a wide band, varying from an eighth to a sixth of the cell diameter, of dense white ash concentrated at the periphery of the cell. The tissue spaces immediately about the ganglion cells are filled with mineral residue not unlike, in quality and quantity, that seen in the neurones.

It seems evident from these observations that like conditions of surrounding medium do not obtain in nerve fibers of the frog sciatic and in the cells of the sympathetic ganglia of the same animal.

11468

Direct Action of Estrone on the Mammary Gland.

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Within the past decade, the hormonal control of mammary growth has been greatly clarified due to the availability of: (1) pure estrogenic compounds which by themselves cause growth of the nipple and ducts as well as a slight degree of alveolar development, (2) progesterone which in proper combination with estrone (or other estrins) causes complete lobular development such as occurs in pregnancy, and (3) mammotropin, the pituitary lactogenic hormone which causes functional growth and lactation in the alveoli or milk-secreting units in glands developed by estrin or estrin-progestin.

[According to some investigators, the mammary glands of hypophysectomized animals do not respond as well (or at all) to estrin, and for this reason, Turner and co-workers[1, 2] have proposed that the sex hormones merely stimulate the pituitary which in turn secretes 2 mammary-stimulating substances, mammogen I which induces duct development and mammogen II causing lobule-alveolar growth.] These investigators have extracted a fat-soluble substance from the pituitary, which they identify with mammogen I because it causes mammary duct development in male and female mice. They[3] also obtained lobule-alveolar growth in castrated female mice by injecting fresh pituitaries from pregnant cattle. Because this material caused alveolar proliferation over and above the duct development induced by their extracts these investigators have postulated a second mammogen.

1 Lewis, A. A., Turner, C. W., and Gomez, E. T., *Endocrinol.*, 1939, **24**, 157.

2 Lewis, A. A., and Turner, C. W., *Mo. Agr. Exp. Sta. Bul.* 182, 1939.

3 Mixner, J. P., Lewis, A. A., and Turner, C. W., *Anat. Rec.*, 1940, Suppl., **76**, 43.

The findings of the Turner laboratory might be interpreted on the basis of earlier investigations[4, 5] showing that estrin and progestin could be extracted from the pituitary, but it has been claimed that mammogen I, although weakly estrogenic, has mammary-stimulating potency greater than the purified estrins now available.[2] While these findings are in themselves interesting and significant, they in no way support the contention of the Missouri group that the ovarian hormones act only indirectly upon the mammary gland. In fact, suggestive evidence to the contrary has been at hand for some time now.[6] In each of 3 women, MacBryde obtained good development in the breast rubbed with estradiol in ointment (25,000 I.U., daily for 2 weeks) while the contra-lateral breast rubbed only with the ointment base showed much less growth. We have carried out in male rabbits a similar experiment designed to sustain the theory of the direct action of estrin, with the advantage over the human work reported, of being able to know more precisely the histologic nature of the growth response.

Experimental. Preliminary experiments were carried out in which the approximate amount of estrin was determined that could be expected to act only on the gland locally treated, and also the amounts of hormone that would be sufficient to allow for some absorption and therefore remote action on the glands not treated directly. And then, 2 groups of three, 2-month-old male rabbits, weighing approximately 2 kg were treated with 2 distinct levels representing threshold and sub-threshold doses of estrone.

The estrone* in sesame oil was rubbed into the skin immediately around the nipples on the left side, while sesame oil alone was administered in like manner to the nipple regions on the right side. Three of the rabbits had an accessory or ninth nipple and this was left untouched, thus serving as a further control. A single drop of oil (approximately 0.03 cc) was applied with a medicine dropper directly to the nipple. The oil usually spread out over a skin area not greater than 3.0 cm and this was rubbed with the end of the finger and gently massaged between the thumb and forefinger. The 3 rabbits in Group 1 were treated with an estrone preparation containing 100 I. U. per cc or 3 I.U. per drop, while the preparation used in Group 2 contained 10 I.U. per cc or 0.3 I.U. per drop. In all 25 drops were administered to each gland (Monday through

[4] Brouha, L., and Simmonet, H., *C. R. Soc. de Biol.*, 1927, **96**, 1275.

[5] Callow, R. K., and Parkes, A. S., *J. Physiol.*, 1936, **87**, 28 P.

[6] MacBryde, C. M., *J. A. M. A.*, 1937, **112**, 1043 (has earlier references).

* Kindly supplied by Dr. Oliver Kamm of Parke, Davis and Company.

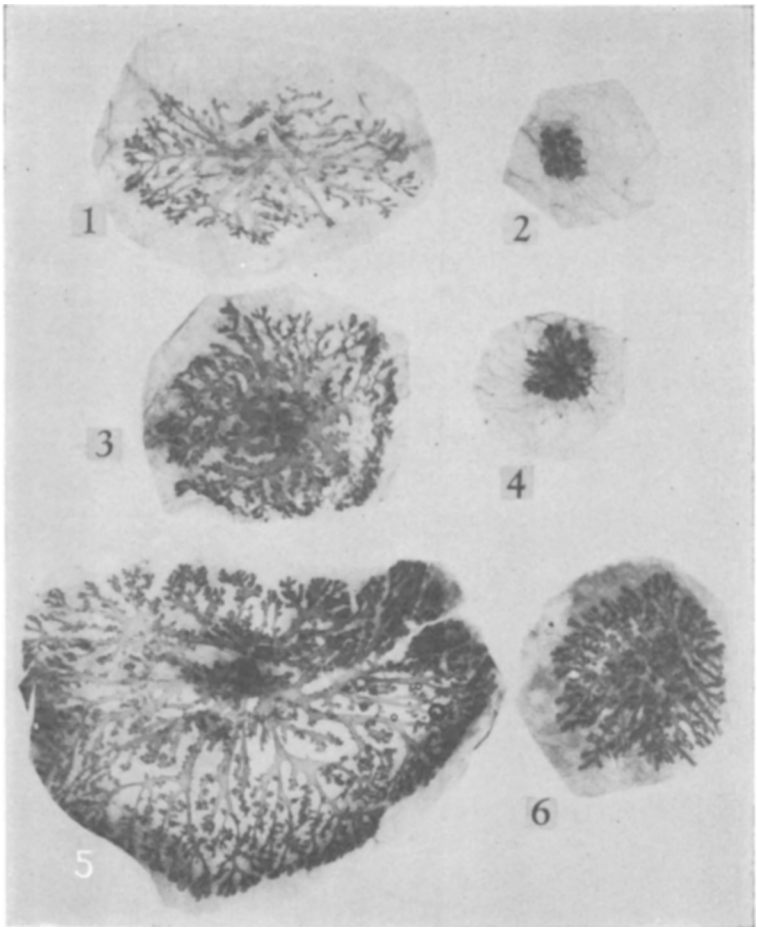

FIG. 1. Typical left mammary spread from male rabbit 1 of group 2. A drop of sesame oil containing approximately 3 I.U. of estrone was rubbed into the skin overlying this gland daily for 25 days. Note evidence of extensive duct growth. All figures × 1.5.

FIG. 2. Typical right mammary spread from male rabbit of group 2. Sesame oil only was rubbed into skin. Gland is rudimentary and resembles that of untreated, normal male rabbits.

FIG. 3. Typical left mammary spread from male rabbit 2 of group 2. A drop of sesame oil containing approximately 3 I.U. of estrone was rubbed into the skin overlying this gland daily for 25 days. Note evidence of extensive duct growth.

FIG. 4. Typical right mammary spread from male rabbit 2 of group 2. Sesame oil only was rubbed into skin. Gland is rudimentary and resembles that of untreated, normal male rabbits.

FIG. 5. Typical left mammary spread from male rabbit 3 of group 2. A drop of sesame oil containing approximately 3 I.U. of estrone was rubbed into the skin overlying this gland daily for 25 days. This gland shows more extensive duct growth as well as some alveolar development.

FIG. 6. Typical right mammary spread from rabbit 3 of group 2. Although only sesame oil was rubbed into skin overlying this gland, enough estrone was absorbed on the left side to circulate and cause the duct growth shown here.

Friday for 5 weeks), making a total of approximately 75 I.U. per gland or 300 I.U. per animal (4 glands) in the first group, and 7.5 I.U. per gland or 30 I.U. per rabbit in the second group.

Results. It became apparent after 2 to 3 weeks of treatment that the nipples receiving the 3 I.U. estrone per dose were growing at a faster rate than the controls as well as those receiving the 0.3 I.U. estrone. At the end of the fifth week, the estrone-treated nipples of both groups were all considerably larger than their contra-lateral controls, but the control nipples of Group 1 were larger than the control nipples of Group 2 indicating that some of the estrone was being absorbed and probably circulated to the contra-lateral glands. At necropsy spreads were made of 53 mammary glands. These were stained *in toto* with alum carmine and cleared in methyl salicylate. None of the glands taken from the animals in Group 1 showed development greater than that seen in normal males of the New Zealand White strain used. Two of the 3 rabbits treated with higher doses of estrone showed duct growth only in the glands rubbed with the hormone, Figs. 1 and 3, the sesame control and untreated glands all being within the normal limits (Fig. 2 and 4). The third animal of this group was more responsive to estrone for not only did its estrone-treated glands (Fig. 5) show better development than the estrone-treated glands of the other rabbits, but its control glands (Fig. 6) also showed considerable development. As in the case of the nipples in both groups, this indicated that some estrone was being absorbed and circulated. However, the striking difference between the hormone-treated and control glands provides just as good proof of the direct action of estrone as the all-or-none effect observed in the other animals.

Ruinen[7] had attempted this same experiment in 1932, but used a much larger dose of hormone (100 units daily of menformon). With such an excess, he obtained equal development in the mammary glands of both sides. As the author admits, his findings still did not preclude the possibility of a direct action of estrin on the mammary gland.

Summary. The proper dose of estrone in oil rubbed into the skin over the rudimentary mammary glands of young male rabbits caused growth only of those glands, and not of the control glands treated with oil. Such evidence supports the view that estrogenic substances are directly mammary-stimulating.

[7] Ruinen, F. H., *Acta Brev. Neerl.*, 1932, **2**, 161.