CHAPTER XV

Hormonal Control of Mammary Growth

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For any exposition of the part played by hormones in the development and functioning of the normal mammary gland we find ourselves presented with an array of established or partly attested scientific facts, collected mostly during the last two decades and providing a body of evidence already sufficiently unequivocal to be of some value to the clinician and to warrant optimism regarding their future application to the practical problems of dairy husbandry. On the other hand there are still issues of fundamental importance which are held in doubt, or around which rival theories spread an enlivening controversy. Broadly, two main aims may be attributed to research in lactational physiology: to find the hormonal mechanisms governing the development of the mammary gland itself, and to determine the influences controlling the secretion and ejection of milk from glands so formed. A useful and unartificial classification of our present knowledge may well be based on a separate consideration of these complementary paths of research, and the present chapter has been written with this plan in mind.

I. Morphology of the Mammary Gland

It is essential for a proper evaluation of studies on the growth and differentiation of mammary tissue to consider first the typical morphological structures of which the glandular parenchyma is composed and to relate certain well-defined stages in the development of these

structures to corresponding phases in the reproductive life of the animal. The literature dealing with the microscopic anatomy of the gland has been ably summarized by Turner (155), whose publication, although dealing primarily with development in the bovine, ranges extensively to provide a most valuable contribution to comparative anatomy and incidentally deserves to be far more widely known.

This author, while stressing the variations to be found in the development and gross anatomy of the mammae of different species, testifies to the uniformity of the histological structure of the parenchyma. Whether one considers species as distinct as man and the marsupial *Dasyurus viverrinus*, or the cow and the rabbit, the same basic type of tissue structure is to be observed, though in the monotremes or egg-laying mammals—we may instance the duck-billed platypus—the apparatus has unusual characteristics and offers in many respects a more primitive version of the general form. There are two structures of major importance: the alveoli (acini), which are the secretory organs, and the ducts which act as channels through which the secretion passes to the external orifice of the gland. Questions of the endocrine influence on mammary growth largely resolve themselves into observations on the proliferation or regression of these structures in response to different stimuli.

The duct system may be compared with a complicated arborescence, at the ends of whose smallest members, by analogy the twigs, are to be found the bulbous alveoli. Secretion from the alveoli flows from smaller to larger ducts, which in high-yielding species such as the cow derive their names—intralobular, interlobular, intralobar, and interlobar—from their internal or interconnective relationships with morphologically recognizable aggregations of alveoli known as lobules and lobes. The latter units are given their individual character by the surrounding connective tissue, thinner in the case of the lobules, which are composed of alveoli, than in the lobes, which are composed of lobules. In smaller laboratory animals such as the rat, a more usual duct nomenclature is that distinguishing the major primary duct and secondary, tertiary, quaternary, and terminal ducts arising from its subsequent, and usually dichotomous, branchings. The terminal and lateral buds on the smaller ducts of the undeveloped gland represent the anlagen of the future lobules and each secondary duct with its bud-like growths constitutes a potential lobe. Histologically the alveoli and all but the finest ducts may be distinguished from one another by their single-cell and double-cell epithelia, while the ducts, again excluding the finest, have an outer layer of connective tissue of which there is no counterpart of comparable thickness enveloping the alveoli. It is contended that some secretory activity is a function of those cells of the fine terminal ducts which lie

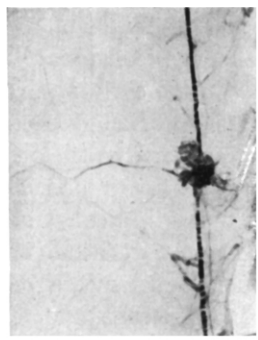
closest to the alveoli, and a transition to a single-cell epithelium has been demonstrated in these areas, as has also the presence of a relatively simple Golgi apparatus (168). These facts suggest secretory powers shared only in negligible degree, if at all, by the double-cell epithelia lining the larger ducts (134), while the report of pronounced vacuolization of the cytoplasm of duct cells during late pregnancy in rats (135) also lends support to the theory of terminal duct secretion. Jeffers (60) has furthermore commented upon certain parallelisms to be noted in the degeneration of alveolar and some duct epithelial cells in the lactating rat. This question is more fully discussed at the beginning of the next chapter.

The history of proliferation and regression in the mammae of any single species cannot be taken as typical of all; differences are primarily not of kind but of the relative importance of the various phases of growth which are customarily recognized as contributing to full development and function, phases dependent upon and owing their variable nature to the still more fundamental and characteristic reproductive history of the species. It may however be less confusing, bearing this proviso in mind, first to consider mammary growth as it can be observed throughout the normal life history of a single species, deferring a consideration of such points as are of interest in a comparative sense until the basic hormonal mechanisms have been discussed.

The rat presents a species suitable for illustration in that it has been investigated in greater detail than most other mammals and also since it exemplifies a sufficiently representative selection of the possible growth phases associated with mammary development in other species. The main details of its postnatal sexual life may be divided into (*1*) a period of inactivity lasting for about seventy days from birth to puberty (79), (*2*) periods of estrous activity in the virgin animal, when an estrus cycle is evinced with a mean length variously computed at between 4.6 and 6.2 days (120); (*3*) periods of gestation—approximately 22 days—and of ensuing lactation, when apart from a single estrus occurring one day after parturition estrous manifestations are in abeyance for about thirty days, and (*4*) periods of resumed estrous activity in the postparturient adult whose litter has been weaned, a process normally taking place when the young are about 21 days old. Finally, sterile copulation, mechanical stimulation of the cervix, and other agencies can induce a pseudopregnant period in the rat lasting for twelve to thirteen days.

We may note here that the figure illustrating this section (Fig. 1) depicts the rabbit, rather than the rat, gland; this figure has been used so that readier comparisons may be made with the experimental glands of rabbits shown later in the text.

Myers, in a series of communications (103–105) has made an intensive study of the changes observed in the mammary glands of the female and of the male albino rat from birth to puberty. His observations show that at birth the female glands have a mammary apparatus comprising second-



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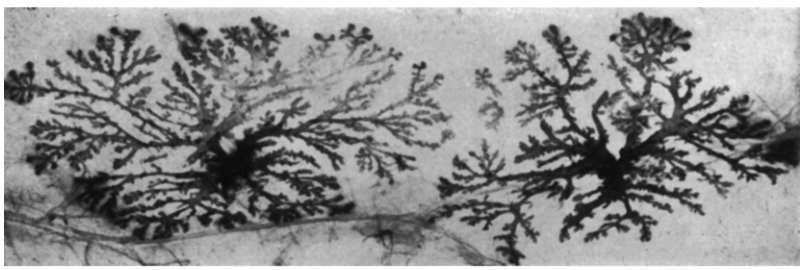

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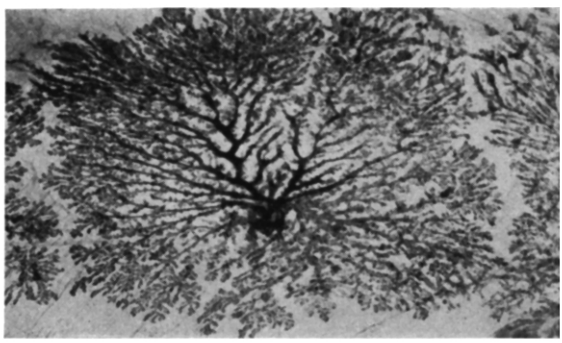

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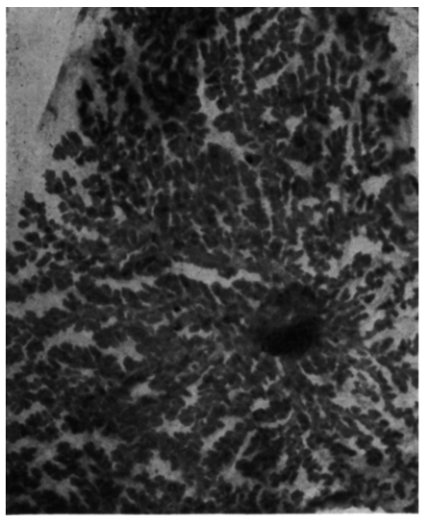
FIG. 1.—Development of the female rabbit mammary gland (× 1). *1*. Prepubertal. *2*. During first estrus. *3*. From an ovariectomized adult receiving estro-

ary, tertiary, and terminal ducts, all with lumina, as well as the partially closed primary duct. The ducts ramify in a single plane parallel to the surface of the skin, except in the case of the second inguinal gland, where obstruction by the hind limbs or the external genital organs even at this



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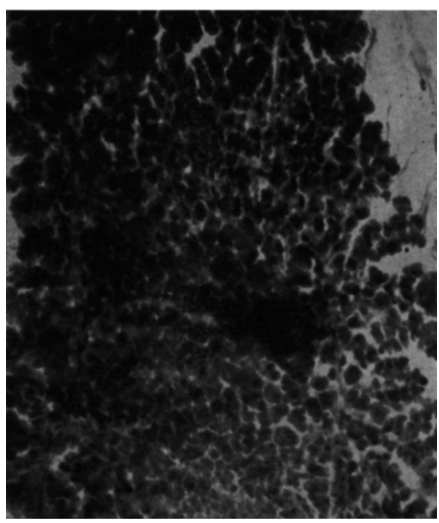

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gen injections. *4*. On twelfth day of pseudopregnancy. *5*. On twenty-third day of normal pregnancy. (From Parkes, 121,122.)

early stage compels a multiplanar development. Lateral and terminal buds are also apparent. Allowing for a marked variation in the degree of development in different individuals, it was clear that the branching and extension of this immediately postnatal duct system proceeded only slowly for the first four weeks; an interesting change during this period, however, was the formation at two weeks of a continuous, but still very minute, channel between the primary duct—and by implication the whole duct system—and the exterior. Development, still by duct extension, is far more rapid during the fifth week when separate gland systems begin to overlap, while in all glands the growth is no longer confined to one plane only. A second phase of greatly increased growth takes place at the ninth and tenth weeks, and, whereas the earlier sudden proliferative burst was not connected by Myers with any concomitant physiological stimulus, this later enhanced activity could be ascribed with confidence to the onset of puberty. At no stage during the prepubertal changes were true alveoli found and we may regard this period as one of extensive duct formation only, though "outpouchings" from the smallest ducts were deemed an early indication of lobulation.

Further growth changes are imposed upon the duct system at each recurrent estrous cycle (149), when the main evidence is a rapid formation and sprouting of lateral buds on the smaller ducts. This activity is followed, however, by regressive changes during the next pro-estrous period, and the net growth over the complete cycle is probably small and of very minor importance only as regards alveolar development.

The formation of this secretory tissue is however abundantly evident during the first half of pregnancy. Weichert and Boyd (170) found conspicuous lobule development in rats killed on or after the ninth day following conception, but noted also that there was little further increase in the absolute amount of secretory tissue during the second half of the gestation period, though the onset of alveolar secretory activity toward the end of pregnancy, with attendant distension of the alveolar lumina, resulted in an apparent extension of the parenchyma at this time. Roberts (135), in an earlier study on mammary changes in the rat during the second half of pregnancy, had reached similar conclusions, stating that there was little further development during this period, in which the glands, when sectioned, resembled "small islands of glandular tissue in a lake of fat." Cytologically he found no evidence of mitosis in the alveoli during the period studied and concluded that the gland had "already reached its maximum growth, so far as number of cells is concerned, by the thirteenth day of pregnancy." From this time until parturition the changes are those associated with the hypertrophy of the formed glandular elements preparatory to active secretion, and with the first incidence of secretion

itself. The cytological study by Weatherford (168) showed agreement with the contention that mitotic activity has practically ceased in the alveoli midway through pregnancy; but Jeffers (60), contrary to this view, claimed that mitotic figures are frequent during the second half of pregnancy and that hyperplasia therefore continues throughout this time.

Maeder (90) has shown that the histological picture remains constant throughout lactation, and is the same in all respects as that seen in the incipient secretory phase at the end of pregnancy. After weaning, however, definite changes were noted by this author on the third day, and the involution of the parenchyma continued progressively from this time until the thirteenth day when the gland was judged to resemble closely that of the adult virginal resting gland, once again being composed only of ducts. These observations substantiated an earlier and less detailed report by Myers and Myers (102).

The appearance of lobule-alveolar tissue is also to be seen in pseudopregnancy resulting from sterile copulation or mechanical stimulation of the cervix, and in the opinion of Freyer and Evans (31) the greatest proliferation during this time is qualitatively similar to that on the eleventh day of a normal pregnancy, though they judged the quantitative significance to be somewhat less. Selye and McKeown (142) have reported that they too have never failed to detect alveolar development in rats pseudopregnant as the result of the suckling stimulus provided by litters from other does (143), and Jeffers (61) has supplemented their observations by a cytological study of the mammary glands of rats used in such experiments, expressing her opinion that the glands could have supported lactation comparable to that occurring after the birth of a normal, full-term litter.

A study of the male rat gland from birth to ten weeks of age (104) has shown a development parallel with that of the female gland for the first five weeks; after this, growth is much slower but may still continue to some extent even after puberty. In contradistinction to most other species, both the extension of the duct system and alveolar development have been observed in mature males (4,88,165), the adult gland having a dense, compact development, while it is interesting to note the absence of nipples and the rudimentary condition of the second inguinal glands in all males examined.

II. Early Investigations

The earliest inquiries directed toward the elucidation of the mechanisms controlling the growth of the mammary gland have been adequately appraised in earlier reports (154,159), and we note here only the general

progress of thought in this field prior to the more modern work utilizing pure hormones or purified extracts with clearly defined endocrine properties.

The original, and somewhat intuitive conception of neural control was overthrown at the turn of the century by experiments involving nerve section and transplants of mammary tissue (see Turner, 154, for review). The alternative possibility of hormonal mediation gained ground rapidly and was upheld as a reasonable physiological hypothesis by the growing appreciation of similar work in other fields, dating from the classic experiments of Berthold, Brown-Séquard, and others in the nineteenth century (see Chapter I of this volume). The close association of mammary growth with pregnancy naturally focused the attention of workers upon the fetus, placenta, and ovaries as the probable active agencies of control, and ovariectomies and ovarian grafts gave encouraging and provocative results. Aqueous extracts of organs associated with the animal's reproductive life, for reasons easily surmized, were disappointing in their action—see for example the pioneering studies of Lane-Claypon and Starling (62)—and further advance awaited the first use in 1912 of lipide extracts (57). Progress remained comparatively slow, however, owing to the indefinite character of the extracts used, which did not permit satisfactory comparisons, either quantitative or qualitative, of one experiment with another. The development of an easy assay of estrogenic potency by the Allen and Doisy vaginal smear test, following the indicative experiments of Stockard and Papanicolaou, and the subsequent isolation and characterization of the estrogenic hormones, quickly brought a greater objectivity into this realm of accumulating, yet unassessable, data, and we may regard these two achievements as demarcating the years of modern inductive work upon which our newer knowledge of the role of estrogens in mammary growth is founded. The progress made in the years between these separate advances resulted from the use of ovarian, placental, and particularly urine extracts (52,64,159) of tested estrogenic potencies. Such preparations gave the first clear indication of the relationship between the power to develop the mammary gland and the estrogen content of the active substances used, and emphasized the need for pure ovarian hormone preparations as an essential step in the furtherance of research on the development of the gland.

Apart from the ovary as a whole, the participation of the corpus luteum in mammary growth had been suspected from an early stage. The proliferation of alveoli in pregnancy, in pseudopregnancy, and, in some species such as the dog, during metestrus could be correlated with the presence of active corpora lutea in the ovaries, but again the experi-

mental approach to the problem was hampered by inadequately characterized materials, until the preparation in 1929 by Corner and Allen of active corpus luteum extracts, and the isolation two years later of crystalline progesterone.

The role of the anterior pituitary, now believed to influence fundamentally the development of mammary tissue, has been the subject of more recent research and its "history" is still that of contemporary endeavor and will fall naturally into the scope of this review, as will also the more scant knowledge we have of the involvement of the thyroid and other endocrine glands.

III. The Ovarian Hormones and Mammary Growth

A comparison of the growth caused by the purified estrogenic hormones, estrone and estriol, and by a crude extract of estrogenic hormones from pregnant cows' urine (161) showed no significant differences in the amount or type of growth produced. The effects of these preparations were studied with the aid of three species, rabbit, rat, and mouse, and in each case it was clear that duct growth alone was being influenced and that the active principles in the urine extract were biologically similar to, and possibly identical with, the pure estrogens used in the experiment.

The animals used in these, and in similar studies having the same aim, were of both sexes. When using females it is of course obligatory, in attempting experimental growth, to use animals spayed prior to puberty so that normal duct growth shall have been inhibited by deprivation of the natural ovarian estrogen supply; alternatively, in species such as the rabbit which show no appreciable development of the glands in the prepubertal stage (159), the use of immature unspayed females should be possible within this limited period. On the other hand, males of some species—since the male gland responds to the same stimuli and, apart from the male ruminants (26), is equipotential with the female gland, while not being under the direct influence of the female gonad—frequently present more suitable experimental subjects; this is especially so in such cases as the mouse in which the male gland remains a mere rudimentary duct system throughout the normal life cycle (162). Castration has been shown unnecessary in several species, normal unoperated adults providing the best experimental subjects.

An extension of the work on duct growth, usually with estrone as the estrogenic hormone, confirmed for all species studied the basic importance of these substances for this particular type of development, a uniformity contrasting markedly with the species differences encountered in the ability, or inability, of estrogens to influence the formation of lobule-alveolar tissue. Concise summaries of the work bearing on both these

aspects of estrogen activity have been given by Nelson (107), Turner (156), Folley (21), and Petersen (124), and of laboratory animals, it will be sufficient to note in any detail here two of the most extreme instances: the mouse, for which a pure duct response has been postulated, and the guinea pig, which by contrast gives complete mammary development after estrogen treatment.

Turner and Gomez (163) demonstrated that 10 rat units (R.U.) of estrone administered daily to male mice would cause progressive growth of the duct system only, and that this growth was still to be seen after 100 days' treatment; they concluded that the normal response of this animal would seem to exclude any lobule-alveolar formation. The results were confirmed by Gardner *et al.* (36), whose male mice, on treatment with estrone, developed glands comparable in size to those of the virgin female animal. Even in this species, however, occasional reports (74) testify to the sporadic appearance of small isolated groups of alveoli in individual cases, though true lobule formation has not been observed as the result of physiological doses of estrogen in normal strains. An abnormal development in which the duct growth was stunted, but lobules of alveoli were formed, was reported by Gardner *et al.* (38) as the result of long-continued injections of estrone into both cancer-susceptible and cancer-resistant strains, but the growth was admittedly atypical; even so the appearance of an alveolar epithelium, which was in some areas showing secretory powers as the result of simple estrogen treatment, cannot readily be discounted in any estimation of the role of estrogens in the development of mammary tissue in this species. Similar results were obtained by Bonser (6), while evidence was adduced by Burrows (8), who painted the skins of male mice with different estrogens, that small differences in chemical structure could lead to appreciable differences in the biological responses evoked. This investigator detected alveolar growth in a proportion of cases on all treatments, but noted particularly that estrone seemed to be mainly active in extending the duct system, whereas estradiol gave far greater alveolar development and had only a relatively slight effect on the ducts.

More uniform results have been given by work on the guinea pig, and, following reports by earlier workers (52,63) claiming definite lobule-alveolar augmentation and development equal to that seen in pregnancy in ovariectomized females and in normal males as a direct result of estrogen treatment, Turner and Gomez (163) found that 20 R.U. of their pregnant cows' urine preparation injected daily for 40 days into adult males caused a glandular response similar to that seen in a female pregnant for 33 days. Even injections of only 1 R.U. over a similar period gave clear indication of alveolar proliferation. Similar results were

obtained with females which had been spayed before puberty. These results were further supplemented by the work of Nelson (108), who, like Laqueur and co-workers (63), was able to report complete development of the gland judged by the spontaneous induction of lactation when treatment was curtailed; excellent corroboration was afforded by his histological studies.

Of the other animals investigated it is thought that the normal response of the rat and the rabbit may be deemed primarily one of duct growth, with a slight degree of lobule proliferation in some cases (160, 165), while simple duct extension is postulated for the cat and dog (158,164). As instanced in the case of the mouse, however, complete agreement among workers has rarely been achieved for any species, and, while some small part of the disagreement may well be ascribed to differences of dosage, of the chemical nature, or purity, of the estrogen preparations used, or the varying age of the experimental animals at the time of treatment, a survey of the literature suggests the participation of intrinsic factors, to be considered in due course in this review, and of which our present knowledge is most inadequate.

First, however, and in further agreement with the instances of equivocal experiments already given, we may note some conflict of opinion regarding the effects of estrogens on the mammary gland in primates. In an undetailed report Turner and Allen (157) claimed lobule formation as the result of the long-continued treatment of a single normal male rhesus monkey. This was confirmed by Gardner and Van Wagenen (39), who also obtained similar development, following a shorter injection period, in the case of one spayed immature female. A similar female given prolonged treatment developed glands resembling those of normal pregnant animals in type, though rather smaller in size. Folley *et al.* (22) on the other hand, who also used the rhesus monkey, obtained less uniform results, only one of thirteen male monkeys and two of four ovariectomized females showing any alveolar formation in response to estrone injections; in these the response was only slight and rendered less significant by the presence of a condition of gynecomastia, with alveolar development, in one of a series of five normal untreated males which was also examined. The work provided some grounds for believing the female gland of this species to be more responsive to estrogenic stimuli than the male. It is perhaps of significance in assessing these various observations that the experimental periods chosen by Folley and co-workers were either much shorter (up to two months), or much longer (one year or more) than that found favorable for alveolar development in the male by Gardner and van Wagenen (six months). Gardner (33) has more recently reported complete morphological development in

young male and female monkeys given estradiol benzoate injections for periods of approximately eight months, and has also obtained an indication of an inhibitory effect on mammary growth when the weekly dosage exceeds 0.4 mg.

The recent discovery (15) of synthetic products chemically distinct from but biologically similar in their action to the natural estrogens and the production of these substances both cheaply and in quantity have led to an immediate extension of the investigations involving estrogens to cover the major domestic animals of importance to the dairy industry. Lewis and Turner (74,75) confirmed the power of diethylstilbestrol to act upon the mammary glands in very much the same way as do the natural estrogens when administered to mice, rats, rabbits, and guinea pigs, and it will be legitimate therefore for us to include studies with this and similar synthetic products in our consideration of estrogenic influence.

de Fremery (29,30) had already reported mammary growth in the virgin goat following percutaneous inunction of the udder region with an ointment containing estradiol monobenzoate, and his results were confirmed by similar inunction experiments in which diethylstilbestrol was used as the active substance (25,26). Administration of this synthetic hormone by injection in oil, by implantation (Fig. 2), or orally has also caused mammary growth sufficient to maintain abundant lactation in this species (74,77,78). It is of particular interest to observe the successful use of an ovariectomized female in the experiments conducted by Lewis and Turner (77), as also in the first encouraging report of similarly induced udder growth—inferred from the occurrence of lactation—in the bovine (167). The implication that progesterone, at any rate from an ovarian source, is not required for full glandular development in these two species will be more fully considered in due course. Confirmatory evidence of mammary growth in the bovine following treatment with synthetic estrogens has since been given by Reece (128), Folley and Malpress (23,24), Hammond and Day (50), and Parkes and Glover (123), among others. In all these reports the evidence for lobular as well as duct growth cannot be open to doubt, both the resulting measure of lactation, in some cases equalling that of normal parous animals, and the macroscopic appearance of whole udder slices bearing clear witness to this end. In most cases, too, it seems likely that luteal influence was excluded, since the ovaries were hypoplastic during treatment (23,50). A histological study by Mixner and Turner (101), however, has suggested that in virgin female goats the development may not always be truly normal following diethylstilbestrol treatment, and in any case both goats and cows have shown wide individual variations in response to estrogens; growth of udder tissue following the same treatment has

ranged from a complete absence of response to the formation of full-sized glands. In many cases it would seem that these two species offer a parallel to the complete mammary growth following estrogen stimulation which has been noted for the guinea pig, a development that clearly cannot, without qualification, be explained on any simple theory whereby estrogens are held responsible for the promotion of duct growth alone. It must however be remembered that diethylstilbestrol, on which our main conclusions for the cow and goat are based, may differ from the

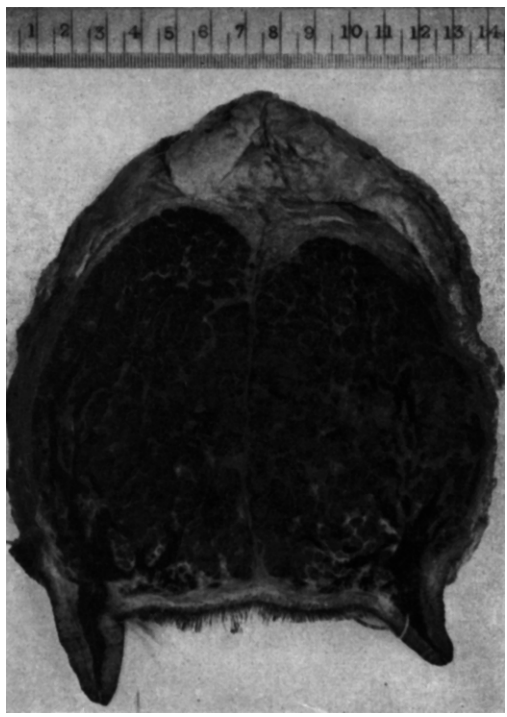

FIG. 2.—Section through whole virgin goat udder, showing glandular development following subcutaneous implantation of synthetic estrogen tablets. (Scale in centimeters.)

natural estrogens in the ability to proliferate lobular tissue (77), a hypothesis that receives support from the lobule formation observed by Mixner and Turner (101) as a result of diethylstilbestrol and estrone injections given to ovariectomized mice, and from the work of Burrows already quoted (8), but against which we may cite the mammary growth obtained by de Fremery (30) in the virgin goat given injections of estradiol benzoate.

It will be useful at this point to consider experiments designed to

throw light on the role of progesterone in mammary tissue formation, before attempting to give any general conclusions on the relative importance and significance of the ovarian hormones in the parenchymatous development of the gland. The complementary relationship existing between the ovarian hormones in their action upon the uterus, as evidenced by the proliferative and progestational phases of endometrial development, is now deemed to have a near parallel in the associated phenomenon of mammary growth. We have seen that only rarely does the fullest development of mammary tissue follow simple estrogen treatment in males or ovariectomized females, the guinea pig providing the only well-attested case for which both alveolar and duct formation in similar amounts to that occurring in normal pregnancy can be regularly obtained. The larger domestic animals seem to be subject to much individual vagary of response, a result which, were estrogens the only hormone needed for full mammary development, we should not *a priori* expect. Attempts to develop alveolar growth by progesterone treatment alone, however, proved in vain in many species, and early optimistic reports by Nelson and Pfiffner (114), who claimed lobule development in male and spayed female guinea pigs, rabbits, and rats in response to injections of corpora lutea extracts, were later withdrawn (108) on suspicion that some estrogen had in fact been present in the relatively crude extracts used. Turner and Schultze (165) injected lipide extracts of corpora lutea into castrated male and female rats and found no lobule or end bud formation, results which were reproduced for the rabbit (160) and for the guinea pig (163); for the latter animal it was observed that the extract was ineffective even after a preliminary treatment with estrone. An interesting, yet anomalous, result was that reported by Gardner and Hill (37), who found an extension of the duct system in male mice, both castrated and noncastrated, after injections of highly purified extracts of progesterone. The presence of slight estrogen contamination, although unlikely in quantities sufficient to affect the result, was not absolutely excluded as a possibility. This work has been extended by the observations of Mixner and Turner (98), who obtained lobule-alveolar growth in castrated female mice by injecting high doses (3–7 mg.) of progesterone alone. Conflicting reports have been given by workers using the rat as an experimental animal, and in the view of Selye (138) negative responses recorded earlier by himself and co-workers (139), and by Astwood *et al.* (4) may be ascribed to the insufficiently large dosages given. In his later work (138), as also in that of Reece and Bivins (129), 15 mg. progesterone given daily over a period of ten days to mature ovariectomized rats induced definite lobule-alveolar development. There is disturbing evidence, however, that the degree of proliferation might depend on the

time of ovariectomy relative to the time of starting treatment, decreasing as this postoperative period increases (138). This would imply a possible participation of residual unmetabolized and unexcreted estrogens in the positive responses. Doses of a similar order given to mature ovariectomized female rhesus monkeys, for periods of approximately one month, have also led to an increase in the amount of lobule tissue present in biopsy specimens (51). Further studies are clearly desired both to confirm these results and to extend the use of these comparatively high doses of the purified hormone to the investigation of the proliferative power of progesterone when given alone in other species.

The occasional reference to the inability of a preliminary treatment with estrogens to prepare the gland for an active response to subsequent progesterone injections is in sharp contrast with the results obtained with simultaneous injections of these two hormones. Here the reports have been most uniform, attesting to an alveolar response, resulting from the hormonal synergism, superimposed on the customary estrogen duct stimulation. Turner and co-workers (158,160,163,165), for the rabbit, rat, mouse, and cat, and Anselmino *et al.* (2) and MacDonald (87), for the rabbit, have all helped to establish firmly this synergistic relationship which results in the formation of glands very similar indeed to those of normal pregnant animals; in the case of the guinea pig, in which estrogen alone can give full lobule formation, the supplementary treatment with progesterone failed to alter the character of the tissue, or to induce a more extensive development (108).

More recently attention has been directed to the proportions in which the two hormones have to be given in order to achieve an optimal result. In particular, Lyons and McGinty (83), using male rabbits given a standard daily dose of 120 I.U. estrone, studied the effect of daily doses of progesterone varying from 0.25 to 8 I.U. Synergism was maximal with 1 I.U. (*i.e.*, 1 mg.), although not productive of the full development to be observed in pregnant animals (Fig. 3). Further experiments were therefore performed in which the progesterone dose was kept constant at this optimal level, but the estrone dosage varied from 30–960 I.U. daily (137). The best results, although still not fully equivalent to the proliferation in the glands of pregnant animals two to three weeks after conception, were given by those groups receiving 240 and 960 I.U., but, as male animals were used whose glands were of course initially more rudimentary than in the female, it is probable that the development did represent very closely the natural conditions of mammary development for this species (Fig. 4). A similar study in which ovariectomized virgin female mice were used has been reported by Mixner and Turner (98). With a constant daily estrogen dose of 133 I.U. these authors observed a

satisfactory growth of lobules with 1.0 to 1.5 mg. progesterone daily. They further showed that, if progesterone administration was held at 1 mg. daily, optimal synergism was given over an estrone range of 40–133 I.U. Unfortunately their range of estrone doses jumped from 133 to 1200 I.U.; at the higher value the lobular response, although suboptimal, was still evident. It would seem therefore that the relative amounts of

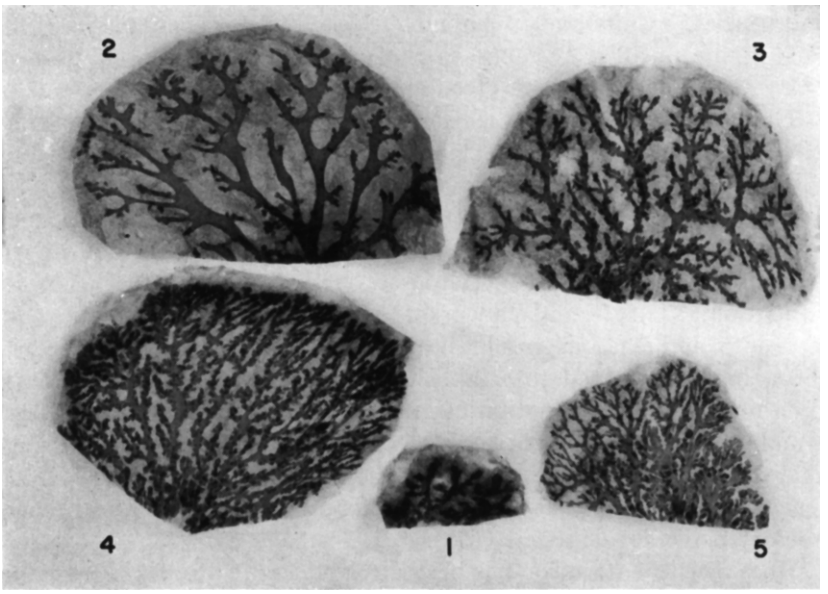

FIG. 3.—Experimental development of the rabbit mammary gland. Figures represent approximately one half of a male rabbit mammary spread after eighteen injections given over a 28-day period (× 1.5). Single injections: 1. None. 2. 120 I.U. estrone. 3. 120 I.U. estrone and 0.25 I.U. progesterone. 4. 120 I.U. estrone and 1 I.U. progesterone. 5. 120 I.U. estrone and 8 I.U. progesterone. (From Lyons and McGinty, 83.)

the two ovarian hormones required to evoke the fullest mammary response is of the same order for the mouse as for the rabbit. Since the international unit of estrogens is equivalent to 0.1 μg. estrone, we may regard the evidence from these two species as suggesting a proportional relationship of about 40:1 (progesterone:estrone), by weight, for the best mammary development.

It is of great interest, in view of the proportionality found necessary for effective synergism in mice and rabbits, to contrast the authors' unpublished and as yet incomplete results on the simultaneous implantation of goats with progesterone and estrogen tablets, with similar work, in which the hormones were injected, carried out by Mixner and Turner

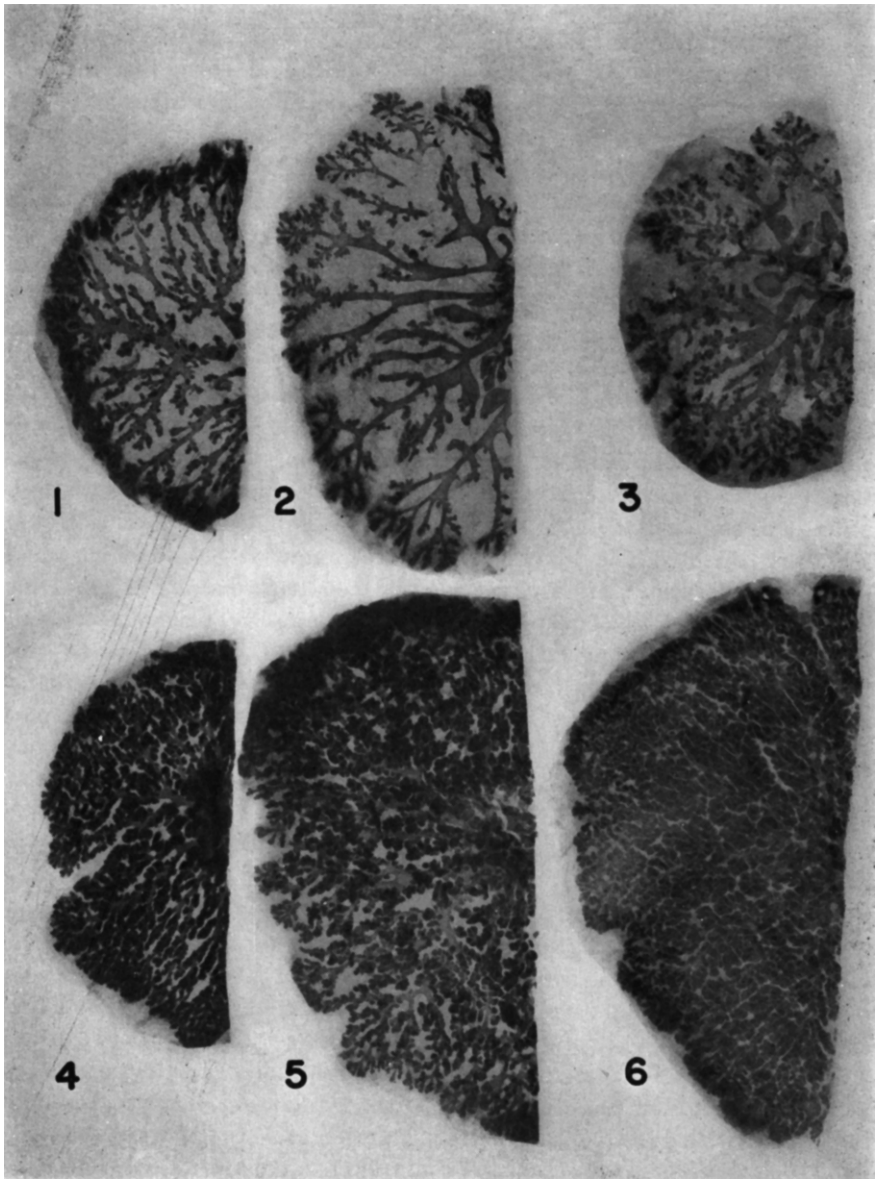

FIG. 4.—Experimental development of the rabbit mammary gland. Figures represent approximately one half of a male rabbit mammary spread after 25 injections given over a 35-day period (× 1.5). Single injections: *1*. 30 I.U. estrone. *2*. 240 I.U. estrone. *3*. 960 I.U. estrone. *4*. 30 I.U. estrone and 1 I.U. progesterone. *5*. 240 I.U. estrone and 1 I.U. progesterone. *6*. 960 I.U. estrone and 1 I.U. progesterone. (From Scharf and Lyons, 137.)

(101). In the former experiments the gravimetric ratio of progesterone absorbed to estrogen absorbed was never in excess of 9:1, and more usually about 2:1. The estrogen used—hexestrol—has, like diethylstilbestrol, been variously computed to have a biological activity 2.5 times as great as (15), or equal to (19), that of estrone, and, although as shown by Emmens (18) too much reliance should not be placed in comparisons of one estrogen with another, since the relative activities may vary with the assay used or even the technique adopted, the possibility remains that the effective ratios were smaller still. The highest mean daily absorption of progesterone for these animals was about 5 mg. It is perhaps not surprising, therefore, that no evidence of increased alveolar development could be observed even after 100 days in those animals given the dual hormonal treatment, when compared with controls receiving the estrogen alone. Mixner and Turner (101) however were able to report development after sixty days corresponding with that of midpregnancy, in goats receiving 20 or 30 mg. of progesterone daily, and diethylstilbestrol sufficient to give a ratio of progesterone:diethylstilbestrol of 200:1 by weight. The difficulty, when working with goats in such small experimental groups, of ensuring adequate controls, having in mind the very wide variations of response given after simple estrogen treatment, necessarily prevents any strict conclusions being drawn from these contrasted results, but they do suggest a possible uniformity in the quantitative synergism of the ovarian hormones in their effect on mammary growth in different species.

Using the semisynthetic progestational hormone—pregneninolone or ethinyltestosterone—together with estrone, Mixner and Turner (96) have demonstrated a synergism for mammary growth in spayed female mice very similar indeed to that shown by the natural hormone itself. There have recently been other reports too, indicating that progesterone is not even the only naturally occurring hormone which may develop the lobule-alveolar system. In particular the involvement of the steroid adrenocortical hormones must be considered a real possibility, and further work in this field is urgently required. A relationship between the adrenal cortex and the mammary gland had been inferred by some authors as the result of clinical observations on the association of cortical tumors in the male with gynecomastia, but no experimental studies were available until van Heuverswyn *et al.* (54) obtained extensive duct growth in unoperated or castrated male mice, following the injection of 4.0 mg. desoxycorticosterone acetate on alternate days over a period of sixteen days. The response was greater than that given by estradiol benzoate given similarly in daily doses varying from 0.016 to 0.666 mg. No lobule-alveolar growth was recorded by these authors. Nelson *et al.*

(111) have reported mammary growth in young male guinea pigs after similar injections, and since lactation started in some cases when treatment was stopped we may reasonably infer that alveolar tissue had been formed. Speert (146) also found active lobule-alveolar growth in the glands of two adult ovariectomized rhesus monkeys given daily 10 mg. desoxycorticosterone acetate for 13 and 24 days. Five mg. given daily for 30 days failed to stimulate growth in a third monkey, however. Mixner and Turner (99), comparing the power of various steroid substances to produce lobule-alveolar growth in virgin ovariectomized mice given supplementary estrogen treatment, rated desoxycorticosterone acetate to be one third as active as progesterone, and also noted that acetoxypregnenolone—another related steroid—had approximately one sixteenth of progesterone's activity. Reports on the effect of adrenalectomy on underfed (9) and normally fed (133) rats given sodium chloride therapy have agreed in part. Thus, increased growth compared with glands from control animals was observed insofar as the number of lateral and end buds were concerned, though the area of the glands was affected only in the first-mentioned experiments. The mechanisms responsible for these changes are in doubt, though direct ovarian implication would seem unlikely since the same changes were seen in normal-adrenalectomized and spayed-adrenalectomized animals. In a more recent investigation Cowie and Folley (13) have failed to confirm this proliferative effect of adrenalectomy, in fact the mammary gland area was significantly decreased by the operation.

Leaving aside any question of pituitary mammogenic activity, which according to modern theories depends on preliminary evocation by ovarian and possibly other related hormones, we may fairly assess control of mammary growth as originating in ovarian and adrenal relationships. It will be evident that any attempt to form a concise theory of this development will be baffled by two recurrent observations: first, the variation among species encountered in the experimental growth of the gland, ranging from the mouse, which apparently requires both estrogen and progesterone for the development of a potentially secretory parenchyma, to the guinea pig, for which estrogen alone has been deemed sufficient; and second, the variation within species, for examples of which we may instance the alveolar development following estrogen treatment in certain strains of mice, and the very wide differences in the mammary response of heifers and cows given simple exogenous estrogen applications. On the other hand, certain facts regarding the hormonal regulation seem to be cardinal for almost all the mammals which have been relatively intensively studied. Chief among these we may note the ability of estrogens to cause, at least, an extensive duct growth—though

the inability hitherto to increase in any marked degree the gland of the male goat by such treatment must be recorded as an exception (30,77)—and the probable power of estrogen and progesterone, provided they are administered simultaneously and in the correct proportions, to ensure a full mammary development in normal or ovariectomized females, as also in the males of most species investigated. Here again we must cite the evidence that the guinea pig gland can proliferate fully without any intervention of progesterone at all. We are therefore posed with the question: do true species variations exist in the qualitative hormonal influences required to form the mammae of different animals—a theory which may most easily explain observed experimental data, but which on purely *a priori* grounds might well be unconvincing—or might there be an alternative hypothesis whereby common hormonal factors are held responsible for duct and alveolar growth, respectively, whatever the species, and a concordancy of the established results is sought on the basis of quantitative rather than qualitative differences?

The arguments in favor of the first view may be fully appreciated by any direct reading of the experimental inconsistencies to be observed in reports relating to different species, and it is the possible alternative that we shall consider here.

A warning was first sounded by Folley (21), who pointed out that, since the isolation of progesterone from a concentrate of ox adrenal glands by Beall and Reichstein (5), none of the experiments purporting to have obtained alveolar development in males and immature or ovariectomized females as the result of estrogen treatment alone could in fact be interpreted with certainty in this way. The realization that at least one of the adrenocortical hormones—desoxycorticosterone—also has very pronounced progesterone-like properties in its action on the mammary gland has further focused attention on the adrenal cortex as an alternative source of hormones stimulating lobule-alveolar proliferation. It is interesting, too, to note the excretion of pregnanediol by rabbits after injections of desoxycorticosterone acetate (55), and the later report of a similar conversion in man and the chimpanzee (56). The intermediates in this change remain hypothetical but it is not impossible that some of them might also possess, and be of sufficient permanence in the tissues to exert, progestational powers. Similar biologically active substances could be envisaged as arising in the anabolism or catabolism of the other steroid cortical hormones; our knowledge of steroid metabolism is, however, so small at the present time that we cannot with any confidence assume that the metabolic pathways are the same for all species, or even if this should be so, that the rates of conversion and destruction have any species uniformity. Reports of the progestational activities of adrenal

extracts from horses, cattle, and pigs have been recorded (10,20). It will be readily appreciated, therefore, that progesterone itself can no longer be considered a specific hormone for the mediation of alveolar growth, even though the evidence is still strongly in favor of its premier role in this respect, but that the adrenal gland must be henceforth regarded as having supplementary powers in this function. This conception immediately suggests a possible explanation for the interspecies differences found in experimental studies, since quantitative variations alone in the amount of progesterone-like substances produced by the adrenal cortex in different species could presumably condition widely divergent mammary responses to simple estrogen treatments. Further studies on the metabolism of the adrenal steroids and on the biological properties of intermediary substances, especially if undertaken with a view to the comparative biochemistry of these changes, should greatly clarify the confused picture which has until recently been presented by the hypothesis of simple ovarian control of mammary hyperplasia, and might possibly establish a complete absence of lobule-alveolar activity on the part of estrogens for all species. These considerations will of course be unaffected by any conception of pituitary participation in mammary growth, provided such mediation is itself under ovarian control.

Since it would seem from various studies already quoted that the estrogen:progesterone-like substance ratio has to fall within certain defined limits for optimal synergism, the path and rate of metabolism of estrogens, and of their excretion, might also account for response variations among species. Here again our present knowledge of metabolic changes is far from complete, though recent work would seem to show that the pathways and excreted products may well vary among species (150); and also within species, depending on the presence or absence of other hormones, amount of estrogenic hormone present, and other fluctuating criteria (see reviews by Doisy *et al.*, 16, and Pincus and Pearlman, 126, for admirable and recent summaries). We can therefore carry our argument one stage further, to afford a reasonable explanation of the intraspecies variations obtained by different workers using different strains of the same laboratory animals, or by all workers in their own experiments when using the larger domestic mammals.

For, where two factors, the metabolism of both the endogenous and administered hormones in their qualitative as well as quantitative aspects, are unknown, and the potential supplementary endogenous progestational activity of the adrenal gland is a matter of pure conjecture, uniformity of response will probably not readily be obtained by uniformity of treatment; it is perhaps in accord with this view that

undoubtedly the largest intraspecies variations so far recorded have been those found following estrogenic treatment of the larger mammals—cows and goats—whose widely different potentialities under normal conditions are frequently suspected, at least in part, to be hormonal in origin. The prolonged hypoplastic condition of the bovine ovary under estrogen stimulation (23) will of course make extraovarian sources of progesterone of supreme importance, and despite the presence of ovaries in the treated animals a condition tantamount to ovariectomy is in fact imposed.

IV. The Anterior Pituitary Gland and Mammary Growth

Probably the most lively interest at the present time in connection with the endocrine control of the mammary gland revolves round the role of the pituitary and the controversial question of the existence of specific mammogens secreted by this gland. It would be premature in the present state of our knowledge to attempt any definite statement, but we may recognize three distinct viewpoints in favor of which evidence exists, and for which further confirmation is still being sought. These are, first, the mammogenic theory propounded by Turner and collaborators (73,101), which claims that the ovarian hormones, although essential for the growth of the gland, do not exert their effects directly, but indirectly through the mediation of the anterior pituitary gland. It is held that the pituitary, following stimulation by estrogens and progesterone, secretes specific "mammogens" of its own, which are in fact the hormones acting directly on the undeveloped mammary structures. Two mammogens are postulated, one evoked by estrogen stimulation and known as the "duct growth factor," the other by progesterone and related substances, or estrogen and progesterone together and known as the "lobule-alveolar growth factor." It will be clear that this modern hypothesis, if accepted, will in no way render invalid the earlier work on the mammary function of the ovarian hormones, but that these substances will retain their fundamental importance as primary agents of growth. Secondly there is the more conservative attitude which holds that those who support the mammogenic theory have failed to sustain their claims by clear-cut experiment, and that all effects so far observed are explicable on the basis of direct ovarian control; and lastly there is the intermediate view (131), more recently expressed, which attributes growth to a synergistic relationship between the ovarian hormones and pituitary mammogens.

It may be that species differences, age differences, and like factors may here again be basically responsible for much of the disagreement among the results of separate groups of workers. Recent experiments on the effects of steroids on the mammary glands of hypophysectomized

rats by Leonard (67) and Smithcors and Leonard (145) may be interpreted as indicating that the age of the experimental animal, both absolute and relative to the time of hypophysectomy, may greatly affect the results obtained; but it seems more likely that confusion arises from problems of experimental technique and in particular the fact that most of the critical experiments to test the involvement of the pituitary necessarily demand the use of hypophysectomized animals. In such work the danger of incomplete hypophysectomy presents a very real difficulty. Gomez *et al.* (46) have shown that, in the presence of residual fragments amounting to such a small total as 2% of the excised gland, the mammary response of hypophysectomized male mice following estrogen treatment could be changed from a negative to a positive response essentially the same as that found in intact animals similarly treated. Substantially the same results have been reported by Gomez and Turner (44) for the guinea pig, rat, rabbit, cat, and ground squirrel. The authors in consequence stressed the danger of interpreting positive responses in hypophysectomized animals treated with estrogens as evidence of an absence of pituitary mediation in mammary growth changes, unless a rigorous postmortem histological examination of the *sellae turcicae* of the experimental subjects had established with certainty the thoroughness of the operation.

Further, it is well known that hypophysectomy necessarily involves other changes in the experimental animal affecting its general well-being and its fundamental endocrine relationships, so rendering it in many respects abnormal. As a result of this, and in contradistinction to the attempts to attribute the positive responses of hypophysectomized animals following estrogen treatment to incomplete hypophysectomy, some workers have adduced evidence that, in completely hypophysectomized animals, negative responses to estrogen may be due to general depressive effects of the operation, such as a lowering of the plane of nutrition. Astwood *et al.* (4) tested this possibility by injecting two groups of intact young rats with estrone for a period of fourteen days; one group was placed on a restricted diet, approximating that consumed by hypophysectomized animals, so that weight was lost throughout the experimental period, while the other group was fed normally and gained weight. The latter group showed a typical extension of the duct tree following treatment; comparable growth was not seen in the case of the poorly fed animals. The authors suggested therefore that the arrest of body growth following hypophysectomy might explain the observed failure of estrogen to prevent the regression of the mammary gland encountered under these conditions in the immature rat. Nathanson *et al.* (106), developing this theory, found that hypophysectomized rats

treated postoperatively and simultaneously with estradiol benzoate and a pituitary growth complex preparation which was itself inactive mammogenically, showed graded mammary responses which directly paralleled the weight increases following the operation; but, since, even so, the degree of development never approached that given by intact animals treated with estrogen alone, they were inclined to assign some mammogenic activity to the hypophysis itself, despite the clear "nutritional" effect. Samuels *et al.* (136), on the other hand, in experiments in which the weight of hypophysectomized rats was maintained, not by injections of growth hormone but by forced feeding coupled with desiccated thyroid administration, failed to demonstrate any such connection between the plane of nutrition and mammary development; they concluded that Nathanson *et al.* had achieved their results solely by virtue of some factor, present in the pituitary extract they used, having either mammogenic activity or an essential metabolic function evinced only in the presence of estrogens. Trentin and Turner (153) have observed an inverse relationship between the food intake level of normal male mice and the amount of estrogen required to produce a minimum duct growth response. They interpret this, in the light of other known depressive effects of inanition on the secretory activity of the pituitary, as indicating pituitary participation in ovarian–mammary relationships. Their contention, however, that, if the action of estrogen on the mammary gland were direct, the response would, if anything, be increased by inanition, is based on the imperfect analogy that inanition may lead to a heightened sensitivity of certain pituitary-controlled glands to hormonal stimuli, and must be regarded as most dubious.

Finally, attempts at replacement therapy by different pituitary fractions have all too frequently involved difficulties of interpretation and comparison owing to the variable or even uncertain composition of the extracts used, and the crippling doubts in any case regarding the reality of their mammogenic potencies.

It will be clear therefore that attempts to solve the riddle of pituitary mammogenic function are fraught with difficulty and rarely permit any conclusions which can be accepted without some reservations. In particular, the imperative need for checking the thoroughness of pituitary removal in experiments involving hypophysectomy may be deemed to throw a cloud of suspicion over much of the early work in this field, which remains one of speculation for the scientist and will probably require some additional, new experimental approach before its enigmas are fully solved.

The conflicting evidence accumulated from the attempts to determine the action of ovarian hormones, particularly estrogens, on the mammary

glands of hypophysectomized animals, and the effects of pituitary implants and extracts on normal and hypophysectomized animals has been ably reviewed by several authors (21,107,156). More recent reports in which these methods have been used have failed to clarify the problem, as may be seen by reference, for example, to the work of Reece and Leonard (131), who were unable to stimulate any mammary development in hypophysectomized rats treated with estrogen alone, and to the impressive study of Fredrikson (28), who using hypophysectomized rabbits found that treatment with estradiol monobenzoate and progesterone caused just as much glandular development, both of ducts and alveoli, as could be expected in normal rabbits similarly treated, thus fully confirming the earlier work of Asdell and Seidenstein (3) on this species.

An important step in the history of this investigation was taken when Gomez *et al.* (47) reported that, if male hypophysectomized guinea pigs were each given an implant of one male rat pituitary daily for twenty days, extensive alveolar development could be produced comparable with that given by injections of estrone into normal guinea pigs, provided only that the pituitary implants were obtained from rats previously injected themselves with estrogen. This work, apparently providing positive evidence of a pituitary factor essential for mammary development and formed as a result of estrogen stimulation, provided the germ of the mammogenic theory. The authors distinguished it from the lactogenic, thyrotrophic, and adrenotrophic hormones, preparations of which, under similar conditions, had failed to give any commensurate mammary response (44). Confirmatory evidence was produced (45) when duct and alveolar growth were stimulated in immature, spayed female rabbits and rats by injections of fresh pituitary material obtained from cattle in the first half of pregnancy. Similar injections of pituitary powder from nonpregnant heifers failed to stimulate such growth; again there seemed therefore to be an interdependence between the production of "mammogens" and the ovarian hormones. Since the latter experiments were conducted using ovariectomized animals as the test subjects, a tentative conclusion could also be drawn that the action of mammogens on the mammary gland was unaided and did not involve any synergistic relationship with ovarian steroids. However, these results have not been unfailingly reproduced by other workers; Nelson (109), for instance, using hypophysectomized female rats and adult male mice obtained the same mammary growth following implantation of pituitaries from untreated rat donors, as he did from those of estrogen pretreated animals; while Reece and Leonard in similar experiments (130), also reported the same degree of development in both cases. The latter authors supported the claim for the existence of a specific hypophyseal mammogenic hor-

mone on the grounds that their implanted animals always showed a greater mammary development than unimplanted hypophysectomized controls.

Seeking to develop their mammogen theory, Turner and colleagues formulated an assay technique for their duct growth factor—mammogen I (73). Normal male albino mice were found to give a suitable biological response to subcutaneous injections of fresh macerated anterior pituitary tissue taken from pregnant cattle, involving the appearance of thick ducts with side branches and large club-like end buds. On the basis of these observations a mammogenic mouse unit was defined as the amount of tissue or extract, given subcutaneously once daily for six successive days, which would produce definite signs of development in one or more glands of 50 ± 10% of a minimum of ten male albino mice weighing 15–25 g., the glands being removed on the seventh day. In its present form, however, the absence of a clear increase in the percentage of positive responses attendant upon an increase in dosage must be regarded as an unsatisfactory feature of the test; and it seems inadequate to explain a decreased response at higher levels by reference to the established and supposedly parallel stunting effect of large amounts of estrogens on the mammary gland. In the latter case we are considering an over-dosage beyond the limit of positive biological response; in the former the apparent inability to reach even a threshold value in the case of about 40% of the mice involved in a given test. It is, in any case, confusing to compare the varying effect that an increasing dose of a substance may have on individual animals with the fluctuations in the percentage evocation of response in a group of animals. The possibility of refractoriness in some of the mice seems more plausible, or indeed that endogenous factors are influencing the assay to a variable extent in different individuals. With the aid of the assay method an increase in the mammogen I content of cattle hypophyses during the first half of pregnancy was demonstrated, and values were also obtained for the hypophyses of nonpregnant cattle, for pregnant rabbits, and male rabbits pretreated with varying amounts of estrone. Since in many cases, however, the stringent conditions of the assay appear to have been disregarded and only the merest approximations to a correct value obtained—deduced questionably from responses falling outside the 50 ± 10% range, and from test groups of less than ten mice—further work will be required before the findings can be accepted without reserve.

Attempts to identify the mammogen duct growth factor with any of the known pituitary principles have met with little success. Interest has mainly centered around possible associations with the growth hormone or lactogenic fractions, and the results of Nathanson *et al.* (106)

already quoted might be construed as evidence in favor of participation of the growth hormone in mammogenic responses, in synergism with estrogens. Reece and Leonard (131) also found that for the hypophysectomized rat the growth hormone preparation used seemed to supply the necessary substance enabling the mammary glands to respond to estrogen treatment. Gardner and White (40) on the other hand have demonstrated mammary growth in hypophysectomized male mice following the simultaneous injection of estrogen and purified prolactin preparations, and Gomez (42) also was able to report extensive duct growth in hypophysectomized, castrated guinea pigs, provided the treatment with prolactin and estrogen was sufficiently prolonged and the dose of pituitary principle sufficiently high. He was inclined however to attribute the response to distinct mammogenic factors present in his prolactin preparation. Supplementary data accrued from this and other work in which pituitary extracts were used, showing that the significant amounts of other pituitary hormones frequently present were ineffective in causing any mammary proliferation. Gardner and White (41), however, using hypophysectomized male mice, reported, contrary to most other workers, some mammogenic activity resulting from concurrent injections of estrogen and a pituitary extract having marked adrenocorticotrophic activity. The "lactational growth" observed by Lyons (80) and restricted to those sectors of proliferated lobules which he directly injected with lactogenic hormone preparations has been more fully considered in the next chapter, but should be noted in the present context.

The claim that the pituitary duct growth factor, unlike other hormones from this gland, is soluble in fat solvents (72,73), has more recently been reinvestigated. It is now believed (151) that the factor does indeed resemble other pituitary principles in that the activity after extraction of the fresh glands resides in the protein fraction. The significance of much work published on the mammogenic properties of lipide extracts of pituitaries is now therefore rendered of small account. Greep and Staveley (48) first drew attention to the error of attributing duct growth powers to lipide extracts when, using such solutions obtained from cattle pituitaries which were themselves able to induce duct growth and end bud formation in spayed and hypophysectomized immature female rats, they found that the mammogenic activity of the original whole tissue had not been extracted, but in great part was still present in the tissue residues.

It will be appreciated that, in view of the discrepancies and general lack of agreement bearing on fundamental aspects of the mammogen theory so far as it relates to the duct growth factor, any premature

dogmatism regarding its postulates must be dangerous. It remains a pressing field for further research particularly for the revision of assay techniques and the better characterization or isolation of the active principle.

The literature concerning mammogen II, the lobule-alveolar growth factor, has been reviewed by Mixner and Turner (101). A preliminary report (93) demonstrated the ability of injections of fresh anterior pituitary material from cattle to cause lobule-alveolar growth in young ovariectomized virgin female mice. Development akin to that seen in pseudopregnancy was obtained in some cases. A tentative but unsatisfactory assay technique was developed using spayed virgin mice as the test animals (94). They were injected under specified conditions with the pituitary material under assay, but the alveolar responses were irregular and the method was superseded by one in which the pituitary injections were given with simultaneous estrogen administration. In its final form (101) the test still makes use of the nulliparous spayed mouse weighing between 12 and 18 g. as assay animal, which is injected subcutaneously once daily for ten days with the material on test and with 7.5 I.U. of estrone. Glands are removed on the eleventh day, and a mouse unit is defined as the total amount of material required per mouse to ensure lobule-alveolar growth, comparable with that of glands taken from mice four to eight days pregnant, in 50 $\pm$ 10% of a group of ten test animals. Under these revised conditions the test is far more sensitive and a clear relationship exists between the dose of pituitary material injected and the percentage of positive responses obtained. A parallel is thus offered to the proportionality shown when graded doses of progesterone and some other related compounds are given, together with estrogen, in place of the pituitary injections (94)—circumstantial evidence that the lobule-alveolar factor is secreted as a direct result of progesterone activity. An attempt to explain this sensitizing action of estrogens within the framework of the mammogen theory has led to the suggestion (98) that it might be due to an accessory and direct action of the estrogen on the stromal tissue of the mammary gland, whereby an increased vascularity and hyperemia is caused, leading to a greater permeability of the blood vessels and a heightened mammogen concentration in the region of the developing parenchyma. This view is supported by the demonstration of a similar function of estrogens in other fields—*e.g.*, the hyperemic reaction of the uterus (53), or the sexual skin of monkeys (11). It was also thought that estrogens might, in addition, have a special direct sensitizing effect on the mammary gland which would make it more responsive to stimulation by mammogenic factors. There is evidently a close connection between the ability of estrogens to

enhance the effect of the lobule-alveolar factor in intact mice and their action in promoting an increased duct response to various pituitary preparations in hypophysectomized mice (40), and the alternative possibility that in both cases it is the pituitary preparation which is sensitizing the mammary gland to the direct action of estrogens must not be overlooked.

An attempt to characterize the lobule-alveolar factor has shown that it is probably protein in nature but distinct from the lactogenic, thyrotrophic, and gonadotrophic hormones (92), though this report must be considered in its relation to the evidence presented by Lyons (81) on the ability of purified lactogenic hormone preparations to maintain a normal duct system with a few alveoli in hypophysectomized female rats, and of crude lactogenic preparations (containing also adrenotrophin and growth hormone) to cause an incomplete lobule-alveolar development in similarly operated animals. No other data are available to test its relation to the growth, adrenotrophic, or mammary duct growth hormones. In view of the possibility of adrenocortical hormone participation in lobule-alveolar growth, some measure of the adrenocorticotrophic hormone content of pituitary preparations assayed for mammogen II activity would clearly be of interest in order to establish that pituitary lobule-alveolar activity is not in fact an indirect function of the gland, mediated by the cortical steroids. This view receives some support from the work of Cowie and Folley (13) showing that improved duct and alveolar development follows the treatment of castrated male rats with ox anterior pituitary extracts, provided only that the adrenal glands are not removed. No difference in the responses of adrenalectomized and nonadrenalectomized female rats given similar treatment was observed, however. That the alveolar growth is not caused by the presence of progesterone in the anterior pituitary extracts has been shown by Trentin *et al.* (152).

We may note in this connection that since duct growth has been observed in normal and castrate male mice following treatment with progesterone alone (37), adrenal participation might also be contributory to positive responses in the mammogen I assay.

An interesting application of the mammogen II assay was reported by Mixner and Turner (97), which might seem to offer independent evidence of the validity of the mammogenic theory. An increase of 10°C. from 25° to 35° in the temperature to which the mice were subjected during assay was found to cause a great decrease in the response to injections of progesterone and estrogen, whereas the response to pituitary extract and estrogen was unaffected. The authors interpret these results as indicative of a decreased ability of the mouse pituitary to secrete mammogen II in response to progesterone stimulation at the

higher temperature. It is a pity, however, that the percentage positive responses at the lower temperature—progesterone treatment, 51.7, pituitary treatment, 86.7—were not more nearly comparable.

The large body of facts now accumulated having relevance to the mammogen theory permits no final statement to be made of its validity. One might hope for further enlightenment to be shed on this rather confused picture if experiments were conducted on assay animals subjected to adrenalectomy before pituitary preparations were tested. Particularly difficult to explain by the tenets of the mammogen theory are the many instances reported of localized glandular development following inunction of single mammae with ointments containing estrogens. MacBryde (86) noted greater growth in human breasts so treated compared with the contralateral control breasts treated with the ointment base only. Confirmatory reports were published by Lyons and Sako (85), who, using young male rabbits, noted greater duct growth in the estrogen-treated glands; in one case, although the same differential growth effect was found, the control gland too had developed to a certain extent, presumably indicating a better absorption of the estrogen in this animal or alternatively a greater sensitivity to the hormone (Fig. 5). Speert (147) and Chamberlin *et al.* (11) have demonstrated identical effects for young male monkeys, the latter authors drawing attention to their resemblance to the localized responses following the application of estrogens to the sexual skin of *Macaca mulatta*. The use of gonadectomized male and female guinea pigs (110) has shown that in this species too, unilateral growth follows unilateral percutaneous administration of the hormone.

In view of this general agreement regarding the action of locally applied estrogen in normal or castrated animals, the observations of Leonard and Reece (70) on the effect of similar treatment given to castrated, hypophysectomized rats are of great interest in their bearing on the validity of the mammogen theory. In no case in which hypophysectomy was complete was any mammary growth seen in the estrogen-inuncted gland, or its control, a fact clearly arguing some pituitary involvement. Rats weighing, apparently, about 100 g. were used in these experiments and treatment was delayed for three weeks following the operations; it would be interesting, in consequence of a later report (67), in which a greater response to estrogen injections in seventy-day-old rats was demonstrated when treatment was begun immediately after hypophysectomy rather than at the end of a postoperative recovery period, if the work could be repeated observing these more favorable conditions.

The suggestion that a local hyperemia in those glands inuncted with

estrogen conditions the differential response in normal animals has been put forward by Lewis *et al.* (71) and by Mixner and Turner (98) to explain the unilateral effect. They point out that mammogen I was

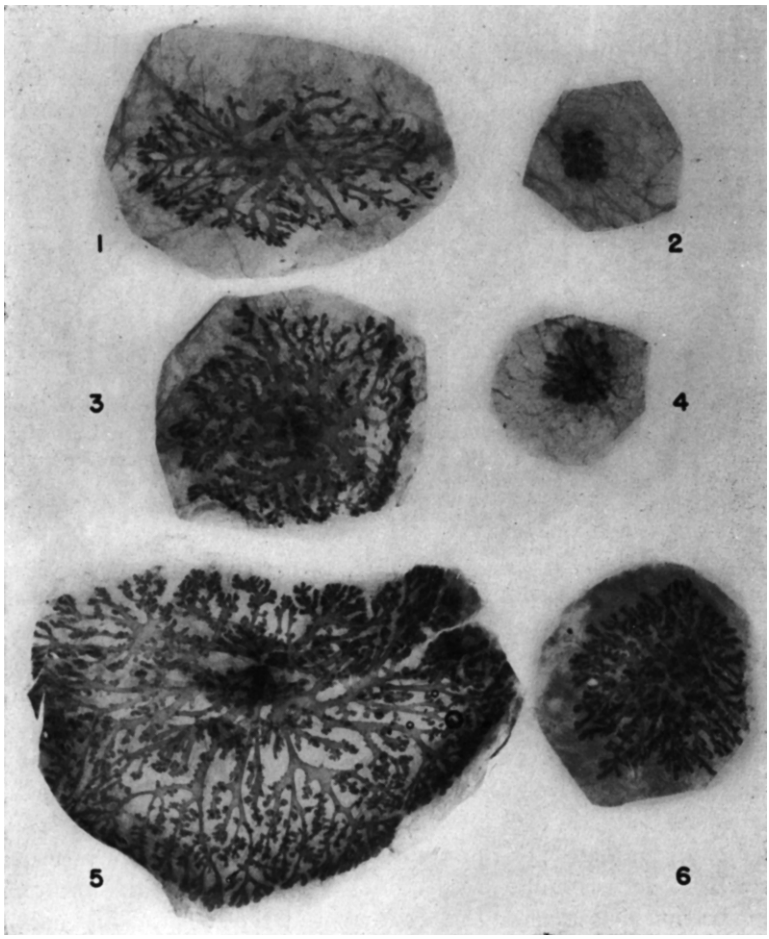

FIG. 5.—Mammary glands from left (1,3,5) and right (2,4,6) sides of three male rabbits. Left glands inuncted for 25-day periods with estrone in sesame oil; right glands inuncted over the same periods with sesame oil alone (× 1.5). (From Lyons and Sako, 85.)

found present, at least in cattle hypophyses, at all stages studied and that a local hyperemic condition might allow an enhanced effect of the mammogen, leading to more pronounced hyperplasia of the treated gland. If this were so, rubefacients other than estrogen might also allow

increased mammogen activity when applied percutaneously; but attempts to demonstrate this using turpentine have so far been unconvincing (76,95).

It is clear that the results of unilateral stimulation, while difficult to reconcile with the theory of direct mammogen stimulation on the normal unsensitized gland (Fig. 6A), harmonize better with the view that mammogens act directly on the estrogen-sensitized gland (Fig. 6B), and perhaps best of all with the view that growth follows direct estrogen

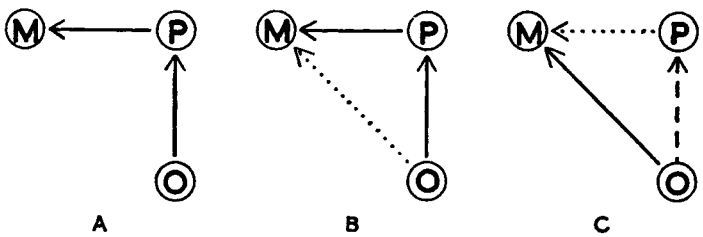

FIG. 6.—Diagrammatic representation of possible interrelationships between ovarian and anterior pituitary hormones in the control of mammary growth. M: mammary gland. P: anterior pituitary. O: ovary. For explanation see text.

———— trophic hormonal pathways.
. . . . . "sensitizing" hormonal pathways.
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action on a gland sensitized by some pituitary factor, for which "mammogen" would scarcely be an adequate name (Fig. 6C). Whether or not the secretion of such a pituitary-sensitizing factor depends on estrogen stimulation of the pituitary would not affect the last interpretation of these facts.

This synergistic, or "sensitizing" concept of ovarian-hypophyseal interrelationship was first clearly enunciated by Reece and Leonard (131) as the most satisfactory interpretation of their own results, which showed that while growth hormone administered to hypophysectomized male rats would stimulate slight duct development, the simultaneous administration of estrogen greatly increased the effect. The enhanced activity following estrogen treatment was accompanied by an adverse effect on the body weight of the rats, and in consequence the authors were unable to attach great significance to merely nutritional factors, and inclined rather to the view that " . . . either estrogen facilitated the mammogenic effect of the growth hormone or that the growth hormone facilitated and was responsible for the effect of estrogen." Remembering also Leonard's observation (67) on the ability of estrogens to stimulate mammary growth in hypophysectomized rats only when injections are begun immediately after hypophysectomy, it becomes still more likely

that pituitary involvement in mammary growth is a matter of inducing a sensitive condition in the undeveloped parenchyma rather than one whereby the secretion of specific mammogens directly, by themselves, causes an extension of tissue structures. The gland may thus be "potentiated by pituitary factors" rather than "developed by mammogens," such potentiation comprising an induced ability to respond to estrogenic substances. Whether this ability is wholly dependent upon presensitization by the pituitary is not yet clear, though the literature on the use of hypophysectomized animals in studies on mammary growth would suggest that it is so, and that positive results following estrogen stimulation in such animals—where they cannot be explained by other factors already mentioned—may be due to an incomplete dissipation of the sensitizing effect at the time estrogen treatment was begun. In particular the power of very small traces of residual pituitary tissue in incompletely operated animals to allow normal growth in response to estrogen treatment (46), is more plausibly explained by ascribing to the fragments the function of maintaining an already existing potentiating effect upon the mammary gland, rather than that of producing, under estrogen stimulation, sufficient "mammogen" to provide a normal mammary response by direct hormonal action. Lewis and Turner (73) have reported mammogenic activity in cattle pituitaries at all stages of the animals' growth, even when no glandular development is taking place. Possibly this anomaly could be explained by more rapid destruction of the circulating mammogen at certain periods when mammary growth is not taking place, by a mechanism preventing mammogen release from the pituitary at these times, or by the lack of sufficient estrogen to sensitize the gland to mammogen activity; but it may be doubted whether these explanations are as satisfactory as those based on the theory of sensitization by the pituitary. For in the latter theory pituitary mediation is of itself latent, only becoming apparent in the presence of estrogens, while the mammogen theory presupposes a hormone, or hormones, which if present could induce all the growth changes in the absence of other aid.

On the other hand it is difficult to explain the absence of response in rats weighing more than 70 g. and given estrogen immediately after hypophysectomy (67), by reference to the "sensitization" theory, though it may well be merely that the rate at which the potentiating effect disappears is linked with the animals' general development. It is known for instance that for mice (32) successful estrogen treatment may be delayed for a longer period following hypophysectomy than is the case for rats. It is also of interest to note that definite phases may be distinguished in the young intact rat regarding its mammary response to

estrogen, and that for the first two weeks of life estrogen has no demonstrable effect (4).

Smithcors and Leonard (145) have shown that for progesterone, too, although mammary stimulation followed treatment of the hypophysectomized rat, it was not optimal and only occurred if treatment began immediately after the hypophysectomy. Combined estrogen and progesterone injections induced greater growth than either hormone alone in animals given immediate treatment, but growth never reached that shown by normal animals. Gardner (32) also has reported mammary growth in hypophysectomized mice following treatment with progesterone, and observed improved stimulation when progesterone and estradiol dipropionate were given simultaneously. Identical results were obtained when progesterone was replaced by desoxycorticosterone acetate in the latter experiments, in both cases the hormone, or combination of hormones, being administered for periods of 12–15 days and from 1–89 days after the hypophysectomy. In the absence of estrogen, however, the mammary response was uncertain and only found in a small proportion of the subjects (progesterone 1 in 4, desoxycorticosterone acetate 2 in 7), and, in view of the entirely negative results of Chamorro (12) for adult hypophysectomized male mice given injections of desoxycorticosterone acetate, it might be of interest to know the age, sex, and time of injection relative to the operation, of the animals which gave these positive responses. It seems possible that some factor depending on the sex of the animal might be influencing the results—perhaps the presence or absence of endogenous estrogens. Apparently, however, mice, compared with rats, as already noted, suffer a slower postoperative change in the sensitivity of the mammary gland to various steroid hormones, for, in those experiments in which supplementary estrogen was also given, almost all the animals responded with mammary growth irrespective of the length of the postoperative period.

Desoxycorticosterone has uniformly failed to induce new growth in the glands of hypophysectomized rats even when given with estrogen (70), or given alone under conditions favorable for estrogen or progesterone stimulation (145).

V. The Androgens and Mammary Growth

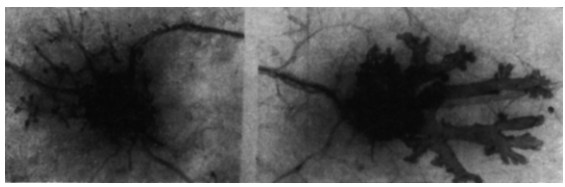
Evidence, summarized by Folley (21) and others, has frequently been obtained since the original experiments of Selye *et al.* (141), that testosterone and also androgens semisynthetically produced may be active in causing mammary growth. Alternative theories may thus be presented to explain the considerable mammary growth normally seen in males of certain species, such as the rat, and perhaps the abnormal growth—

gynecomastia—occasionally seen in males of other species, for example the human and the monkey. Either these animals can produce substances having estrogenic activity—we may note that estrogens, possibly of adrenal origin, occur in male urine (14)—in which case we could explain mammary growth in males in the same terms as for females; or growth is the result of androgenic stimulation and must be regarded as a distinct, though related, problem to that of normal female development. Bottomley and Folley (7) have suggested that, in view of the experimental demonstration of the gynecogenic properties of the male sex hormones so far as mammary growth is concerned, there is no need to postulate the first alternative. These authors themselves reported active duct proliferation in castrate male guinea pigs in response to Δ^5-*trans*-androstenediol, testosterone propionate, and 17-methyltestosterone, but in contrast to the results of Astwood *et al.* (4) and Reece and Mixner (132) with rats injected with testosterone, alveolar development, and that very slight, was only seen after treatment with the first of these substances. *cis*-Androsterone and other androgens used had no clear proliferative effect on the mammary structures, in agreement with earlier and later results obtained by other workers on the rat (113,127). van Heuverswyn *et al.* (54) extended these results to mice, finding extensive development of the duct system after normal animals were injected with testosterone, androstenedione, or dehydro*iso*androsterone, but practically no effect with androsterone. Dehydroandrosterone was later shown to be more active than testosterone in eliciting good alveolar responses from spayed female mice, when both hormones were given in conjunction with estrogen injections (99). Van Wagenen and Folley (166) found dilatation of the ducts of preadolescent ovariectomized female rhesus monkeys following injections of testosterone propionate, but no extension of the duct system or alveolar development unless alveoli were present before treatment was started (Fig. 7).

The importance of estrogen in promoting an enhanced response to androgens was anticipated by the work of Laqueur and Fluhmann (66, see also 65), who found a dependence of the mammary response of adult rats to testosterone propionate upon the functional state of the ovaries at the time the first injection was given. Animals whose treatment began during estrus gave a greater response, involving alveolar as well as duct development, the animals injected in diestrus showing mainly duct extension and only slight alveolar proliferation. Only feeble responses were given, too, by immature rats, a result supported by the later work of Forbes (27) on the development of the mammary glands as the result of testosterone propionate pellet implantations.

McEuen *et al.* (89), using male rats, demonstrated that, as in the

case of estrogen stimulation, an intact hypophysis was necessary for any mammary response to injected androgens. Endogenous androgen produced by injecting chorionic gonadotrophin was also ineffective in promoting growth after hypophysectomy, while the fact that injections of anterior pituitary gonadotrophic extracts permitted normal growth in the hypophysectomized animals to continue added to the evidence in favor of an essential pituitary factor participating in the androgenic growth effects. These results, illustrating the importance of the pituitary, were confirmed by Noble (119) for the ovariectomized-hypophysectomized adult female and the hypophysectomized immature female rat treated with testosterone propionate.



1 *2*

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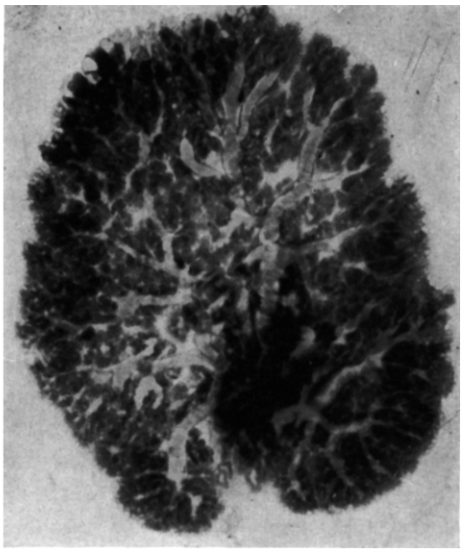
FIG. 7.—Effect of testosterone propionate on mammary glands of preadolescent female rhesus monkeys. *1*. Control gland (monkey A). × 2. *2*. Gland after injection of 2000 mg. testosterone propionate over 65 days (monkey A). × 2. *3*. Control gland (monkey B). × 2.



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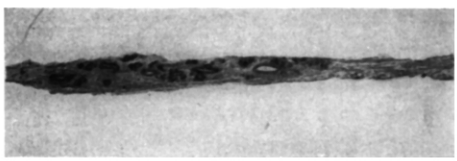

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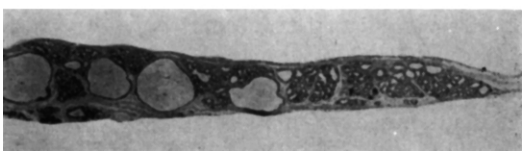

FIG. 7.—*4*. Gland after injection of 388 mg. testosterone propionate over 65 days (monkey B). × 2. *5*. Photomicrograph of section of control gland (monkey B). × 6.5. *6*. Photomicrograph of section of gland after testosterone propionate injection (monkey B). × 6.8. (From Van Wagenen and Folley, 166.)

VI. The Thyroid and Mammary Growth

Early experiments in which normal lactation, and presumably therefore normal mammary growth, was shown by thyroidectomized parturient bitches (17) and rats (115), or in which thyroidectomized and thyroidectomized, gonadectomized male and female rats were observed to give

marked mammary development following estrone injections (112) led to a tentative belief in the absence of any strongly characteristic effect of the thyroid on the growth of the mammary gland.

This view was not in strict accord, however, with the results given by studies on the development of the gland under conditions of hyperthyroidism. Weichert and Boyd (170), for instance, found a striking stimulation in the glands of thyroid-fed pregnant rats compared with normal pregnant controls, the degree of lobule-alveolar formation being far superior by the ninth day of pregnancy, and the differential rate of development being maintained throughout the gestation period. The authors expressed the opinion that the effect was due to endocrine factors rather than to any general influence of an increased metabolic activity. A later report (171) revealed a difference in the histological picture presented by the mammary glands of rats in which pseudopregnancy had been induced by hyperthyroidism (169), and glands taken from animals in which pseudopregnancy was the result of sterile copulation with a vasectomized buck. There was therefore an apparent ability on the part of the thyroid hormone to influence, whether directly or indirectly, the normal development of the gland. The possibility of mediation via an effect on the ovary was perhaps supported by the inability of thyroid feeding to alter in any significant manner the glands of adult ovariectomized rats, but since the adult animals used failed to evince signs of mammary growth even when the thyroid feeding was supplemented by estrone injections, or in response to estrone injections alone, it is doubtful whether these experiments can be regarded as a critical test of ovarian participation in the particular thyroid function under discussion.

The increase in the area of branching ducts in the glands of intact male mice when desiccated thyroid was added to the normal rations (34) did, however, again suggest gonadal involvement, since castrated male mice similarly treated failed to show such growth. Since the adrenal glands also hypertrophied as the result of the thyroid treatment, it seemed that these glands too might be taking some part in the enhanced mammary reaction, but since a similar hypertrophy occurred in the castrated animals this explanation could not be regarded as wholly satisfactory.

Mixner and Turner (100), under the conditions of their mammogen II assay test, found the lobule-alveolar response of spayed female mice given simultaneous progesterone and estrogen injections to be decreased by thyroidectomy and increased by thyroid feeding. The authors were inclined to attribute these results to the stimulatory action of thyroxine on growth, whereby the normal optimal growth rate could be accelerated by increased thyroid activity, or decreased by hypoactivity of the gland.

Confusing results obtained by Leonard and Reece (69) and Smithcors and Leonard (144) may conceivably have their interpretation in a distinction based on sex difference, but the results in these two publications emphasize the ambiguities obscuring this branch of inquiry. The first authors, using young female rats, found that groups subjected to thyroidectomy, spaying and thyroidectomy, or spaying, thyroidectomy and estrogen injections, all showed a greater degree of mammary growth—comprising thickened ducts and an increase in lateral and end buds—compared with appropriately treated unoperated control animals; simultaneous thyroxine injections given to rats treated otherwise as in the third, estrogen-injected group partially checked this differential growth. Explanation of these results must at present remain purely conjectural, and, in view of the largely opposed results given by male rats treated similarly (144), some confirmatory evidence is needed. The male animals showed inhibited duct development after thyroidectomy but an increased lobule-alveolar response on the limited, but thickened, duct systems present. An interesting feature of the experiments was the augmented alveolar development when estrogen injections were given to thyroidectomized, castrated animals, equivalent to that given under similar conditions by testosterone propionate injections. Possibly alterations in the metabolic pathways of steroids may be involved here, as the result of the thyroxine deprivation.

Other studies, relating to the thyroidectomized bovine, have indicated that such operative treatment may result in subnormal mammary growth during pregnancy (148), and that udder development will not follow diethylstilbestrol treatment unless myxedematous symptoms are first removed by suitable supplementary thyroid feeding (125).

We are thus in the somewhat anomalous position of holding evidence that hypothyroidism and hyperthyroidism can both stimulate increased mammary growth, and further that hypothyroidism can cause duct-stunting effects *pari passu* with alveolar stimulation. The various conjectural hypotheses put forward to explain the foregoing results are for the most part lacking all but the slenderest experimental support, but it would seem reasonable to suppose that two distinct mechanisms might well be contributing to the inadequately studied sequelae of abnormal thyroid functioning. The first, necessarily linked in our consideration with inanition effects, depends upon alterations in the general systemic metabolism, and we may suppose that in this way growth changes, such as are implicated in mammary development, may be inhibited or accelerated despite conditions which, from the standpoint of hormonal activity, might be quite normal. Secondly, the smooth interrelationship of the endocrine systems themselves may be upset, and we could formulate

a complicated series of influences at work which would purport the thyroid hormone to be active not merely directly or indirectly through one other gland, but at a multiple remove via intermediate effects on the gonads and the pituitary. It would not then be surprising if paradoxical results should arise in investigating this problem, since the condition of these further participating glands might itself have a very important modifying effect on the outcome of any experiment.

VII. The Placenta and Mammary Growth

The various attempts to induce mammary growth experimentally, which have been enumerated above, all fail fundamentally to reproduce the conditions of most active natural growth—that is, growth during pregnancy—since all overlook certain concomitants of pregnancy which might be deemed to play some part in the growth process: possible neural effects resulting from the distension of the uterus, for instance; hormonal effects deriving from the fetus or the uterine endometrium; or trophic secretions arising from the placenta. We may suppose that some of these might exert at least a modifying effect upon the progress of mammary growth during normal pregnancy, and in fact there is a considerable amount of experimental evidence that the last of these influences—that of the placenta—has indeed a major importance.

Selye *et al.* (140) observed that, if the embryos and the ovaries were removed from rats in the middle of pregnancy, the mammary glands remained in a well-developed but nonsecretory condition, provided only that the placentae were retained intact. This work was confirmed for the mouse by Newton and Lits (117), who further demonstrated a continuation of growth under placental influence during the second half of pregnancy in mice from which fetuses and ovaries had been removed. This, it may be noted, is in conflict with the generally accepted view that proliferation of the parenchyma is completed during the first half of pregnancy in this species, and there would seem to be a distinct possibility that mammary hypertrophy—accompanying the incipient secretory activity of late pregnancy—or the inhibition of regressive changes might have been responsible for the "hyperplasia" which the authors inferred.

The authors were able to conclude from their experiments that the presence of placentae in the uterus has a positive effect upon mammary development, which is independent of any ovarian action and which is not the result of uterine distension. They were not able to define the trophic agency more accurately, but considered the direct action of an internal secretion of the placenta or endometrium to be a distinct possibility. This view, as opposed to that of a neural or indirect hormonal

stimulus mediated by the pituitary, gained very strong support from the later studies of Gardner and Allen (35) and of Newton and Beck (116), whose results, to be considered together with the supplementary study of Newton and Richardson (118), showed that removal of the fetuses coupled with hypophysectomy at midpregnancy was only followed by involution of the mammary glands of mice if the placentae were also lost. Those animals retaining adequate placental tissue were found to have a mammary development at the nineteenth day slightly in excess of that seen in normal mice on the twelfth day of pregnancy.

Discussing the nature of this placental action, the authors tentatively reject the theory of its dependence on a secretion of the ovarian hormones, an opinion which receives confirmation from the work of Lyons (82) on rats, spayed and hypophysectomized after one third of the gestation period had been completed, and then injected daily with estrone or progesterone or a combination of these hormones. Only those animals receiving the last treatment, and a few injected with progesterone alone, showed the presence of placental tissue at full term, and these animals alone showed any extensive mammary development. The other animals, in which resorption was complete, mainly showed a mammary system consisting of ducts only. If, as has been shown under experimental conditions, the anterior pituitary is essential for the mammogenic activities of the ovarian hormones, it would be correct to conclude from Lyons' work that the placenta is able to assume those functions hitherto postulated for the pituitary, at any rate during the latter part of pregnancy; and further that, among the mechanisms responsible for mammary growth in the normal pregnant animal, the placenta may provide the "potentiating" mammogenic factor of which need is abundantly manifest. Such a view would indicate that the pituitary plays a subsidiary role in this respect, having assumed an exaggerated importance in the consideration of hormonal mechanisms responsible for mammary growth merely by reason of the very abnormal conditions under which this problem has until recently been approached. It seems quite possible that in most of the experiments showing the importance of pituitary mammogenic function this gland has been acting vicariously for the absent placentae of normal pregnancy. We are reminded in this connection of a similar shared ability of the pituitary and chorionic tissues to secrete gonadotrophic substances.

Still more recently Leonard (68) has presented further results testifying to the importance of placental tissue for mammary development or at any rate the prevention of mammary regression in the rat—at least during the later stages of pregnancy. Rats were subjected to various operative procedures at the thirteenth day of pregnancy, involving the

removal singly or in all possible combinations of the pituitary, the ovaries, placentae, or fetuses. It was observed that whatever the surgical removals might otherwise be, the retention of the placentae was never associated with any marked regression of the mammary gland, but usually with active development, while removal of the placentae, even when ovaries and pituitary were kept intact, invariably led to severe regression of the gland. There was, however, fairly clear evidence that the placental mammogenic effect was enhanced in the presence of the pituitary and ovaries and somewhat reduced by their removal. The author concluded that his results "indicate that the placenta of the rat is an endocrine organ and that the active principle(s) work synergistically with hormones of the hypophysis and ovaries to control mammary growth during the second half of pregnancy."

Another explanation of these results, in which the pituitary and the placenta are envisaged as serving separate ends, is the assumption that continued mammary growth in the presence of placental tissue and following hypophysectomy is due to a continuance for a period after operation of the pituitary-sensitizing effect upon the mammary gland, and that the placental role is not one of growth stimulation at all, but rather one of maintaining in functional state the nonsecreting (insofar as copious postparturient lactation is absent) but potentially secretory tissue. Such a view would be consonant with the suggestion, already tentatively advanced, that the placenta might prove an agent of mammary hypertrophy but not of true hyperplasia.

VIII. Comparative Aspects of Mammary Growth Control

Although in the earlier part of this chapter the rat was taken as a prototype to illustrate the changes in mammary growth throughout the stages of a mammalian life cycle with several fairly characteristic sexual manifestations, it will have become clear that this generalization, made for convenience, will necessarily have to be modified in greater or less degree as one passes in review from species to species, by reason of the variations encountered in the underlying sex histories of different animals. This comparative aspect of the problem has been well reviewed by Turner (156), and it is here intended merely to consider the fundamental secretory relationships which may be held responsible for the various types of development observed.

Despite the recent evidence indicating a complex, multiple glandular control of mammary growth, it remains a basic postulate of all theories that growth results from hormonal stimuli initially set in train by one or both of the ovarian hormones, and, although the simple theory of direct stimulation of the gland by estrogens or progesterone may have to be

supplemented by other views, ovarian quiescence or activity does in fact, whether directly or indirectly, control the proliferation of ducts and lobules. This control, we have already seen, may be shared, in a degree to which we can as yet give no quantitative estimate, by the adrenal gland, but in all probability only by virtue of the occurrence in that organ of substances identical with, or very closely akin to, the ovarian hormones themselves. From this dependence upon ovarian function spring the possibilities for wide variations in both the quantitative and qualitative aspects of normal mammary development in different species, and particularly is this so in those stages of growth, between puberty and the first pregnancy, when fortunately mammogenic effects may be observed most unambiguously in relation to estrous or menstrual cycles.

By contrast with the rat or the mouse in which the luteal phase is usually considered very short, or even absent, it might be expected that animals having estrous cycles with a definite luteal phase such as the cow, for which the normal cycle is of 21 days, or primates, with a menstrual cycle of about 28 days, would show a relatively greater alveolar development during the course of each cycle in response to the presence of an actively secreting corpus luteum for considerable periods. Such development has been observed; and, since alveolar development was never found in rhesus monkeys which had undergone anovulatory cycles but only in those showing clear evidence that ovulation had occurred, the importance of a lengthy luteal phase for this type of development during the sexual cycle was substantiated for this species (22). However, we may suppose that the presence of ripening follicles at this time will be continually affecting the progesterone: estrogen ratio, rendering it optimal for mammary growth only for a small proportion of the time during which an active corpus luteum is present. The conditions are thus not so favorable for growth as those observed during pseudopregnancy in some other mammals when a prolonged existence of the corpus luteum, in the absence of further follicular growth, does lead to the formation of relatively more extensive lobule-alveolar tissue. In the rabbit, for instance, very extensive growth of the mammary alveolar system has been observed after sterile mating (1,49), while the complete mammary development following spontaneous infertile ovulation in the dog, is also linked closely with the persistence of the corpus luteum in this species during metestrus (91).

Another peculiarity which may be noticed in the rabbit is an absence of the waxing and waning effect in duct growth frequently seen in animals experiencing a regular succession of estrous cycles. The rabbit shows a more steady growth in the period preceding its first pregnancy, correlated no doubt with the state of continuous estrus and a less fluctuating estrogen secretion.

Apart from differences thus based on ovarian rhythms there remain the possibilities of variations in the actual hormonal stimuli required to give rise to mammary tissue, and though these agencies would appear to be the same in a qualitative sense for most species studied—and in all probability for all—it may well transpire that wide quantitative divergencies exist in the relative amounts of the hormones needed for optimal growth and in the relative importance of the contributions of individual glands toward this end.

IX. The Control of Nipple Growth

In contrast to the complexities of the hormonal control of the parenchymatous tissue of the mammary gland, it has become increasingly apparent that nipple, or teat growth is governed by simpler physiological mechanisms, and, although a variety of sex hormones can effect this development, their action in all cases would seem to be direct and not supplemented or modified by any pituitary function. Thus Lyons and Pencharz (84) have found that the nipples of male guinea pigs show very much the same rate of growth following estrogen injection, whether or not the animals are previously subjected to hypophysectomy, and despite the fact that development of the mammary gland could only be obtained in the intact animals. These results were confirmed and extended to include the female guinea pig by Gomez and Turner (43). For the male of this species Bottomley and Folley (7) demonstrated the dependence of teat growth on the endocrine activity of the testes, since castration stopped growth; in the young intact animal growth was found to proceed isometrically, keeping pace with the rate of body growth in general. These authors tested a number of androgens for their power to cause accelerated growth of the teat in both normal and castrated guinea pigs and noted the greater efficacy of the unsaturated androgens in this respect. Further evidence of the absence of any pituitary function in nipple growth was given by Noble (119), who found that this growth in the female rat, both adult and immature, was promoted by testosterone propionate injections irrespective of any previous hypophysectomy or ovariectomy. This gynecogenic action was accordingly attributed by the author to direct stimulation of the nipple by the androgen.

There are distinct indications that, with teat growth as for mammary gland development, species differences may deny us any thoroughly comprehensive explanation of the hormonal mechanisms involved, for Folley *et al.* (26) have failed to observe any cessation of normal teat growth following castration in the young male goat. This may suggest an altered sensitivity to androgens *vis-á-vis* the male guinea pig, or alter-

natively the presence of subsidiary mechanisms—involving possibly the adrenal gland—which can be called into action when the normal stimulating hormone source is removed. The authors' demonstration of phases in teat growth in the caprine, coinciding with the onset and cessation of the breeding season, is of great interest since it implies an inhibitory function of progesterone, or of estrogen in large amounts, upon the rate of growth observed during anestrus. Unlike the young male which shows an isometric teat growth rate, the female goat exhibits positive allometry at an early age. The facts presented above for this species, together with the observation that positive allometric growth can be induced in males by appropriate treatment with estrogen, suggest that a dual mechanism may be responsible for normal growth in the female—one part, whose nature remains unknown, being responsible for a basal isometric rate of development as in the normal male and the other, probably a direct estrogen action, causing a superposed accelerating effect and responsible for the observed allometry. Cessation of teat growth in the breeding season could then be explained by inhibition of the first "isometric" mechanism involving as a dependent effect failure of the second estrogenic stimulation.

In conclusion reference may be made to numerous papers by Jadassohn and co-workers on the so-called "nipple-test"—differential growth effects produced by various sex hormones, when applied to the nipples of guinea pigs (58,59).

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