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## The follicular hormone of the hen ovary.

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The demonstration that the follicular hormone from ovaries of swine would satisfactorily substitute for the hormonal function of the ovaries of non-pregnant rats and mice[1,2] has led us to attempt its extraction from other sources. So far we have obtained it from large, normal follicles of ovaries of swine, cattle, and sheep and from cystic follicles of swine and man, and have injected it into mice, rats, guinea pigs, rabbits, monkeys and human patients. These experiments seem sufficient to establish the expectation that this active substance is not species specific at least among mammals.

After trials in several laboratory mammals we have come to the conclusion that the rat, with both ovaries removed, is the best test animal. It has the following advantages: (1) the time required for the growth reaction of the tissues of the genital tract to the growth stimulus of the injected hormone is short, 36 to 48 hours, thus returning a decisive test after a few injections of a small amount of extract; and (2) the course of the experiment can be followed accurately by microscopic examination of the cell content of the vaginal smear, thus making it possible to use a test animal repeatedly.

As shown in our earlier work, the hormone is present in liquor folliculi from which all cells have been removed. The conclusion drawn was that it is secreted by the follicle cells under the influence of the ovum as the dynamic center.[2] Theoretically, it should be produced in the ovaries of all animals in which there is cyclic growth in the genital tract while the ova are ripening.

Our first tests of material from hens were of extracts made by Dr. Doisy of fresh eggs from the market. These tests returned several negative results and only one questionable positive result. We have since run five distinct series of tests upon hen's ovaries, follicles of different sizes, and mature eggs are listed in Table I, experiments 1 to 5.

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[1] Allen, E., and Doisy, E. A., *J. Am. Med. Assn.*, 1923, lxxxi, 819.

[2] Allen, Doisy, Francis, Gibson, Robertson, Colgate, Kountz, and Johnston, *Am. J. Physiol.*, 68, 138; *Am. J. Anat.*, (in press).

TABLE I.

The source of the follicular hormone of the hen ovary.

| Material extracted | Volume of material | Volume of alcohol | Volume of oil | Number of injections | Results |
|---|---|---|---|---|---|
| Exp. 1. Follicles | cc. | cc. | cc. | | |
| Small | 54 | 270 | 6 | 3 | + |
| Medium | 84 | 420 | 6 | 3 | — |
| Large | 93 | 558 | 6 | 3 | — |
| Exp. 2. Follicles | | | | | |
| Small | 180 | 720 | 6 | 3 | + |
| Medium | 55 | 220 | 6 | 3 | + |
| Large, and eggs from tube | 180 | 720 | 6 | 3 | — |
| Exp. 3. Follicles | | | | | |
| Small | 200 | 800 | 7 | 3 | + |
| Medium | 175 | 700 | 7 | 3 | + |
| Large | 200 | 800 | 7 | 3 | — |
| Exp. 4. Follicles | | | | | |
| Small | 140 | 280 | 10 | 3 | + |
| Medium | 420 | 1680 | 10 | 3 | +short |
| Large | 340 | 1360 | 10 | 3 | — |
| Exp. 5. Fresh eggs | | | | | |
| 18 yolks | | 1200 | 7 | 3 | — |
| 24 whites | | 1300 | 7 | 3 | — |
| Exp. 6. Chick embryos, incubated 5 days. | | | | | |
| 20, with membranes | 33 | 130 | 8 | 3 | — |
| 20 whites | 190 | 570 | 8 | 3 | — |
| Exp. 7. Chick embryos, incubated 10 days. | | | | | |
| 10, with membranes | 85 | 340 | 7 | 3 | — |
| 10 yolks | 130 | 520 | 7 | 3 | — |
| 10 whites | 110 | 440 | 7 | 3 | — |
| Exp. 8. Chick embryos, incubated 14 days. | | | | | |
| 6 with membranes | 100 | 400 | 7 | 3 | — |
| 6 yolks and whites | 75 | 300 | 7 | 3 | — |

The procedure of preparing and testing extracts was as follows: (1) the ovaries from laying hens were torn from the dorsal body wall and the larger follicles clipped off and separated into classes according to size (average diameter); (a) small follicles, less than 15 mm., (b) medium sized, 15 to 35 mm., and (c) large, 35 mm. to full sized yolks. A few eggs taken from the oviduct were also included in the latter class. (2) These classes of follicles were extracted separately with 95 per cent alcohol for 2 to 4 days, the alcoholic extract filtered off and evaporated, and the residue dissolved in Mazola oil. This product, although

crude chemically,[3] is satisfactory for tracing the hormone to its source. (3) The test animals, spayed rats, were given three 1 cc. injections of the oil preparation at 3 to 4 hour intervals. (4) The course of the experiment was followed by microscopic examination of smears of the vaginal contents as described in earlier papers.[4] The details of a typical test are given in Table II.

TABLE II.

A typical test of extracts; experiment 3 as listed in Table I.

| Animal | Extract of follicles | Control smear 4/7 3 P. M. | 4/8 Injections | | | 4/10 Test smears | | | Results |
|---|---|---|---|---|---|---|---|---|---|
| | | | 10 A. M. | 2 P. M. | 5 P. M. | 10 A. M. | 4 P. M. | 4 P. M. | |
| 5 | Less than 15 mm. | D | 1 cc. | 1 cc. | 1 cc. | Ea O | O | $M_2$-D | + |
| 5-NL | 15 to 35 mm. | D | 1 cc. | 1 cc. | 1 cc. | O | O-$M_1$ | $M_2$ | + |
| 4-NR | Larger than 35 mm. | D | 1 cc. | 1 cc. | 1 cc. | D | D | D | — |

D, operative diestrum; smear of leucocytes indicating absence of growth and slow atrophy of the vaginal epithelium.

O, estrous; smear of cornified epithelial scales indicating that growth of a thick estrous vaginal wall has been induced, (Ea, early phase of this period).

$M_1$ and $M_2$ metestrum; degenerative period during which removal of tissue resulting from hormonal action is accomplished by leucocytic infiltration.

[3] Doisy, E. A., Allen, E., Rolls, and Johnston, *J. Biol. Chem.*, 1924, lix, 43.

[4] Allen, E., *Am. J. Anat.*, 1922, xxx, 297; 1923, xxxii, 293.

Leucocytes were the chief consituents of the control smears on the day before injections were begun (stage D, column 3, Table II), thus checking the condition of operative diestrum or period of complete absence of growth in the vagina of the test animal. Three injections were given on the following day, April 8. As it usually requires 40 to 48 hours for the full reaction of the tissues of the genital tract to the growth stimulus of the hormone,[2] the first test smear was made on the morning of April 10 (column 7). Cornified epithelial cells made up the smears of animals 5 and 5-NL, indicating that full estrous growth or the addition of 8 to 12 layers plus the stratum corneum had been induced in the vaginal epithelium (stage 0, column 7, Table II). The smear of animal 4-NR was composed chiefly of leucocytes, indicating a negative result. Smears made at 4:30 P. M. of the same day confirmed the results of the morning examination. These details of the tests are more fully described and illustrated in earlier papers. The results of these series of tests are listed in the last column in Tables I and II.

The fifth series in Table I shows negative results obtained from extracts of whites of 24 eggs, and yolks of 18; these being fresh fertile eggs from the poultry department of the university.

We have also tested extracts of chick embryos and their membranes, and the whites and yolks, at five, ten and fourteen days of incubation with negative results (experiments 6, 7 and 8, Table I).

Histological examination of the walls of the follicles which contain the hormone show them to have from 1 to 4 or 6 layers of follicle cells in which mitotic proliferation is usually active. The absence of any accumulated secretion equivalent to the liquor folliculi of the mammalian follicle shows that the liquor is not necessarily a fundamental factor in the secretion of the follicular hormone.

To summarize briefly: (1) The ovarian follicular hormone can be extracted from ovaries of laying hens. (2) Injected into spayed rats it causes estrous growth in the genital tract. (3) Extracts of full sized follicles, eggs from the oviduct, fresh eggs (also whites and yolks separately), and 5, 10 and 14 day embryos with their membranes have given negative results. (4) These experiments establish the expected non-specificity of the follicular hormone as concerns birds and mammals.