ZOOLOGY

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Assay of Chicken Pituitary Glands by Means of Radioactive Phosphorus[1]

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It is often of interest to determine the gonadotropin content of the anterior pituitary gland under normal and experimental conditions by means of a biological assay. The newly-hatched cockerel has been the assay animal of choice in our laboratory. Breneman *et al.*, (2) observed that weight changes in the testes of the cockerels after gonadotropin stimulation served as an excellent end-point. More recently a new end-point, the uptake of radioactive phosphorus (P^{32}) by the testes, has been shown by Breneman *et al.* (3) to be sensitive to small amounts of purified samples of FSH, LH, and whole pituitary glands. This paper presents some further studies on whole pituitary material using the P^{32} assay.

Materials and Methods

The experimental animals used in these investigations were Single-Comb White Leghorn chickens. The assay birds were received from the hatchery when one day old, were injected on day two, and autopsied when three days old. They received no food or water. The "donor" birds were killed at appropriate times and their anterior pituitary glands removed, weighed, and stored in acetone until they were assayed for their gonadotropin content. At assay time, the pituitary glands were air dried, ground, and diluted with distilled water to the desired concentration. This pituitary material was then injected in two equal doses into the cockerels 24 and 12 hours before the scheduled autopsy time. Three microcuries of P^{32} were administered to each assay bird 18 hours before autopsy time. At autopsy, the testes were removed, cleaned, weighed, and each pair was placed on a separate planchet. The testes were allowed to air dry for 48 hours and then were counted in a Baird-Atomic general purpose multiscaler. The end-point was the counts per minute per milligram of wet testes tissue. There were 12-20 birds in each assay series.

Results

One study was directed at determining the levels of gonadotropic material present in normal male chickens and capons over a period of time from the age of 20 to 207 days. Caponizations were performed when the birds were 3-5 days old. Table I compares the pituitary weights of the two groups of birds. The capon anterior pituitary glands were always heavier than the control glands.

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TABLE I. Anterior Pituitary Gland Weights

Age Days	Average Weight, mgs. Controls	Capons
20	1.88	2.02
40	4.48	6.23
60	5.83	10.28
80	8.03	13.50
102	8.55	14.70
123	8.78	19.40
146	9.40	17.90
175	9.30	22.54
207	11.62	19.74

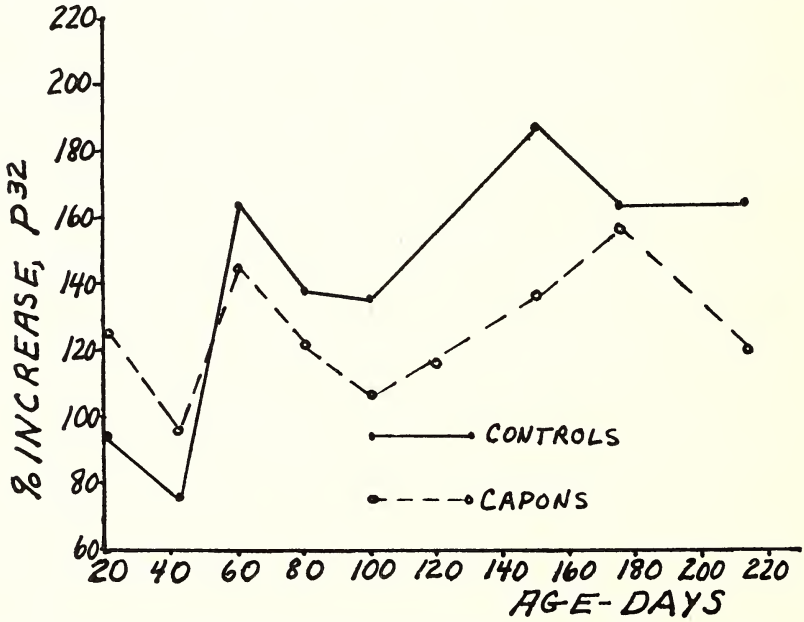

Figure 1. Gonadotropic Content of Normal Male Birds and Capons.

Figure 1 records the changes in gonadotropin content over the 200 day period. Each point on the graph represents a series of assay birds in which each bird received 1.0 mg. of donor pituitary gland. The basis of comparison of the different groups was the per-cent increase of P^{32} obtained by each series of birds over the control series for that particular assay. The capon pituitary glands at 40 days of age contained more gonadotropin than did the control glands. This is in agreement with results of Breneman and Mason (1). After 40 days, the control glands contained more gonadotropin per unit pituitary weight than did

the capons. This was also reported by Herrick *et al.* (4) using testes weights.

Other experiments were performed to note the effects of sex hormones on the gonadotropic content of capon pituitary glands and to further test the sensitivity of the P^{32} assay. Figure 2 illustrates the effects of repeated doses of 75 μgm. estradiol[2] on 50 and 75 day old

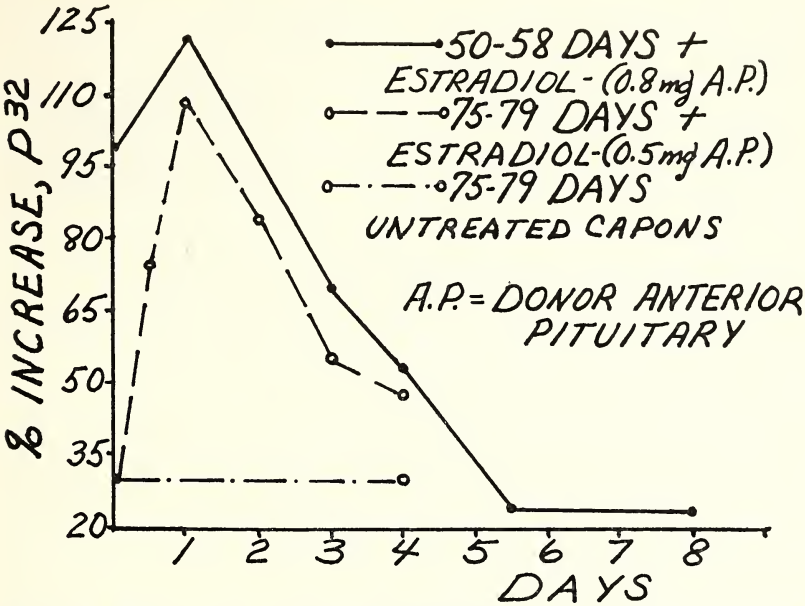

Figure 2. Effect of 75μgm. Estradiol dipropionate on the Gonadotropic Content of 50-58 and 75-79 Day Old Capons.

capons. In both cases there was an initial increase in gonadotropic content after one injection followed by a steady decrease with subsequent injections of the hormone. The assay was sensitive to changes within 12 hours after the first injection.

Figure 3 records the results obtained when 100 μgm. testosterone propionate[3] were administered to 52 day old capons. A gonadotropin increase was noted within 12 hours after the first injection. This was maintained until after the third injection when the content showed a further increase. Also to be observed in Figure 3 are the results obtained from administering 75 μgm. estradiol and 100 μgm. testosterone together to 68 day old capons. The curve is similar to the one obtained from administering testosterone alone.

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2. Estradiol dipropionate courtesy of Dr. A. A. Renzi, CIBA Pharmaceuticals, Inc.

3. Testosterone propionate courtesy of Dr. J. C. Siegrist, Schering Corporation.

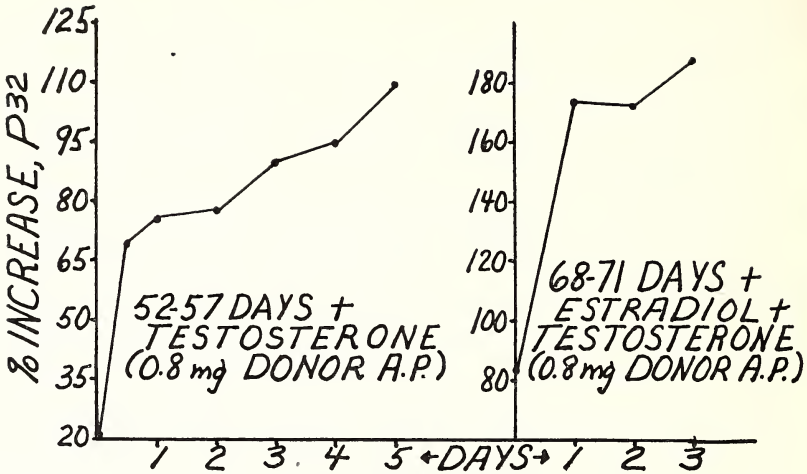

Figure 3. A. Effect of 100 μgm. Testosterone on the Gonadotropic Content of 52-57 Day Old Capons. B. Effect of 100 μgm. Testosterone + 75 μgm. Estradiol on the Gonadotropic Content of 68-71 Day Old Capons.

Summary

Although not specific for a particular gonadotropin, the method described in this paper utilizing the P^{32} uptake by the testes of newly-hatched cockerels is a sensitive assay and one that can be of use in determining the total gonadotropin content of pituitary glands under normal and experimental conditions.

Literature Cited

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4. HERRICK, R. B., W. H. MCGIBBON, W. H. MCSHAN. 1962. Gonadotropic Activity of Chicken Pituitary Glands. Endo. **71**:487-491.