under these circumstances was fully as low as that reached under conditions when the animals convulsed. The data obtained with group C was averaged with similar data obtained with group B (graph 5).

When the animals of group A were kept at 33°C during both the 18-hour fasting period and the 7-hour experimental period it was found that their blood sugar level was consistently higher than when they were kept at a temperature of 20°C during these periods. (Graphs 1 and 2.) This may be due to a lack of complete acclimatization to the lower temperature. Recent data indicate that 3 to 4 days are required for the full acclimatization to desert temperatures by man.[9] Similar experiments with group B produced similar data. These data were not averaged with those obtained with group A because of occasional sporadic deviations in blood sugar values. This was also true of the data obtained with the rabbits of group C.

When the animals of group A were kept at 33°C during the 18-hour fasting period and at 20°C during the 7-hour experimental period the blood sugar level at first was inclined to follow the level maintained when these animals were kept at 20°C during both periods and then approached the level obtained when these animals were kept at 33°C during both periods. (Graph 3.) When the animals of group A were kept at 20°C during the 18-hour fasting period and at 33°C during the 7-hour experimental period a similar reversal of the blood sugar level, but in the opposite direction, took place. (Graph 4.) Similar data were obtained with the animals of group B.

The data which were obtained with group B when kept at 33°C during the 18-hour fasting period and at 20°C during the 7-hour experimental period were averaged with those obtained under similar conditions with the rabbits of group C. (Graph 5.)

Phenomena associated with the failure of the rabbits of group C to convulse when kept at 20°C during the 7-hour experimental period after they had been kept at 33°C during the 18-hour fasting period, are suggestive of similar phenomena associated with the behavior of parathyroidectomized animals under the influence of a sudden change in the acid-base balance of the blood. Because of the base deficit caused by overventilation at 33°C the rabbits would be subject to an uncompensated acidosis at 20°C until the kidneys had restored the acid-base balance to a normal state.

Just as an increased acidity restrains the onset of tetany in parathyroidectomized animals it appears to restrain the inclination of normal animals to convulse under the influence of insulin hypoglycemia. An inhibiting effect upon the convulsive response after insulin injections due to an atmosphere with a high partial pressure of CO_2 has been reported.[10]

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14486

Androgen Assay on Three-Day-Old Male Chicks.*

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The capon comb growth method is recognized as the standard procedure for the biologic assay of androgens. However, when a great many assays are being performed constantly the difficulties of maintaining large numbers of such birds diminishes the practicability of this method.

Frank and his associates have developed an assay method based on the comb growth of the immature chick. The progress in devel-

oping the method was described in a number of reports[1-4] with a final detailed description of the procedure and method of calculation.[5] Before this detailed report appeared a number of investigators had used the immature chick for assay purposes[6,7,8] but the results were unsatisfactory and the method was considered unreliable. This is believed by Frank *et al.*[9] to be due to failure to adhere strictly to the details of the procedure specified by them.

We were led by the reports of Frank *et al.* to use the immature chick for androgen assays; our procedure was developed on the basis of their earlier communications and therefore differs in certain respects from the method finally outlined by these authors.

Method. Single Comb White Leghorn chicks are purchased from one source. The chicks are shipped on the first day of life and arrive at the laboratory the following morning. The assays are started the next day (*i.e.*, when the chicks are from 48-72 hours old). Although a slight variation (less than 24 hours) in age is unavoidable, the influence of this factor is minimized by performing control assays with each batch. Only male chicks are used, obviating the necessity for statistical correction for differences in comb growth response of males and females. Repeated checks by autopsy have demonstrated the reliability of the selection of "sexed" cockerels by the hatchery (within 5%).

On arrival, the chicks are placed in a thermo-regulated brooder. Frank *et al.* emphasize the importance of constant lighting conditions but recent reports[10] indicate that it is the temperature rather than the light that is of importance in this connection. The cockerels are kept in a room with ample daylight, but since control groups are run with each batch, the results are not influenced by variations in this factor.

Vehicle for Hormone Solution. The androsterone‡ and the extracts to be assayed are dissolved in ether. The ether solution is applied directly to the lateral surface of the comb by means of a fine needle and microsyringe or tuberculin syringe fitted with a micrometer screw. The applications are best accomplished by having one worker hold the comb firmly while the other manipulates the syringe so that the ether solution does not spread beyond the comb before drying. Applications are made once daily for 7 days and the animals are killed on the following day, the 8th day of the experiment or the 10th day of life. For the past 2 years we have employed ether rather than oil as a vehicle for applying the hormones following a suggestion made by Koch[11] for the assay of small amounts of androgens on capons. Since the ether evaporates quickly, the danger of the solution spreading beyond the comb or being smeared from one animal to another is avoided. Frank *et al.* prevent smearing from one group to another by isolating each group, but this obviously does not prevent transfer from one animal to another within the same group. When performing large numbers of assays we have not been able to individualize each group because of lack of space, but control experiments have demonstrated that this is not

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TABLE I.
Comb-Bodyweight Ratios of Chicks Treated with Androsterone.

Dosage of Androsterone: γ per cc	0	5	10	20	50	100	250
γ total dose	0	1.75	3.5	7.0	17.5	35.0	87.5
1.	26.9* ± 2.3† (8.2)‡	61.6 ± 16 (25.4)	—	76.3 ± 10 (13.1)	—	—	—
2.	27.4 ± 5.1 (18.5)	45.4 ± 7.7 (16.9)	54.9 ± 7.1 (12.9)	78.8 ± 10.4 (13.3)	—	—	—
3.	23.6 ± 3.4 (14.0)	36.3 ± 3.9 (11.0)	44.2 ± 4.0 (9.0)	76.6 ± 9.4 (12.2)	—	—	—
4.	23.7 ± 4.1 (17.2)	46.9 ± 11.7 (24.9)	64.0 ± 11.2 (17.5)	69.9 ± 10.9 (15.5)	—	—	—
5.	26.1 ± 6.3 (24.1)	53.5 ± 11.1 (20.7)	68.9 ± 15.6 (22.6)	—	—	—	—
6.	23.8 ± 3.6 (15.1)	44.5 ± 5.8 (13.0)	57.3 ± 8.4 (14.6)	67.4 ± 9.5 (14.0)	—	—	—
7.	22.1 ± 4.1 (18.5)	—	—	44.6 ± 7.7 (17.2)	59.9 ± 5.8 (9.6)	92.4 ± 18.4 (19.9)	123.8 ± 26.2 (21.1)
8.	25.2 ± 1.5 (5.9)	—	44.4 ± 5.9 (13.2)	56.0 ± 11.1 (19.8)	80.7 ± 15.8 (19.5)	—	—
9.	28.1 ± 6.3 (22.4)	36.9 ± 3.6 (9.7)	52.5 ± 8.4 (16.0)	64.9 ± 5.6 (8.6)	—	—	—
10.	23.4 ± 4.3 (18.3)	31.6 ± 5.9 (18.6)	39.5 ± 6.5 (16.4)	48.7 ± 9.8 (20.1)	68.1 ± 14.8 (21.7)	89.1 ± 11.2 (12.5)	—
11.	22.2 ± 1.9 (8.5)	41.1 ± 4.4 (10.9)	61.1 ± 14 (22.9)	78.6 ± 13.2 (16.8)	—	—	—
12.	27.2 ± 4.9 (18.0)	—	—	57.1 ± 12.0 (21.0)	81.5 ± 23.4 (28.7)	116.3 ± 25.4 (21.8)	159.7 ± 22.3 (13.9)
13.	20.5 ± 5.2 (25.3)	32.3 ± 9.8 (30.3)	45.1 ± 9.9 (21.9)	—	70.3 ± 13.5 (19.2)	—	—
14.	26.7 ± 5.7 (21.3)	48.2 ± 13.3 (27.5)	62.6 ± 12.3 (19.6)	82.9 ± 18.8 (22.2)	—	—	—

* Comb-body weight in mg per 100 g.

† Standard Deviation $\sigma = \sqrt{\frac{\Sigma (\bar{x})^2}{n-1}}$, $n = 10$

‡ Coefficient of variation.

necessary when ether is used as the vehicle.

Standardization. The androsterone controls are assayed on groups of 10 chicks each, at 3 or more dosage levels within the range of satisfactory response (Table I). The unknown is similarly assayed on groups of 10 chicks, whenever possible using more than one dilution, depending upon the amount of material available and the expected response. In addition, on one group of 10 cockerels, only the vehicle, ether, is used.

Results. In this report are presented the results of assays of the androsterone control solutions in 14 experiments involving 2800 chicks, 620 of which received various dosages of androsterone. The pertinent data are presented in Table I.

The average combweight in mg per 100 g bodyweight of the controls receiving the vehicle (ether) only, varied from 20.5 ± 5.2 to 27.0 ± 5.1 at the end of the experimental period. In 10 experiments in which each bird in one group received a total of 3.5 γ of androsterone, the average combweight per 100 g bodyweight varied from 39.0 ± 6.5 to 64.0 ± 11.2 mg. Variations of the same magnitude were observed at all dosage levels employed.

We do not know the cause of these variations in sensitivity of the comb response. However it is clear that the differences in average combweight response in the different experiments are the result of a true variation in sensitivity and are not due merely to a higher original combweight. If the latter were the case the groups showing the highest comb-body weight ratios among the ether controls would also show the highest values at the

various dosage levels. The slope of the curves would be the same in all experiments. However there is no such parallelism between comb-body weight ratios of controls and treated animals, and consequently the slopes of the curves vary. Therefore, corrections for initial bodyweight cannot correct for these variations. For these various reasons the use of a composite standard dose-response curve gives less accurate results than the construction of individual dose-response curves for each assay.

Dose Range. The total dosages used in the 14 experiments ranged from 0.175 γ to 87.5 γ. Not all dosage levels were employed in each experiment. A dosage of 1.75 γ (5 γ per cc) was used in 11, 3.5 γ (10 γ per cc) in 11, 7 γ (20 γ per cc) in 12 and 17.5 γ (50 γ per cc) in 5 experiments. The other dosage levels were employed in less than 5 experiments. Most of the curves are linear within the dosage levels employed, but some show a flattening at the higher dosage levels.

In a number of experiments not reported in this paper, in which testosterone and methyl testosterone were also employed, testosterone was found to give a response of about the same magnitude as androsterone, while methyl testosterone assayed somewhat higher.

Summary and Conclusions. Androgen assay by means of the comb growth response of the immature cockerel was found satisfactory. The method described is based upon that of Frank and his associates, but in addition to minor modifications, differs from the latter in the following respects: (1) male chicks only are used; (2) ether is employed as the vehicle for application of the hormone; (3) individual dose-response curves are constructed with each assay.

14487

Excretion in the Dog of Androgens and Estrogens in the Bile Following Injections of Androgens.*

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Testis transplants into the mesentery[1,2] and testosterone pellets implanted in the same situation[3] or in the spleen[4,5] fail to exert androgenic activity in castrate male rats, as evidenced by the lack of repair of the prostate and seminal vesicles. It has been concluded from such experiments that testosterone is inactivated by the liver.

We have shown in previous communications[6,7,8,9] that large amounts of estrogenic hormones both of exogenous and endogenous origin are excreted in the bile. Because of these observations it was decided to study the excretion of androgen and estrogen in the bile following injection of androgenic hormones in the dog. In this species administration of

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