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Onset of Follicular Atresia Following Hypophysectomy of the Laying Hen.* (26587)

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In the laying hen hypophysectomy results in rapid degeneration of all ovarian follicles which have entered the final stage of greatly accelerated growth. Atresia may be macroscopically visible in such follicles as early as 12 hours after pituitary removal(1).

A recent and unexpected observation in this laboratory has indicated that of the 8 to 14 rapidly developing follicles usually found in the ovary of a laying hen, those of intermediate size (5-10 g) are most resistant to atresia after hypophysectomy and that they remain ovulable for several hours after collapse of the mature follicle(2). In the present report the relationship of follicle size to onset of atresia is further examined.

Materials and methods. Thirty-two regularly laying White Leghorn hens, kept in individual cages and subjected to a constant 14-hour-light day, were hypophysectomized midpoint in a clutch of 4 or more eggs.

The birds were killed for examination of the ovaries at intervals of 6 to 72 hours after operation. All follicles in the final stage of accelerated growth (0.09 g or larger) were removed from the ovaries, weighed and examined individually for macroscopically visible signs of atresia. The follicles of each ovary were divided into 5 classes according to size, and percent of atretic follicles in each class was calculated.

Results. The results establish conclusively that follicles of intermediate size are the most resistant to degeneration following pituitary removal (Table I, Fig. 2). Atresia was first detected in the smallest follicles, but by 18 hours after hypophysectomy mature follicles (15 or more g) were the most heavily affected. By 24 hours all follicles in 4 out of 6 hens were completely atretic. In the remaining birds there were one or 2 follicles in the 5- to

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TABLE 1. Effect of Hypophysectomy on Rate of Follicular Atresia of Laying Hens.

Hr after operation	No. of hens	% follicles atretic in weight class (g)					
		15+	10-14.9	5-9.9	1-4.9	.4-.99	.09-.39
6	6	0	0	0	0	0	19.7
12	6	50.0	10.0	20.0	1.2	0	47.5
18	6	100.0	57.0	20.0	0	0	60.7
24	6	100.0	100.0	67.0	100.0	100.0	100.0
48	3	100.0	100.0	100.0	100.0	100.0	100.0
72	3	100.0	100.0	100.0	100.0	100.0	100.0

10-g class which were still intact. The rapid collapse and resorption of the ovarian follicles is shown in Figs. 1, 2, 3, and 4.

Discussion. On the basis of size of follicles, their probable growth rate and their assumed needs for hormonal support, it would have seemed logical to postulate that the largest follicles should have become atretic first, followed by those of lesser size and ending with atresia of the smallest ones. There is no obvious explanation for the unexpected relationship between follicular size and the order in which atresia affects them. Since rate of follicular growth, or more specifically rate of deposition of yolk components at the surface of the growing ovum, is not uniform throughout the period of 7 to 10 days required for the rapidly developing follicle to attain maximum size(3), the possibility arises that intensity of metabolic activity within a follicle at time of hypophysectomy may determine the speed of post-operative regression. Present knowledge of fluctuations in metabolic activity during the accelerated growth period, however, does not fully support such a correlation.

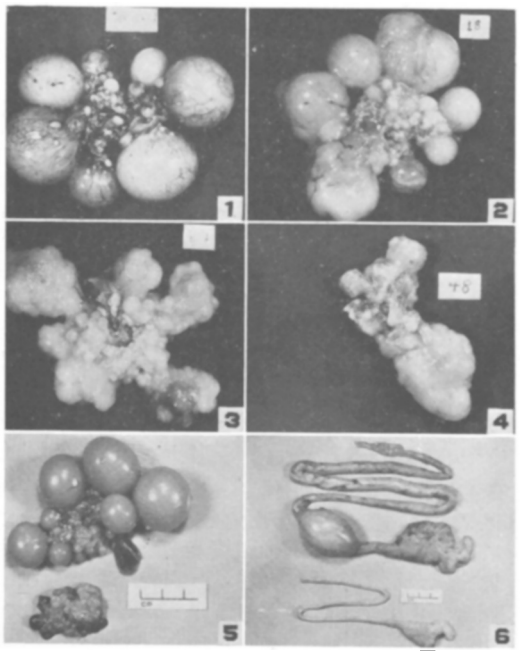

FIG. 1. Ovary of normally laying hen.

FIG. 2, 3, 4. Effect of hypophysectomy 18, 24 and 48 hr after operation. Note in Fig. 2 that the largest and smallest follicles are atretic while medium sized ones are not.

Fig. 5,6. Comparison of ovaries and oviducts of normal hen and hypophysectomized hen 6 days after operation.

Smith(4) has recently shown that rate of follicular growth (percent change in weight per hour) is maximum in follicles entering the phase of accelerated growth and decreases in an approximately linear fashion as follicle size increases. Permeability of the follicle wall, which presumably regulates the transfer of yolk materials to the ovum, follows a similar pattern of change. Using the rate of transfer of Evans' blue dye from the blood to yolk as a third measure of metabolic activity, Smith interprets his results to mean that the transfer rate increases to a maximum in follicles weighing 2-3 g, then decreases somewhat linearly with increasing size. These methods give, at best, an approximation of metabolic activity of follicles. With these facts in mind, the present results indicate that follicles exhibiting the greatest metabolic activity are the first to show signs of post-operative degeneration. However, onset of atresia in these follicles (0.09-0.39 g) is highly variable and roughly 40% of them are still intact 14 hours after hypophysectomy. Mature follicles, which according to Smith's standards are the least active metabolically, are the next group to show a high percentage of atresia. Once initiated, atresia rapidly spreads to all follicles in this class, and by the eighteenth hour all mature follicles have reached an advanced state of regression. Finally, the follicles which are

most resistant to atresia (5-10 g) had been developing at an intermediate and slowly declining rate which shows no sharp demarcation from the growth rate of follicles in adjoining weight classes.

Summary. Ovarian follicles in the laying hen were found to exhibit a differential rate of atresia following hypophysectomy which is dependent on follicle size. Atresia appeared first in the smallest of the rapidly developing follicles at 6 hours post-operatively and spread to include all mature or nearly mature follicles by 18 hours. Follicles weighing 5 to 10 g remained intact for as long as 24 hours.

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A Thermostable Cytotoxic Factor in Normal Human Serum Active Against Landschutz Ascites Tumor Cells.* (26588)

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During previous studies on the cytotoxicity of various agents on Landschutz ascites cells *in vitro*(1,2,3) it was found that fresh normal human serum (NHS) showed a marked cytotoxic effect. Bolande and Todd(4) have reported a similar cytotoxic effect on HeLa cells and Willheim *et al.*(5,6) and Bolande and McClain(7) on Ehrlich ascites cells (EA). According to the latter authors normal human serum contains a thermolabile nondialysable cytotoxic factor active against both Ehrlich cells and Sarcoma 180 cells. They also found that heating to 56°C for 30 minutes and fixation of complement eliminated toxicity for the cells studied.

The present paper reports further information as to the nature of this cytotoxic factor (e.g. that it is stable to 56°C) and as to its mechanism of action (e.g. that it is absorbed by tumor cells and interferes with anti-tumor cell heterologous antibody action).

Materials and methods. Ascites cells (A) were obtained from white mice (local strain) infected with Landschutz ascites tumor. The tumor cells were maintained by weekly passages. Using aseptic precautions, the ascitic fluid was removed 7 days following infection. The cells were washed with Hank's balanced salt solution (BSS) and diluted to a concentration of approximately 5×10^7 cells per ml.

Sera and complement reagents. Pooled normal human serum (NHS) obtained from at least 8 normal donors was stored at −20°C. Serum reagent lacking complement (C′) components C'_1 and C'_2 ($R_1 + R_2$) was prepared by heating NHS at 56°C for 30 minutes which was then designated as NHS 56. Serum reagents lacking properdin (P) $C'_3 + C'_4$ (RP, $R_3 + R_4$) were prepared according to the method described by Kabat and Mayer(8) and Pillemer *et al.*(9). Complement titrations were performed according to the method described by Kabat and Mayer(8) using anti-sheep hemolysin (Difco). C'_{50} unitage of complement was used exclusively.

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