ject might have been due to an increased filtered sodium. However, there were no changes in endogenous creatinine clearance in the other subjects exhibiting natriuresis (H.F., O.J.), nor in those in whom sodium excretion decreased or did not change. One might speculate that angiotensin acts directly on the renal tubule causing a diminished reabsorption of sodium. However, this hypothesis is difficult to reconcile with the fact that adrenal secretion of aldosterone is elevated in hypertensives and is further increased by administration of angiotensin(2, 9). Another hypothesis might be that the natriuresis induced by angiotensin represents the effect described in the dog(10), wherein a rise in the kidney perfusion pressure causes increased sodium excretion without changing the glomerular filtration rate.

Summary. Angiotensin II was administered at rates of 0.3 to 0.6 μg per minute over 6 to 24 hours to 6 subjects with hypertension of various etiologies under conditions of positive and negative sodium balance. Angiotensin produced hypernatriuresis under condition of positive sodium balance. The effect was most marked in those subjects having a diastolic blood pressure above 110 mm Hg.

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1. Nijensohn, C. M., *Semana Med.*, 1957, v205, 111.
2. Genest, J., *Canad. M. A. J.*, 1961, v84, 403.
3. Dahl, L. K., *Essential Hypertension—An International Symposium*, 1960, p53.
4. Bossnes, R. W., Taussky, H. H., *J. Biol. Chem.*, 1945, v158, 581.
5. Croxatto, H., Barnafi, L., Passi, J., *Science*, 1952, v116, 507.
6. Masson, G. M. C., del Greco, F., Corcoran, A. C., Page, I. H., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 631.
7. Peart, W. S., Brown, J. J., *Lancet*, 1961, v1, 28.
8. del Greco, F., *Fed. Proc.*, 1954, v13, 34.
9. Laragh, J., Angers, M., Kelly, W. G., Lieberman, S., *J.A.M.A.*, 1960, v174, 234.
10. Selkurt, E. E., *Circulation*, 1951, v4, 541.

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Ovarian Weight Response to Varying Doses of Estrogens in Intact and Hypophysectomized Rats.* (26805)

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A dose-response relationship was demonstrated for injected diethylstilbestrol and ovarian weight stimulation in hypophysectomized immature rats after a 4-day treatment interval(1). A recent study has shown that the greatest increase in ovarian weight occurs after about 8 days of treatment with large doses of estrogen in both intact and hypophysectomized immature rats(2). The following experiments were carired out to evaluate ovarian weight response after 8 days of injection with varying doses of diethylstilbestrol or estradiol.

Materials and methods. Intact or hypophysectomized Sprague-Dawley rats, 23-25 days old, were utilized in groups of 5 animals each. Hypophysectomized rats were received on the second post-operative day from Hormone Assay Laboratories, Chicago, Ill. Cortisone acetate (2.5 mg) was administered upon arrival and estrogen injections were begun on the following day. Daily injections of 4, 20, 100, 500, or 1000 μg of either diethylstilbestrol or estradiol, dissolved in 0.1 cc peanut oil, were given for 8 days. Uninjected control animals were caged separately. The animals were asphyxiated with natural gas 24 hours after final injection. The ovaries were dissected free, weighed on a Roller-Smith torsion balance, and prepared for microscopic examination.

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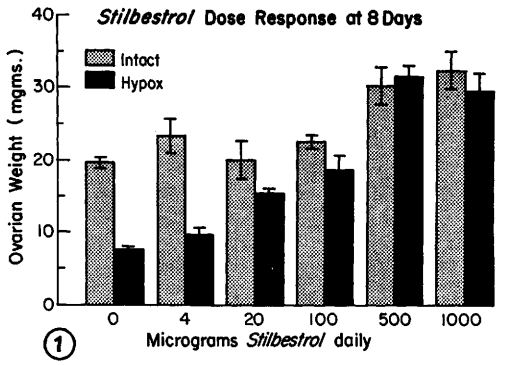

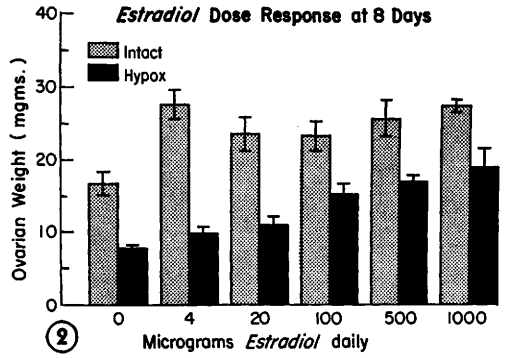

FIG. 1. Avg ovarian weights of rats treated with varying doses of diethylstilbestrol for 8 days. Stand. error of mean indicated by vertical bars.

FIG. 2. Avg ovarian weights of rats treated with varying doses of estradiol for 8 days. Stand. error of mean indicated by vertical bars.

Relative follicle size was estimated by examination of randomly selected fields in several ovarian cross-sections. An eyepiece grid was utilized which outlined an area of 1 sq mm at 100 power magnification. All follicles within this area were counted and the average number of follicles per grid area was determined for each dose level of stilbestrol.

Results. Fig. 1 depicts average ovarian weights of rats treated with varying doses of diethylstilbestrol. Step-wise increases in ovarian weight were produced in hypophysectomized rats with increasing doses of estrogen. The greatest response was observed with 500 μg, although this did not differ significantly from the average weight attained with 1000 μg doses.

No clear-cut sequential pattern of ovarian weight stimulation was detected in the intact animals subjected to doses of 100 μg or less. However, the 2 largest doses brought about significant increases which were comparable to ovarian weights observed in hypophysectomized animals treated in the same manner.

Average ovarian weight response to estradiol injection is illustrated in Fig. 2. Slight step-wise increases were produced in hypophysectomized rats and the sequence was similar to that obtained with diethylstilbestrol. However, the weights attained after treatment with the larger doses of estradiol were not as great as those produced by comparable doses of stilbestrol.

In intact rats, all the test doses of estradiol increased ovarian weight over control level; but no definitive dose-response pattern was apparent. The smallest and largest doses were equally effective.

Histologically, ovarian response to injected estrogen was similar to that reported previously. Many solid follicles were present, distributed uniformly throughout the ovary and some of the larger follicles showed the peculiar peripheral degeneration that others have described(1,2,3,4).

The averages of several counts of the number of follicles present within a 1 sq mm area of the ovaries from each test group of hypophysectomized rats are listed in Table I. Since the fields examined consisted only of follicles with a very slight amount of interstitial tissue intervening, one could assume that the average follicular cross-sectional area approached the reciprocal of the number of follicles counted. By estimating follicle size in this manner, it appears that the 20 and 100 μg doses produced comparable increases; and 500 and 1000 μg produced another similar increment (Fig. 3). No counts were done on the untreated control ovaries since the interstitial component between follicles occupied

TABLE I. Follicle Population per sq mm Ovarian Cross-Section after Diethylstilbestrol Treatment. Hypophysectomized rats.

Daily dose (μg)	Mean No. of follicles/mm²
4	30.2
20	20.8
100	19.2
500	11.3
1000	11.7

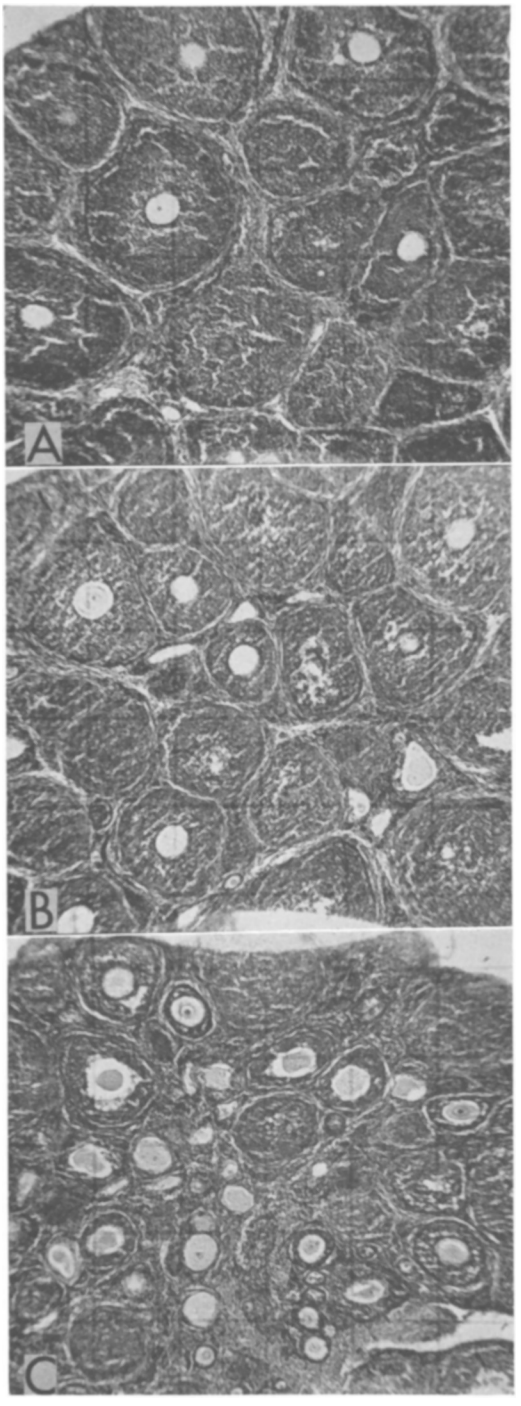

FIG. 3. Photomicrographs of ovaries from hypophysectomized immature rats.
(A) 8 days treatment with 1000 μg stilbestrol daily.
(B) 8 days treatment with 4 μg stilbestrol daily.
(C) Untreated control.

an inordinate amount of the grid area.

Discussion. The dose-response relationship observed after 8 days of stilbestrol treatment was similar to that previously reported for 4 days of treatment(1). However, after 8 days treatment, higher average ovarian weights were noted with the large doses in hypophysectomized rats. Also, as has been previously suggested, stilbestrol is more effective in stimulating ovarian growth than is the natural estrogen, estradiol(1,4).

The presence of the pituitary body undoubtedly accounts for the lack of a dose-response relationship in intact rats. Bradbury reported that small doses of estrogen can cause release of gonadotrophins by the pituitary of immature rats, and thereby indirectly increase ovarian weight(5). However, it is felt that the resulting enhancement of ovarian growth after injection of large doses of estrogen in both intact and hypophysectomized rats is due to a direct estrogen stimulation of the ovary. This proposed direct effect is further documented by the results in hypophysectomized animals where a dose-response relationship is manifest.

Williams found a graded ovarian response to increasing doses of estrone (up to 200 μg), although ovarian weights did not exceed 11 mg(4). He proposed that the ovarian growth was due to granulosal proliferation, resulting in greater follicle size. Other investigators have shown that small doses of estrogen (less than 1 μg) cause slight increases in follicle size(6). The microscopic findings of the present study support the observation that ovarian response to exogenous estrogen is granulosal proliferation. This granulosal response is apparently directly proportional to the dose of estrogen administered, as documented by increasing follicle size. Enhanced follicular growth is in turn reflected in the ovarian weights which rise as the estrogen dosage is increased.

Summary. A dose-response relationship was demonstrated between ovarian weight and injected estrogen in hypophysectomized immature rats. No definitive pattern of response was evident in intact animals treated similarly. In hypophysectomized animals, increases in follicular size were produced as

estrogen dosage was raised. The follicular growth was characterized by granulosal proliferation, resulting in many solid follicles. Apparently, ovarian weight increments resulting from increasing estrogen doses are reflections of this enhanced follicular growth.

1. Payne, R. W., Hellbaum, A. A., *Endocrinology,* 1955, v57, 193.

2. Smith, B. D., *ibid.,* 1961, v69, 238.

3. Simpson, M. E., Evans, H. M., Fraenkel-Conrat, H. L., Li, C. H., *ibid.,* 1941, v28, 37.

4. Williams, P. C., *Proc. Royal Soc. Series B,* 1944, v132, 189.

5. Bradbury, J. T., *Endocrinology,* 1947, v41, 501.

6. Croes-Buth, S., Paesi, F. J. A., de Jongh, S. E., *Acta Endocrinol.,* 1959, v32, 399.

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Competition of Antigens as Influenced by Spacing of Heterologous Antigen Injections.* (26806)

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Decreased responses to some antigens following injection of 2 or more antigens have been observed by a number of investigators (1-5). In some cases a crowding out has been shown to occur when an antigen is injected into an animal actively undergoing antibody formation against a previously injected antigen(6-9). Benjamin and Witzinger(7) found that a preliminary injection of horse serum inhibited the formation of hemolysin to sheep cells. Glenny *et al.*(8) found a crowding out of the response to diphtheria toxoid in guinea pigs when the toxoid was injected simultaneously with, or 2, 4, or 6 days after, an injection of normal horse serum. Frolova *et al.*(9) observed a reduction in precipitin response of guinea pigs to ox serum injected 7-14 days after sensitization with horse serum.

In some cases a more rapid response to one antigen in a mixture has been postulated as leading to a crowding out of antibody production against other antigens in the inoculum(1-3). Adler(2) found that simultaneous injection of rabbit serum or hemocyanin with rabbit immune globulin into guinea pigs impeded antibody production against the rabbit immune globulin and that the degree of this suppression was related to the intensity of antibody response to the competing antigens. Abramoff and Wolfe(3), in quantitative studies of the primary response to bovine serum albumin (BSA) and human gamma globulin (HGG), at a 20 mg per kilogram body weight (mg antigen/KBW) dosage level, observed that the chicken system responds earlier to HGG than it does to BSA and the HGG is thus able to crowd out the response to BSA when these antigens are simultaneously injected. However, Abramoff(10) has shown that the selectivity of HGG could be reduced during simultaneous injection of BSA and HGG, at the 40 mg/KBW dosage level, if the animals had been presensitized to BSA. Under these conditions the responses to both of these antigens were significantly reduced.

The present investigation is concerned with further clarification of the mechanisms by which one antigen interferes with antibody production against a second antigen during primary antibody responses. Responses of chickens to single, simultaneous, and spaced injections of BSA and HGG at a dosage level of 40 mg/KBW have been studied.

Materials and methods. Eighty-two New Hampshire Red Chickens (74 males, 8 females) of approximately 34 weeks of age and weighing 2300-4000 g were used in these

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