phoresis. This, indeed, happened on the 6th day where the patient's condition was much worse and she was not relieved at all by injection of epinephrine and not as much as usual by the electrophoretic technic.

It is the impression of the writer that with further development and analysis of the technic here disclosed, more prolonged and effective action may be obtained than that observed with hypodermic administration.

Mrs. Henriette Gettner has been kind enough to render her able assistance. Schieffelin & Company have kindly supplied the epinephrine base.

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Dose-Response Relationship of Androsterone by Direct Application to the Capon's Comb.*

SAM R. HALL AND L. P. DRYDEN. (Introduced by M. H. Friedman.)

From the Bureau of Dairy Industry, U. S. Department of Agriculture, Washington, D. C.

Fussgänger[1] first discovered that the capon comb is very sensitive to the direct application of dissolved androgen. McCullagh and Osborn[2] use this method for the determination of androgens in the blood of men. Butz and Hall have taken advantage of the sensitivity of this method in a recent study[3] wherein they showed that more of the androgen in bull's urine was found in the "cholestenone" than in the "cholestanone" fraction when the Anchel-Schoenheimer fractionation procedure was applied. None of the above authors has described fully the methods or the dose-response relationship for androsterone.

In an attempt to standardize the conditions in the use of this method and to understand the factors that influence the response, more than nine hundred individual tests have been made over a period of a year and a half. We have reported elsewhere[4] that season, previous use,

* This research was supported by an appropriation from Bankhead-Jones funds (Bankhead-Jones Act of June 29, 1935).

1 Fussgänger, R., *Mediz. Chem. Abhandl. Chem. Forschungstätten der I. G. Farbenin*, 1933, p. 213.

2 McCullagh, D. R., and Osborn, W. O., *J. Biol. Chem.*, 1938, **126**, 299.

3 Butz, L. W., and Hall, S. R., *J. Biol. Chem.*, 1938, **126**, 265.

4 Hall, S. R., and Hunt, John D., *Proc. Am. Physiol. Soc.*, 1939, in press.

age after 6 months, and initial comb size were not detectable factors in the response. Continuous artificial light tried only in the fall of the year caused lessened response.[5]

The capons used in the experiment here reported were all of one strain, single comb, White Leghorn, all hatched at the same time and caponized at between 28-32 days of age. They were housed together and were on the same diet. The androsterone† was dissolved in tricaproin and so made up that the desired amount was contained in ⅛ cc.‡ A 1 cc tuberculin syringe was used. When filled, it is, of course, sufficient for 8 applications and since the administration is repeated for 5 days on birds picked from the group at random, it is felt that the error in measuring ⅛ cc in the syringe graduated in 32nds is within the limits of the accuracy claimed for this method.

Following Gallagher and Koch,[6] length plus height was used as an index of comb size. Measurements were made with a transparent millimeter ruler. All but one barb had previously been removed from the combs by cautery and the backs of the combs cut smooth so as to eliminate any doubt regarding the points for measurement.

As a further check on our measurements the length and height of the comb on 20 unstimulated birds were determined twice on the same day with a mean difference of .1 mm between the 2 readings. Moreover as a further precaution in order to determine if comb manipulation and the tricaproin might not cause slight size increases in the comb, 13 birds were treated with the oil daily and the size changes noted on the sixth day. There was an average decrease of .5 mm with a S. E. of $\pm.3$; the decrease is most likely due to their continued regression following a use 3 weeks previously. It is probable that an average increase of 1 mm in 12 birds is significant.

"Slips"§ occasionally occur in our flocks. They appear most often at about the age of 4 to 5 months but have often been seen past a year and a half old. We can confirm Hooker and Cunningham[7] in their conclusion that at least in these older birds this condition arises spontaneously and is not the result of incomplete castration. They are easily recognized and after they appear are, of course, not used.

[5] Hall, S. R., and Dryden, L. P., *Proc. Am. Physiol. Soc.*, 1939, in press.

† Supplied by the Ciba Company through the courtesy of C. C. Haskell.

‡ We have evidence that for a given dose the volume of the vehicle is an important factor in the response and must be controlled.

[6] Gallagher, T. F., and Koch, F. C., *J. Pharm. Exp. Therap.*, 1935, **55**, 97.

§ A term long used by poultrymen to designate unsuccessfully caponized birds. To avoid introducing a new word, we are using the term here to include also those cases in which complete gonadectomy seems to be followed by the production of new testicular tissue.

[7] Hooker, C. W., and Cunningham, Bert., *Anat. Rec.*, 1938, **72**, 371.

TABLE I.

Date	No. of birds	Androsterone in gamma	Mean increase, mm	Stand. Dev.	Standard error
Oct. 31	6	.125	1.33	±1.21	±0.49
" 31	11	.250	3.73	±1.21	±0.37
" 31	11	.500	4.67	±1.83	±0.53
" 3	16	.500	4.69	±1.62	±0.41
" 3	16	1.000	6.25	±2.21	±0.55
" 3	16	2.000	8.50	±2.22	±0.56
" 3	16	4.000	11.13	±2.42	±0.60
" 3 (Injection)	15	100.000 (½ cc)	4.80	±2.15	±0.55

We, however, see no justification for discarding data obtained from them before they "slip" as did Gallagher and Koch.[6] In reviewing our data on individual capons, we find that, as a rule, those which "slip" past 10 months or more of age have not been above the average in sensitivity previous to the time the comb and wattles become brilliantly red and fail, as they do, to regress following stimulation.

Table I gives the data for increasing doses of androsterone. The hormone was administered once a day for 5 days and the comb size increase determined on the sixth day.

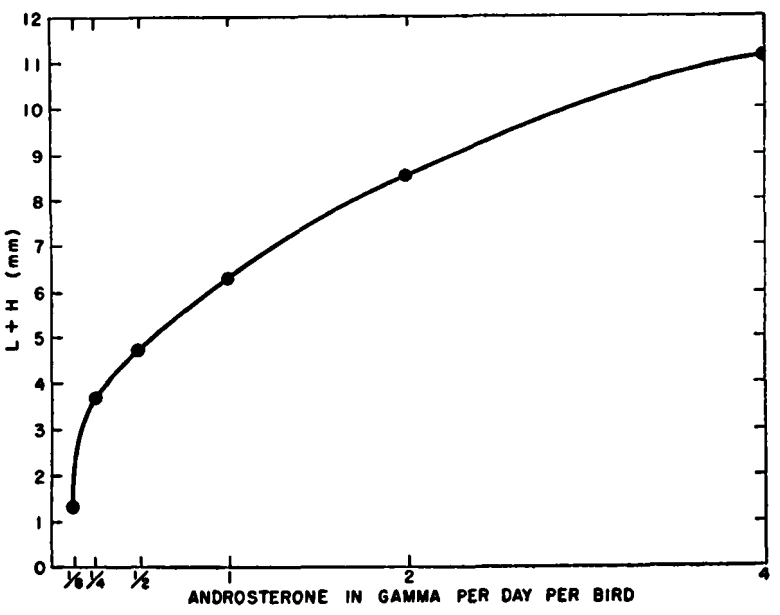

FIG. 1.

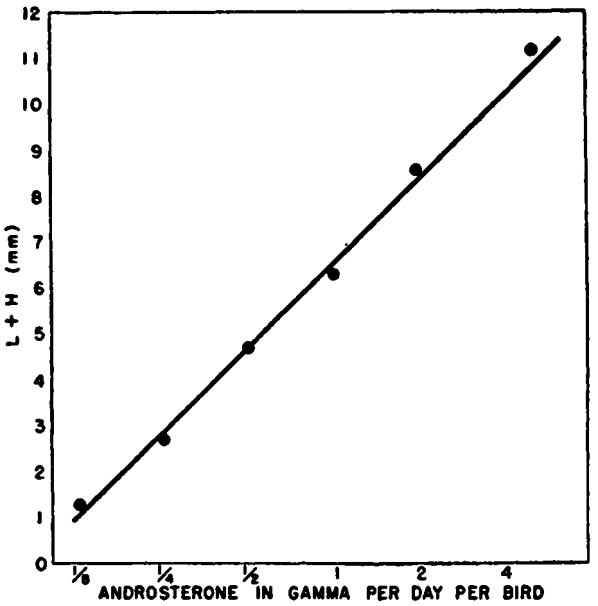

FIG. 2.

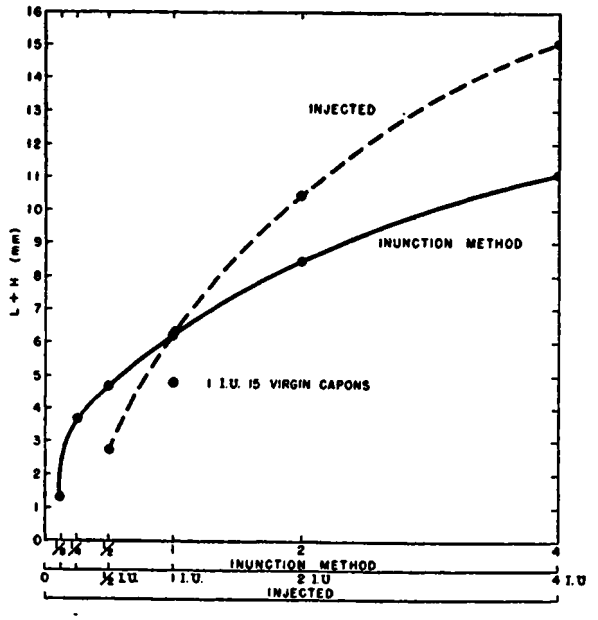

FIG. 3.

Fig. 1 gives the dose-response curve.|| Sixteen capons 6-months-

|| This curve is for androsterone in tricaproin. In work to be reported with L. W. Butz, we find the dilution factor to be important in the assay of crude

old that had not been used before were employed for all the points except .125 and .25 gamma. These were obtained 3 weeks later on 6 and 11 capons respectively. These 2 groups were so made up from the birds previously used only once that they should be equal to an average of the entire group. To find out if we were safe in combining data from "fresh" capons with those from birds previously treated, 11 of the capons selected as above were run in the second experiment on .5 gamma; these 11 gave a response identical with the first. (See Table).

From Fig. 2 it is seen that the curve is logarithmic from .125 to 4 gamma. The best fit is from .25 to 2 gamma.

Fig. 3 compares the curve in Fig. 1 with a curve obtained by injection. The latter curve is obtained from Greenwood, *et al.*[8] For comparison, the average response obtained from the injection of 100 gamma androsterone into 15 of our "virgin" capons is included.

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Chemical Composition and Vitamin Content of Royal Jelly.*

R. M. MELAMPY AND D. BREESE JONES.

From the Bureau of Entomology and Plant Quarantine, Department of Zoology, Louisiana State University, and the Bureau of Chemistry and Soils, United States Department of Agriculture.

In the colony of the honeybee (*Apis mellifera* L.) there are two castes of females, the queen, whose function is limited to reproduction, and the workers, who carry on all the other activities of the colony. The physiological process by which one female larva develops into a worker and another becomes a queen is assumed to be determined by its diet. For the first 2 days after hatching all female larvae apparently receive the same diet and the physiology of their development is similar. This diet is royal jelly, a secretion of the pharyngeal glands of the workers, and it is fed to the larvae at fre-

extracts of bull and ram urine and presumably of extracts of other materials or fractions containing relatively large amounts of extraneous material. In one extract of ram urine, we were consistently unable to promote comb growth above 4.5 mm regardless of the concentration. For such materials we propose as an end point the maximum dilution that will cause unmistakable comb growth (1 to 2 mm).

[8] Greenwood, Blyth, and Callow, *Biochem. J.*, 1935, **29**, 1400.

* A contribution from the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, in cooperation with the Louisiana State University, and the Bureau of Chemistry and Soils, U. S. Department of Agriculture.