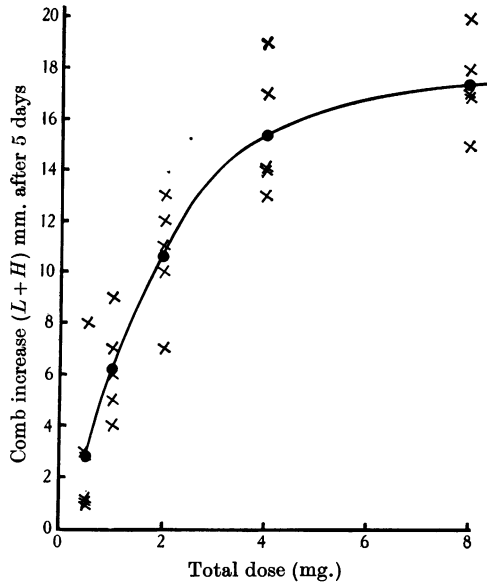


Fig. 1. Relation of increase of size of comb to the total dose of androsterone given over 5 days. Series A. × Individual birds; • mean values for groups of 5.

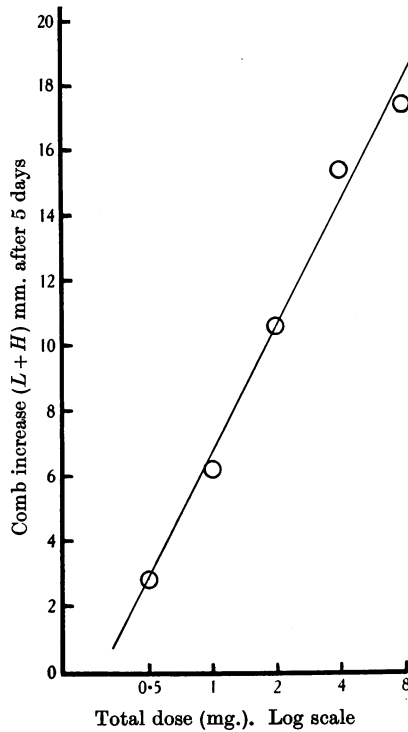


Fig. 2. Relation of increase of size of comb to log(total dose).

for a group of 5 capons, or ± 0.7 for a group of 10 capons. In comparing the responses in two groups of 5 therefore a difference of not less than 3.0 mm. is required in order to be statistically significant.

The curve drawn through the means in Fig. 1 obviously gives the elements of a standardisation curve; its shape suggests that the relation between dose and response is logarithmic, and when, as in Fig. 2, the response is plotted against the logarithm of the dose, it is seen that the points lie approximately along a straight line. The observations embodied in this (log dose)/(response) line were all made during the same week, and it is well known that such lines vary from time to time in both position and slope—an important factor in assay work. They can, however, always be reorientated by giving two different doses of a standard preparation at the same time as an assay is made.

From the line in Fig. 2 we obtain graphically $\frac{du}{dx} = \frac{12.8}{1}$, where u = comb response in mm. and x = logarithm of the dose in mg. The standard error of ± 1.0 in the mean response of a group of 5 capons will thus represent an error of about ± 0.08 in the determination of the logarithm of the dose. This is equivalent to an error of about $\pm 18\%$ in the determination of the dose. With groups of 10 capons the error would be reduced to about $\pm 12\%$.

Nature and volume of medium. Two tests were designed to examine the possible influence of changes in the medium in which the hormone is dissolved. In series B (Table III and Fig. 3) the actual amount of hormone injected daily was the same as in group 3 of the first experiment, namely 0.4 mg., but the volume of oil in which it was administered was increased from 0.2 to 1.0 ml. In series D (Table III and Fig. 3) the amount of hormone was again kept the same but the medium altered, both in nature and quantity, being a solution in 10% aqueous alcohol, given in a daily dose of 4 ml. Five birds were used for each test, their average comb size and body weight being approximately the same as those for the groups in series A. An examination of the responses obtained in these two experiments, compared with that obtained from a similar amount of hormone injected into group 3 of the first experiment shows that the increase in comb size of 12.4 mm. resulting from the administration of the aqueous-alcoholic solution (series D) is nearly twice that obtained in series B (7.4 mm.) with the increased volume of oil, while that in series A (which will be shown later to approximate closely to the average of all increases obtained from this dosage) is intermediate between them.

It is clear then that in any procedure for the standardisation of the hormone it is necessary to consider not only the nature of the solvent employed but also the volume.

Site of injection. In the next experiment (series C, Table III and Fig. 3) the same dosage was adopted as in A 3, but the injections were given subcutaneously instead of intramuscularly. It will be seen from Fig. 3 that the reactions obtained in the two experiments follow similar courses, the response elicited by subcutaneous injection being slightly but not significantly less than by intramuscular injection.

Heat stability of androsterone. Data concerning the effect of subjecting the hormone to heating at 150° for 24 hours are to be found in Table IV. Control and heated solutions of androsterone were administered in the same concentration and dosage as in A 3. The birds in this experiment had been used previously (series C and D respectively) and, 17 days after the final injection, the comb size in both groups had regressed to approximately the same extent.

Table III. (*Fig. 3.*)

No. of bird	Body weight g.	Comb size (mm.)				Increase in		Comb size (mm.)			
		18. ii. 35	9. iii	12. iii	14. iii	3 days	5 days	16. iii	19. iii	24. iii	29. iii
<i>Series B. Effect of volume of oil medium.</i>											
0.4 mg./ml. Total dose 2 mg.											
E. 165	2650	57	58	60	61	2	3	62	62	62	60
304	2900	67	67	73	76	6	9	78	75	74	71
325	3400	73	74	80	84	6	10	83	82	82	81
327	2650	70	71	76	80	5	9	81	76	77	75
346	2450	72	70	73	76	3	6	75	74	72	69
Average	2810	67.8	68.0	72.4	75.4	4.4	7.4	75.8	73.8	73.4	71.2
<i>Series C. Effect of subcutaneous injection.</i>											
2.0 mg./ml. Total dose 2 mg.											
E. 144	3050	69	69	73	78	4	9	77	74	74	73
156	3200	73	72	75	81	3	9	82	81	80	79
274	2500	62	63	68	73	5	10	73	68	66	65
640	3050	64	66	71	78	5	12	78	76	74	73
902	2300	71	72	76	80	4	8	78	77	76	75
Average	2820	67.8	68.4	72.6	78.0	4.2	9.6	77.6	75.2	74.0	73.0
<i>Series D. Effect of aqueous-alcoholic medium.</i>											
0.1 mg./ml. Total dose 2 mg.											
E. 11	1900	68	70	74	81	4	11	83	82	77	75
75	3100	68	69	74	82	5	13	85	86	83	80
77	3200	72	72	78	87	6	15	85	83	82	82
102	2600	66	64	69	74	5	10	73	71	70	68
244	3400	65	66	74	79	8	13	77	76	75	74
Average	2840	67.8	68.2	73.8	80.6	5.6	12.4	80.6	79.8	77.4	75.8

Note. Series B, C and D may be compared with group 3, Series A (Table II), where the amount of androsterone injected was the same.

Table IV.

Series E. Effect of heating androsterone in oil solution.

No. of bird	Comb size (mm.)				Increase in		
	30. iii. 35	1. iv	2. iv	4. iv	2 days	3 days	5 days
Group 1. Heated solution. Total dose 2 mg.							
E. 165	60	66	68	72	6	8	12
304	71	75	79	83	4	8	12
325	81	84	87	93	3	6	12
327	75	81	83	88	6	8	13
346	69	72	75	77	3	6	8
Average	71.2	75.6	78.4	82.6	4.4	7.2	11.4
Group 2. Control. Total dose 2 mg.							
E. 144	73	77	78	83	4	5	10
156	79	82	87	93	3	8	14
274	65	70	74	81	5	9	16
640	73	77	80	84	4	7	11
902	75	79	81	83	4	6	8
Average	73.0	77.0	80.0	84.8	4.0	7.0	11.8

In this case the close parallelism between the comb responses with control and heated solutions shows definitely that the latter solution had not lost activity.

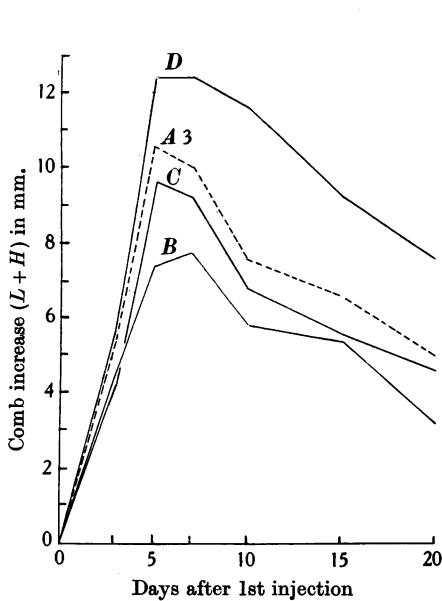


Fig. 3.

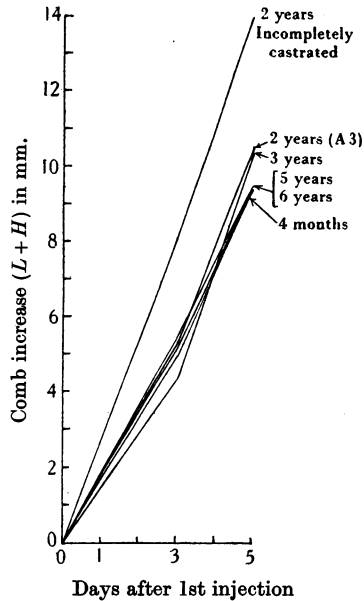


Fig. 4.

Fig. 3. Growth and regression of combs under varying conditions of administration of androsterone (2 mg. in 5 days). Groups of 5 birds. A 3, control; B, dilute oil solution; C, subcutaneous injection; D, aqueous alcoholic solution.

Fig. 4. Effect of age of bird and of incomplete castration (upper curve) on comb growth. (Dose 2 mg. in 5 days.) Series G.

Age of bird. The possible effect of age of the capon on the amount of comb-response induced was considered in the next experiment. Age groups of 2, 3, 5 and 6-year-old capons as well as one of 4 months were subjected to the same treatment as group A 3 (0.2 ml. daily for 5 days of a solution containing 2 mg. of androsterone per ml.). The small number of older birds available from which to select subjects for these tests made it necessary to include only 4 birds in each of the two oldest groups instead of five as in other experiments. The 2-year-old birds, group G 4, included in this test had been previously discarded, because, although the combs in point of view of size fell well within the range of the completely castrated birds their appearance and colour suggested that castration had not been satisfactorily complete. This suspicion was confirmed when the results of the test became available and it was seen that, whilst the responses obtained from the other age groups fell relatively closely to one another, that from the 2-year-old group was significantly larger (Table V and Fig. 4).

If, however, series A 3, which is more strictly representative of the 2-year-old birds, is used for comparison, it is found that the increase obtained after 5 days is just above that of the 3-year-old group (10.6 mm. to 10.4 mm. respectively) which in turn exceeds somewhat the increase elicited in the remaining three groups; these small differences are not significant.

Table V. (Fig. 4.)

Series G. Effect of age of birds.

No. of bird	Comb size (mm.)			Increase in		Body weight g. 29. iii
	29. iii. 35	1. iv	3. iv	3 days	5 days	
Group 1. 6 years old						
A. 292	70	75	79	5	9	2750
333	76	80	84	4	8	3050
462	79	84	87	5	8	2650
695	63	71	76	8	13	3000
Average	72.0	77.5	81.5	5.5	9.5	2862
Group 2. 5 years old						
B. 599	63	65	69	2	6	2100
641	65	71	77	6	12	3050
687	59	69	75	10	16	2650
974	81	83	85	2	4	3000
Average	67.0	72.0	76.5	5.0	9.5	2700
Group 3. 3 years old						
D. 129	72	77	85	5	13	2950
130	63	67	71	4	8	3400
456	69	74	81	5	12	3100
935	71	74	77	3	6	2850
953	66	71	79	5	13	2900
Average	68.2	72.6	78.6	4.4	10.4	3040
Group 4. 2 years old						
E. 5	71	81	88	10	17	2300
270	78	86	92	8	14	2700
552	71	76	82	5	11	2600
679	77	86	92	9	15	3150
712	71	79	84	8	13	2400
Average	73.6	81.6	87.6	8.0	14.0	2630
Group 5. 4 months old						
	11. iv. 35	14. iv	16. iv			11. iv
G. 1	64	68	73	4	9	1400
—	72	77	81	5	9	1850
3	71	77	81	6	10	1450
4	64	70	73	6	9	1400
5	69	74	79	5	10	1700
Average	68.0	73.2	77.4	5.2	9.4	1560
Av. for all groups	69.8	75.4	80.4	5.6	10.6	2540

(Total dose: 2 mg. of androsterone, 2 mg./ml.)

Weight of birds. If the possible effect of initial body weight on the magnitude of the comb response is examined it might be considered on *a priori* grounds that the concentration of the hormone in the body fluids is a factor in determining the amount of response and that therefore an inverse relationship would exist between the comb increase obtained and the body weight of the bird. However, no such relationship can be discovered in our experiments. Moreover,

in the age series (Table V) the 4-months-old group, which has an average body weight of 1560 g. compared with an average body weight of from 2630 to 3040 g. in the older groups (2 years to 6 years old), produced a comb increase which was not above the average for the dosage and indeed was slightly lower.

Initial size of comb. An analysis of the responses obtained from the relatively large number of 2-year-old capons with a view to determining whether any relation exists between the initial comb size and the magnitude of the comb reaction, in previously untreated birds, leads, if we exclude the age group G 4 for reasons explained above, to the conclusion that no consistent relation exists between them.

Previous treatment. In order to determine the effect of previous treatment on the magnitude of subsequent responses, 26 days after the cessation of the first experiment all the birds in series A were subjected to a further series of androsterone injections. The dosage was the same in all groups, being a daily injection of 0.2 ml. of a solution containing 2 mg. of the hormone per ml. On the previous occasion only one group (A 3) received this dosage, two of the others (A 1 and A 2) having had lower doses and two higher (A 4 and A 5).

When these results (Table VI and Fig. 5) are examined for evidence of any "priming" effect, it is found that 25 capons receiving 2 mg. gave an average increase in comb size of 11.3 mm., compared with 10.6 mm. given by a group of 5 birds a month before; the difference is not significant. The maximum differences between the groups are barely significant, and any correlation with the degree of previous priming is disposed of by the low response in group 5, unless it can be speculatively suggested that the responses in groups 3 and 4, which show a difference on the verge of statistical significance, indicate an optimum priming effect.

Rate of comb growth. The rate of growth of the combs in the first experiment (Table II and Fig. 6) where different amounts of androsterone were used, presented no exceptional features. When comb measurements were made 3 days after the initial injection it was found that the increase in comb size was roughly related to the amount of hormone injected. The measurements at 5 days indicated that increased responses were obtained with increasing dosage in the growth period between 3 and 5 days except in the case of the maximum dosage where the response to the two highest doses was similar. The lack of a directly proportional response of the comb to the amount of hormone injected is suggested by the amount of growth in the various groups at 3 days but is well marked in the amount of growth occurring in the succeeding period.

In this series, where the comb measurements were recorded after 3 and 5 days from the initial injection, a point of note is that those groups receiving the lower doses showed a distinct lag in response during the first period, suggesting that a definite latent period exists before the influence of the hormone can be expressed in the comb, or that a threshold of stimulus had to be attained before the hormone could prove effective. (The stimulation provided by the higher doses was apparently sufficient rapidly to overcome this inhibition or to mask it.) In this respect the data for the daily measurements at the second test of this series (Table VI) are of interest. As was expected with the moderate dosage used the increase obtained 24 hours after the initial injection was small and between 24 and 48 hours comparatively large. On the 4th and 5th days the daily increment in the size of the comb approached that obtained at 48 hours, but on the 3rd day the figure recorded was distinctly low. Since there appears no obvious reason why such a lag in the amount of the response should occur at this time the question arises whether it can be explained by errors in measure-

Table VI.

Series F. Effect of previous treatment. (Fig. 5.)

No. of bird	Comb size (mm.)								Increase in days						
	29.iii.35	30.iii	31.iii	1.iv	2.iv	3.iv	4.iv	5.iv	1	2	3	4	5	6	7
Group 1															
E. 99	69	70	74	77	81	84	84	81	1	5	8	12	15	15	12
214	67	68	70	72	77	77	79	78	1	3	5	10	10	12	11
232	68	69	72	72	74	75	77	76	1	4	4	6	7	9	8
399	60	61	64	64	67	69	69	69	1	4	4	7	9	9	9
533	73	73	76	78	78	80	81	79	0	3	5	5	7	8	6
Average	67.4	68.2	71.2	72.6	75.4	77.0	78.0	76.6	0.8	3.8	5.2	8.0	9.6	10.6	9.2
									Daily increase						
									3.0	1.4	2.8	1.6	1.0	-1.4	
Group 2															
E. 153	73	72	75	76	77	80	80	78	-1	2	3	4	7	7	5
182	62	64	66	66	69	72	72	71	2	4	4	7	10	10	9
275	75	75	79	81	83	89	88	87	0	4	6	8	14	13	12
330	83	83	86	88	91	94	95	95	0	3	5	8	11	12	12
653	56	56	60	60	62	64	64	63	0	4	4	6	8	8	7
Average	69.8	70.0	73.2	74.2	76.4	79.8	79.8	78.8	0.2	3.4	4.4	6.6	10.0	10.0	9.0
									Daily increase						
									3.2	1.0	2.2	3.4	0.0	-1.0	
Group 3															
E. 10	85	88	92	96	99	101	101	98	3	7	11	14	16	16	13
227	57	58	61	62	64	66	68	66	1	4	5	7	9	11	9
317	78	79	84	87	90	95	96	93	1	6	9	12	17	18	15
350	70	70	73	74	78	80	79	77	0	3	4	8	10	9	7
403	70	71	73	76	79	82	82	81	1	3	6	9	12	12	11
Average	72.0	73.2	76.6	79.0	82.0	84.8	85.2	83.0	1.2	4.6	7.0	10.0	12.8	13.2	11.0
									Daily increase						
									3.4	2.4	3.0	2.8	0.4	-2.2	
Group 4															
E. 126	63	64	70	73	76	80	80	79	1	7	10	13	17	17	16
145	83	83	87	88	92	93	96	94	0	4	5	9	10	13	11
187	81	81	83	84	88	91	92	89	0	2	3	7	10	11	8
328	74	75	79	81	83	86	88	84	1	5	7	9	12	14	10
385	74	75	78	80	83	88	89	85	1	4	6	9	14	15	11
Average	75.0	75.6	79.4	81.2	84.4	87.6	89.0	86.2	0.6	4.4	6.2	9.4	12.6	14.0	11.2
									Daily increase						
									3.8	1.8	3.2	3.2	1.4	-2.8	
Group 5															
E. 24	86	88	93	95	98	100	101	98	2	7	9	12	14	15	12
230	79	81	84	84	87	92	91	88	2	5	5	8	13	12	9
412	78	80	82	84	86	87	88	89	2	4	6	8	9	10	11
536	74	75	77	80	84	85	85	83	1	3	6	10	11	11	9
585	72	72	74	76	79	82	82	80	0	2	4	7	10	10	8
Average	77.8	79.2	82.0	83.8	86.8	89.2	89.4	87.6	1.4	4.2	6.0	9.0	11.4	11.6	9.8
									Daily increase						
									2.8	1.8	3.0	2.4	0.2	-1.8	
Total av. (25 birds)	72.4	73.2	76.5	78.2	81.0	83.7	84.3	82.4	0.8	4.1	5.8	8.6	11.3	11.9	10.0
									Total daily increase						
									3.3	1.7	2.8	2.7	0.6	-1.9	

(Total dose: 2 mg. of androsterone, 2 mg./ml.)

ment, but this seems unlikely since for this reading the amount of increase recorded is consistently low for all the groups. (It is true that in group 3 it approaches a higher level but equally true that it is lower than the daily

observations made immediately preceding and following it.) Whatever its meaning, the observations made force us to the conclusion that it is a real phenomenon.

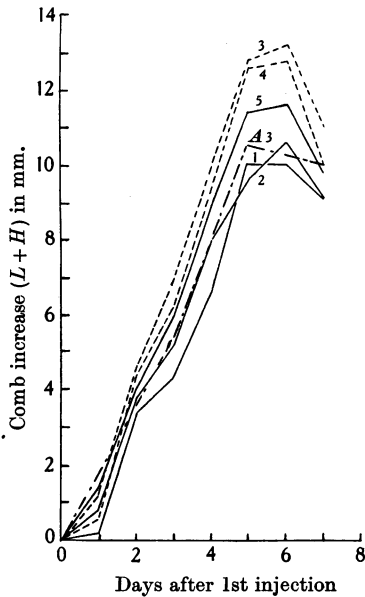


Fig. 5.

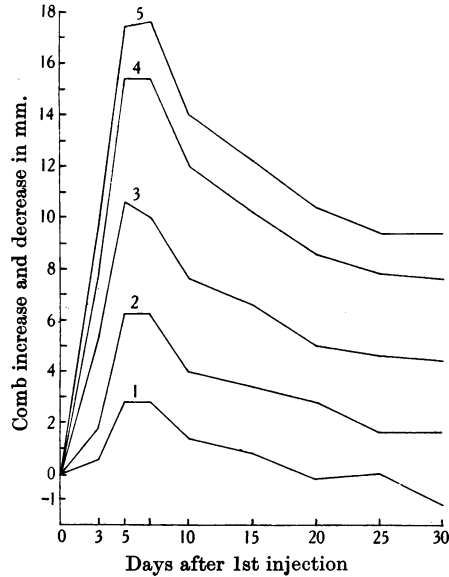


Fig. 6.

Fig. 5. Effect of previous treatment on growth of comb of groups of 5 birds receiving 2 mg. of androsterone over 5 days. Series F.

Fig. 6. Growth and regression of combs in groups of 5 birds receiving different doses. Series A. Group 1, 0.5 mg.; group 2, 1 mg.; group 3, 2 mg.; group 4, 4 mg.; group 5, 8 mg.

Rate of comb atrophy. The behaviour of the capon's comb after treatment has ceased may also be studied briefly in the same two tests. In series A measurements were made on the 7th and 10th days from the commencement of the experiment and thereafter at 5-day intervals for a further 20 days (Table II and Fig. 6). In series F the daily measurements were continued until the 7th day. For the former group the section of the curve under consideration may be conveniently divided into two parts, that between the 5th and 7th days, which shows comparatively little change, and the remainder of the curve, characterised by an initial phase of rapid regression which however is not maintained. The individual curves, though exhibiting some variability, suggest that the rate of regression decreases as the comb size becomes progressively smaller. The decline also appears to be greater in groups receiving the higher doses and this is particularly evident in the first sharp fall in the size of the comb (between the 7th and the 10th days). Nevertheless the inter-group divergence in regression rate is slight and it is clear that, whilst the combs of the first group have returned to their original state by the 20th day, a considerable time must elapse before those of the birds receiving the higher doses do so. In fact by the 30th day the average comb size in the birds of group A 5 had only regressed to the level attained by A 3 at its maximum.

With regard to that part of the curves between the 5th and 7th days, whereas A 3 exhibited a slight decrease the other groups remained stable at their maximum level. It is interesting to compare these results with those for series F. Here, daily measurements were recorded, and while group 1 showed a definite increase in the size of the comb on the 6th day, the others were more or less stable at the maximum size. By the 7th day however all had regressed to a size below that obtaining on the 5th day, the fall in the majority of the groups being greater than that in A 3.

SUMMARY.

1. A quantitative study has been made of the response of the comb of the Brown Leghorn capon to crystalline androsterone, and the effect of certain variables has been investigated.
2. In order to minimise the effect of individual variation, groups of five birds of similar initial comb size and body weight were used. These have been kept under constant conditions, and the technique of comb measurement has been carefully standardised.
3. Daily injections were made for 5 days, and the response was measured in terms of the increase in maximum length *plus* height of the comb.
4. The relation between the amount of hormone injected and the response of the comb measured on the day after the 5th injection is not linear when doses greater than 2 mg. of androsterone over 5 days are administered, corresponding to an increase of about 10 mm. in length *plus* height, but the response is directly proportional to the logarithm of the dose between the limits 0.5 and 8 mg. over 5 days.
5. The results are considered in relation to the formulation of a satisfactory technique for the assay of the male hormone. Careful measurement on selected groups of 5 birds yields results which imply a standard error of about $\pm 18\%$ in the determination of the dose.
6. The amount and nature of the solvent influence the response.
7. The responses to subcutaneous and to intramuscular injection are similar.
8. The differences in response with groups of widely differing ages (4 months to 6 years) are negligible.
9. It has been shown that in certain cases, probably as the result of the presence of a fragment of testis, unusually large responses are obtained. It is suggested that, as a precaution, birds should be used not earlier than 12 months after castration.
10. No relation between body weight and comb response could be demonstrated, nor could any consistent relation between initial comb size and amount of growth be found.
11. It is important to fix the period of the assay, since the amount of comb growth on successive days is not regular.
12. Only when a relatively small amount of comb growth is induced can regression of the comb to its pre-injection level be expected within a month, but after this period the responses to a new series of doses, whilst slightly irregular, do not show any significant effect of "priming".
13. The activity of androsterone is unaffected by heating the solution in arachis oil at 150° for 24 hours.

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