

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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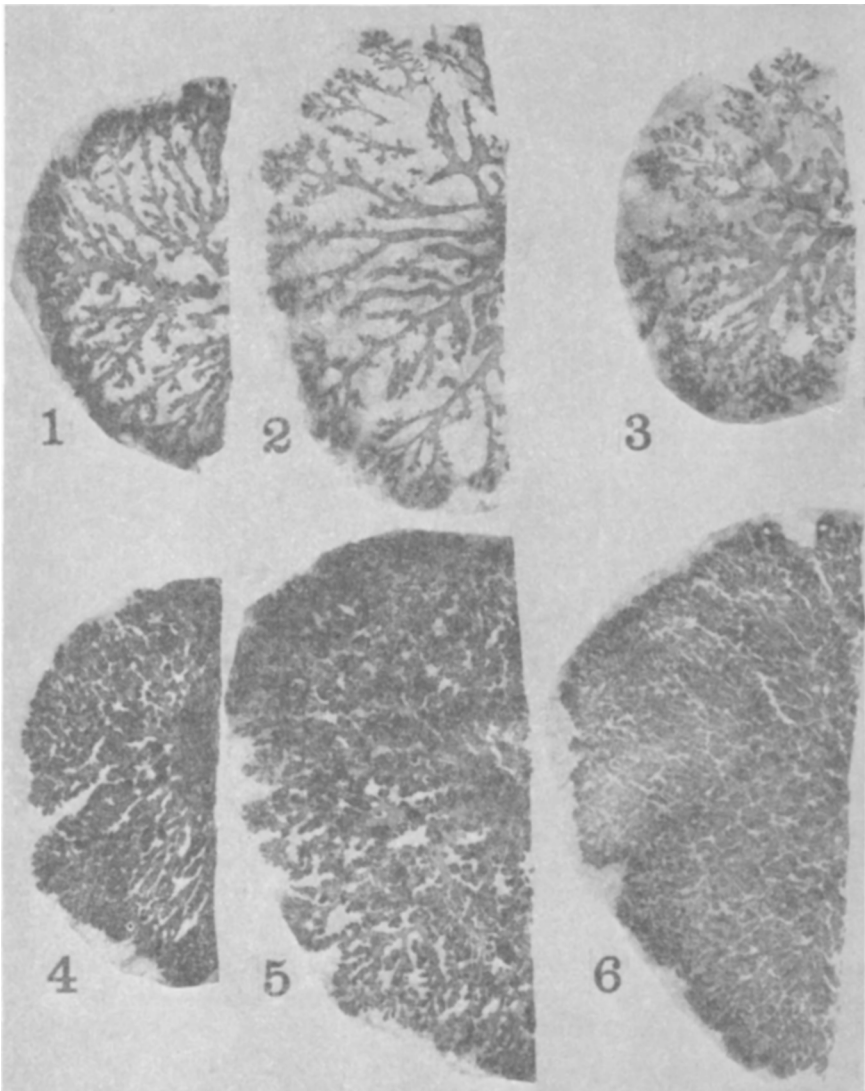
The estrone and progesterone were generously supplied by Parke, Davis and Company, Detroit, Michigan.

handled as previously described. For comparative purposes, a series of mammary spreads were obtained from females of the same strain of New Zealand White rabbits at weekly intervals during their first pregnancy.

Results. The mammary reactions to E or E plus P were quite constant within groups, care having always been taken to compare glands taken from the same region. The 30 and 60 I.U. daily doses of E induced a mammary growth that was somewhat less extensive (Fig. 1) than that obtained with the 120, 240, and 480 I.U. levels which were all about equally effective (Fig. 2) with an optimum at 120 I.U. The 960 I.U. level was excessive as indicated by relatively less duct extension as well as by cyst-formation observed in this group (Fig. 3). This tendency to cyst-formation or sacculatation in the larger ducts was evident even in the 240 I.U. level as may be seen in Fig. 2.

When 1 I.U. of P was given simultaneously with the different levels of E, prolactational proliferation was observed in every case, although considerable variation in the number and completeness of the lobules was encountered. In the groups receiving 1 I.U. of P plus 30 and 60 I.U. of E respectively, good lobule-formation was observed (Fig. 4), but the duct system had not extended itself as much as in the rabbits receiving the higher levels of E (Estrone) along with P (Progesterone). This finding was consistent with the results obtained with 30 and 60 I.U. of E alone, where inferior duct growth was produced. In the groups receiving the 4 higher levels of E plus P, duct growth was extensive and apparently maximal for the routine used since all 4 levels produced duct systems approximating each other in length. However, it was observed that the groups receiving 1 I.U. of P plus 240 and 960 I.U. of E respectively showed better lobule formation than those receiving 1 I.U. of P plus 120 and 480 I.U. of E respectively. In the latter 2 groups there were more incomplete lobules in which alveoli seemed to be still in process of multiplication and the final "grape-cluster" arrangement had not yet been attained. In glands from animals receiving 240 I.U. of E plus 1 I.U. of P complete lobules predominated but some incomplete lobules were also present, as may be seen in the lower quarter of the gland shown in Fig. 5. The glands from rabbits receiving 960 I.U. of E plus 1 I.U. of P showed the greatest lobular proliferation (Fig. 6), and in striking contrast to the animals receiving 960 I.U. of estrone alone showed neither cyst formation nor inhibition of duct extension.

In none of the experimental groups did the results parallel pre-



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

Daily treatment with estrone and progesterone injected subcutaneously in oil 5 days each week for 5 weeks was as follows:

- FIG. 1. 30 I.U. of E.
- FIG. 2. 240 I.U. of E.
- FIG. 3. 960 I.U. of E.
- FIG. 4. 30 I.U. of E plus 1 I.U. of P.
- FIG. 5. 240 I.U. of E plus 1 I.U. of P.
- FIG. 6. 960 I.U. of E plus 1 I.U. of P.

cisely the complete prolactational proliferation attained between the second and third weeks of pregnancy, but the groups receiving 240 I.U. of E plus 1 I.U. of P and 960 I.U. of E plus 1 I.U. of P most closely approximated that type of growth. The main difference in the gland shown in Fig. 6 and that of a 2-3-week pregnancy gland is revealed at the periphery where lobule formation in the experimental animal is incomplete and growing club-endings are prominent. At the third week of pregnancy, adjacent mammary glands usually have overlapped at their peripheries and club-endings have been transformed into complete lobules. However, it should be remembered that at the beginning of pregnancy an extensive duct system already exists whereas at the beginning of the injection period in our male rabbits an extremely rudimentary gland was present (Fig. 1 of preceding paper). Another difference had to do with the overlapping of lobules within a gland—a process which gives rise in part to the increase in thickness of the gland in the latter half of pregnancy. In Fig. 6 and to a lesser degree in Fig. 5 this overlapping may be seen, but again it was not quite as extensive as in the glands of 3-week pregnant rabbits.

Summary. Six groups of immature male rabbits were injected 5 days weekly for 5 weeks with 30, 60, 120, 240, 480, and 960 I.U. of estrone respectively. The best mammary duct growth occurred in the animals receiving 120 I.U. The 30 and 60 I.U. animals showed slightly less extensive duct systems, and the higher levels caused cystic changes in the main ducts.

The same 6 levels of estrone were injected into 6 other groups of male rabbits along with 1 I.U. of progesterone daily for a similar period. In every case, lobular growth of the mammary glands occurred and no tendency toward cyst formation was noticed even in the animals receiving the highest level of estrone. The mammary glands of the animals receiving the 2 lowest levels of estrone, plus progesterone had not developed as extensively as the others, but showed good lobule formation on a relatively shorter duct system. The best prolactational proliferation was observed in the groups receiving 1 I.U. of progesterone plus 240 and 960 I.U. of estrone respectively. Only the presence of incomplete lobules at the periphery of these glands made possible their differentiation from glands taken from rabbits in their second to third week of pregnancy—a period when prolactational proliferation is usually maximal.