

mammalian and aves antigonadotropic sera is like that found in serological relationships where interclass specificity and interorder aspecificity are noted in the study of classical antigen-antibody reactions. The results reported herein suggest that the gonadotropic complex of chicken hypophysis is immunologically distinct from that observed for the mammalian gland and give further support to the theory that antigonadotropic substances are immune bodies.

Summary. Sheep antigonadotropic rabbit serum, aspecific in nature, although effective in inhibiting the gonadotropic action of extracts of mammalian tissues and fluids did not prevent the typical effect of gonadotropic extracts made from chicken hypophyses. Chicken antigonadotropic rabbit serum, while inhibiting the gonadotropic action of chicken pituitary glands, was ineffective against gonadotropic extracts of sheep pituitary glands. The possible relationship of these experiments are discussed in regard to the nature of the antihormone mechanism.

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Effects of Estrone and Progesterone on Male Rabbit Mammary Glands. I. Varying Doses of Progesterone.

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Turner and Frank¹ showed that whereas estrogen caused growth of the duct system and slight lobule proliferation in the male rabbit mammary gland, the combination of estrogen and progestogen caused lobule-alveolar growth simulating that seen in pregnancy. The hormones used were impure, but were assayed for rat units of estrogen and rabbit units of progestogen and judged by the results, were uncontaminated, one with the other. Until recently few investigators have had at their disposal sufficient progesterone with which to investigate this problem further, and, as far as we are aware, the doses of estrone and progesterone that will synergize best to cause optimal mammary growth have not as yet been satisfactorily determined in any animal form. In the preliminary in-

¹ Turner, C. W., and Frank, A. H., *Mo. Agr. Exp. Res. Bull.* 174, 1932.

vestigation reported herein, an attempt was made to determine the approximate dose of progesterone that would function optimally with a given daily dose (120 I.U.) of estrone in causing prolactational* growth of the male rabbit mammary gland.

Experimental. Immature, New Zealand White male rabbits weighing approximately 1.5 kg at the beginning of the experiment were treated in groups of 3 with 18 daily doses (Monday through Friday, from 2/28/39 until 3/23/39) of the following:

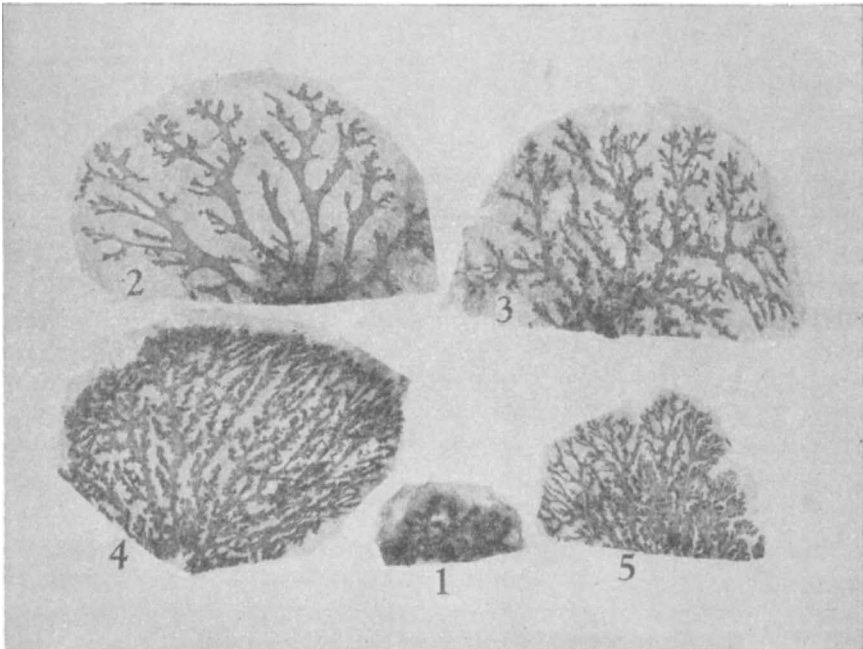
Group 1, 120 I.U. of estrone (theelin); Group 2, 1 I.U. of progesterone; Group 3, 120 I.U. of E and 0.25 I.U. of P; Group 4, 120 I. U. of E and 1 I.U. of P; Group 5, 120 I.U. of E and 4 I.U. of P; Group 6, 120 I.U. of E and 8 I.U. of P. The 2 hormones were given separately, subcutaneously, in peanut oil. On 3/28/39, 5 days after the last injection, a biopsy specimen of the second left (thoracic) mammary gland was taken from each animal, fixed in formol, stained *in toto* with alum-carmin and cleared in methyl salicylate.

Results. The mammary spreads from Group 1, showed that the dose of 120 I.U. of E produced good duct growth with almost negligible alveolar formation (Fig. 2). Those from Group 2 (1 I.U. of P) showed but little more development than that seen in normal immature rabbit glands (Fig. 1). The duct system of the glands from animals in Group 3 (120 I.U. of E plus 0.25 I.U. of P) was as extensive as that seen in animals treated with 120 I.U. of E alone, but the main ducts were narrower and, as though in compensation, more alveolar buds were present. Thus, just as progesterone in some experimental animals prevents the estrogen-induced uterine dilatation and stimulates a proliferation of luminal and glandular epithelium, so also in the rabbit it counteracts an estrogen-induced mammary duct dilatation and permits instead an extensive alveolar proliferation. The amount of incomplete lobule formation shown in Fig. 3 was found typical of all rabbits in this group and probably may be interpreted as barely minimal prolactational proliferation in response to a low dose of progesterone. The glands from animals in Group 4 (120 I.U. of E and 1.0 I.U. of P)

* The term prolactational is used to indicate the additive effect of progesterone on the mammary gland (lobule-alveolar growth) in the same sense that progestational is used for the progesterone effect on the rabbit uterus. In the rabbit usually by the third week of pregnancy, maximal prolactational growth is attained through a rapid multiplication of alveoli in large complex lobules. The term lactational growth is used for the enlargement of individual alveoli that takes place at parturition (sometimes before) under the direct influence of hypophyseal lactogenic hormone.

showed the best evidence of synergism obtained in this preliminary experiment (Fig. 4), although judged on the basis of a set of glands taken at different stages of pregnancy, they could not be said to show maximal prolactational proliferation. Interesting results were obtained in Groups 5 and 6, where 2 of the animals in Group 5 and all in Group 6 showed only scanty alveolar development and an inhibition of the duct growth (Fig. 5). The third animal in Group 5 showed good prolactational development with no inhibition of duct growth, indicating that 4 I.U. of progesterone approximated the border-line inhibiting dose when given with 120 I.U. of estrone by this particular routine.

Summary. Eighteen doses of 0.25, 1.0, 4.0 and 8.0 I.U. of crystalline progesterone were given, simultaneously with 120 I.U. of



All figures represent approximately one-half of a male rabbit mammary spread fixed in formol and stained in alum-carmin. $\times 1.5$.

Treatment was as follows:

FIG. 1. None. Control gland.

FIG. 2. 18 subcutaneous doses of 120 I.U. estrone in oil over a 28-day period. Duct growth with almost no alveolar development.

FIG. 3. 18 subcutaneous doses of 120 I.U. estrone and 0.25 I.U. progesterone over same period. Very little prolactational proliferation.

FIG. 4. 18 subcutaneous doses of 120 I.U. estrone and 1 I.U. progesterone over same period. Fair prolactational proliferation.

FIG. 5. 18 subcutaneous doses of 120 I.U. estrone and 8 I.U. progesterone over same period. Inhibited duct growth and only slight alveolar development.

estrone, to immature male rabbits during a period of 4 weeks. Of these 4 levels of progesterone, the 1.0 I.U. dose synergized best although the prolactational proliferation induced was not maximal. The 4.0 and 8.0 I.U. doses of progesterone were apparently inhibiting as shown by the relatively poor mammary growth obtained.

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Effects of Estrone and Progesterone on Male Rabbit Mammary Glands. II. Varying Doses of Estrone.*

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In the preceding paper it was shown that of 4 different levels of progesterone, only one (1 I.U.) functioned well with a given daily dosage of estrone (120 I.U.) in producing prolactational growth of male rabbit mammary glands. A lower dose was insufficient and higher levels were inhibitory. The inhibition of estrin effects by progestin has received considerable attention as far as uterine and vaginal reactions have been concerned, but almost no work of a similar nature has been done on the mammary gland. It is also well known that estrogen may inhibit the progestin effect on the uterus, but again almost no information is available regarding estrogen inhibition of the progestin effect on the mammary apparatus.

Experimental. Continuing our attempt to determine the optimal combination of estrone and progesterone for prolactational proliferation of male rabbit mammary glands, the daily dose of progesterone found best in the preceding experiment (1 I.U. subcutaneously in sesame oil) was held constant while estrone was given simultaneously, though separately at the following levels to 6 groups of 3 rabbits each: 30, 60, 120, 240, 480, and 960 I.U. daily subcutaneously in sesame oil, 5 days weekly (Monday through Friday) for 5 weeks. These 6 levels of estrone alone were injected likewise into 6 groups of 3 rabbits each. On the day following the last injection, mammary glands were taken at biopsy or necropsy and

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