CHAPTER 3

Hormonal Regulation of Mammary Gland Growth

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I. Introduction

A. General Features of Mammary Gland Growth

The mammary glands belong to the group of organs serving the important task of reproduction. Like other organs of the reproductive system the mammary glands grow but slowly before puberty. Thereafter, in females the size of the glands increases rapidly and a dense arborization of the ducts and in some species a development of alveoli make their first appearance. During the fertile age, a waxing and waning of mammary gland structures has been observed in a number of species to occur concomitantly with cyclic changes of the ovaries, uterus and vagina. During gestation, in many species only then, the mammary glands develop adequate amounts of differentiated structures which become functionally active and produce the nourishment necessary for the offspring after birth. These facts brought forward by a

great number of investigators working on a variety of animal species have been recognized long ago to indicate that mammary gland growth is closely linked to the development of other reproductive organs. Since, at the turn of the last century, it became clear that the integrative linkages among different parts of the reproductive system could not be explained by nervous mechanisms, attention was directed towards the actions of hormones.

Fifty years ago, little was known about the physiology of reproduction, endocrine organs, the nature and actions of hormones and the reactivity and reaction pattern of effector organs. The clarification of the role played by hormones in the development of the mammary glands has been, and still is, dependent upon the progress made in the whole, diverse field of endocrinology. A comparison between the first (1910) and the latest (1952, 1956) edition of F. H. A. Marshall's textbook "The Physiology of Reproduction," as well as between reviews about mammary gland development and function of Turner (1939) and of Cowie & Folley (1955) may provide many examples of the correctness of this statement. In the following survey it can then hardly be avoided sometimes to digress into parts of the general field of endocrinology at first sight not closely connected with the present subject.

The hormones believed to be involved in the co-ordination of the development of the mammary glands with that of other parts of the reproductive system are the gonadotrophic hormones of the anterior-pituitary gland and placenta, and the steroids produced in the ovaries, testes, adrenal cortex and placenta. These are the chief concern of this paper. As may be seen below (Section III) the responsiveness of mammary gland tissues to the stimulating actions of these hormones appears to be dependent upon the metabolic state of the experimental animal. Hormones with powerful actions upon the general metabolism, such as the hypophysial growth hormone, insulin and the glucocorticoids of the adrenal glands, will be discussed with regard to their possible importance for normal growth of the mammary glands.

Before a report of experimental work is given, mention should be made of the structure of the mammary gland after birth. (For embryonic and foetal development, see Raynaud, Chapter 1. A detailed description of the histology of the mammary gland is given by Mayer & Klein in Chapter 2.) As indicated above, the amount as well as the type of the tissue constituting this gland varies during the life cycle of an individual. Before puberty the gland may consist only of a few short ducts confined to the nipple area. In some species a more extensive arborescent duct system is found already during that period. A development into a compound tubular gland occurs in females usually at puberty. By the formation of alveoli arising from the sides and terminal portions of the ducts, the transformation into a compound tubulo-alveolar gland is accomplished. Highly developed mammary glands showing numerous alveoli are found during pregnancy, pseudopregnancy and in some species

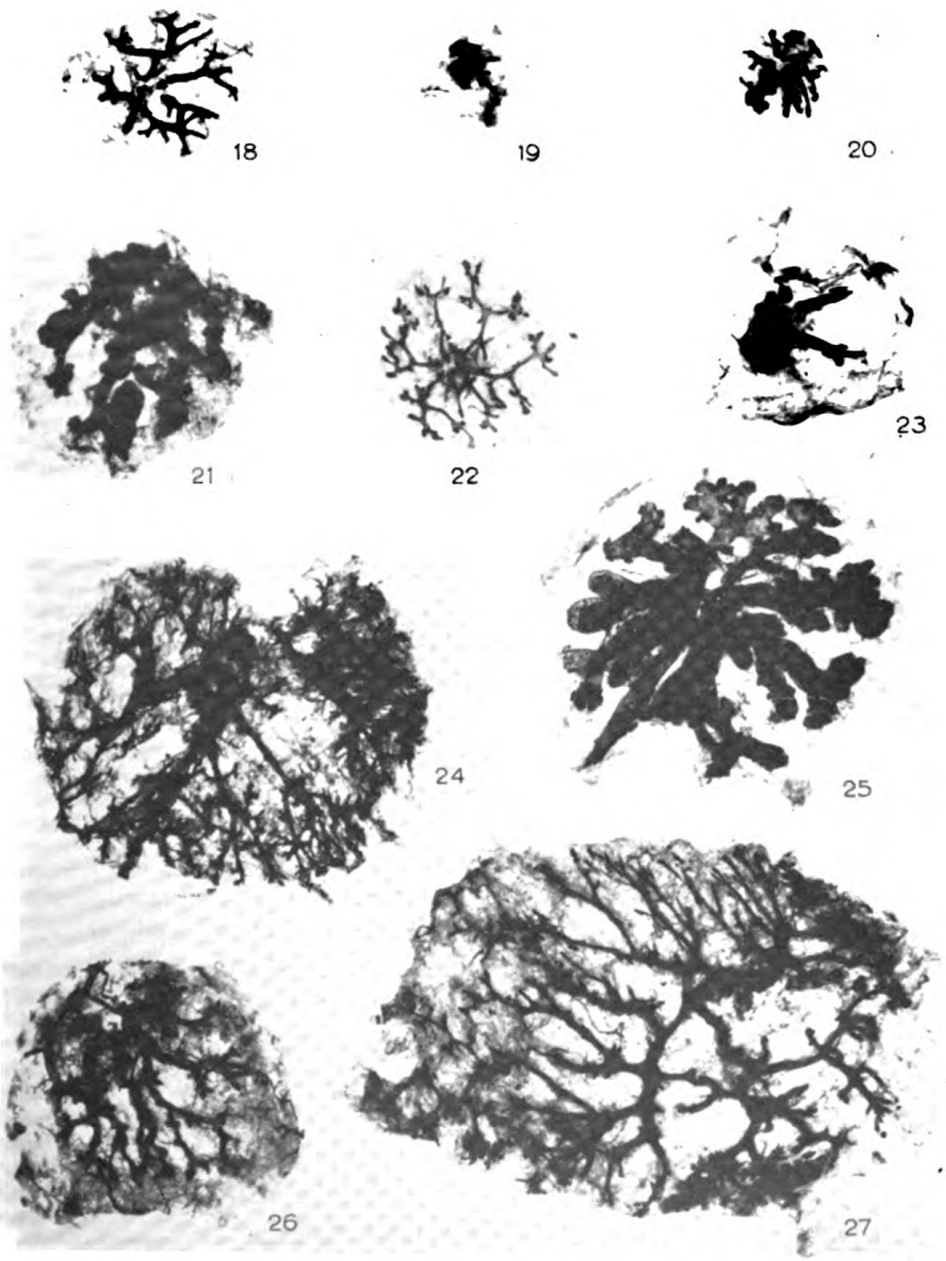


FIG. 1. Whole-mount preparations of mammary glands of ten mature male monkeys. Note individual variation. (From Speert, 1948.)

during the luteal phase of the sexual cycle. For a detailed account of the growth of the mammary glands during the post-natal life of females and males of various species the reader is referred to earlier reviews (Turner, 1939, 1952; Geschickter, 1945; Folley 1952a), to a study on calves and lambs (Wallace, 1953) and to Chapter 2 of this book. To those who are not too familiar with the hormonal control of sexual cycles the masterful, short treatise of Corner (1946) is recommended.

A closer study of the processes outlined above shows that the mammary gland readily reflects a variety of bodily changes in the growth patterns of its tissues. Variations in its appearance have been found between and within species. During the period from birth to puberty the mammary glands of males and females are equally undeveloped in rabbits; in rats a marked growth occurs, but it is not the same in both sexes, and in mice duct growth takes place in females, but not in males. In the fertile age the mammary glands differ in males and females, but considerable variations have been found between the glands of mature males of one and the same species, e.g., in the monkey (Fig. 1). In adult virgin females the structures constituting the mammary glands vary from one species to another according to the type and stage of sexual cycles. The amount of tissue present at a certain stage may be remarkably different, however, in females of the same species and even in glands of one and the same individual. A similar variability has been found for the abundant growth typical of pregnancy or pseudo-pregnancy, although an accurate assessment of the amount of tissue present at this stage is very time-consuming and can rarely be adequately made. That involutionary changes also vary as to time and extent seems hardly necessary to mention.

The variability of the mammary gland makes it an intriguing subject for study. Certainly, it is not accidental that Starling turned his attention to this organ at the time he published his classical work on secretin, the first chemical excitant to receive the name "hormone" and to be classified as such (Bayliss & Starling, 1902; Lane-Claypon & Starling, 1906). On the other hand, the experimenter is confronted with difficulties arising from the variations presented by the mammary glands of his material. In the following Section methods aiming to reduce the fallacies from these and other difficulties will be discussed. The methods of study of the histology of the mammary gland have been described in the preceding Chapter of this book.

B. General Remarks on Methods of Investigation

For studies of the hormonal mechanisms regulating mammary gland growth the classical methods of endocrinology are generally used. These include the ablation of endocrine glands and the repair of deficiencies by means of tissue extracts, purified active principles and pure substances.

Since a small part of an endocrine organ can maintain a considerable functional activity, it is of paramount importance to remove the entire organ when deficiency signs are investigated. For anatomical reasons this may be difficult to achieve. In rats and other species accessory adrenal glands may be present in various sites at some distance from the adrenals. Therefore, in spite of extirpation of both adrenal glands, such animals are not devoid of functional adrenal-cortex tissue. The organ most difficult to remove without leaving remnants behind is the pituitary gland. Therefore, it is necessary to control the success of a hypophysectomy by means of microscopic examination of serial sections through the hypophysial capsule and adjacent tissues including the median eminence of the tuber cinereum of the brain. Adequate methods for the extirpation of the pituitary gland in various species have been developed, and detailed descriptions may be found in the relevant literature (cf. Selye, 1947; Pickford, 1939, for the dog; Jacobsohn & Westman, 1940 for the rabbit; Haeger *et al.*, 1952, for the cat; P. E. Smith, 1954, for the monkey; Lostroh & Jordan, 1955, for the mouse; Brolin *et al.*, 1956, for the rat).

Purified active principles and, certainly, tissue extracts may contain biologically active substances interfering with the effectiveness of the agent to be studied. The majority of anterior-hypophysial hormone preparations so far available in sufficient quantities to workers in biological research belong to the group of purified active principles. Therefore, information about the source of material and the biochemical procedures used for the preparation, as well as about contamination with biologically active agents is essential for the evaluation of results. On suitable biological test-objects the content of hormone(s) in a preparation is estimated by comparing the activity with that of a standard. The principles of biological assays and the mathematical treatment of their results have been described and discussed (see Gaddum, 1953). The advances made during the last two decades in the field of biological standardization (Burn, 1937; Emmens, 1948, 1950; Burn *et al.*, 1950; Bliss, 1951) have been of importance for biological research, and thus for research on mammary gland growth. The potency of hormone preparations is now generally defined by international standard units and determined with great, or at least known precision. The usefulness of statistical methods for the design of an experiment and the analysis of data obtained has been recognized. The importance of a control of "experimental variables" (Ingle, 1951) has been emphasized, but the limitations of a rigid control in a wider type of experimentation on an inductive basis have also been pointed out (Emmens, 1948).

The latter points are pertinent for experimental work on mammary gland growth. It is well known that the growth and activities of reproductive organs are susceptible to actions of "exteroceptive factors" such as light,

temperature, diet and emotional changes (Marshall, 1936, 1956; Hammond, 1954; Amoroso & Matthews, 1955). Breeders of large domestic animals are well aware of the importance of environmental factors (Nichols, 1944). Evidence obtained from investigations into the mechanisms involved in the mediation of the control of reproductive functions by factors of that kind indicates an action via the anterior-pituitary gland (Harris, 1955; Benoit & Assenmacher, 1955). The mammary gland is an integrated part of the reproductive system, and the structure of this gland is variable. It is obvious then that attention should be paid to possible effects of extrinsic factors stimulating or inhibiting the release of hormones acting upon the mammary gland. Environmental and nutritional factors may also influence the responsiveness of the mammary gland to hormones regulating its growth (see Section III).

As the most effective method to reduce variations of responses remaining in spite of controlled experimental conditions, the use of genetically closely related animals is generally recommended. However, observations made on one particular, highly inbred strain of, for instance, mice may reveal features different from those in another inbred strain, F_1 hybrids or randomly bred animals of the same species (Grüneberg, 1943; Mühlbock, 1948; Mixner & Turner, 1957). Moreover, it can hardly be taken for granted that variations of a particular response are less in an inbred strain than among randomly bred animals (Biggers & Claringbold, 1954; Claringbold & Biggers, 1955; Nagai *et al.*, 1957). In this situation the choice of animals depends upon the problem to be studied. Often, the investigator is, for practical reasons, forced to work with a heterogeneous material, and variations must be accounted for in the design of the experiment and the evaluation of results.

Qualitative changes such as the development or disappearance of alveoli, as well as marked alterations in the quantity and extension of ducts can be distinguished clearly enough to allow conclusions. However, it is often necessary to base the judgement upon a comparison between corresponding glands of one and the same animal, one of the glands being removed before the experiment and the corresponding one of the other side being examined at the end of the experiment. Estimations of minor changes cannot be made with sufficient accuracy without the use of quantitative methods including a statistical treatment of data (Folley, 1952b, 1955; Flux & Munford, 1957). These methods developed in recent years are laborious, but the advantages are obvious, especially when histological methods allowing studies of structural details are applied as well (Benson *et al.*, 1957).

The necessity to control the experimental conditions and to apply quantitative methods in the evaluation of mammary gland responses have not always been appreciated in the past. In the following Sections dealing with investigations into hormonal actions on the mammary gland attention will

be paid to factors considered in this Section. Many papers not mentioned here are discussed by authors quoted in this Chapter, which does not aim at a complete presentation of the pertinent literature.

II. Investigations into Actions of Hormones on the Mammary Glands of Animals with Intact Pituitary Gland

A. Ovarian Hormones

After puberty, when the hormonal activities of the ovaries are established, the tissues of the mammary glands grow markedly. In species which do not ovulate spontaneously (e.g., the rabbit) ovarian activity promotes a state of constant oestrus in virgin females, and the mammary glands are composed predominantly of ducts. When ovulation has been induced by mating the oestrous female with an intact or vasectomized male, corpora lutea are formed in the ovary, and abundant growth of alveoli takes place in the mammary glands. This alveolar growth occurs irrespective of whether ovulation is followed by pregnancy or pseudo-pregnancy. Observations of mammary gland growth are actually responsible for the introduction of the term "pseudo-pregnancy." Hill & O'Donoghue (1914) studied the reproductive cycle in the Australian native cat, a marsupial, *Dasyurus viverrinus*, which ovulates spontaneously. The most striking changes found by the authors in this species during the period following ovulation without subsequent fertilization were those of the mammary glands which hypertrophied in the same way as those of pregnant animals. When the state of pseudo-pregnancy has come to its end, that is when the luteal function of the ovary ceases, the alveoli of the mammary gland regress rapidly. An involution of all mammary tissues can then be obtained by removal of both ovaries.

Findings of that kind indicated that mammary gland growth is stimulated by ovarian hormones, duct growth being promoted by oestrogens and alveolar formation by progesterone. Experiments with ovarian extracts and, later, with pure oestrogenic and progestogenic substances injected into animals with undeveloped mammary glands have been performed in various species. There is now an impressive body of evidence confirming the stimulatory action of oestrogenic substances on the growth of ducts and the dependence of alveolar formation upon progesterone administered either together with oestrogens or alone in high doses. In many species the male mammary gland is equipotential with the female gland in its response to ovarian hormones.

Inconsistencies with this reaction pattern have been observed, however. These concern mainly the action of oestrogenic substances. (For a detailed review, see Folley & Malpress, 1948; Mayer & Klein, 1948; Folley, 1952a.) The following account deals with observations made under conditions in

which an effect of oestrogens on the animal's own ovaries can be excluded or seems unlikely (cf. Desclin, 1952). The possibility that oestrogens exert some effect on the pituitary gland, which, in turn, influences the mammary glands either directly or via target organs (ovaries, adrenals), will be discussed later.

Besides stimulating the growth of ducts, oestrogenic substances have in some species been found to evoke a development of alveoli. This effect seems to present itself most strikingly and constantly in male as well as in female guinea-pigs. Until recently it was believed that complete mammary development could be elicited in this species by injection of oestrogenic substances alone. However, Benson *et al.* (1957) were able to show with improved methods that, over a wide dose-range of oestrone, a considerably more extensive lobule-alveolar growth could be obtained by the addition of progesterone.

The situation concerning the goat appears to be similar. In this animal as well as in the bovine animal, sheep, pig and horse, growth of mammary gland tissues and lactation have been induced by administration of oestrogenic substances alone (see Cowie *et al.*, 1952; Desclin & Derivaux, 1953; Folley, 1956). One of the links in the chain of events leading to the production of milk in these animals was assumed to be the formation of alveoli. Lobule-alveolar tissue was found in goats after prolonged treatment with oestrogens, but it did not appear normal. Compared with glands of normal lactating goats the main abnormalities in experimental glands have been described as (1) an enlargement of the alveolar lumina, especially in the periphery of the gland, (2) a folding of the epithelium of alveoli or ducts or both, this folding being present also in alveoli distended with milk, and (3) the presence of immature lobules distributed randomly within the gland (Cowie *et al.*, 1952). These features indicate that the secretory surface of mammary glands stimulated by oestrogen administration is less than of glands developed during a normal pregnancy (see Fig. 2). The results of studies of the amount of milk yielded by artificially developed glands reflect this condition (see also Meites, Chapter 8).

Many data are available showing that in ruminants udder growth and milk production induced by oestrogens alone or together with progesterone are highly variable and generally much inferior to those obtained under normal conditions (see, Cowie *et al.*, 1952; Hancock *et al.*, 1954; Lambourne, 1956). Attempts to improve the situation, which, incidentally, may be of practical importance, have recently been more successful. By additional administration of progesterone, with due regard to the oestrogen/progesterone ratio, it was possible to induce efficient lactation in cows (Turner *et al.*, 1956) and goats. In the goats a development of the udder closely resembling that occurring under normal conditions, both with regard to external appearance and internal

structure, was demonstrated (Benson *et al.*, 1955). Apparently, neither in ruminants nor in guinea-pigs do oestrogenic substances alone promote an optimal development of the mammary gland. In fact, in the goat, an abnormal growth of lobule-alveolar tissue was changed towards normal by the administration of sufficient amounts of progesterone.

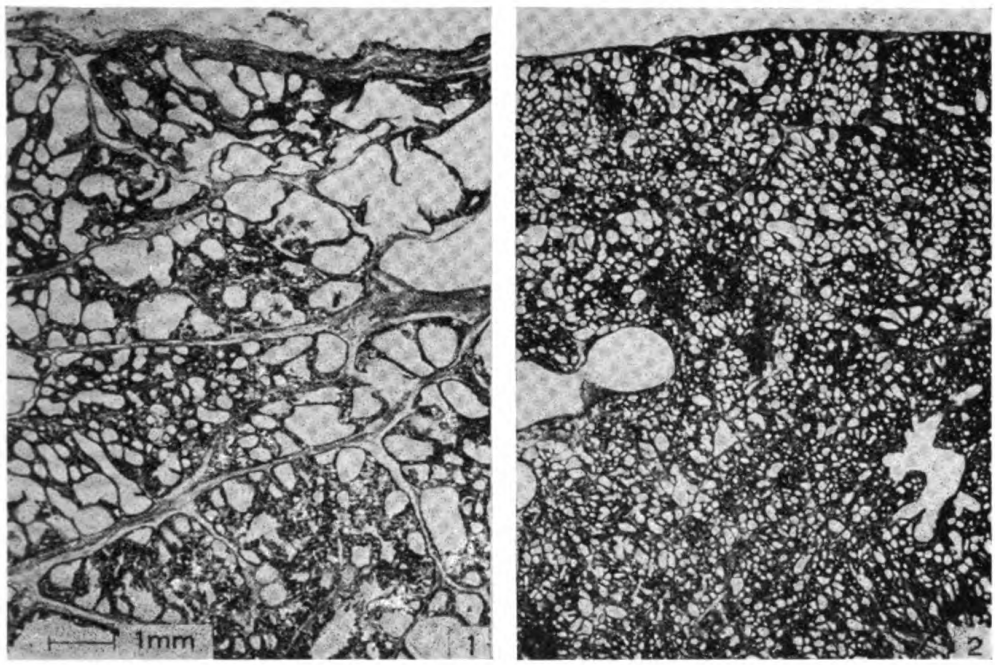


FIG. 2. Sections through the udders of two goats. Left (1) abnormally large alveoli resulting from treatment with oestrogen. Right (2) normal appearance of alveoli during lactation after pregnancy. (From Cowie *et al.*, 1952.)

Development of alveoli after prolonged treatment with oestrogenic substances was also observed in monkeys (see Folley, 1952a). From the well illustrated work of Speert (1948) it appears that the mammary gland of the female Rhesus monkey can react in a similar manner to that of the guinea-pig except that alveolar formation is not as readily induced. The combined actions of oestrogenic substances and progesterone seem, however, to be necessary to promote optimal growth of mammary gland tissues in this species as well as in those mentioned hitherto.

In laboratory animals such as the mouse, rat and rabbit a slight proliferation of lobules has sometimes been described to occur after administration of oestrogenic substances. In these species it may be difficult, especially in whole-mount preparations, to differentiate true alveoli from a cluster of small ducts (cf. Richardson, 1947). Work on highly inbred strains of mice

may be expected to lead to varying results. Nevertheless, it is interesting that a formation of alveolar lobules can occasionally be evoked in the mammary glands of these species (e.g. Daane & Lyons, 1954; T. C. Smith, 1955). Prolonged administration of high doses of oestrogens to laboratory animals results in abnormal growth of mammary tissues including stunted growth of ducts, cystic dilatation of lobules and marked secretion (Gardner *et al.*, 1935–36; Frazier & Mu, 1934–35; Astwood *et al.*, 1937; Fauvet, 1940). A confirmation of these observations, which are interesting with regard to changes found after oestrogen treatment of cows and goats, may be found in many investigations published later than those mentioned (see Fig. 3).

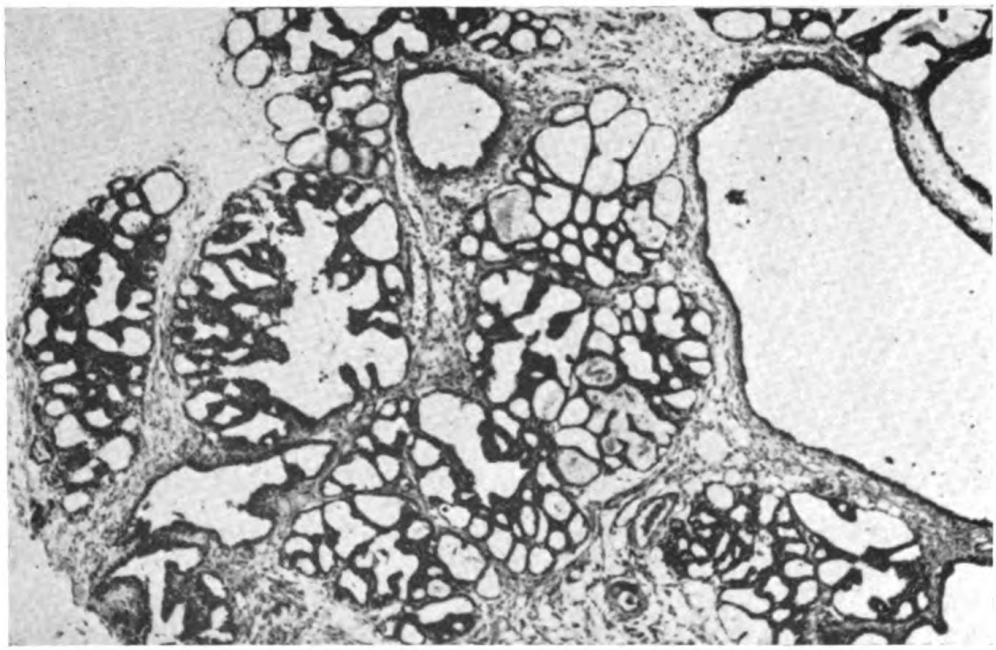


FIG. 3. Section through a mammary gland of a rabbit, ovariectomized when infantile and later injected with oestrogen. Note the large alveoli. (From Fauvet, 1940).

The experience gained from studies of the effect of oestrogenic substances on the growth of the mammary gland reveals changes varying with dose, length of treatment, species, and to some degree with the type of preparation (Folley, 1952a), from simple growth of ducts to a development of alveoli and even stunted, highly abnormal growth of ducts and lobules. The results obtained from work on progesterone and related substances are more uniform. Small doses of progesterone have rarely been found to exert an effect on the mammary gland, except when administered together with oestrogenic substances. With high doses of progesterone given alone during long periods, alveolar development has been obtained in several species (see Folley,

1952a). Geschickter (1945) and Speert (1948) observed this effect in monkeys castrated from 37 days to 4 years before the beginning of progesterone treatment. Benson *et al.* (1957) report the stimulation of lobule-alveolar growth in ovariectomized guinea-pigs injected with 2400 μg progesterone daily.

Since in the intact female a complete development of mammary gland tissues occurs in pregnancy and during the luteal phase of the sexual cycle, that is when both ovarian hormones are secreted into the blood stream, information about the effect of oestrogenic substances plus progesterone seems to be of special interest. It is well established that both hormones together are more effective than either alone in promoting growth of all mammary gland tissues. A synergism is assumed, but few attempts have hitherto been made with adequate methods to determine the doses producing the optimum effect—which, certainly, is a difficult and laborious task.

The only analysis penetrating into the intricate relationships between dose and response has been performed on guinea-pigs (Benson *et al.*, 1957). The authors used elaborate methods both in the design of the experiments (factorial) and the assessment of growth of tissues constituting the mammary gland (quantitative and semi-quantitative). A wide range of doses was tested, and optimal growth responses were obtained with 1000 μg progesterone plus 10 to 15 μg oestrone. From the total result it is concluded that within a species the absolute quantities of oestrone and progesterone are far more important for the effect produced in the mammary glands than the dose ratio. "The ratio of progesterone to oestrogen *per se* is of no importance since altering the dose levels but maintaining the ratio can give entirely different growth responses." This finding is in agreement with observations of the combined actions of the two hormones on, e.g., the uterus of the rabbit. Courrier (1950), reviewing the effects produced by oestrogens plus progesterone on reproductive organs other than the mammary glands, emphasizes that the type of interaction between the two hormones varies not only with dose levels but also with effector tissues and animal species. This statement appears to be true for the mammary gland as well (for review and discussion of data obtained from other species see Benson *et al.*, 1957). With regard to variations of the responses of effector tissues it should, however, be mentioned that Lyons (1951) found the same doses (1 μg oestrone plus 4 mg progesterone) adequate in maintaining pregnancy in oophorectomized hypophysectomized rats and in stimulating lobule-alveolar development of the mammary glands of castrated rats.

Since the guinea-pig represents extreme conditions in so far as the type of response to stimulation with oestrogens is concerned, further work on other laboratory animals seems desirable. A clarification of the interactions of the two ovarian hormones on the mammary gland tissues might be of value for

the elucidation of the mechanisms involved in the actions of these hormones on the mammary gland cells (Roberts & Szego, 1953).

Investigations like that on the guinea-pig can hardly be performed on large domestic animals. For practical reasons, increased knowledge about substances and doses necessary to promote optimal growth of mammary tissues in bovine animals, sheep and goats may be required, however. In view of the importance of doses it is interesting that Turner *et al.* (1956) working on cows apparently started from the same considerations as Lyons (1951) studying rats. Turner *et al.* (1956) added 100 μg oestradiol benzoate daily to a dose of progesterone (100 mg per day) found previously to be adequate in maintaining pregnancy in dairy heifers from which the corpora lutea had been removed. The milk yields were reported as satisfactory. The cows in these experiments were not ovariectomized. Undesirable side effects, such as occur after oestrogen treatment alone, were absent.

As mentioned before, in a number of species, the mammary glands of the males can, with ovarian hormones, be stimulated to growth and differentiation in about the same manner as those of females. The effect produced in the mammary glands of male and female animals by administration of male sex hormones will now be discussed.

B. Hormones of the Testis

Except for two species belonging to monotremes no species seems to be known in which the mammary glands of males become equally well developed as those of females. In some species the male mammary gland remains undeveloped throughout life and in others a fairly extensive duct system may be found at puberty. In adult male rats the presence of alveolar lobules has been described. These lobules disappear after castration (for references see Folley, 1952a; also Ahrén & Etienne, 1957). In the mature monkey and in man the growth of the male mammary gland is restricted, but its structural pattern varies considerably. Extensive arborization of ducts, and lobules of alveoli may be present (Speert, 1948). The growth of ducts, at least in rats, seems to be co-ordinated with general body growth (Cowie, 1949; Silver, 1953a). Evidence obtained from experiments on castrated male and female monkeys and rats injected with male sex hormones indicates that the development of alveoli, which disappear after castration, represents an effect of androgens on the mammary gland (cf. Speert, 1948; Folley, 1952a). The results of investigations on other species including man are equivocal. The substances used, the doses and length of treatment varied (Geschickter, 1945; Folley, 1952a; Flux, 1954a). The male sex hormones comprise a considerable number of biochemically related substances with similar biological effects. However, the potencies of these androgenic substances present

in various organs and body fluids are not the same (Dorfman & Shipley, 1956).

A stimulating effect of testis hormones on the growth of the mammary glands in man is indicated by pathological conditions. Abnormal growth of the mammary glands, gynaecomastia, has been observed in about 10 % of adult men suffering from neoplasms of the interstitial cells of the testis (Leydig cell tumour), a condition in which androgenic substances are produced in excess (Soffer, 1956). In view of the profound actions exerted by androgens on the accessory sex organs of males (cf. Mann, 1954; Parkes, 1955; Dorfman & Shipley, 1956), it seems interesting that, in many species, a response of the mammary glands to hormones produced by the testes can, under normal conditions, not be detected.

In the preceding section it was mentioned that oestrogenic substances stimulate growth in male mammary glands presenting normally a restricted system of ducts. Other organs and tissues, notably the accessory sex glands, also show abnormal features in males subjected to treatment with oestrogenic substances. Oestrogens are present in the testes and adrenal glands and excreted into the urine of males. The possibility that such substances are circulating in the blood of males must be taken into account. Interactions between oestrogenic and androgenic substances seem relevant to the growth pattern of the mammary gland under normal and pathological conditions. Synergistic as well as antagonistic effects have been observed on a variety of tissues (Emmens & Parkes, 1947; Ferguson & Visscher, 1953) including the mammary gland (Speert, 1948; Arhelger & Huseby, 1951). Regarding interactions between oestrogenic substances and progesterone it was pointed out that the type of interaction is dependent upon the levels and ratios of doses, the effector tissues and animal species (Section IIA). The same seems to be true for oestrogens and androgens, but it is considerably easier to demonstrate an antagonism than a synergism (Emmens & Parkes, 1947). In mice, Arhelger & Huseby (1951) observed that the growth of the mammary glands evoked by low doses of oestrone and oestradiol in castrated males was inhibited in non-castrated males. The hormones produced by the testes counteracted the stimulating effect of oestrone and oestradiol, a result which is in agreement with the antagonism between oestrogens and androgens found by other workers. However, studies on the mammary glands of monkeys indicated a synergism (Speert, 1948).

From the data at hand it seems clear that the effect exerted by androgenic substances on the mammary gland varies in different species. The stimulating effect of oestrogens may be depressed by androgens. The result of interactions between these two hormones seems to be dependent upon the quantity and ratio in which the substances are administered. Ovarian hormones, as reported in Section IIA, except for very high doses of oestrogens,

stimulate the growth of mammary gland tissues. The combined effect of oestrogens and progesterone indicate a synergism. The actions of androgenic substances are apparently different from those of ovarian steroids, but perhaps similar to those exerted by certain hormones of the adrenal cortex. These will be considered in the following Section.

C. Hormones of the Adrenal Cortex

The importance of adrenal-cortex hormones for mammary gland growth has been studied in a variety of experiments on adrenalectomized animals. The results obtained from earlier investigations have been conflicting (cf. Folley, 1952a,c), and a detailed description will not be given here. Apart from difficulties in assessing the developmental state of the mammary gland correctly, failures to control experimental variables may have contributed to the divergency of results. It is well known that external factors are far more important for the general condition of the adrenalectomized animal than for the intact one. The amount and composition of the food, for instance, may reasonably be expected to influence mammary gland responses more in the absence than in the presence of the adrenal glands. An analysis performed on pairs of rats joined together in parabiosis indicated that adrenal cortex hormones are not obligatory stimulators of mammary gland growth (Jacobsohn, 1949). The same conclusion may be drawn from experiments on adrenalectomized and/or hypophysectomized, castrated rats injected with oestrone, progesterone, prolactin and growth hormone (Lyons *et al.*, 1955), though later experiments of these authors point to a "permissive" action of adrenal-cortex steroids (see Section III).

The capability of the adrenalectomized animal to maintain homeostasis and to meet increased metabolic demands is severely impaired. The disabilities caused by the ablation of the adrenals are ubiquitous and in many respects poorly understood (cf. Engel, 1954). For studies of the effect exerted by adrenal cortex hormones on the mammary glands the adrenalectomized animal can hardly be regarded as an ideal test object. Experiments with administration of adrenal-cortex steroids appear to be more informative provided that the doses used are not high enough to promote profound changes in the intermediary metabolism of the experimental animal. The substance so far most extensively studied with regard to mammary gland growth is 11-deoxycorticosterone, usually as acetate (DCA). This compound has been extracted from adrenal glands, but it seems doubtful whether it is secreted (if at all) *in vivo* in amounts sufficient to attain physiological significance (Hechter & Pincus, 1954; Farrell *et al.*, 1954; Dorfman, 1957). DCA was, however, the first adrenal cortex steroid available for study. The general actions of DCA concern mainly the electrolyte and water metabolism. In the

mammary glands DCA stimulated, with few exceptions, growth of ducts and, together with oestrogens, also of alveoli (Folley, 1952a; Flux, 1954b).

The corticosteroids found in the venous effluent of the adrenal glands in greatest amounts are cortisol and/or corticosterone (Hechter & Pincus, 1954). The effect of C_{11}-oxygenated corticoids on the growth of the mammary gland has been studied in rats and mice. In castrated rats and mice injected with cortisone a stimulation of mammary growth was not observed. In rats cortisone and cortisol enhanced the development of alveoli and stimulated secretion under conditions in which ovarian hormones were active as well. In intact mice cortisol promoted a slight development of acini and secretion (Sparks *et al.*, 1955). In castrated mice the effect of small doses of oestrogens was found to be inhibited by cortisone and a number of related compounds (Flux, 1954b). The observations of Flux suggest an antagonism similar to that found between oestrogens and male sex hormones. However, the discrepant results obtained from studies on rats and mice may also be due to a greater sensitivity of mice to the adverse metabolic actions of the substances studied. Investigations available at the time were reviewed and discussed by Ahrén & Jacobsohn (1957). Munford (1957) in experiments on mice similar to those of Flux (1954b) confirmed that mammary gland growth was inhibited by high doses of cortisol acetate, low doses had a stimulating effect, however.

The investigations dealt with in the present Section have been performed on animals with intact pituitary gland. The hormones concerned are produced by organs controlled as to growth and function by the anterior-pituitary gland. It is known that hormones produced by hypophysial target organs can act in turn upon the anterior-pituitary gland and alter its functional activity (Everett, 1950; Benoit & Assenmacher, 1955; Harris, 1955; Long, 1956; Soffer, 1956; Donovan & Harris, 1957). Actions exerted by the pituitary gland cannot be excluded when this organ is present. In the following Section the effect produced by gonadal and adrenal-cortex hormones on the mammary glands of hypophysectomized animals will be reviewed. In addition investigations into the actions of known anterior-hypophysial hormones will be discussed.

III. Investigations into Actions of Hormones on the Mammary Glands of Hypophysectomized Animals

A. Gonadal Hormones

One of the consequences of the removal of the pituitary gland is an atrophy of the gonads. This atrophy is due to the disappearance of gonadotrophic hormones, the stimulators of the development and endocrine functions of the ovaries and testes. The mammary glands of hypophysectomized

animals are atrophic, and the question that arises at first is whether the failing endocrine functions of the gonads alone are responsible for the lack of mammary gland growth in hypophysectomized animals. The experimental approach to this problem, according to classical endocrinological methods, appears at first sight obvious and easy. The experience gained from numerous investigations into the effect of replacement of gonadal hormones in hypophysectomized animals reveals, however, considerable complications. Experimental errors due to failures in the surgical removal of the entire pituitary gland and to difficulties in the accurate assessment of mammary gland changes impeded the clarification of the problem. From a critical evaluation of the available evidence it may be said that mammary gland growth is absent or considerably reduced and abnormal in animals supplied with ovarian hormones but deprived of their pituitary gland. The effect of male sex hormones seems to be similar, but not always the same as that of ovarian steroids (Folley & Malpress, 1948; Jacobsohn, 1954; Ferguson, 1956). In hypophysectomized rats injected with androgenic substances, the growth of ducts and alveoli is considerably reduced. Mitoses and an increase in the size of epithelial cells lining the inner wall of ducts as well as secretion do, however, occur. Since the epithelium is proliferating in ducts the growth of which is limited, and since these ducts are often distended with secretion, the mammary glands appear distorted and abnormal (Leonard, 1943). In view of observations of epithelial proliferation in the mammary glands of monkeys treated with androgens (cf. Folley & Malpress, 1948) it would be interesting to know whether this effect persists after hypophysectomy in monkeys as well as in rats.

In an analysis of the cause(s) of the failure of the mammary glands of hypophysectomized animals to respond to the stimulating actions of ovarian hormones, factors other than hormones with specific actions on the mammary gland should be considered. Ablation of the pituitary gland includes the removal of anterior-hypophysial hormones regulating a great variety of essential processes of the general metabolism, directly or via endocrine target organs. Rapid and effective adaptations to meet special demands by shifting the directions of the intermediary metabolism of protein, carbohydrate and fat are hardly possible in the hypophysectomized animal. General body growth is retarded or inhibited and homeostasis is easily disturbed (cf. Engel, 1954). Early attempts to clarify the role of the general metabolism by studying the reaction of the mammary glands after force feeding or underfeeding failed. Mammary gland growth could be stimulated by ovarian hormones neither in force-fed hypophysectomized rats nor in underfed rats or mice with intact pituitary gland (cf. Folley & Malpress, 1948, Ferguson, 1956).

B. Ovarian Hormones and Long-acting Insulin

Salter & Best (1953) reported that treatment with long-acting insulin increased the voluntary food intake and altered the intermediary metabolism of hypophysectomized rats. The insulin treatment resulted in, amongst other things, nitrogen retention and body growth. Experiments designed according to the findings of Salter & Best (1953) showed that injections of long-acting insulin combined with a carbohydrate-rich diet *ad libitum* and administration of oestrone and progesterone promoted mammary gland growth in hypophysectomized castrated female and male rats (Ahrén & Jacobsohn, 1956; Ahrén & Etienne, 1958) (see Fig. 4). This observation is in agreement with results obtained with underfed rats and mice

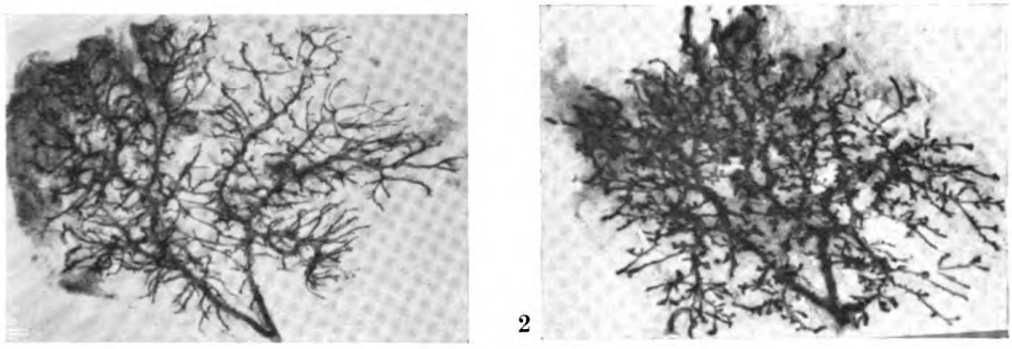


Fig. 4. Whole-mount preparations of mammary glands of two hypophysectomized rats injected with oestrone and progesterone. (1) Atrophic gland in spite of injections. (2) Growth and differentiation obtained by additional treatment with long-acting insulin. (From Ahrén & Jacobsohn, 1956.)

(Ferguson, 1956) with intact pituitary gland. Both types of experiment indicate that the response of the mammary gland to the stimulating actions of ovarian hormones is dependent upon the state of the general metabolism. However, this only holds true provided that the effect of insulin is not exerted locally on mammary gland tissues, a possibility that remains to be investigated (for the effect of insulin on the *in vitro* metabolism of mammary-gland slices, see Folley & McNaught, Chapter 12). With regard to the question whether ovarian hormones act directly on mammary gland tissues or via the pituitary gland, it is interesting that mammary gland growth could, by means of insulin treatment, be evoked by oestrone and progesterone in the absence of hypophysial hormones. That ovarian hormones can exert a direct action is evident also from experiments of a number of authors showing in several species that oestrogens applied to the skin overlying one mammary gland produce growth of that gland, but not of others. Such localized mammary gland growth has not been reported to occur in

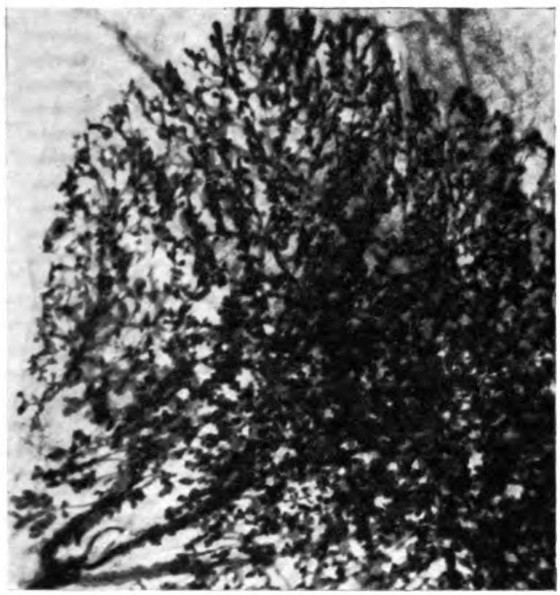
hypophysectomized animals (cf. Folley & Malpress, 1948), but has since been observed in hypophysectomized rabbits. Intramuscular injections of oestradiol monobenzoate into hypophysectomized rabbits resulted in slight, stunted mammary growth and some secretion in the mammary glands (Jacobsohn, 1954). A similar reaction was observed in hypophysectomized rabbits after local application of about 0.05 ml of oestrone daily in arachis oil in a concentration of 10 μg per ml during about 3 weeks. The mammary gland underlying the area of the skin on which the oestrone solution was dropped showed slight, but distinct growth. The corresponding gland of the other side treated with arachis oil remained undeveloped (Jacobsohn, unpublished).

C. Ovarian Hormones and Cortisone

Authors studying the hormonal control of mammary gland growth in hypophysectomized animals agree that the metabolic abnormalities prevailing in such animals render the interpretation of results difficult (e.g., Mayer & Klein, 1948; Jacobsohn, 1948; Nelson, 1954). The extent to which alterations of the metabolic state interfere with mammary gland growth has not been clarified. The general metabolic state is probably more important than realized hitherto. This is indicated by the effect of cortisone on the mammary glands of hypophysectomized rats (Ahrén & Jacobsohn, 1957). As mentioned in Section II C, the effect of cortisone administered to rats with intact pituitary gland revealed itself by an enhancement of alveolar formation and secretion in mammary glands, the growth of which was stimulated by ovarian hormones. In hypophysectomized rats injected with cortisone, either alone or together with oestrone and progesterone, the reaction of the mammary glands turned out to be different, both as to quality and quantity. An abnormal increase in size and number of epithelial cells arising from the inner wall of ducts was regularly found (Fig. 5). After prolonged treatment many ducts had their lumen obstructed by enlarged epithelial cells. A restoration of these abnormalities towards normal was achieved by additional treatment of these rats with long-acting insulin. The result appeared to be dependent upon the ratio and level of the doses of cortisone and insulin. For details and discussion of the part played by the failure of the hypophysectomized rat to counteract the metabolic actions of cortisone see Ahrén & Jacobsohn (1957).

D. Ovarian and Pituitary Hormones, Chiefly Prolactin and Growth Hormone

As mentioned above, ovarian steroids stimulated mammary gland growth in hypophysectomized rats treated with insulin. Insulin administered alone



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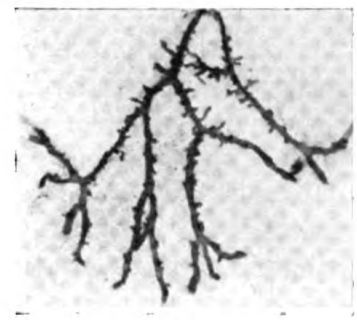

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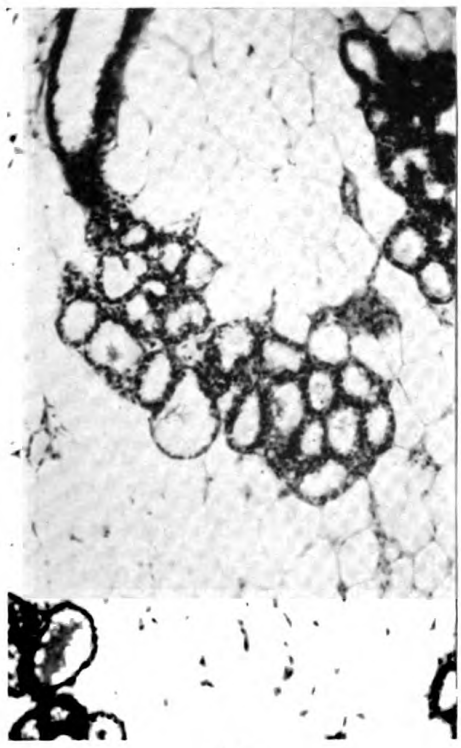

(c)

(d)

FIG. 5. Whole mounts (a, b) and sections (c, d) of mammary glands of ovariectomized rats injected with oestrone, progesterone and cortisone. Growth, differentiation and secretion in the glands (b, c) of the rat with intact pituitary gland. Failure of growth, but thickening of ducts (a) and epithelial proliferation (d) in the glands of the hypophysectomized rat. (From Ahrén & Jacobsohn, 1957.)

did not produce an effect detectable with the methods used (Ahrén & Jacobsohn, 1956). This finding emphasizes the importance of ovarian hormones, but it does not exclude the possibility that hypophysial hormones contribute to the stimulation of mammary gland growth occurring in intact animals. In fact, in view of observations mentioned in Section IV C, and on the basis of extensive investigations of, foremost, Lyons and co-workers (Lyons, 1951; Lyons *et al.*, 1955) this possibility has to be seriously considered. The aim achieved by these authors was to promote in hypophysectomized gonadectomized rats mammary gland growth similar to that found normally in mid-pregnancy (Fig. 6). The substances used were oestrone, progesterone and highly purified preparations of growth hormone and lactogenic hormone. The duration of the period of injections corresponded approximately to half the length of pregnancy in rats. The results obtained by Lyons and his group were

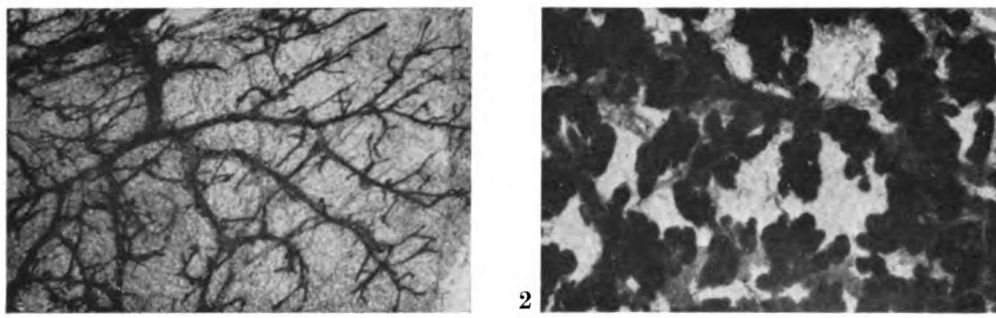

FIG. 6. Whole mounts showing part of mammary glands of two hypophysectomized castrated rats injected with oestrone and progesterone. (1) Non-responsive atrophic gland. (2) Growth and differentiation obtained by additional treatment with prolactin and growth hormone. (Lyons *et al.*, 1955).

The reproductions shown in this figure as well as in Fig. 7 were made from photographs given to the author by Dr W. R. Lyons.

confirmed and extended by Nelson (1951, 1952). This author found, amongst other things, that injections of human chorionic gonadotrophin (HCG) and pregnant mare serum (PMS) into rats hypophysectomized on the 8th day of pseudo-pregnancy promoted further mammary gland development provided that prolactin was administered as well. The gonadotrophic hormones stimulated the ovaries to secrete oestrogens. Progesterone secretion was maintained by prolactin, which, presumably, in addition acted directly on the mammary gland. That endogenous ovarian hormones can stimulate mammary gland growth in hypophysectomized rats treated with insulin is indicated by experiments of Bengtsson *et al.* (1957) using HCG and PMS in high doses.

From the results reported by Lyons and Nelson it may be concluded that, at least in the rat, growth hormone and prolactin are involved in the mech-

anisms leading to mammary gland growth stimulated by ovarian hormones. Investigations into possible actions of other, more or less purified hypophysial factors, notably Turner's "mammogenic hormones" are reviewed and discussed by Folley & Malpress (1948); Nelson (1954); Cowie & Folley (1955); Folley (1956). The effect of relaxin on mammary growth has been studied by T. C. Smith (1954).

For the interpretation of the part played by the two anterior-pituitary hormones shown to enable the mammary gland of the hypophysectomized rat to grow, four points seem relevant: (1) Growth hormone and prolactin exert powerful actions on the intermediary metabolism (Randle, 1957; Foà, 1956). (2) Prolactin applied locally has been reported to elicit a circumscribed effect on mammary gland tissues of rabbits, mice and rats (Lyons, 1942; Mizuno *et al.*, 1955; Ferguson, 1956; Lyons *et al.*, 1957). Mammary gland tissue obtained from mice in mid-pregnancy and cultured *in vitro* could be maintained and kept secreting by addition of mammotrophin (prolactin) plus cortisol to the medium (Elias, 1957). (3) Treatment with insulin alters the general metabolism of hypophysectomized rats and renders the mammary gland responsive to stimulating actions of ovarian hormones (Ahrén & Jacobsohn, 1956). (4) Few other species have been investigated.

(1) In the introductory section it was pointed out that the structure of the mammary gland varies. The organ is sensitive and reflects bodily changes in the growth patterns of its tissues. This feature revealed itself strikingly in the reaction of the mammary glands of rats to cortisone (Ahrén & Jacobsohn, 1957). The type of changes produced in the mammary gland by this or related substances may then be regarded as an indication of the prevailing state of the body. From this point of view, the metabolic actions of the preparations of growth hormone and prolactin in experiments such as those of Lyons *et al.* (1955) are attested by the effect of cortisol consisting in an enhancement of secretion and, possibly, alveolar formation. To be sure, the authors neither overlooked nor denied the metabolic actions of their pituitary preparations. Insulin was less effective than the pituitary hormones in counteracting the metabolic effects of the glucocorticoids used by Ahrén & Jacobsohn (1957) and Lyons *et al.* (1955), respectively. This situation is not surprising since, under similar conditions, growth hormone was found to be more effective than insulin in increasing protein synthesis (Scow, 1957).

(2) In rabbits with intact pituitary gland and adrenals, milk secretion can be evoked in one sector of a suitably developed mammary gland by injection of prolactin into one of the main ducts (Lyons, 1942). The specificity of this effect has recently been confirmed by Bradley & Clarke (1956) who, using Lyons's method of application, obtained milk secretion with prolactin, but not with other preparations of pituitary hormones, including growth hormone. Localized growth of mammary gland tissues after intraductal injections

of prolactin has been reported to occur together with milk secretion (Lyons, 1942) or sometimes alone (Mizuno *et al.*, 1955). Pituitary hormone preparations other than prolactin were not tested with regard to growth stimulating properties after intraductal injection.

In hypophysectomized mice a local growth effect has been described after injections of prolactin under the skin near one of the nipples. When oestradiol was injected in addition, but into another site, the mammary gland belonging to the nipple area injected with prolactin developed more than other glands. Mammary gland growth occurred bilaterally, however. Secretion was not observed (Ferguson, 1956).

In two abstracts it is reported that localized mammary gland growth occurred in the neighbourhood of pellets implanted under the skin of hypophysectomized castrated male and female immature rats. The pellets contained oestrone, progesterone, growth hormone and prolactin individually or in all possible combinations. The type of growth seen in the mammary glands that reacted was about the same as that observed after injections of the various hormones (Lyons *et al.*, 1956, Lyons *et al.*, 1957).

None of the observations reported under point (2) is incompatible with the view that prolactin and growth hormone exert some action directly on mammary gland tissues. To clarify the question whether the effects of prolactin and growth hormone are independent of other hormones and the metabolic state, experiments on hypophysectomized animals in which intraductal injections can be made seem desirable. This concerns especially the growth hormone. As to prolactin, a corroboration of its effect on growth seems to be required (cf. also Meites & Sgouris, 1953). For reasons inherent in the methods, the results obtained from the work on hypophysectomized mice and rats are difficult to analyse. The experiments of Lyons and co-workers on rats were presented in detail at the Laurentian Hormone Conference in 1957 (Lyons *et al.* 1958). The observations presented at this conference support the notion of local actions of growth hormone and prolactin and indicate a contribution of adrenal-cortex steroids. They also show that ovarian hormones are obligatory stimuli for the growth of specific mammary gland structures. Further evidence of a direct action of a pituitary hormone is provided by the observations on tissue cultures (Elias, 1957).

(3) The changes observed in the mammary glands after treatment of hypophysectomized rats with insulin and cortisone (Ahrén & Jacobsohn, 1957) suggest that the effect of insulin is due to its metabolic actions. As pointed out above, experiments designed to inquire into the possibility of a direct effect of insulin on mammary gland tissues are not available. With regard to similarities of effects of prolactin, growth hormone and insulin on the responsiveness of the mammary glands of hypophysectomized rats to ovarian hormones it is interesting that both pituitary hormones have been reported

to enhance the secretion of insulin from the pancreatic islets (cf. Foà, 1956; Randle, 1957).

From the comments on (1) and (2), and from (4) it appears that further investigations including other species and, if possible, isolated organ preparations are desirable to clarify the ways by which prolactin and growth hormone elicit their influence on mammary gland growth. That experiments like those commented on under (3) should be extended and repeated by other workers is obvious. When the effect of insulin treatment on the responsiveness of the mammary glands of hypophysectomized animals can be regarded as an established fact, a discussion whether ovarian hormones stimulate mammary gland growth solely via the pituitary gland will become superfluous. The same applies to the view that oestrogens sensitize the mammary gland towards growth-promoting pituitary hormones (for a detailed discussion of possible interrelationships between ovarian and anterior-pituitary hormones see Folley & Malpress, 1948).

According to the third alternative, discussed by Folley & Malpress (1948), oestrogens act directly on a mammary gland sensitized by some pituitary factor(s). Present knowledge about the mode of action of hormones upon effector cells is scanty (Hechter, 1955; Levine, 1957). In the rat, studied most extensively, or exclusively, with regard to interactions of pituitary and ovarian hormones, mammary gland growth fails to occur after hypophysectomy and administration of oestrogens. Characteristic features concerning the relationships between dose and response have not been established for the combined actions of prolactin, growth hormone and oestrogens on the mammary glands of hypophysectomized animals. It can hardly be accepted as certain that a "sensitization" as defined in pharmacology is brought about by pituitary hormones, and this word should at present better be avoided in connexion with mammary gland growth. Folley (1956) discussed the possibility that prolactin has mammogenic properties, other pituitary hormones playing a "permissive" or synergistic role. The evidence available at present emphasizes the part played by ovarian hormones and the role of the general metabolism. It also indicates that prolactin and growth hormone are contributory factors in the stimulation of mammary gland growth in intact animals. Prolactin and growth hormone are probably not acting in the same manner (cf. Lyons *et al.*, 1955). The mechanisms by which the two pituitary hormones exert their actions on the mammary glands seem to involve changes in the intermediary metabolism, but actions exerted directly on mammary gland tissues may also occur.

The effect of adrenocorticotrophic hormone (ACTH) on the growth of the mammary glands of hypophysectomized animals does not seem to have been studied recently, but a "permissive" role of adrenal-cortex steroids appears likely from the experiments of Lyons *et al.* (1958). That thyrotrophic hormone

(TSH) is of minor importance is indicated by an investigation of Chen *et al.* (1955), who observed mammary gland growth in athyroid, hypophysectomized, adrenalectomized, castrated rats injected with oestrone, progesterone, prolactin and growth hormone.

For a detailed review of the role played by the thyroid gland in mammary gland growth see Folley (1952a).

E. Placental Hormones

With regard to the fact that the mammary glands are in many species fully developed only during pregnancy, investigations into the role of placental hormones seem of special interest. To design and perform experiments aiming at a penetration of this problem is difficult. In laboratory animals

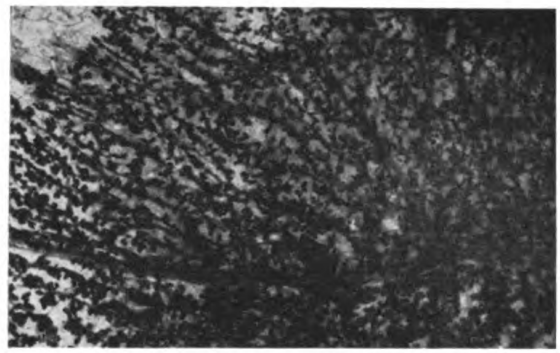

Fig. 7. Whole mount showing part of a mammary gland of a hypophysectomized, ovariectomized rat treated with oestrone, progesterone and extracts or implants of four 12-day rat placentae daily during 6 days. Lobule-alveolar growth. (From Ray *et al.*, 1955.)

(mice, rats, guinea-pigs and rabbits) hypophysectomy at the beginning of pregnancy results in abortion. In mid-pregnancy the mammary glands are already developed. When the pituitary gland is removed at that stage, pregnancy may continue to term, but the period remaining for studies of changes in the mammary glands is short. In addition a beginning secretion, such as occurs during the second half of pregnancy, may obscure the picture. A distinction between growth and distension of already existing structures is hardly possible under such conditions. In view of these limitations the results of earlier investigations on rats and mice (cf. Folley, 1952a) suggest an action of placental hormones on the mammary gland, but whether this action concerns the growth or secretion appears uncertain.

Experiments providing evidence that the rat's placenta contains a factor with luteotrophic, mammotrophic and lactogenic properties have been described by Ray *et al.* (1955). These authors were able to promote mammary

gland growth in hypophysectomized, ovariectomized rats injected with oestrone progesterone and placental extracts (or implants) (Fig. 7). The effect of the placental factor appeared similar to that of the pituitary hormones, prolactin plus growth hormone, studied extensively by Lyons and his group (see above). With regard to its effect on the pigeon's crop sac the placental factor was found to be considerably less active than pituitary lactogenic hormone. However, the potency was high with regard to the luteotrophic, mammotrophic and lactogenic effects.

Notwithstanding the fact that the demonstration of a biologically active agent in an organ does not prove that the agent is normally secreted into the blood and carried to an effector organ, it seems tempting to regard the observations of Ray *et al.* (1955) as a key to a better understanding of conditions prevailing in pregnancy and certain pathological conditions in man.

The importance of placental hormones for the growth of the mammary glands during pregnancy in primates is indicated by an investigation of Agate (1952) on rhesus monkeys. Agate obtained most of his material from rhesus monkeys hypophysectomized during pregnancy and studied by P. E. Smith (1954, 1955). Ten of the twenty animals of P. E. Smith went to term, six babies were delivered living, two died during parturition and two others were stillborn. The interval between hypophysectomy and parturition varied between 20 and 131 days and exceeded 58 days in six animals. The completeness of the removal of the pituitary gland was thoroughly controlled. Agate (1952) examined the mammary glands of most of these monkeys and found good development of alveolar lobules at term, secretion appearing during the first few days after delivery. The size of the glands was somewhat less than in intact monkeys at term. The extent of lobular development was not correlated with the time between hypophysectomy and delivery. "Monkey 801, which was hypophysectomized on the 32nd day of pregnancy and delivered 131 days later, showed just as extensive lobular development as 774 which was hypophysectomized on the 102nd day and delivered 59 days later."

Speert (1948), who studied the growth of the mammary glands of intact rhesus monkeys during pregnancy, reports that hardly any changes occurred during the first month. Alveolar proliferation was observed during the 2nd and 3rd months and appeared most conspicuous during the 3rd month. In this situation, at least some of the monkeys supplying the material for Agate's investigation should have been hypophysectomized before alveolar growth had begun. As indicated by the bright colour of the sex skin and the weight and microscopic appearance of the adrenal glands (P. E. Smith, 1955) oestrogens and adrenal-cortex hormones were present in the blood of the hypophysectomized pregnant monkeys. The question arising then is whether these hormones alone could have stimulated the mammary gland growth.

I have not found reports of studies of mammary gland growth in hypophysectomized monkeys treated with gonadal and adrenal cortex steroids. It appears unlikely, however, that the results would be very different from those obtained from other species. The role played by the pituitary gland of the growing foetus must be considered, but does not seem to be important for the growth of the maternal mammary glands (cf. Silver, 1953b; P. E. Smith, 1954, 1955).

From the experience gained by Ray *et al.* (1955) it seems tempting to assume that the placenta of the monkey as well as that of the rat can produce some factor(s) supporting mammary gland growth in a manner similar to that of pituitary lactogenic hormone and growth hormone. It is well known that the placenta of primates secretes gonadotrophic hormones. HCG was shown to prolong the luteal phase of the menstrual cycle in monkeys (Hisaw, 1944) and women (cf. Fried & Rakoff, 1952). In the species in which it is produced chorionic gonadotrophin has a luteotrophic effect. A possible action upon the mammary glands is difficult to reveal without removing the gonads and adrenal glands. Gynaecomastia induced by treatment with HCG in boys suffering from cryptorchidism, may, but need not, be solely due to an increased production of gonadal hormones. The same applies to the gynaecomastia occurring in men suffering from chorionepithelioma. These tumours produce excessive amounts of HCG, and it is interesting to find the following statement in a discussion of this disease of the testis (Soffer, 1956): "The increased excretion of estrogens demonstrated in some patients suggests that breast enlargement may be an effect of estrogenic stimulation. It is apparent from the meagre studies at hand that this cannot always be true and that the precise mechanisms involved in the production of gynecomastia are in need of clarification."

The observations of Hisaw (1944) and Fried & Rakoff (1952) indicate that HCG in maintaining the functional activity of the corpus luteum prevents the menstrual breakdown of the endometrium and assists in creating favourable conditions for the implantation of a fertilized egg. In the monkey (Hartman & Corner, 1947) and in the woman (cf. Courrier, 1945) the ovaries can be removed at the 25th day and between the 1st and 2nd month of pregnancy, respectively, without interrupting its course. The luteotrophic effect of HCG is important only at the beginning of pregnancy. So far no answer can be given to the question why HCG is secreted into the blood of pregnant women during the whole length of pregnancy (though at lower levels in the last 6 months). In view of the various indications of a contribution by placental hormones to mammary gland growth, it seems tempting to expect that the obscure role played by the placental factor(s), HCG, in pregnant women might be clarified by intensified studies of the role played by the placenta in mammary gland growth.

IV. Comments to Observations on Mammary Gland Growth under Physiological Conditions

This last section is devoted to the question whether observations made on intact animals during the various stages of the life cycle can be explained by hormonal effects revealed in experimental work on mammary gland growth. Hormones observed to influence mammary gland growth are produced in hypophysial target organs (e.g., gonadal hormones) and in the anterior-pituitary gland (e.g., growth hormone, prolactin). Mammary gland growth is dependent then on the functional activity of the anterior-pituitary gland, and knowledge of its control seems necessary for an understanding of the regulation of mammary gland growth.

A. Brief Outline of the Control of Anterior-pituitary Function

Anterior-pituitary hormones regulate directly or via other endocrine organs the general metabolism, and they stimulate the development and activities of the gonads. The function of the anterior pituitary, serving vital processes, should be expected to be safeguarded by several mechanisms which, according to prevailing conditions, can co-operate with each other or replace each other. The clarification of the mechanisms regulating the activities of the pituitary gland is difficult. A well-defined supply of secretomotor nerves, which might be stimulated or interrupted experimentally, has not been found. The anterior-pituitary gland secretes, as far as we know, six different hormones, none of which can be detected or assayed with any accuracy in the venous outflow from the gland. The effects produced by the six hormones differ. *A priori*, it seems unlikely that the secretion of all these hormones is regulated in exactly the same manner. Our understanding of the control of anterior-pituitary function is based on experimental inquiries using an indirect approach. Considering this situation, but in view of the great variety of ingeniously designed investigations, it may be said that the activities of the anterior-pituitary gland are generally regulated: (1) by the central nervous system, (2) by the hormones of hypophysial target organs, and (3) perhaps by hormones of other endocrine glands (cf. Benoit & Assenmacher, 1955; Harris, 1955; Fortier, 1956; Long, 1956; Donovan & Harris, 1957).

(1) The cerebral control is apparently the most effective and differentiated one, and enables the anterior-pituitary gland to adjust its secretions rapidly to ordinary as well as extraordinary needs. The region of the brain studied most extensively with regard to its importance for the anterior-pituitary gland is the hypothalamus, but other parts are implicated too. The hypothalamic control is probably mediated by a specific system of blood vessels

(hypophysial portal vessels) connecting, via the pituitary stalk, the median eminence of the tuber cinereum of the brain with the anterior-pituitary gland.

(2) Relationships between the output of hormones from the anterior-pituitary gland and the blood level of hormones produced by hypophysial target organs are obvious, but complicated. The site of action of the target gland hormones, which are distributed throughout the body by the general circulation, may be either the central nervous system or the pituitary gland. In general, the secretion of hypophysial trophic hormones is suppressed when the concentration of their target gland hormones is high, and vice versa. The target gland hormones may cause secondary effects on other trophic hormones. A few examples may be given. Cortisone depresses not only the output of ACTH, but also the activity of the thyroid gland, probably by suppressing the secretion of TSH. Administration of thyroxine depresses the secretion of TSH, but the adrenal glands hypertrophy at the same time (Harris, 1955; Donovan & Harris, 1957). The secretion of oestrogens by the ovaries is stimulated by the combined actions of the two pituitary gonadotrophins, follicle-stimulating hormone (FSH) and luteinizing or interstitial-cell-stimulating hormone (LH or ICSH). Oestrogens have been reported to cause a release not only of FSH and LH, but also of the third hypophysial gonadotrophic hormone, luteotrophic hormone (LTH) or prolactin (Everett, 1950; Desclin, 1952). Oestrogens are believed to stimulate the secretion of increased amounts of ACTH. It is doubtful, however, whether this effect is primary or secondary to an inhibition of the production of adrenal-cortex steroids (Vogt, 1955). The experiments of Vogt are of special interest since the activities of the adrenal glands were studied by determination of the content of cortical steroids in the venous effluent from the adrenal glands. In rats injected with hexoestrol the adrenal glands were enlarged and appeared microscopically like actively secreting glands, but the output of hormones was found to be inhibited. Subsequent studies of Holzbauer (1957) revealed that the actions of oestrogens on the pituitary-adrenal system are even more complicated and showing differently according to the demands on the activities of the adrenal cortex.

(3) Evidence suggesting a participation of hormones of the adrenal medulla and the neurohypophysis in the control of anterior-pituitary function has been put forward. The role played by these hormones cannot, however, be regarded as clarified. The claim was made repeatedly that neurohypophysial hormones are instrumental in the hypothalamic control, but so far an agreement about the nature of the hormonal factor(s) carried by the hypophysial portal vessels to the anterior-pituitary gland has not been reached (Benoit & Assenmacher, 1955; Harris, 1955; Long, 1956; Fortier, 1956; Cowie & Folley, 1957; Donovan & Harris, 1957).

From this brief outline it is apparent that the functional activity of the anterior-pituitary gland is controlled in part by the central nervous system (1 and 3). The hormonal control of the anterior-pituitary gland seems to involve a highly intricate system of interactions (2). The growth of the mammary gland may then be regarded as dependent upon the central nervous system. However, the question whether the growth patterns of mammary gland tissues are influenced, under certain conditions, by hormones released from the anterior-pituitary gland by target-gland hormones can often be answered by assumptions only.

In the following remarks concerning the control of mammary gland growth from birth to puberty the activities of the anterior-pituitary gland will be considered.

B. Mammary Gland Growth from Birth to Puberty

The period from birth to puberty may for the present purpose conveniently be divided into three stages: (1) A short period after birth in which effects of hormones circulating in the maternal and foetal blood may still be present, (2) the nursing period in which maternal hormones may reach the offspring with the milk, and (3) the period after weaning until puberty. Under natural conditions the young are not weaned abruptly. The transition from (2) to (3) occurs gradually. The end of the third period may be difficult or impossible to determine accurately especially when, as in males, no outward sign (opening of the vaginal orifice, occurrence of first oestrus or menstrual bleeding) indicates that the gonads have reached maturity.

The developmental state at parturition varies considerably in different species of mammals. In the oviparous monotremes, e.g., the duck-billed platypus and in the viviparous marsupials, the Australian native cat and the Virginian opossum, the hatched or new-born young are extremely undeveloped. Guinea-pigs, on the other hand, look like miniatures of adults and are able to supply themselves with ordinary food from the 1st day of their life. A great variety of intermediate stages are known, and it is clear that bodily changes will be more or less comprehensive during extra-uterine life depending on the state reached at birth. Many factors, a good deal of them unknown, are operating to bring about a development of the reproductive organs to functional activity. With regard to mammary gland growth two questions are pertinent: (a) Are the hormones known to be involved in the stimulation of mammary gland growth produced before puberty, and at what levels? (b) Are the effector organs (gonads, sex accessory glands, mammary glands) responsive to the stimulating actions of hormones, and what is the degree of responsiveness?

Detailed studies of these questions were made, e.g., by Price (1947) on sex

accessory glands of rats. It has long been known that gonadotrophic hormones are not released into the blood of young animals in amounts sufficient to stimulate ovarian growth and function. Observations of Harris & Jacobsohn (1952) indicate that early in life the stimuli originating in the brain, but not the capacity of the anterior-pituitary gland to secrete gonadotrophic hormones, are absent or insufficient. Ovarian hormones are secreted before puberty (from 14 days onwards in rats, cf. Price, 1947), but the mammary glands of rats and mice appear to be unresponsive at an early age, the growth of the glands being isometric with body surface. A response of the mammary glands of suckling rats to oestradiol dipropionate could be elicited by administration of anterior-pituitary extracts (cf. Folley, 1955). In man, a responsiveness of the mammary glands to stimulating (most likely maternal oestrogenic) hormones is indicated by the well-known hypertrophy and secretion (witch's milk) in the breasts of many male and female babies during the 1st or 2nd week after birth (Geschickter, 1945). Similar conditions seem to occur in sheep (Wallace, 1953). The possible influence exerted on mammary glands of suckling young by hormones present in the milk cannot be regarded as clarified yet (cf. Folley, 1955). Since rats are very undeveloped at birth and it is difficult to raise them on an artificial diet, studies of this question should be performed on other species.

As mentioned in the introduction to this Chapter, mammary gland growth is restricted or absent before puberty, a situation that may well be explained by the failure in the production of stimulating hormones in adequate amounts as well as by a partial inability of the mammary glands to react. It should, however, be mentioned that systematic studies of the factors indicated in the present Section are few.

C. Mammary Gland Growth During the Fertile Age

In the species studied, the regulation of mammary gland growth by ovarian hormones is well attested by observations of the whole reproductive system at different stages of the sexual cycles and during pregnancy. The observations are mentioned in the prefatory Section (for literature see Section IA) and will not be repeated here. The maintenance of mammary gland structures during the period of nursing is probably best explained as an effect of anterior-pituitary hormones, chiefly prolactin (cf. Section III D; Lyons, 1942; Cowie & Folley, 1957). Provided that suckling young are present, the mammary gland structures developed during pregnancy are maintained and lactation continues even when both ovaries of the mother have been removed (cf. Hartman & Corner, 1947; Mayer & Klein, 1949; Desclin, 1953; see also Cowie, Chapter 4).

In view of the evidence available from systematic studies of

other reproductive organs, notably the placenta (Amoroso, 1952), it should be said that present knowledge about the mammary gland is chiefly derived from observations and experiments on selected breeds of domesticated laboratory or farm animals. The possibility that the responsiveness of the mammary glands to hormonal stimuli varies in different species should be kept in mind. So far, the ferret seems to be a rare exception, in that growth of mammary ducts does not occur during the long period of oestrus which is clearly indicated by reactions of the sex accessories (Hammond & Marshall, 1930). On the other hand, males of monotremes seem exceptional because their mammary glands develop equally as well as those of the females. The structure of these mammary glands is, however, primitive. Since only the female, of at least the duck-billed platypus, cares for the sucklings, it appears unlikely that the offspring profit from the development of the male mammary glands (Fleay, 1951). Systematic investigations on members of different classes of mammalia, including wild relatives of the animals studied, would perhaps reveal that differences in the responsiveness and reaction patterns of the tissues constituting the mammary glands are more common. From what is known about other reproductive organs in the evolution of viviparity (Amoroso, 1952; Medawar, 1953), it seems reasonable to assume such a situation. In conclusion it appears apt to recall the inspired words of F. H. A. Marshall (1936) in his Croonian Lecture: "At present the most reasonable way of regarding the matter is to suppose that the sexual hormones were originally derivatives from the sterols which are widely distributed in living tissues, that the hormones at first had no particular physiological significance, but that in the course of evolutionary progress the parts of the body concerned, the uterus and the vagina and the male accessory sexual glands as well as the secondary sexual structures, have developed the capacity to respond to the chemical substances which have thus acquired the character of specific hormones in the manner originally postulated by Starling (1905)."

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