The Hormonal Control of Mammary Growth and Lactation

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The foremost questions still asked about mammary endocrinology concern the substances referred to as mammogens and lactogens. In this report, the term mammogen will be applied to any hormone acting singularly, or in synergism with other hormones, to induce any of the three main phases of mammary growth, namely: (*1*) ductal; (*2*) ductoalveolar (lobular); or (*3*) lactational. A lactogen may be said to be a hormone which, individually or in combination with other hormones, induces milk formation. There may be many mammogens and lactogens such as: (a) endogenous substances secreted by the pituitary, ovary, testis, adrenal, placenta, tumors, etc.; (b) dietary components; (c) substances formed in the body by enzymatic action on substrates not themselves mammogenic or lactogenic (e.g., sterol derivatives). Some hormones may be both mammogenic and lactogenic (e.g., mammotropin and certain corticoids). Some, like the estrogens, are mammogenic, and also indirectly lactogenic, in the intact animal, which permits a wide play of interactions and synergisms. And yet one encounters some difficulty in proving a solo mammary stimulating role for any hormone.

In the experiments to be reported, the hormones used have been restricted to two ovarian substances, estrone (E) and progesterone (P); to the adrenocorticoids, deoxycorticosterone acetate (DCA) and either cortisol acetate or prednisolone acetate (Pred-ac); and to the anterior pituitary hormones, somatotropin (STH, growth hormone) and mammotropin (MH, prolactin). The latter protein has, until now, resisted all attempts to break it down into separate fractions with lactogenic, mammogenic, luteotropic, or crop sac-stimulating activities. The pure protein has all of these functions, at least. The MH and STH were prepared by one of us (C.H.L.) and assayed by the other two authors in hypophysectomized rats used for detecting histologically each of the six well-known anterior pituitary hormones. The STH preparations contained 0.5–2% MH and less than an estimated 0.5% of ACTH (adrenocorticotropic hormone), TSH (thyroid-stimulating hormone), FSH (follicle-stimulating hormone), ICSH (interstitial cell-stimulating hormone). Where possible these minor contaminations were controlled by removing their target organs or by testing the contaminant in a range of doses for such positive responses as were obtained. The MH showed less than an estimated 0.5% of ACTH, TSH, FSH, and ICSH. In doses 200 times the amount of STH required for a positive tibial test, MH imitated that hormone in this respect, even when boiled to destroy STH. Activities

ascribed to MH have finally been checked with 1% solutions boiled for 30 minutes at pH 7.0.

Male and female, immature and adult Long-Evans rats were used in the eight experiments to be reported, and the results obtained are not said to apply to any other rat form or other animals. The test animal is particularly well suited for this type of experiment because after parapharyngeal hypophysectomy probably little, if any, functional pars tuberalis tissue persists, and because accessory adrenocortical tissue is found only infrequently.

In order to present the morphologic picture as completely as possible by showing the effects of the above-mentioned hormones on the main stages of mammary growth, this report will be given in three sections under the following headings: (I) Ductal Mammary Growth; (II) Lobuloalveolar Mammary Growth; (III) Lactational Mammary Growth. In some instances, the hormones were introduced locally as indicated in the right abdominoinguinal subcutaneous area over mammary glands 4 and 5, in the form of compressed pellets or in 2% butanol in saline. Otherwise, all injections are referred to as systemic, having been made subcutaneously in the dorsal, thoracocervical region. The right and left abdominoinguinal glands were studied routinely, and in some cases the thoracocervical glands were also examined. Glands were spread on filter paper, fixed in 10% formalin, stained *in toto* in alum carmine, and mounted in a polyester plastic. Sections of representative reactions were also prepared for higher power microscopic examination.

I. Ductal Mammary Growth

For many years, estrogens were considered to be the hormones concerned with mammary duct growth. When it was learned that this hormone is only mammogenic in the presence of the anterior pituitary (A.P.) or its extracts, three new theories were presented, namely: (a) Ovarian E stimulated the A.P. to secrete a ductal mammogen; (b) the mammogenic activity of E was potentiated by some A.P. factor; (c) E "sensitized" the mammary ducts so that they responded to a pituitary mammogen. The theories were illustrated by Folley (14) at the 1951 Laurentian Conference, and the discussion did not resolve the problem. Other questions presenting themselves before and after that time have been (a) which of the known anterior pituitary hormones are mammogenic; (b) which of these are lactogenic; (c) which of the ovarian and adrenal steroids are mammogenic, lactogenic, or antilactogenic.

Experiment 1. The Negative Mammogenic Effect of Estrone in Hypophysectomized, Oöphorectomized Rats

In many experiments on hypophysectomized rats, we have never been able to show that estrone is mammogenic (20, 21, 23). Further attempts

were made using local application of E in the form of cholesterol pellets or giving the hormone systemically as follows: three rats were hypophysectomized on day 30 and after 2 weeks injected daily, systemically with 10 μg. E; two rats were hypophysectomized on day 30, and after 2 weeks oöphorectomized and given a 12-μg. pellet of E locally, then sacrificed after 13 days; four rats on the same operative schedule received a 25-μg. pellet of E, and three, a 0.25-μg. pellet. Finally, three rats on this schedule received a 500-μg. pellet of E, and all died within 1 week. In no case was there any evidence of mammary stimulation. All glands had remained in the regressed condition observed in young rats after hypophysectomy or after the double operation. Figure 1 (Plate I) shows an average developmental state of a normal 31-day-old female rat; Fig. 2 shows a regressed gland, 2 weeks after hypophysectomy; Fig. 3 shows even further regression in spite of the presence of a 12-μg. pellet of E during the 13-day postoperative period in an hypophysectomized, oöphorectomized rat.

Experiment 2. Duct Growth Induced in Hypophysectomized, Oöphorectomized Rats with STH and Estrone

Although E may have no solo mammogenic effect in hypophysectomized rats, its combination with A.P. extracts and particularly those with good somatotropic activity leads to ductal proliferation (23, 27, 30). Slight mammogenic effects have been demonstrated in hypophysectomized-oöphorectomized rats with impure STH (30). It is difficult to ascribe the duct growth in such experiments to a solo STH mammogenic effect because the adrenals were present. These organs not only retain some slight function after hypophysectomy but are available for stimulation by corticotropic activity of contaminating ACTH, or by STH itself. Previously we had been unable to demonstrate solo mammogenic activity in potent STH preparations even with adrenals present (23). In retesting this point a 2-mg. systemic dose of STH that permitted hypophysectomized rats to grow as rapidly as normal controls was employed with and without a 1-μg. systemic dose of E adequate to cause cornification of the hypophysectomized rat's vagina. Six rats hypophysectomized on day 30 and oöphorectomized on day 44 received 2 mg. of STH daily for 1 week, and three rats on the same schedule received also 1 μg. of E daily. A lower daily systemic dose of STH (0.05 mg.) was also tried alone, in five similarly prepared rats. Two groups of three rats received this dose of STH plus either a 25-μg. or a 500-μg. pellet of E in cholesterol placed in the usual mammary site. Tests were also made in four hypophysectomized, oöphorectomized rats exposed to a single locally placed 6-mg. pellet of STH, and in five rats similarly treated but also given 1 μg. of E systemically for a 13-day period.

The results are shown in Table I and in Figs. 4–11 (Plates I and II). The 50-μg. systemic dose of STH alone, and with 500 μg. E, was ineffective (this dose of E being toxic if not lethal to hypophysectomized rats). However, the low systemic dose of STH did synergize with the 25-μg. pellet of E to cause slight ductal growth in the right and left mammary glands. All rats showed evidence of systemic absorption of the E by vaginal cornification. The E must have been released at varying rates from the 15 mg. of cholesterol with which it was compounded; but from a 25-μg. dose in a 1-

TABLE I

Mammary Duct Growth Induced in Hypophysectomized, Oöphorectomized Rats with STH and Estrone Injected Daily Systemically or Implanted Locally as a Pellet[a]

Group	No. of animals	Body weight change (gm.)	STH (mg.)	Estrone (μg.)	Duct growth					
					Right			Left		
					++	+	—	++	+	—
1	5	+10	0.05	—			5			5
2	3	+15	0.05	25[b]		2	1		1	2
3	3	— 7	0.05	500[b]			3			3
4	6	+26	2.0	—	2	4		2	4	
5	3	+24	2.0	1	2	1		2	1	
6	4	+26	6.0[b]	—		4				4[c]
7	5	+23	6.0[b]	1	4	1			4	1[c]

[a] Pituitaries removed on day 30; ovaries on day 44. Groups 1–5 treated days 44–50. Groups 6–7, days 44–56.

[b] One pellet on right.

[c] Slight increase in number of lateral ducts.

PLATE I

The figures represent typical areas of the right or left abdominoinguinal mammary glands (numbers 4 or 5, numbering from the cephalic end). The glands, after fixation, in 10% formalin, *in toto* staining in alum carmine, and plastic embedding, were photographed at × 10 magnification. As reproduced in this volume, all of these plates have been reduced by one-fifth. E = estrone; STH = somatotropin (growth hormone).

FIG. 1. 31-day-old normal female Long-Evans rat. Note evidence of duct proliferation in club ends and lateral branches.

FIG. 2. Untreated rat 14 days after hypophysectomy on day 31. Gland had regressed to a bare duct system.

FIG. 3. Rat, hypophysectomized on day 30; a 12-μg. pellet of E in cholesterol had contacted gland for 13 days. No evidence of duct growth.

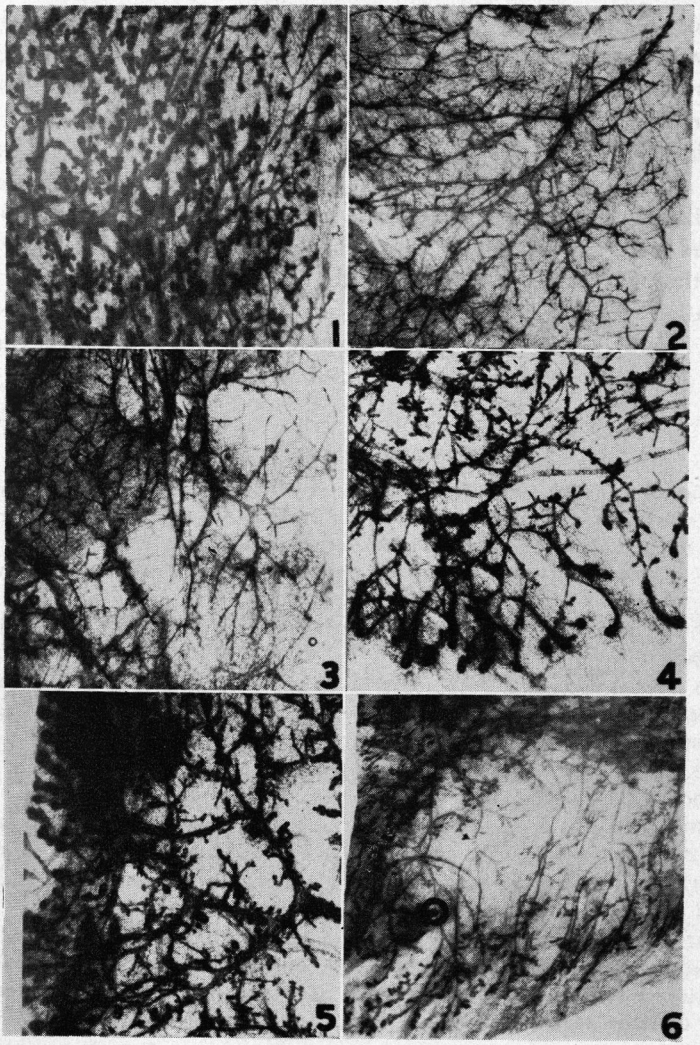
FIG. 4. Rat, hypophysectomized and oöphorectomized on day 30 and injected daily, systemically, with 2 mg. STH for 7 days. Note fair end-club development.

FIG. 5. Same as Fig. 4 except that 1 μg. E was added to the STH. Good end-club proliferation.

FIG. 6. Rat, hypophysectomized and oöphorectomized on day 30. A 6-mg. pellet of STH had contacted this gland for 13 days. Note many small lateral branches.

week period, the daily release probably approximated 1 μg., judging by comparison with uterine and vaginal reactions to a 1-μg. systemic dose. More work must be done with these critical levels of the two hormones that evoke the first signs of ductal proliferation.

The 2-mg. systemic and 6-mg. local doses of STH induced ductal proliferation with or without benefit of E, showing either that STH is indeed a solo mammogen or that the adrenal cortex (or another intermediary) may be stimulated to secrete a hormone that could imitate E in synergizing with



STH. The adrenal cortices of hypophysectomized Long-Evans rats given high levels of STH are usually slightly heavier than those of their controls. In the present series the adrenals showed histologically a slightly better glomerulosa than the controls, although the sudanophobic zone had persisted. Figure 4 shows good ductal proliferation in response to seven daily systemic injections of 2 mg. STH, and Fig. 5 shows some improvement especially in the bulbous club ends attained by adding a daily systemic dose of 1 μg. E. Figure 6 shows a greater mammogenic effect on the right glands contacted by a 6-mg. STH pellet than that seen in the left glands (Fig. 7). The most noticeable effect seems to have been on the small lateral ductal outgrowths from the main long ducts. Clusters of these ductules were formed as though preparing for lobule formation. That this is a local and direct effect is shown by the reduced response in the contralateral glands. The glands not directly contacted by the STH pellet showed borderline effects, due presumably to the absorbed and systemically distributed STH. A possible synergism with a DCA-like hormone from the adrenals could not be ruled out; but granting that such a substance may be important in "potentiating" STH as a mammogen, the fact still remains that a far greater stimulation was obtained in the areas of contact with the STH pellet. This is also shown in mammary glands from rats that received the 6-mg. STH pellets and daily systemic injections of 1 μg. E. In all of the rats treated

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PLATE II

The figures represent typical areas of the right or left abdominoinguinal mammary glands (numbers 4 or 5, numbering from the cephalic end). The glands, after fixation in 10% formalin, *in toto* staining in alum carmine, and plastic embedding, were photographed at a × 10 magnification. E = estrone; STH = somatotropin (growth hormone).

FIG. 7. Control gland contralateral to that shown in Fig. 6. Note comparatively few side branches.

FIG. 8. Distal ducts with good end clubs in gland from rat, hypophysectomized and oöphorectomized on day 30, treated daily, systemically, for 13 days with 1 μg. E and given one 6-mg. pellet of STH in the region of this gland.

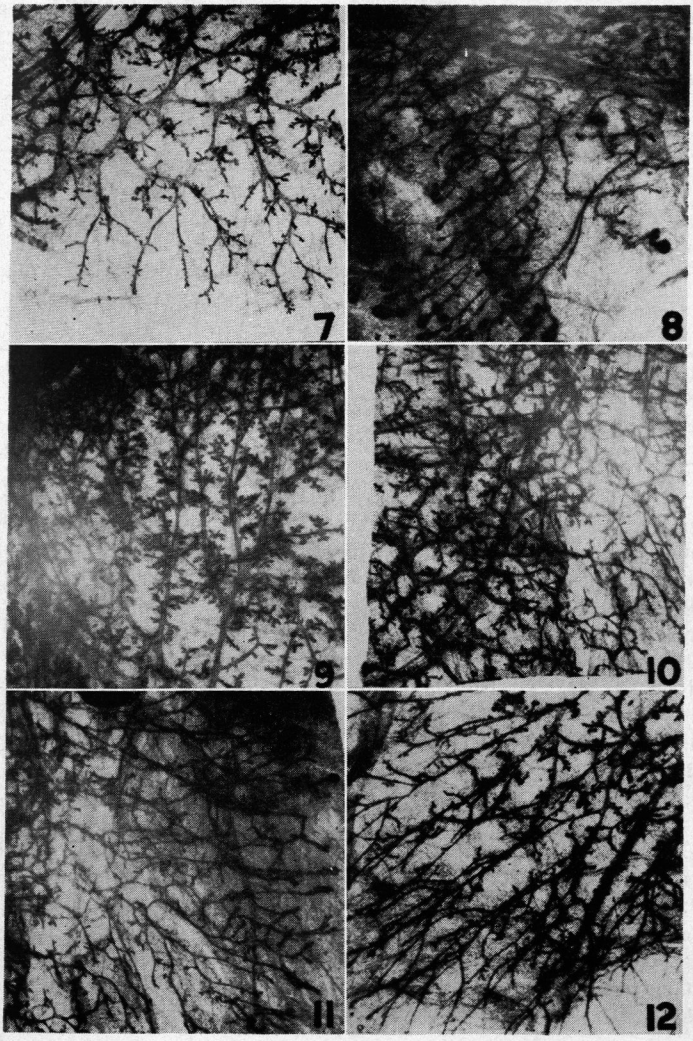
FIG. 9. More central area (near nipple) of the same gland shown in Fig. 8. Note abundance of short lateral ducts.

FIG. 10. Same area from gland contralateral to that shown in Fig. 9. No pellet contracted this gland, which shows few side branches in comparison with preceding figure.

FIG. 11. From same rat, showing distal ducts in contralateral, unimplanted gland. Bulbous club ends are not as prominent as in the right gland (Fig. 8).

FIG. 12. Gland from rat hypophysectomized on day 30, oöphorectomized and adrenalectomized on day 44. Treated daily, systemically, for 7 days with 2 mg. STH. Very little evidence of stimulation, but the duct atrophy is not as striking as that in the average operative control.

with this combination, the STH-contacted glands (Figs. 8 and 9) showed greater ductal proliferation than the contralateral glands (Figs. 10 and 11). The principal effect produced by adding the E seems to have been the formation of larger and more numerous club ends. From these experiments it may be concluded that physiological levels of E (1 μg. systemic) and STH (2 mg. systemic, 6-mg. pellet) restored the atrophic mammary gland seen after hypophysectomy and oöphorectomy to a normal appearing, rapidly proliferating condition typical of the prepubertal female rat, with the quali-



fication that an atrophic but weakly functioning adrenal cortex may have also provided a contributing factor.

Experiment 3. Duct Growth Induced in Hypophysectomized, Oöphorectomized, Adrenalectomized Rats with STH, Estrone, and DCA

The qualification left in the previous experiment had to be answered in the triply operated rat. And the question of how DCA exerts its mammogenic

TABLE II

Mammary Duct Growth Induced in Hypophysectomized, Oöphorectomized, Adrenalectomized Rats Injected Systemically with STH, Estrone and DCA[a]

Group	No. of animals	Survivors	Body weight change (gm.)	STH (mg.)	DCA (mg.)	Estrone (μg.)	Duct growth ++	+	—
1[b]	5	1	—13	—	—	—			1
2[b]	4	4	— 7	2	—	—			4[c]
3	5	3	+10	2	—	—			3[c]
4	4	4	— 9	—	0.5	—			4
5	4	4	+18	2	0.5	—		4	
6	3	3	+19	2	—	1		3	
7	3	3	+23	2	0.5	1	3		

[a] Pituitaries removed on day 30; ovaries and adrenals on day 44. Treated: days 44–50.
[b] 1% NaCl to drink.
[c] Borderline reaction.

PLATE III

The figures represent typical areas of the right or left abdominoinguinal mammary glands (numbers 4 or 5, numbering from the cephalic end). The glands, after fixation in 10% formalin, *in toto* staining in alum carmine, and plastic embedding, were photographed at a × 10 magnification. DCA = deoxycorticosterone acetate; STH = somatotropin (growth hormone); E = estrone.

FIG. 13. Gland from rat hypophysectomized on day 30; oöphorectomized and adrenalectomized on day 44. Treated only with 0.5 mg. DCA daily, systemically. Ducts atrophied as in untreated controls.

FIG. 14. Same schedule. Treated with 2 mg. STH plus 0.5 mg. DCA. Good duct proliferation.

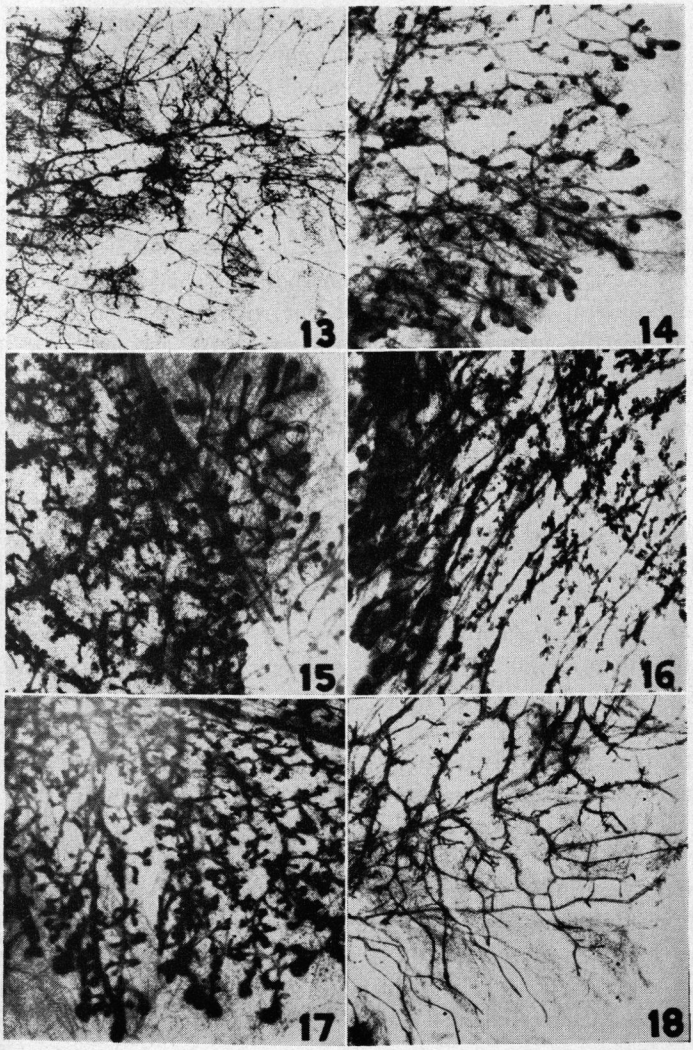
FIG. 15. Same schedule. Treated with 2 mg. STH plus 1 μg. E. Good duct proliferation.

FIG. 16. Same schedule. Treated with 2 mg. STH plus 1 μg. E plus 0.5 mg. DCA. Excellent development of all parts of the duct system.

FIG. 17. Gland from a normal 43-day-old female rat for comparison with Fig. 16. Shows the quality of duct proliferation typical of the 40–50-day-old rat.

FIG. 18. Gland from a rat hypophysectomized on day 30, oöphorectomized and adrenalectomized on day 44 and treated locally for 7 days with daily injections of 1 μg. E plus 0.25 mg. DCA. Shows atrophic, bare duct system.

influence (18, 28, 32) required further investigation. That a pituitary co-factor would be necessary seemed likely from the work of Leonard and Reece (19) and Smithcors and Leonard (31). In an attempt to answer these questions, rats with pituitaries removed on day 30 and ovaries and adrenals ablated on day 44 were treated systemically for 1 week as follows: five received no treatment other than 1% NaCl in the drinking water; four received 2 mg. of STH daily, and also had 1% NaCl to drink; five were injected daily with 2 mg. STH (and these and the remaining groups drank



ordinary tap water, but NaCl was not excluded from their diet); four were injected with 0.5 mg. DCA; four with 2 mg. STH plus 0.5 mg. DCA; three with 2 mg. STH plus 1 μg. E; and three with 2 mg. STH plus 1 μg. E plus 0.5 mg. DCA.

The results are summarized in Table II and illustrated in Figs. 12–17 (Plates II and III). Only one of five rats survived with no treatment except 1% NaCl in the drinking water. The gland from this animal had regressed. Glands from rats receiving only 2 mg. STH with or without NaCl to drink showed borderline stimulation (Fig. 12). Daily doses of 0.5 mg. DCA did not alter the picture of regression seen in glands of untreated rats, 3 weeks after hypophysectomy (Fig. 13). In sharp contrast to this were glands from rats that received 0.5 mg. DCA plus 2 mg. STH (Fig. 14) and those given 1 μg. E plus 2 mg. STH (Fig. 15). Thus a 500-fold dose of DCA imitated the 1 μg. E in the quality of its synergistic effect with STH on ductal growth. The group of rats injected with the two steroids (0.5 mg. DCA plus 1 μg. E) and 2 mg. STH showed mammary development (Fig. 16) that differed but little from that of normal 40–50-day-old females (Fig. 17). An abundance of ductal club ends was considered to be evidence of rapid proliferation. The ducts had not reached the length usually attained in sexually mature rats, but this was also accomplished later by prolonging the injection period from 7 days to 3 weeks.

Experiment 4. Duct Growth Induced in Hypophysectomized, Oöphorectomized, Adrenalectomized Rats with STH Administered Locally and DCA Injected Systemically

As a continuation of experiment 3, a lower systemic dose of DCA (0.1 mg.) and different levels of STH applied locally to the right abdominoinguinal glands were tried. Three triply operated rats also in this series were injected locally with 0.25 mg. of DCA plus 1 μg. of E, and showed again the futility of attempts to stimulate mammary growth directly with these steroids (Fig. 18, Plate III). In Table III may be seen the results obtained with the STH and DCA. Figures on survival after the second operation (oöphorectomy and adrenalectomy) and during treatment give some idea of the mortality rate in these experiments. The difficult period was usually during the first day or two after operation. None of the five rats without treatment, and none of the three on 1 mg. of STH alone lived through the 1-week experiment, although one of the latter group lived within an hour of scheduled necropsy time and provided a mammary gland showing atrophy. Five rats injected with 0.1 mg. of DCA lived but lost about 1 gm. of body weight daily. Their glands showed regression (Fig. 19, Plate IV). The four rats that received 0.1 mg. DCA systemically plus 0.04 mg. of STH

in solution locally showed borderline activity (Fig. 20) that would have to be called positive when compared with the completely negative response to DCA alone. But the difference between the right and left glands was too small to be of any other value than to indicate the approximate level of the minimal effective dose of STH applied locally. This was also suggested in the STH plus E experiments. There was no question about the right and left differences in the 0.2 and 1.0 mg. STH plus DCA groups (Figs. 21, 22) nor in the rats given one 15-mg. pellet of STH locally and 0.1 mg. of DCA systemically (Figs. 23, 24). The 0.2- and 15-mg. doses of STH, again, were large enough to provide some systemically absorbed hormone for synergism with DCA on the contralateral gland. The 0.2 mg. of STH happened to be a good critical amount for showing distinct differences

TABLE III

Mammary Duct Growth Induced in Hypophysectomized, Oöphorectomized, Adrenalectomized Rats Treated Locally with STH and Systemically with DCA[a]

Group	No. of animals	Survivors	Body weight change (gm.)	STH (mg.)	DCA (mg.)	Duct growth				
						Right			Left	
						++	+	—	+	—
1	5	4	+16	1.0	0.1	4			4	
2	5	3	+17	15[b]	0.1	3			3	
3	5	3	+12	0.2	0.1		3			3
4	5	4	— 0.5	0.04	0.1			4[c]		4
5	5	5	— 8	—	0.1			5		5
6	3	0	—	1.0	—			—		—
7	5	0	—	—	—			—		—

[a] Pituitaries removed day 30; ovaries and adrenals day 44. Treatment days 44–50.

[b] One pellet on right; other groups received STH injections on right.

[c] Borderline activity.

on the two sides. Allowing for important assistance from DCA, the difference that must be credited to the local action of STH was seen in the greater proliferation of the lateral duct buds and the better growth of club endings. It may be concluded from experiments 3 and 4 that DCA substitutes for the adrenal gland in the triply operated rat and permits the mammary gland to respond to STH by growth changes similar to those induced by STH in hypophysectomized, oöphorectomized rats. Results of experiments showing that pituitary preparations are mammogenic in the presence of adrenocortical tissue require more careful scrutiny in order to ascertain whether the effects described were comparable to the limited mammary duct response to STH alone or more like the STH plus DCA or E effects. In the latter case, the pituitary preparations might be said to have promoted the secretion of a

DCA-like or E-like compound by the adrenal. In these experiments the importance of removing both ovaries and adrenals has been shown.

Experiment 5. Mammary Duct Growth Induced by Hypophysectomized, Oöphorectomized, Adrenalectomized Rats Injected Systemically with STH, Prednisolone Acetate, and Estrone

The glucocorticoids have not been implicated as mammogens in the sense that DCA has (12) although they have been shown to have lactogenic potency (6, 7, 8, 13, 17). In our earlier work with triply operated rats, it was not possible to show any solo mammogenic effect with cortisol acetate other than a peculiar duct distension in otherwise stunted glands.* Since it was intended eventually to replace the adrenals with better substitution therapy than DCA alone in tests for STH's mammogenic activity, prednisolone acetate was tried alone and with STH and E. The rats were triply operated. Three were injected daily with 0.2 mg. of Pred-ac for 1 week, and three for 2 weeks. Three were injected with 0.2 mg. of Pred-ac plus 2.0 mg. of STH for 1 week, and three for 2 weeks. Three were injected with 0.05 mg. of Pred-ac plus 2.0 mg. of STH for 1 week, and three for 2 weeks. Three rats also received 0.05 mg. Pred-ac plus 2.0 mg. STH for 2 weeks, and 1 μg. of E daily during the second week. The results are shown in Table IV and Figs. 25–28 (Plate V). Pred-ac was not mammogenic at 0.2 mg. for 1 or 2 weeks (Fig. 25). When combined with 2.0 mg.

PLATE IV

The figures represent typical areas of the right or left abdominoinguinal mammary glands (numbers 4 or 5, numbering from the cephalic end). The glands, after fixation in 10% formalin, *in toto* staining in alum carmine, and plastic embedding, were photographed at × 10 magnification. DCA = deoxycorticosterone acetate; STH = somatotropin (growth hormone).

FIG. 19. Gland from a rat hypophysectomized on day 30, oöphorectomized and adrenalectomized on day 44. Treated daily systemically with 0.1 mg. DCA. Glands atrophic.

FIG. 20. Same schedule, but besides the 0.1-mg. DCA systemic injections, this rat also received 7 daily injections of 0.04 mg. STH in the region of this gland. Little or no difference between this and contralateral gland was seen, but both showed less regression than glands from rats receiving DCA alone.

FIG. 21. Similar schedule and treatment except that 0.2 mg. STH was injected locally. Fair duct growth in this right gland as compared with the contralateral gland shown in Fig. 22.

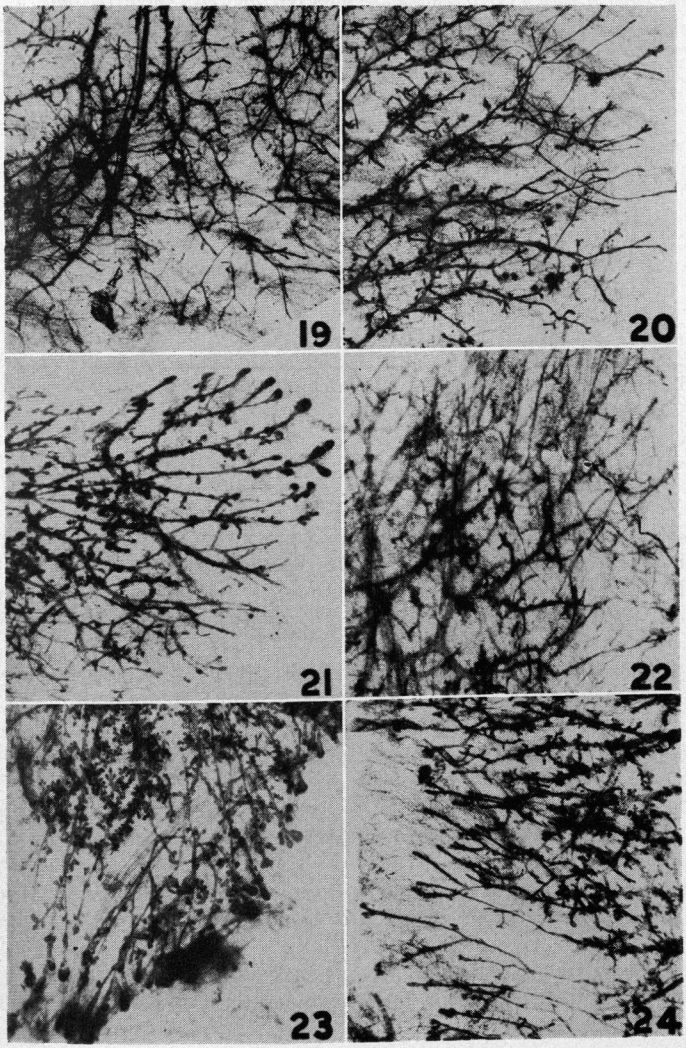
FIG. 22. Left or control gland to the one shown in Fig. 21.

FIG. 23. Same schedule, but the STH was administered in the form of one 15-mg. pellet on the right side. Right gland shows good proliferation of end clubs and side ducts.

FIG. 24. Gland contralateral to that shown in Fig. 23. Slight ductal stimulation.

* Unpublished.

of STH daily the animals fared better in the matter of body weight change, but the mammary glands showed only slight ductal alteration which seemed to be mainly distension with fluid (Fig. 26). However, after 2 weeks on the 0.05-mg. dose of Pred-ac plus 2.0 mg. of STH, the rats showed a 22-gm. body weight increase and slight but definite evidence of ductal proliferation (Fig. 27). The addition of 1 μg. of E to the rats during the second week on this regimen improved the ductal growth considerably, just as it did for the STH plus DCA regimen (Fig. 28). Although predniso-



lone is said to have some mineralocorticoid activity, it is a potent glucocorticoid and an antagonist to STH in a catabolic as contrasted with an anabolic sense,—quite unlike DCA in this respect. A combination of 0.1 mg. of DCA and 0.05 mg. Pred-ac proved later to be good substitution in the triply operated rat. It may be concluded from this experiment that 0.05 mg. of Pred-ac synergized with 2 mg. of STH to promote ductal proliferation; and when 1 μg. E was added to that combination, still better

TABLE IV

Mammary Duct Growth Induced in Hypophysectomized, Oöphorectomized, Adrenalectomized Rats Injected Systemically with STH, Prednisolone Acetate, and Estrone[a]

Group	No. of animals	Body weight change (gm.)	STH (mg.)	Pred-ac. (mg.)	Estrone (μg.)	Duct growth ++	Duct growth +	Duct growth —
1	3	—17	—	0.2	—			3
2	3	— 3	2	0.2	—			3[b]
3	3	+ 3	2	0.05	—			3[b]
4	3	—30	—	0.2	—			3
5	3	+10	2	0.2	—			3[b]
6	3	+22	2	0.05	—		2	1[b]
7	3	+21	2	0.05	1	3		

[a] Pituitaries removed on day 30; ovaries and adrenals on day 44. Groups 1–3 injected days 44–50; groups 4–7 injected days 44–57.

[b] Duct distension.

PLATE V

The figures represent typical areas of the right or left abdominoinguinal mammary glands (numbers 4 or 5, numbering from the cephalic end). The glands, after fixation in 10% formalin, *in toto* staining in alum carmine, and plastic embedding, were photographed at × 10 magnification. Pred-ac = prednisolone acetate; STH = somatotropin (growth hormone); E = estrone.

FIG. 25. Gland from rat hypophysectomized on day 30, oöphorectomized and adrenalectomized on day 44; injected systemically daily for 7 days with 0.2 mg. Pred-ac. Bare duct system.

FIG. 26. Same schedule and treatment except that 2 mg. STH was also injected daily, systemically, for 1 week. Ducts show only slight distension.

FIG. 27. Same operative schedule, but treated for 2 weeks with daily systemic injections of 2 mg. STH plus 0.05 mg. Pred-ac. Slight evidence of ductal growth in the small club ends.

FIG. 28. Gland from rat similarly prepared and treated but with the addition of 1 μg. E daily, systemically, during the second week of treatment. Note better club ends than in Fig. 27.

FIG. 29. Gland from a normal 30-day-old male rat.

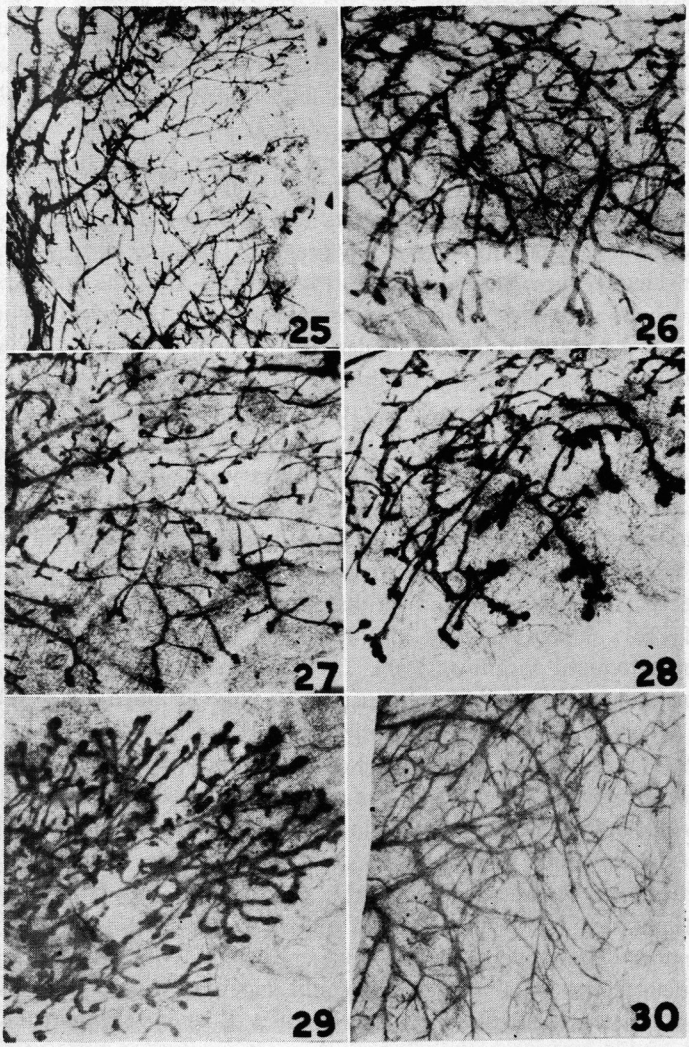
FIG. 30. Gland from a male rat hypophysectomized at 30 days and sacrificed after one month without treatment.

growth was induced. There was no evidence that Pred-ac alone functioned as a duct mammogen.

The main findings in these experiments on mammary duct growth may be summarized briefly as follows:

1. Neither estrone, DCA, nor prednisolone acetate alone are duct mammogenic in the doses used.

2. STH has a direct duct mammogenic effect but requires the help of



a comammogen (e.g., estrone) in inducing normal ductal development in the hypophysectomized, oöphorectomized rat.

3. In the triply operated rat, STH plus E are also effective in stimulating ductal growth, but the addition of a corticoid (DCA especially) was required before the normal ductal growth typical of the prepuberal to puberal ages could be duplicated.

4. The normal, rapid growth of the Long-Evans rat's mammary gland in the prepuberal phase was closely imitated in the hypophysectomized, oöphorectomized, adrenalectomized rat by replacing the ablated organs as follows: (a) the pituitary with 2 mg. of bovine STH; (b) the ovaries with 1 μg. of estrone; (c) the adrenals with 0.1 mg. of DCA.

5. No single hormone has been found capable of inducing complete ductal mammogenesis.

II. Lobuloalveolar Mammary Growth

The term lobulolalveolar (LA) refers to the phase of mammary development seen in the pseudopregnant or pregnant rat. It is true that small alveolar clusters are present in the cycling rat, but the gland of such animals shows poor alveolar development because of the brief, cyclic, luteal phases. In such virgins, large lobules are formed, following the injections of estrogens, progestogens, or androgens, through pituitary-gonadal interactions. In the oöphorectomized rat, E plus P induce LA development similar to that of pregnancy. In the hypophysectomized rat this may be accomplished by E supplemented with pituitary or placental MH. Another combination that will induce LA growth is FSH and ICSH to stimulate the ovary to form E, and MH to activate the luteal cells to secrete P, in which case MH acts with E plus P as comammogens. In the hypophysectomized, oöphorectomized rat, E, P, and pituitary or placental MH must be injected (20, 21, 29). Whenever pure MH has been used for this purpose in the hypophysectomized virgin rat, the LA development has equaled that of early pregnancy, but full development was only attained by adding STH (22). At one time it seemed possible that this limitation in LA growth might be explained by general metabolic debilitation due to the absence of STH. However, there was also a strong likelihood that STH might act directly in this phase of growth just as in the ductal phase. Since LA growth during much of pregnancy is a composite of duct and alveolar proliferation, it would seem reasonable to expect E and STH to continue their stimulation of ductal extension and new formation, with the triad E plus P plus MH acting upon the duct epithelium to transform it into the alveolar secretory units whenever it could supersede the E plus STH combination. In this dual phase of development E and STH may be said to

continue to play their earlier role and also to assume a new assignment of synergizing with P plus MH to induce alveolar differentiation and proliferation. If the roles are separate it would then be a matter of STH competing with MH plus P for E and finally sharing it.

Experiment 6. Lobuloalveolar Growth Induced by Locally Placed Pellets of MH, STH, Estrone, and Progesterone

In attempts to provide answers to these questions, the four hormones (E, P, MH, and STH) previously shown capable of inducing LA growth in hypophysectomized, oöphorectomized, adrenalectomized rats (23) were implanted in the form of pellets in a localized mammary area. Fifteen different pellet formulas were used in order to determine what each hormone accomplished alone and whether it synergized with one, two, or three of the other substances. Cholesterol was used as the binder and filler in order to permit the making of pellets of fairly uniform size and weight, regardless of the number of hormones in any given pellet. Groups of six Long-Evans male rats hypophysectomized at 30 days were treated with one implantation of hormones or cholesterol blanks immediately after operation. Four or five pellets weighing between 15 and 17 mg. and totaling 70–75 mg. were inserted through a trocar subcutaneously near the nipple of the first or second gland of the right abdominoinguinal group. In a few experiments, pellets of one or more hormones were placed on the right and one of different composition on the left, leaving the thoracocervical glands as controls. The estimated total dose of each hormone whether with cholesterol only or with other hormones was: E = 28 μg.; P = 42 mg.; MH = 28 mg.; STH = 2.3 mg. These arbitrary doses can only be justified by the results. It is realized that variables compounded many times enter into this type of experiment. Without further treatment and at the end of one month the rats were sacrificed, and mammary spreads were procured for whole-mount and microscopic study.

The results of the hormonal action on the contacted and contralateral glands are summarized in Table V and illustrated in Figs. 29–38 (Plates V, VI, and VII). Figure 29 shows a gland from a normal 30-day-old male. Slightly better ductal development may be seen in older rats ranging from one month to at least two years of age, and this may be found even after castration. After hypophysectomy, male mammary glands show the same regression as do those of the female (Fig. 30). As indicated in Table V, no one of the hormones induced LA growth (Figs. 31–34). Progesterone in the large 42-mg. dose (at least half of which was resorbed) had a partial maintenance effect causing bizarre parenchymal formations suggestive of secretory distension in some of the ipsilateral glands (Fig. 34). The double

combinations induced no LA growth; and with only one of the triple compounds (E plus P plus MH) was this development achieved, and then only in the contacted parenchyma (Fig. 35). In the E plus P plus STH (Fig. 36) and the E plus MH plus STH ipsilateral but not contralateral glands, evidence of slight ductal proliferation was present. This might be expected because of the availability of E and STH, which hormones must have been more readily released from the more soluble bulk of P and MH than when

TABLE V

Mammary Reactions in Hypophysectomized Male Rats to Pellets Containing Mammotropin (MH), STH, Estrone (E) and Progesterone (P) in Cholesterol or in the Listed Combinations,[a] Implanted on the Right

Group	No. of animals	MH (mg.)	STH (mg.)	E (μg.)	P (mg.)	Lobuloalveolar growth				
						Right			Left	
						++	+	—	+	—
1	5	28	2.3	28	42	5				5
2	4	28	—	28	42	1	2	1		4
3	6	28	2.3	—	42			6[b]		6
4	4	28	2.3	28	—			4[c]		4
5	5	—	2.3	28	42			4[c]		4
6	4	28	—	—	42			4[b]		4
7	4	—	—	—	42			4[b]		4

[a] E; P; MH; STH; E + P; E + MH; E + STH; MH + STH: all negative for LA growth. Hypophysectomized and implanted on day 30. Necropsy one month later.

[b] Partial maintenance of onset state and secretory changes.

[c] Ductal growth only.

PLATE VI

The figures represent typical areas of the right abdominoinguinal mammary glands (numbers 4 or 5, numbering from the cephalic end). The glands, after fixation in 10% formalin, *in toto* staining in alum carmine, and plastic embedding, were photographed at × 10 magnification. Glands are from male rats hypophysectomized at 30 days of age and given dosages of hormones implanted in the area of the right abdominoinguinal mammary glands on the day of operation. Necropsies were performed one month later. MH = mammotropin (prolactin); STH = somatotropin (growth hormone); E = estrone; P = progesterone.

FIG. 31. Dosage: 28 mg. MH. Slight maintenance. No lobuloalveolar growth.

FIG. 32. Dosage: 2.3 mg. STH. Bare ducts; regression.

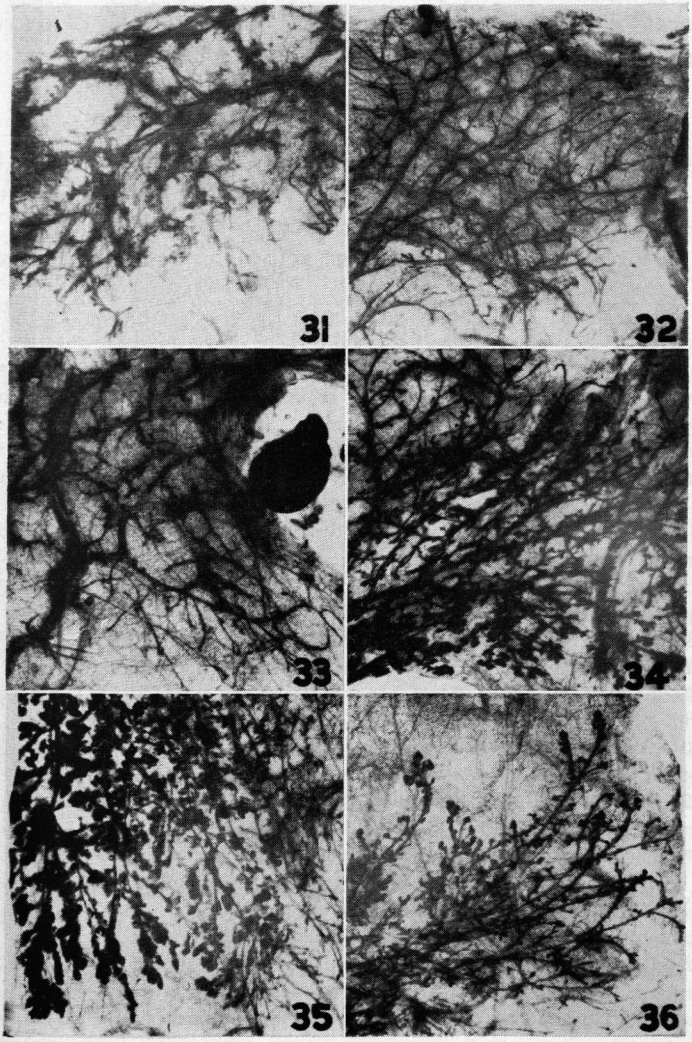
FIG. 33. Dosage: 28 μg. E. Bare ducts; regression.

FIG. 34. Dosage: 42 mg. P. Partial maintenance and some duct distension. No lobuloalveolar growth.

FIG. 35. Dosage: 28 μg. E plus 42 mg. P plus 28 mg. MH. Fair lobuloalveolar growth in half of area shown, which was next to pellet. Lesser to negative reaction in other half.

FIG. 36. Dosage: 28 μg. E plus 42 mg. P plus 2.3 mg. STH. Slight ductal proliferation; no lobuloalveolar growth.

combined with cholesterol. The STH plus E pellets did not induce ductal changes in the amounts released in these experiments, although they did in other combinations (see above). All of the rats that received the four-hormone combination showed excellent LA growth in the pellet areas (Fig. 37), with lesser reactions grading down to a regressing or slightly maintained parenchyma on the left side (Fig. 38), or distal to the pellet on the right. One of the rats in this series showed large lobules typical of late pregnancy in the gland near the pellet and the typical "feathery" lobules



denoting regression from full lobular development in the adjacent gland. It would seem that necropsies were not performed too soon.

It may be concluded that of the fifteen different combinations of E, P, MH, and STH applied directly to the mammary gland, only E plus P plus MH and E plus P plus MH plus STH induced LA growth. The result showing that STH applied directly to the gland with E plus P plus MH enhances the LA growth attained with the latter triad confirms our earlier findings in experiments in which these hormones were injected systemically. Although in previous experiments the presence of the adrenal was unnecessary, the requirement of large doses of progesterone makes it difficult to rule out the possible necessity of corticoids in LA development.

III. Lactational Mammary Growth

The transition from a prolactational to a lactating mammary gland in the rat is a gradual one during the last part of pregnancy, when the two processes seem to compete for dominance. Since MH functions in LA growth, lactogenesis, and corpus luteum activation, one would expect com-

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PLATE VII

The figures represent typical areas of the right or left abdominoinguinal mammary glands (numbers 4 or 5, numbering from the cephalic end). The glands, after fixation in 10% formalin, *in toto* staining in alum carmine; and plastic embedding, were photographed at × 10 magnification. E = estrone; P = progesterone; MH = mammotropin (prolactin); STH = somatotropin (growth hormone); DCA = deoxycorticosterone acetate; Pred-ac = prednisolone acetate.

Fig. 37. Male rat hypophysectomized at 30 days of age and implanted with hormones in the area of the right abdominoinguinal mammary glands on the day of operation. Dosage: 28 μg. E plus 42 mg. P plus 28 mg. MH plus 2.3 mg. STH. Excellent lobuloalveolar growth in area of gland near pellet.

Fig. 38. Contralateral (left) gland from same rat that provided right gland shown in Fig. 37. Partial maintenance and increase in number of small lateral ducts.

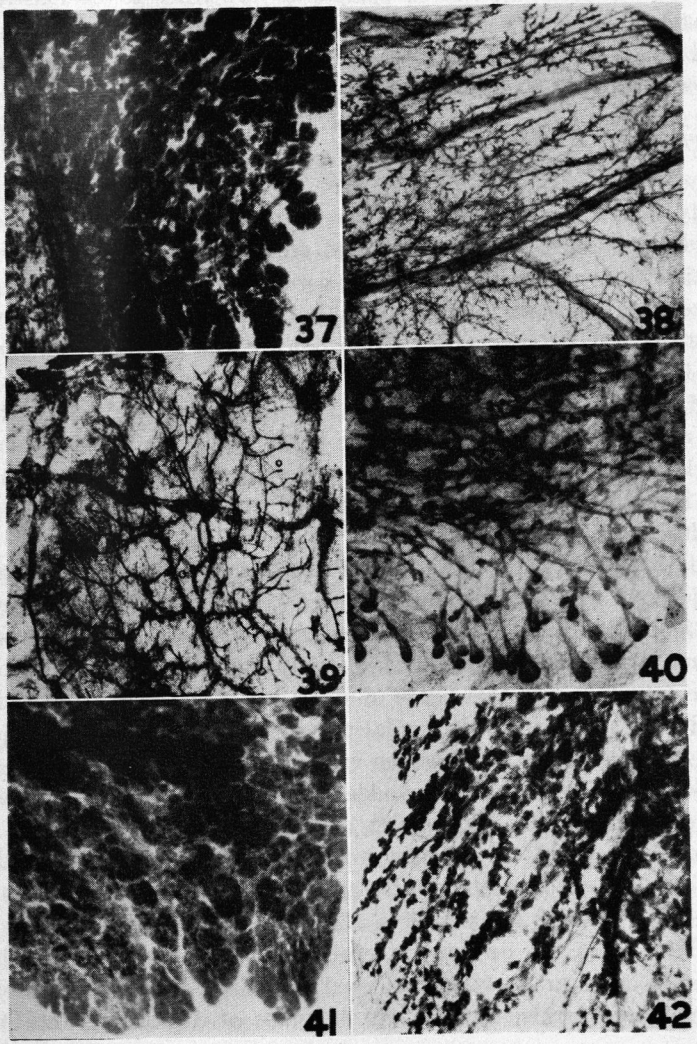
Fig. 39. Gland from a female rat hypophysectomized when 30 days old and maintained without treatment for 21 days.

Fig. 40. Gland from a female rat hypophysectomized on day 30, oöphorectomized and adrenalectomized on day 60, and injected daily, systemically, from day 60–69 with 1 mg. STH plus 0.1 mg. DCA plus 1 μg. E. Necropsy on day 70. Note signs of good ductal proliferation.

Fig. 41. Same schedule and treatment as listed for Fig. 40, followed by 10 days of systemic treatment with 5 mg. MH plus 2 mg. STH plus 1 μg. E plus 2 mg. P plus 0.1 mg. DCA plus 0.05 mg. Pred-ac. Excellent lobuloalveolar growth equal to that of late pregnancy.

Fig. 42. Gland from a rat treated as described in Fig. 41 (second phase = 20 days) and then given 0.1 mg. DCA plus 0.1 mg. Pred-ac systemically for 6 days. Necropsy 1 day later. Note regressing alveoli in lobules that had attained a brief secretory state.

petition for it by the corpus luteum and by the mammary gland's growth and secretory requirements, the first of which also involves progestin. In the rat, the placenta is a potent source of an MH-like hormone (1, 24, 25, 29) which, like its pituitary counterpart, has two highly activated targets during pregnancy, namely, the ovarian luteal tissue and the mammary apparatus. The degeneration of the corpora lutea at the end of gestation gives the mammary gland top priority for MH, and it retains this during suckling, even though a new crop of corpora lutea forms just after parturition.



There has been as much controversy over lactogenesis as over mammogenesis in the past thirty years; but, in the rat at least, the problems may be more easily solved if it be understood that a fall in titer of the ovarian and placental hormones that functioned with the pituitary and adrenocortical hormones in stimulating LA growth turns the force of the pituitary and adrenal upon the mammary gland. The secretion of milk that ensues is also a growth process, involving as it does the addition of secretory cells, especially in the first stages of colostrum secretion, and thereafter a continuous replacement of secreted cytoplasm. In order to demonstrate this last phase of mammary development using the triply operated rat, it was decided to carry through the two previous growth phases in sequence and to follow them with the secretory phase in the same animals.

Experiment 7. Ductal, Lobuloalveolar, and Lactational Growth in Hypophysectomized, Oöphorectomized, Adrenalectomized Rats

Thirty female Long-Evans rats were hypophysectomized at 30 days of age and maintained without hormonal treatment for one month, during which time their average weight increase was 1 gm. They were then oöphorectomized and adrenalectomized at one operation, immediately after which the first of 10 daily subcutaneous injections of the following hormones was given: 1 μg. of E plus 0.1 mg. of DCA combined in sesame oil, and 1 mg. of STH in 2% butanol in saline. Nineteen of the rats survived this procedure with an average weight increase of 0.2 gm. Two rats were sacrificed to check the mammary duct growth, although this had been amply controlled in earlier series. The remaining 17 rats were then treated for 20 days with: 1 μg. E plus 2 mg. P plus 0.1 mg. of DCA combined in sesame oil; and 5 mg. of MH plus 2 mg. of STH combined in 2% butanol in saline. The injections were given daily in the dorsal thoracocervical subcutaneous areas. Since fluids given in these regions may reach the thoracocervical mammary glands and affect them directly, such glands were kept in a separate category; and this phase of study was restricted to the abdominoinguinal glands, presumably stimulated systemically. Of the 17 rats, 4 died after 1–3 days on this new regimen even though they were gaining weight. When 50 μg. of Pred-ac was then added daily to the other five hormones, no more deaths occurred. This sextet of hormones—two from each of the endocrine organs ablated—seemed to provide good substitution therapy. During the 20 days of treatment, the rats gained an average of 58 gm. They appeared like normal females of a slightly younger age, as might be expected because of the one month posthypophysectomy interval. One was sacrificed in order to ascertain that good mammary lobules were developing. The remaining 12 rats, divided into 4 groups of 3, received the following

hormonal treatments for 6 days: group 1, 2.5 mg. MH locally in the right abdominoinguinal gland area; group 2, 0.1 mg. DCA plus 0.1 mg. Pred-ac systemically; group 3, 0.1 mg. MH, locally as in group 1, and 0.1 mg. DCA plus 0.1 mg. Pred-ac systemically; group 4, 2.5 mg. MH, locally as in group 1, and 0.1 mg. DCA plus 0.1 mg. Pred-ac systemically. Deprived of STH, all groups lost weight as follows: group 1, 19 gm.; group 2, 23 gm.; group 3, 24 gm.; group 4, 12 gm. The abundant milk secretion and well-sustained mammary glands in group 4 on the 2.5 mg. of MH plus DCA and Pred-ac probably explains why this group seemed to lose less weight. The prompt and continuous weight loss indicated that the previously injected STH had disappeared rapidly. The experiment was terminated after 6 days because milk could be expressed after 1 day in some rats and because the group that did not receive the corticoids appeared weak and listless even though they lost no more weight than two other groups. Regardless of what may be said about the nonnecessity of STH (or corticoids) in mammogenesis or lactogenesis, it was obvious from this experiment that they must contribute importantly to the maintenance of a rat over a long period, especially when they are in proper balance with each other. The final results of this experiment are shown in Table VI and in typical photographs of the mammary reactions. A gland from a female rat 3 weeks after hypophysectomy (Fig. 39, Plate VII) may be used as an example of the regressed condition of the structure to be stimulated. The first hormonal regimen of E plus DCA plus STH induced the type of ductal proliferation seen in the 30–40-day-old normal rat, with good evidence of club ends pushing peripheralward and side branches growing from the main ducts (Fig. 40). Figure 41 shows a gland from a rat that had been on the first regimen (E plus STH plus DCA) for 10 days followed by 10 days of the second combination (E plus P plus DCA plus Pred-ac plus MH plus STH). It was noticed that two types of growth had been in progress in this gland: (a) ductal proliferation, and (b) alveolar proliferation. These go on together in the pregnant and pseudopregnant rat. The difference between a virginal and prolactational (gestational) gland is not just a matter of alveolar numbers, but is determined by a great increase in ducts, especially the suborders or intercalated ducts such as may be shown to develop in clusters due mainly to STH plus E. The first LA development can be shown in two dimensions fairly well, but the final, complete status requires three. The gland shown in Fig. 41 was thick, and parts of it were found to be layered one upon the other. The right and left glands of the rats that received DCA plus Pred-ac showed a few scattered secretory areas with an over-all picture of regression of alveoli that had attained an early secretory state (Fig. 42). The rats that received 2.5 mg. of MH locally showed some foci

of secretion and partial lobular maintenance in the right (injected) glands (Fig. 43, Plate VIII) and predominantly a regression from an early secretory state in the contralateral glands (Fig. 44). This implies a degeneration of epithelium in distended alveoli comparable to postlactational involution. The animals injected with DCA plus Pred-ac systemically and

TABLE VI

Effects of Local Injections of MH and Systemic Doses of DCA and Prednisolone Acetate on Milk Secretion in Hypophysectomized, Oöphorectomized, Adrenalectomized Rats[a]

Group	No. of animals	Body weight change (gm.)	MH (mg.)	DCA (mg.)	Pred-ac. (mg.)	Milk secretion[b] Right	Left
1	3	—19	2.5	—	—	+	+[c]
2	3	—23	—	0.1	0.1	+[c]	+[c]
3	3	—24	0.1	0.1	0.1	+++	++
4	3	—12	2.5	0.1	0.1	++++	+++

[a] Treatment: E + DCA + STH for 10 days; E + P + DCA + Pred + MH + STH for 20 days; MH + /or DCA + Pred for 6 days (MH locally on right).

[b] + to ++++ = arbitrary, average, qualitative, evaluations.

[c] Most lobules regressing.

PLATE VIII

The figures represent typical areas of the right or left abdominoinguinal mammary glands (numbers 4 or 5, numbering from the cephalic end). The glands, after fixation in 10% formalin, *in toto* staining in alum carmine, and plastic embedding, were photographed at × 10 magnification. MH = mammotropin (prolactin); DCA = deoxycorticosterone acetate; Pred-ac = prednisolone acetate.

FIG. 43. Gland from a female rat hypophysectomized on day 30, oöphorectomized and adrenalectomized on day 60, injected daily, systemically, from day 60–69 with 1 mg. STH plus 0.1 mg. DCA plus 1 μg. E, followed by 20 days of systemic treatment with 5 mg. MH plus 2 mg. STH plus 1 μg. E plus 2 mg. P plus 0.1 mg. DCA plus 0.05 mg. Pred-ac, and then treated with 2.5 mg. MH locally in the right abdominoinguinal mammary region daily for 6 days. Partial maintenance of secretory lobules.

FIG. 44. Uninjected, left gland, contralateral to that shown in Fig. 43. Regressing lobules.

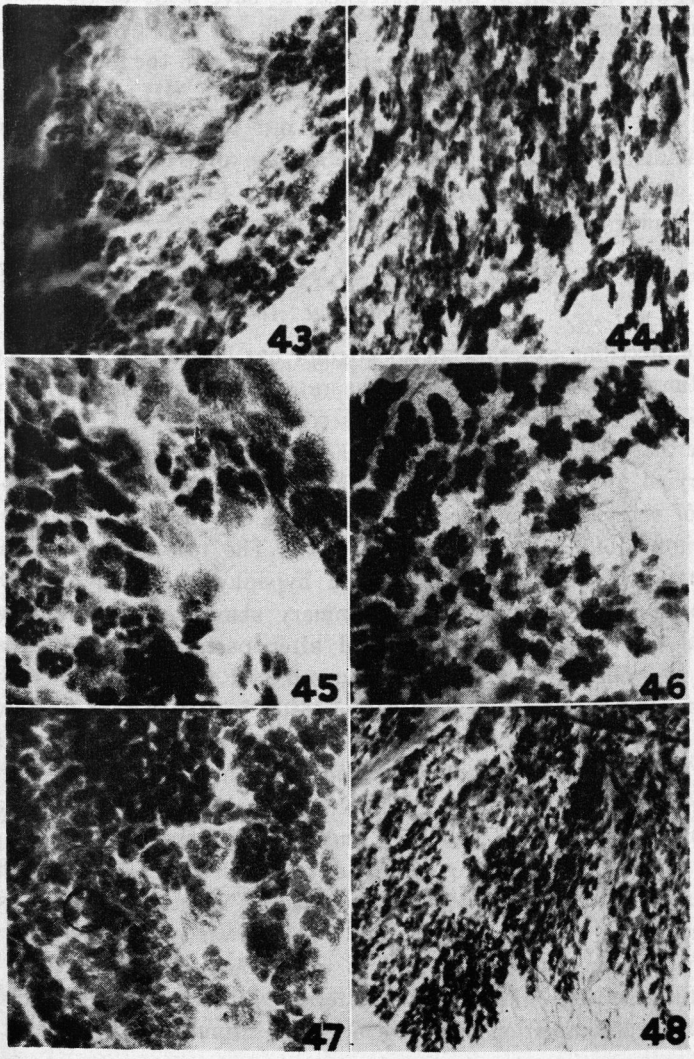
FIG. 45. Same schedule and pretreatment, and then 2.5 mg. MH locally, daily, in area of the right gland and 0.1 mg. DCA plus 0.1 mg. Pred-ac daily, systemically, for 6 days. Fully developed lobules with alveoli filled with milk.

FIG. 46. Uninjected, left gland, contralateral to that shown in Fig. 45. Lobules contain milk; some are well developed and others appear to have begun to regress.

FIG. 47. Same schedule and pretreatment, and then 0.1 mg. MH locally, daily, in area of this right gland and 0.1 mg. DCA plus 0.1 mg. Pred-ac daily systemically for 6 days. Fully developed lobules with alveoli filled with milk.

FIG. 48. Uninjected, left gland contralateral to that shown in Fig. 47. Most lobules show involution from a secretory phase.

with 2.5 mg. of MH locally showed maximal lobular size in the right (injected) glands with alveoli overdistended with milk in some areas. One of these rats showed almost as good a reaction on the left as on the right, but the others showed less lactogenic response in the left glands (compare Figs. 45 and 46). The 0.1 mg. local dose of MH was better for showing differential effects. The rats injected in the area of the right glands with 0.1 mg. MH and with 0.1 mg. of DCA plus 0.1 mg. of Pred-ac systemically showed good secretory responses in the MH-treated loci (Fig. 47). The



contralateral glands showed some areas of maintained secretory lobules (points of predilection) but in general the picture here was one of lobules regressing from a fully developed, secretory state (Fig. 48). Of these several small pilot experiments, this latter group should prove helpful in providing a starting point for a study of the local action of various corticoids in their role of colactogens. It also is obvious that all of these experiments on the local action of mammogens and lactogens lead directly to the use of mammary cultures (11).

It was our intention in this experiment to have some of the rats lactate, which term now implies delivering the milk as well as making it (10). Unfortunately, the nipples did not grow in pace with the glands, and so the experiment can only be said to have shown that MH and the corticoids induced milk secretion in triply operated rats in which ductal growth had been stimulated by the hormonal combination of STH plus DCA plus E and in which lobuloalveolar development had followed injections of MH plus STH plus E plus P plus DCA plus Pred-ac.

Experiment 8. Lactation Induced in Hypophysectomized Rats with MH and Prednisolone Acetate

In the preceding experiments an attempt has been made to delineate hormonal functions in the growth of the rat mammary gland from the rudimentary duct stage to that of a fully secreting gland. As a final experiment to prove that MH and a corticoid are adequate to maintain lactation, it was necessary to use rats prepared in a different way. Reference will be made to one of many lactation experiments carried out in collaboration with Dr. Bintarningsih of Djakarta, Indonesia (3). The test animal was a Long-Evans rat bred for the first time and hypophysectomized on day 12 of pregnancy. In such animals, the mammary gland develops in response to placental and ovarian hormones, and after parturition, secretes a small amount of milk for a day before regressing. Each mother was given six pups—her own or preferably six active foster young that had received one feeding from their own mothers. MH plus STH plus cortisol-acetate (or Pred-ac), and in some instances oxytocin, were injected daily. As shown in Table VII, MH, STH, and cortisol acetate were ineffective alone, as were the combinations of STH and MH, and STH and cortisol acetate. At first it was thought necessary to supply oxytocin in order to have the milk moved along to the nipple by the contractile tissue. However, as Table VIII shows, the mixture of MH plus Pred-ac was quite adequate without oxytocin. Benson and Cowie (2) also found that the milk-ejection mechanism returned after removal of the posterior pituitary, and others (26) have detected a reaccumulation of oxytocin in the infundibular stump. It is also

shown in Table VIII that two sets of three mothers on 0.4 mg. Pred-ac and either 2.5 or 5 mg. MH daily fed their six pups enough milk to enable them to gain 0.8 and 1.0 gm. daily in body weight for a 5-day period. The mothers lost approximately the same amount of body weight. When 1.0 mg.

TABLE VII

Ineffectiveness of STH, MH, and Cortisol Acetate (C) Alone and in Certain Combinations in Inducing Lactation in Hypophysectomized Rats[a]

No. of animals	C (mg.)	STH (mg.)	MH (mg.)	Lactation	Milk in glands[b]
14	—	—	—	0	—
5	1	—	—	0	—
4	—	2	—	0	— to +
3	—	—	10	0	+
3	—	—	5	0	+
9	—	1	5	0	+
4	2	2	—	0	— to ++

[a] Hypophysectomized on day 12 of first pregnancy; 10 daily systemic injections beginning on day of parturition.

[b] At necropsy, 1 day after last injection.

TABLE VIII

Adequacy of MH and Prednisolone Acetate in Inducing Lactation in Hypophysectomized Rats[a]

No. of animals[b]	Average weight change (gm./day)		No. of pups per mother	Pred-ac. (mg.)	STH (mg.)	MH (mg.)
	Mother	Pup				
3	—1.1	+1.0	6	0.4	—	5.0
3	+2.2	+1.1	6	0.4	1.0	5.0
3	—1.5	+0.8	6	0.4	—	2.5
3	+3.2	+0.9	6	0.4	1.0	2.5
4	—0.6	+1.2	4	0.2	—	5.0
4[c]	+2.5	+1.2	4	0.2	0.2	5.0

[a] Hypophysectomized on day 12 of first pregnancy. Systemic injections begun on day of parturition.

[b] Brackets enclose same group of rats treated for two 5-day periods sequentially.

[c] Also received 5 μg *l*-thyroxine daily.

of STH was added to the MH and Pred-ac and the test continued for another 5 days with the same young, the mothers gained 2–3 gm. in body weight, but the pups continued to gain at their previous rate. Another group of four mothers on 5 mg. MH plus 0.2 mg. Pred-ac successfully fed their litters of four pups for 5 days with an average daily weight change of plus 1.2 gm.

for the pups and minus 0.6 gm. for the mothers. They were then given 1 mg. of STH and 5 μg. of *l*-thyroxine in addition to the MH and Pred-ac for the next 5 days. Although this treatment resulted in a 2.5 gm. average daily weight gain for the mothers and a return of oxygen consumption to normal, the pups continued to gain at the same rate as before.

A gain of 1 gm. per day in this period would be considered subnormal for the Long-Evans rat, which should show a 1.5- to 2.0-gm. increase. However, these pups were sustained, and when their eyes opened at about 2 weeks they began eating the mother's diet as well as drinking her milk. Some were allowed to "grow up" and seemed quite normal. Whenever the MH and Pred-ac injections were stopped, all mothers ceased lactating within about 3 days.

The conclusion reached from these two experiments was that MH plus a corticoid constituted the minimal hormonal requirement for lactation (assuming a secretion of oxytocin in our rats). Without a doubt the mothers were restored more toward normalcy by adding STH and thyroxine, but in the rat the role played by these hormones in lactogenesis has not yet been fully determined [see reviews by Folley (15, 16), Cowie and Folley (9), and a paper on the maintenance of lactation in the rat after hypophysectomy by Cowie (5)].

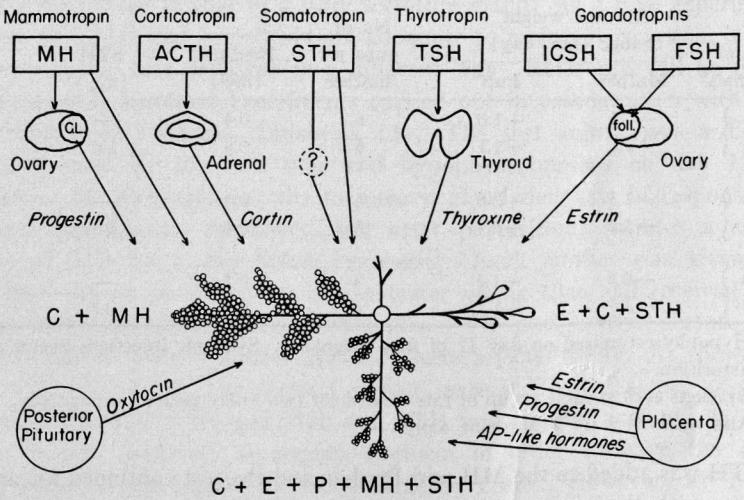


FIG. 49. Schema showing some of the hormones that influence mammary growth and lactation. In the mammary diagram: upper = rudimentary gland; right = prepuberal to puberal gland; lower = gland of pregnancy (prolactational); left = lactating gland.

General Summary

Piecing together the information gained from these and earlier experiments leaves but little doubt that in the Long-Evans rat, at least 5 of the 6 well-identified anterior pituitary hormones play important parts in mammogenesis and lactogenesis. The schema shown in Fig. 49 concentrates this information in a form altered slightly from one that was used earlier (23). FSH (follicle-stimulating hormone) and ICSH (interstitial cell-stimulating hormone) synergize to stimulate the ovary to secrete E (estrin). Estrin synergizes with STH and corticoids (C) secreted under the influence of ACTH (corticotropin) in inducing mammary duct growth. MH (mammotropin, prolactin) activates the corpus luteum to secrete P (progestin), and the prolactational combination of MH plus STH plus E plus P plus C induces full lobuloalveolar development. In the normal rat the placenta synergizes in this phase. For lactogenesis, the decrease in E plus P influence, and the dominance of MH plus C, are important. STH and TSH undoubtedly contribute to the normalcy of a lactating rat, but they are not necessary for lactogenesis. The thyroid and therefore TSH is unnecessary in mammogenesis (4), but the possibility remains that the mammary gland may utilize iodinated compounds independently of the thyroid.

Acknowledgments

The authors wish to acknowledge gratefully the gifts of estrone and progesterone from Parke, Davis and Company; prednisolone acetate from the Schering Corporation; deoxycorticosterone acetate from Ciba Company; cortisol acetate from Merck and Company; and terramycin from Charles Pfizer and Company. This work was done with the aid of Grant 496 from the University of California Research Board. This work was supported in part by a grant (G-4097) from the National Institutes of Health, the United States Public Health Service.

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Discussion

W. O. Nelson: I should like to pay tribute to the elegance of Dr. Lyons' studies. I think only those of us who have attempted to investigate the vagaries of mammary development and function can quite appreciate the painstaking kind of investigations that were involved in this presentation. I think he has, for the first time, shown us the various hormonal factors that are involved in the various aspects of mammary development and function, that is, duct growth, alveolar growth, initiation of lactation, and maintenance of lactation. I fondly hope, that in the process of so doing he has once and for all laid the ghost of the so-called specific mammogenic hormones of Turner that confused our understanding of this subject for quite a number of years. One other point that I think we might consider and speculate about relates to the problem of gynecomastia. This is a very puzzling condition that occurs in the human male, and a condition that cannot always be explained. I think if we apply some of the findings that Dr. Lyons has given us tonight, we may better understand the causes for exaggerated breast growth in men. Certainly it is not simply a matter of whether or not there is estrogen present in normal or excessive amounts. As we have

seen, there are other endocrine factors that are important for the development of both ducts and alveoli. The findings in the rat, of course, may not be directly applicable to other species, but I think the differences will not be differences in the kind of hormones, but rather simply a difference in the amount or relative importance of the various hormones.

W. Lyons: I am not so sure about laying the ghost of mammogens, Dr. Nelson. I am afraid I have stirred up more mammogens. We might be able to explain some of Dr. Turner's mammogens. In animals that were injected with pituitary extracts, with gonads and pituitary but not the adrenals ablated, there was left the possibility of a dual effect of STH plus a DOCA-like substance. I think that you are quite right in saying that, in this species anyhow, we should stop thinking about a solo mammogen unless it would be STH, which seems to be the only hormone that I have tried that has some slight effect of its own in triply operated rats. But it does not produce the normal proliferation such as that which you obtain when you combine it with estrogen.

M. Goldzieher: It may be in place to say a few words about disturbances of mammary growth and development in the human. Of course, in the human we are handicapped because we cannot work on hypophysectomized or ovariectomized females, and we must assume in individuals of normal growth and normal menstrual performances that both the ovaries and the pituitary are functioning properly. Yet, in many of these females, we find inadequate growth and development of the mammary gland, involving not only the duct and the lobular tissue, but also the mammary fat tissue. We find that this abnormality occurs in two types, one affecting both sides more or less evenly, the other limited to only one side whereas the other side is normal or occasionally excessive in development. In all of these cases we have tried for many years to produce relief for the patient, who usually is emotionally affected by these abnormalities. We found that a combination of both topical and systemic administration of various hormones leads to good results. I am not prepared to give you the statistics of our work over many years past, but I shall refer only to the last twelve cases seen in the current year. These patients came to me with absence of mammary development to the extent that the elevation of the nipple above the chest wall was not more than one-half of an inch. At present, the treatment having terminated after anywhere from 6 to 8 months, there is a 3½–4-inch elevation with good glandular development and adequate fat tissue in between the lobules and under the skin. The treatment consisted of the application of an estrogenic ointment to the skin of the breast and of oral administration of estrogen, administration of progesterone in the second half of the cycle, both orally and parenterally, and injections of prolactin in the second half of the cycle. This combination of the three hormones (estrogen, progesterone, and prolactin) seemed to suffice in the human female whose mammary development is inadequate to produce satisfactory growth—a growth which persists after the treatment is discontinued. The results obtained are universally satisfactory except in one area in which we are always unsuccessful: in cases of unilateral stunting of the mammary gland. You cannot bring up a unilaterally stunted gland anywhere near its partner by any kind of treatment. We must conclude therefore that the trouble there is in the target organ, which for some reason or other—it is not for me to speculate why—is incapable of responding to hormonal stimulation. Another point which might be explained the same way is the fact that a woman who has an adequate and perfectly normal menstrual function may show practically complete absence of mammary development. It would seem that her breasts are not responding

to her apparently normal endogenous hormone production; yet the same woman responds nicely to the exogenous administration of hormones, so that it seems that in some cases of bilateral mammary deficiency, we are faced with a quantitative deficiency in responsiveness. Such breast glands seem to require a larger amount of hormones for normal development, but they do respond if they get these larger amounts in a suitable way. One more word about regression of the mammary gland. It is much rarer, but it happens, that a woman is bothered with a hyperplasia of the mammary glands. Of course, sometimes the abnormal size is due only to excessive development of fat tissue, in which case only the plastic surgeon can give relief. If, however, we are dealing with hyperplasia of the mammary tissue, satisfactory involution of the mammary tissue can be obtained by topical administration of testosterone ointment, 2 mg. to the gram of ointment, given over a period of months. This will produce sizable regression of the mammary glands.

W. Lyons: Didn't you try the estrone-progesterone without the prolactin? Was this a sheep or beef prolactin product that you used, and not from a primate source?

M. Goldzieher: It is the Squibb preparation. We have used the addition of prolactin only more recently, and my impression is—and I must say definitely that it is an impression only—that these patients have done definitely better, and I may say that particularly in respect to one case. Usually women who are more advanced in age, in other words past 30, are prognostically in a much poorer category, whereas better results are obtained in the younger women. In this last series of twelve cases, I had a girl of 36 years in whom I got 4-inch development with addition of prolactin to the estrogen-progesterone therapy, and I never obtained anything like it before in her age group.

J. Furth: We are among the newest disciples of Dr. Lyons and learned to appreciate the care and skill in design of experiments to unravel the complexities of mammary gland proliferation and secretion. We introduced a new tool, Dr. Lyons, which we hope will be helpful in understanding these processes. This is the development of monomorphous pituitary tumors composed of cells which have both growth-promoting and lactogenic properties. The remarkable feature of this cell, which we call mammotrope (perhaps we should call it mammo-somatotrope), is that its existence and function depend quantitatively on estrogen. When one grafts it on hypophysectomized-gonadectomized animals that are given stilbestrol in single pellets of from 10 μg. to about 1 mg., there is a parallel increase in the mass of mammotropes, increase in body weight and organ weight (that is, somatotropic effects), and stimulation of the mammary gland with lactation [Clifton, and Furth, J., *Proc. Soc. Exptl. Biol. Med.* **94**, 809 (1957)]. If the ovaries are present in animals bearing these mammotropes, they will become smaller and the uterine horn thinner, so it is evident that these pituitary cells do not have gonadotropic function. The estrogen-induced tumors are at first dependent on estrogen; they will not grow in a normal host but soon gain autonomy. The radiation-induced tumors are autonomous at the start. Autonomous tumors have less secretory power, but they have one advantage; they can be grafted on gonadectomized hosts, causing a marked mammary gland hyperplasia. Now, the question is: How are the various functions of these mammotropic cells explained? Do they secrete two hormones, mammotropic and somatotropic, as your study would indicate, or does the native hormonal molecule possess two activities which in the course of purification are split into two substances, mammary gland-stimulating on the one hand, and growth-promoting on the other. In considering this problem, we are impressed by the observation that in both mice and rats there is this parallelism be-

tween growth hormonal effect and mammotrope mass. Consequently, if we suppose that there are two hormones, we have to conclude that estrogens stimulate somatotropes as well as mammotropes. Lactation is dependent on adrenal corticoids. The mammary glands of animals bearing mammotropic tumors are tremendously enlarged. There is complete development with abundant secretion. These ovariectomized animals can foster-nurse. The puzzles related to these cells can only be answered by chemical isolation of the respective hormones as Li has done and by careful assay studies, such as you have described.

W. Lyons: I know of your very careful work, Dr. Furth, and I have only objected to calling this tumor a mammotrope tumor. I agree that there must be a great deal of mammotropin secreted by your tumors. We can produce mammary glands filled with milk too, but not with any one hormone, not with any two hormones, and I think you must have a multiple-hormone-producing tumor. The only solution is to implant it and assay it for the different pituitary fractions. I would be surprised if you did not find more than mammotropin.

J. Furth: Perhaps it would be better to call the cell mammo-somatotrope, but we can state that it does not have adrenotropin action. The thymuses of animals with medium-sized tumors are normal; nor are there blood changes in the host, as lymphopenia, indicative of ACTH action. The thyroids of these animals are normal—nor are there any gonadotropic effects.

W. Lyons: What are your reasons for not including ACTH? The adrenals were normal, were they not?

J. Furth: No, the adrenals were greatly enlarged, but, as in rats after Amphenone treatment, they are loaded with fat. With the adrenal enlargement due to ACTH stimulation, we would expect involution of the thymus and lymphopenia in the host; these are exceedingly sensitive indices of ACTH action.

W. Lyons: No, not necessarily. In our animals on these corticoid treatments we have checked the thymus and have used doses of cortisol acetate that would not deplete the thymus. You can have a normal-sized thymus and still have lactation. On the 0.1-mg. dose of prednisolone acetate, the thymuses would decrease to about 25% of their controls' weight, but in our combined DOCA (DCA) and prednisolone animals, the thymuses were about 50% of normal, but they were not atrophic and this is also true of the lymph nodes. In our mammary spreads we were able to get some idea of what hormones were acting on the lymphoidal tissue found there. With growth-hormone treatment they were large, and with higher doses of corticoids of the prednisolone type, they were shrunken. But I wouldn't say that you did not have ACTH activity just because the thymus had remained normal in size. Furthermore, somatotropin is a thymotropic substance, and if your tumor secretes enough STH, it will neutralize an ACTH effect and keep the thymus from involuting.

R. Huseby: I wonder if the observations of Dr. Lyons and of Dr. Furth are of necessity in opposition one to the other. It seems to me that in your experiments, Dr. Lyons, where you had the somatotropic hormone implanted locally you presented very good evidence that this hormone by itself can produce mammary gland development. However, when you then gave a small amount of estrogen in addition, this greatly enhanced the growth of the gland. It looks, therefore, as though the situation might be that in the normal economy of the animals some such ingenious cocktail of hormones as you have described is responsible for full mammary gland development and lactation. In Dr. Furth's experiments, on the other hand, he has a pituitary tumor that apparently is producing tremendous amounts of somatotropic and mammotropic

hormones. In this abnormal situation it seems quite possible that the normal economy is by-passed just by the tremendous amount of pituitary hormones that are present, so that now estrogen and progesterone are no longer necessary in order for full mammary gland development and lactation to occur.

W. Lyons: Yes, after estrogen and progestin have served their purposes, they are just in the way. It depends on the type of development you start with. In an intact rat, you have only to give estrin to induce lobular alveolar growth. That treatment brings in pituitary mammotropin, and it brings in the luteal hormone from the ovary. These are interactions that I would like to avoid by "endocrinectomizing" the animal. I would agree that estrogen is certainly not necessary after the ductal and lobular-alveolar growth phases have been achieved. They are inhibitory to lactation then. I think the answer is in the assay of those tumors. After all, we assay individual pituitaries, so a tumor ought to be easy enough to assay in standard hypophysectomized rats or mice.

R. Huseby: Well, is it not true, Dr. Furth, that with your tumor transplant you can start essentially from scratch and get a mammary gland that is extensively developed; that is, having started with a relatively rudimentary gland you get duct growth, then lobuloalveolar development, and finally lactation, just in response to your tumor without the presence of either estrogen or progesterone.

J. Furth: We did not start with fully rudimentary glands, having used animals about 6–8 weeks of age and used them soon after hypophysectomy. We have much experience with adrenotropes; they can also be obtained in pure masses. When grafted on a hypophysectomized animal, they cause atrophy of all target organs, with the exception of the adrenals, which are greatly enlarged. The adrenotropes are not acidophilic, so the concept that there is an ACTH-secreting cell, different from thyrotropes and mammotropes, is well founded. Whether the mammotropes contain a trace of an adrenotropic activity, only special assays and chemical isolation will decide.

R. W. Bates: I would like to ask Dr. Lyons' opinion about the mammogenic and lactogenic hormones which are imputed to be in the urine, referring especially to the recent papers of Hadfield. Are these mammogenic substances any of the six specific hormones which you listed in your talk?

W. Lyons: The pictures that Hadfield has published in English journals show the type of growth that I refer to as ductal with the club ends, and we have shown how you can produce this type of growth in triply operated rats. You can do it with STH and DOCA, you can do it with STH and estrone, or the three of them together. I am sure that you could isolate the estrogens and DOCA-like steroids, maybe aldosterone, from the urines of these cases, and titrate, and know how much of each or both must be injected and possibly a somatotropic substance. Dr. Hadfield thinks he is isolating a mammotropin, a prolactin-like substance, but this hormone from beef or sheep is a poor synergizer with estrone to cause that duct growth in our rats. In very large doses it sometimes imitates it, but I cannot get it to do what STH does at all. There have been attempts to isolate STH from urine, but I don't think very many successful ones.

A. Segaloff: If this really is somatotropin, this would mean that in the amounts that you used even in your local application to get development, and the amounts of urine that Hadfield used, that it ought to be really easy to get out substantial evidence by injecting the crude urine into hypophysectomized animals and measuring the epiphyseal plate. I have not succeeded in getting an increase in the epiphyseal plate by the injection of urine into hypophysectomized rats.

W. Lyons: I have no further explanation of the results of Hadfield's experiments. I tried to find some explanation by showing what we have done in the rat. I do not say that the mammotropin in the ovine and bovine pituitaries is like the human mammotropin. Human mammotropic hormone may produce duct growth with estrin and progestin as Hadfield says. There is something in the urine of human beings that does stimulate the crop sac, but not quite the same as pure bovine or ovine prolactin, as Dr. Bates has definitely shown. I am not too concerned, as you were, about the complete separation of STH and mammotropin. I think it has been well shown that those are two separate hormones. Did you imply that they were not?

A. Segaloff: I think there is a distinct overlap, at least in some activities and it may be that we are getting out one material with a very modest degree of change, in one case predominating somatotropin-like activity and in the other case predominating prolactin.

W. Lyons: If a ratio of 200:1 means overlap, then I would agree with you, but I am afraid that that ratio is too extreme. There is a slight effect on the tibia when you give 2 mg. a day of mammotropin, but you can usually boil that away. The lactogenic hormone can be boiled following a certain procedure and STH cannot.

A. White: I would like to ask Dr. Furth whether he has examined the weight or the histology of the lymphoid tissue of his mice with the so-called adrenotrope tumor over a consecutive period of days. I ask the question because evidence of a failure of lymphoid tissue involution at a particular time may not necessarily establish lack of secretion of ACTH. About fourteen years ago Dougherty and I observed that in the CBA mouse, treated daily with 1 mg. of ACTH, there is a cyclic phenomenon in the lymphoid tissue both with respect to its size and its morphology. During the first 5 or 6 days, one saw a characteristic lymphoid tissue involution. This involution in itself appears to be a stimulus to lymphoid tissue regeneration, and there followed a proliferation of lymphoid tissue, both with respect to size and histology. Then the involutionary phase ensued again, and this cycle was repeated for as long as approximately 30 days in our experiments. At that time, because the ACTH used was derived from hog pituitary glands, the animals became nonresponsive to the hormone and circulating antibodies were demonstrated to the ACTH preparation employed. In these experiments, throughout the length of time when the ACTH was physiologically active, lymphoid tissue did not remain involuted during the entire period. Secondly, I would like Dr. Lyons' opinion as to whether he feels it is correct to continue to use the term "mammotropin" since we now have evidence that there are a number of mammotropins. Under these circumstances could we discard the name "mammotropin" and can we not decide between two possibilities, prolactin (or lactogenic hormone) and the other designation which was suggested by your colleague, Dr. Evans, luteotropin?

W. Lyons: I thought Dr. Astwood created that term "luteotropin." Certainly Dr. Evans showed the first luteotropic effect with crude extracts back in 1920, but I don't think he has used this term for mammotropin. The League of Nations, a long time ago, chose three terms for this particular substance. Luteotropin was not in vogue at that particular time, but prolactin, mammotropin, and galactin were all accepted. That body is no longer meeting, but I hope you will bring this suggestion up with the new group that will eventually decide such points. Mammotropin is the name of a specific hormone. I divide mammary-influencing substances into mammogens and lactogens—two terms that are used quite frequently now by Folley's school and by others. Mammotropin itself is both mammogenic and lactogenic; some of the

steroids are, also. That seemed to be a simplification and is the only reason I use this terminology now. We are going to meet more mammogens as time goes on, as they dig for mud in the Dead Sea and other places.

J. Furth: May I return to the question of Dr. White, related to the adrenotropes? The beauty of working with proteinaceous hormones-secreting tumors is avoidance of antibody production, and thus, as with the animal's own pituitary, the tumor cells discharge the hormone continuously. The first sign noted in animals bearing adrenotropic tumors is that the animals gain weight. This is due solely to obesity. It is marked, even in hypophysectomized animals even before the tumor is palpable. When, at this time, a blood smear is taken, a profound lymphopenia and eosinopenia are found. Never was a mistake made in diagnosing the presence of the tumor from these signs, even without palpation. Lymphopenia and thymic involution persisted. Spleen and lymph nodes remained small. Never did we notice a rebound.

C. D. Kochakian: There was some allusion to the fact that maybe Dr. Lyons' data would put Dr. Turner's "school" into the ghost category. I wonder if Dr. Lyons would be ready to comment on this.

W. Lyons: I did comment on that in answer to Dr. Nelson, and said that I was trying to explain Turner's experiments. I think his mouse experiments provided interesting results, and the question is how to interpret them. The first experiment in this report was included to show that you could obtain the Turner effect in a rat by taking the pituitary and the ovaries out but leaving the adrenals and giving STH, which might have 0.1% contamination of ACTH. Some prefer to believe that STH itself stimulates the adrenal cortex to form a DOCA-like hormone. I am retaining his term and using it a little more broadly.

R. W. Bates: You initiated the sensitive local crop-sac test in pigeons for prolactin, in which microgram or smaller amounts of prolactin were used. You also were the first to induce milk secretion locally in the mammary gland of the rabbit by injection of small amounts of prolactin. Why, in your experiments here, did you use as much as 28 mg. of prolactin? Could you have gotten the same results with much smaller amounts?

W. Lyons: In the lactogenic experiment we used 20–40 μg. or so in the rabbit; in the experiment that I spoke of today, the 100-μg. dose was adequate. It was in the pellet experiment that I used a 28-mg. pellet, but that was to be released slowly over a period of a month, and that was for prolactational development, not for lactation. The doses that we have used for lobular or prolactational development were 0.5–2.0 mg. daily. This amount will stimulate the ovary to form progestin; it will act directly on the mammary parenchyma to change duct epithelium into alveoli. I think that in the rabbit and the rat lactation experiments, the doses were about the same. I think that locally 25–50 μg. will induce lactation in the rat, and that is about where we were with the intraductal rabbit test. In the rabbit we were using an intact animal, and, although there was local lactation, the animal had the benefit of its corticoids. Its adrenals were intact.

L. L. Engel: I would just like to ask Dr. Lyons if he has had any experience with the effect of corticosterone, which is one of the principal secretory products of the rat adrenal, and whether the effects differ from those of prednisolone and DCA.

W. Lyons: I hope somebody will run down the whole list of corticoids in this type of experiment. I know that that is the one which should be tried because of the chromatographic work on the effluent blood of the rat's adrenal. I would like to try it, and some aldosterone, too, if I could get my hands on some.