The Effect of Some Endocrine Factors on Mammary Gland Growth and Lactation

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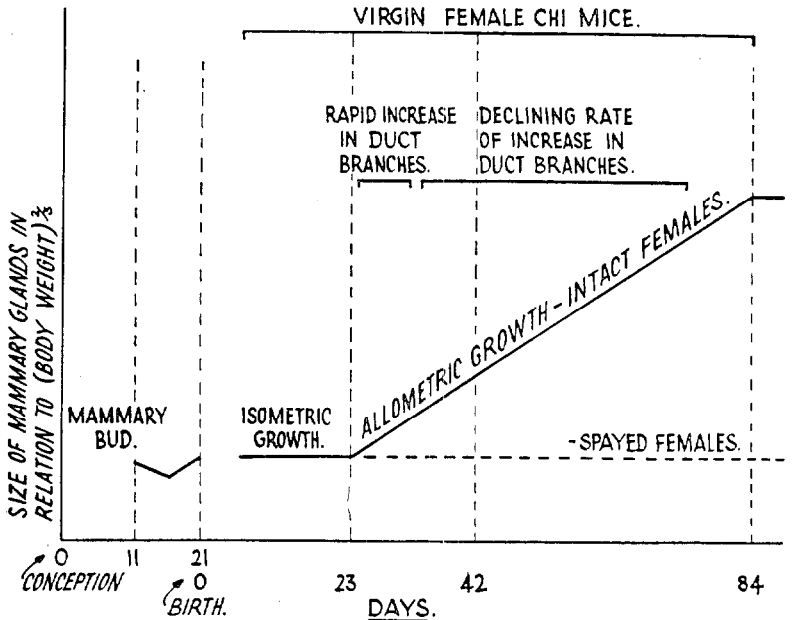
IN this paper a number of "snippets" from recent studies on mammary gland growth and function are presented. Many of the members of the Society have a general knowledge of the part played by the secretions of the endocrine glands in the control of the mammary gland and will have no difficulty in seeing where the parts fall into the general picture. It is hoped that the brief sketch of mammary gland development in the mouse which follows will help those who have no background knowledge of the subject.



MAMMARY GLAND GROWTH IN THE MOUSE

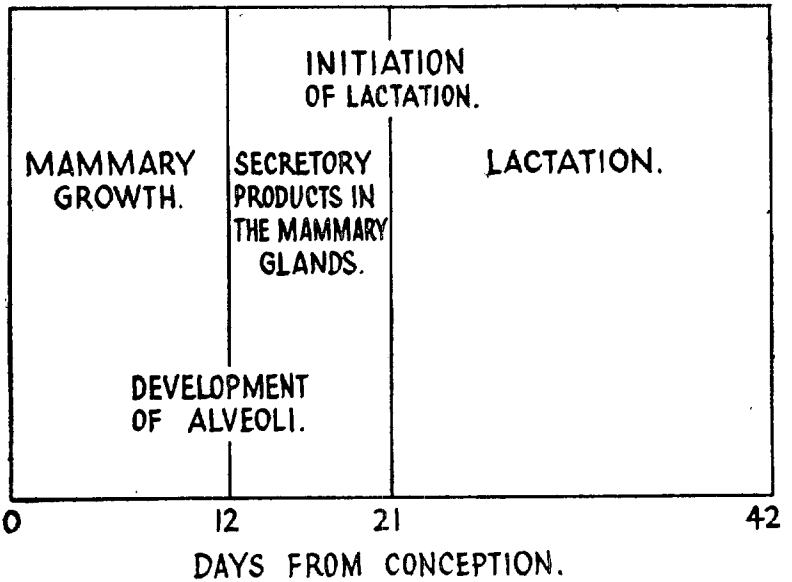
Figure 1 is a somewhat diagramatic representation of the growth of the mammary duct system in the mouse, which, as far as is known, is basically the same as in other mammals.

In the embryo the epidermal thickening (mammary bud) which is the first recognisable mammary gland rudiment is seen at about 11 days after conception. The mammary gland rudiment increases in volume slowly in comparison with the rest of the body until about 16 days, then it begins a period of rapid growth lasting until the 19th day at least (Balinsky 1950).

From about 7 to 23 days after birth, in mice of the CHI strain, log 10 (area of the duct systems) increases at about the same rate as log 10 (body weight) 2·3 (used as a convenient reference base) and

then, in the intact female, it speeds up to about five times that rate. This phase of rapid growth continues until about 84 days of age, after which, in the virgin mouse little more development normally occurs. Removal of the ovaries abolishes the phase of rapid duct growth. The rate of increase in the number of branches in the duct systems of the intact females is very rapid soon after 23 days of age but slows up and is about zero from 70 days of age (Flux 1954a).

While the growth of the duct systems in virgin and spayed female mice has been measured precisely, no such detailed studies have been made of the developing mammary glands of pregnant mice and the basis for the following description is provided by the results of a few rather subjective studies. The stages of development are shown in Fig. 2.



Pregnancy can start as early as 28 days of age. Conception is followed by a rapid increase in the area covered by the ducts and in the number of duct branches and the development on the ducts of alveoli, each of which is made up milk-secreting cells surrounding a lumen which is drained by a small duct. The early development is mostly in the ducts and later it seems that both alveoli and ducts develop together. About half way through pregnancy gland growth is complete and secretory products begin to accumulate in the alveoli and ducts although active lactation does not begin until the young are born. The initiation of lactation seems to be something separate from mammary gland growth brought about by a rapid change in the amounts of certain hormones in the circulation.

ENDOCRINE CONTROL OF MAMMARY GLAND GROWTH

It has long been known that ovarian and anterior pituitary hormones play a major part in the growth and development of the mammary gland (see Folley, 1952, for review of the experiemntal evidence). The factors causing changes in the growth rate of the rudiment in the embryo are not yet known. In the young mouse of the CHI strain it seems that the change from isometric to allometric growth of the mammary glands is brought about by the ovaries beginning to secret oestrogen in adequate amounts since the phase of allometric

growth can be started a few days early by giving injections of oestrogen. Whether the increased ovarian activity is caused by the anterior pituitary beginning to secrete gonadotrophins, or by the ovary becoming sensitive to gonadotrophins already present, or to both is not yet known. Mice of this strain which were treated with a highly purified preparation of anterior pituitary follicle-stimulating hormone showed no response in the ovaries or mammary glands. This may have been because the dose was inadequate, but when material prepared in the same way was used at lower dose rates in mice of another strain (Moon and Li 1952) responses in the ovaries were obtained. Before 15 days of age the mammary glands of the CHI mice did not respond to injections of oestrogen alone but did grow if an unfractionated preparation of anterior pituitary tissue was given in addition. The pituitary hormones involved have not been determined. (Flux 1954a). All that can be said with reasonable certainty about the early stages of mammary growth in the CHI mouse is that both anterior pituitary factors and oestrogen seem to be necessary for the change from isometric to allometric growth.

The mammary duct development of the intact female from 23 to 42 days of age could be well imitated in spayed females of the same age given suitable doses of oestrogen alone, hence it seems that progesterone played little part in mammary gland development up to this stage. This would be expected because only three or four ovarian cycles could have been experienced before 42 days. As mammary development does not increase much after about 80 days in the virgin female it seems that progesterone does not play a major role until after conception. It is interesting to note here that in the dog the mammary glands have been reported to be relatively insensitive to oestrogen, but respond with rapid growth to treatment with progesterone (Trentin, Devita and Gardner, 1952). As only two levels of oestrogen and one of progesterone were used, however, these results can be taken as only suggesting a possible greater sensitivity to progesterone in this species. Wide differences between species in the relative levels of oestrogen and progesterone needed for optimal mammary growth exist, but in most of those which have been tested a combination of both seems to give better results than either alone.

THE ADRENAL CORTEX

The adrenal cortex has frequently been blamed for unexpectedly great development of the mammary glands. This is probably because one steroid of the adrenocortical type (11-deoxycorticosterone or DOC) is known to act synergistically with oestrogen to stimulate mammary development in a manner very similar to progesterone. In addition progesterone has been isolated from adrenal tissue (Beall, 1938, Reichstein, 1938). However, the evidence available at present suggests that neither of these steroids is secreted in appreciable amounts by the normal adrenal cortex. (See Hechter and Pincus, 1954).

From a quantitative point of view the most important steroids which have been found in the blood from adrenal glands are of the type having an oxygen atom or hydroxyl group attached to carbon atom 11 of the steroid molecule [Kendall's compound B (corticosterone)] and Kendall's compound F [17-hyroxy corticosterine (cortisol or, in American parlance, hydrocortisone)]. These steroids, like two others which are similar in the configuration at carbon 11 but not important in the output of the adrenal cortex [Kendall's compound A (11-dehydrocorticosterone)] and [Kendall's compound E (17-hydroxy, 11-dehydrocorticosterone (cortisone)] inhibit oestrone-induced mammary gland growth in mice. DOC which, as mentioned above, acts synergistically with oestrogen to promote mammary growth, has no oxygen or hydroxyl group at carbon atom 11 and in this respect is similar to progesterone which it closely resembles structurally as well as in its effect on the mammary glands. However, there is apparently no simple relationship between the structure of the molecule at carbon

atom 11 and effects on mammary gland growth because Reichstein's compound S (17-hydroxy, 11-deoxycorticosterone), which is like DOC in having no oxygen or hydroxyl group at carbon atom 11 and resembles it qualitatively in many biological activities, inhibits oestrone-induced mammary gland growth (Flux 1954b). There is obviously a need to investigate the recently isolated and very active adrenalcortical steroid aldosterone in the same way. It is known, however, that the spectrum of steroids secreted by the adrenals of the female CHI mouse under the stimulus of injected adrenocorticotrophin (ACTH) has the net effect of inhibiting oestrone-induced mammary gland growth (Flux 1954b).

Under some conditions, however, the adrenal cortex of the mouse does appear to produce mammary growth stimulating secretions. If female mice of some strains are spayed, the adrenal cortices develop neoplastic tissue in the region of the zona glomerulosa after some months. The appearance of this tissue is accompanied by the appearance of enlarged uteri and mammary duct systems. (Woolley 1950, Flux 1954a). There is evidence that such mice secrete unusually large quantities of oestrogen in the urine and it is probable that this has its origin in the abnormal adrenal cortices (Dorfman and Gardner 1944). The adrenal cortex can also produce androgens (see Hechter and Pincus 1954) but the effect these would have is not clear as androgens have been found to stimulate mammary growth in some studies (Bottomley and Folley 1938) and to inhibit oestrogen-induced mammary growth in others (Ahelger and Huseby 1951, Muhlbock 1949).

THE ANTERIOR PITUITARY

It has long been known that anterior pituitary hormones are concerned in mammary gland growth. One group of workers (see Trentin & Turner 1948) has, for many years, postulated the existence of a specific anterior pituitary "mammogenic" hormone, but no compelling evidence for its existence has been forthcoming. Recently Lyons and his colleagues (Lyons, Li, Johnson & Cole 1954) have developed the mammary glands of immature castrate hypophysectomised rats with injections of prolactin, growth hormone, oestrogen and progesterone and induced them to secrete by continuing the injections of prolactin and growth hormone with the addition of 17-hydoxycorticosterone. (Oestrogen and progesterone were discontinued). This work appears to dispose of any necessity to look for a specific "mammogen" except, of course, for the fairly remote possibility that one was present as a contaminant of one of the pituitary hormones used.

PLACENTA

Placental tissues are now known to produce factors with actions very like if not identical with those of the hormones produced by the anterior pituitary and ovary. (For example see Opsall and Lang 1951, who describe identification of ACTH in human placental tissue). These, in some experiments, have been sufficient to maintain pregnancy during the later stages of pregnancy after the removal of the anterior pituitary. (Smith 1954). The contribution of placental hormones to mammary development during pregnancy and to the initiation of lactation is not known and should be investigated.

LACTATION

INITIATION OF LACTATION

There is little point in going over in this paper all the mechanisms suggested in the past for the starting of lactation at parturition (for these see Folley 1953). A recent and apparently very helpful contribution to knowledge on the subject has been made by Meites and Sgouris (1954) who found that in the rabbit the lactation-initiating effect of prolactin was inhibited by a combination of oestrogen and progesterone. The inhibition was relative, i.e., it could be overcome by

increasing the dose of prolactin. It is suggested by these authors that changes in the concentrations of the three hormones at parturition bring about the start of active secretion of milk by the cells of the alveoli. It would be interesting to know the level at which the inhibition takes place (mammary gland secretory cell, anterior pituitary, etc.).

It has been well established that high doses of oestrogen alone inhibit lactation although lower doses are lactogenic (Folley 1952) but because it is known that considerable amounts of progesterone are in circulation in late pregnancy it seems likely that the explanation suggested by Meites and Sgouris is correct than that initiation of lactation is brought about by a change in the level of oestrogen only in the circulation. Progesterone alone does not inhibit established lactation in the rat (Folley 1942, Folley & Kon 1938).

ESTABLISHED LACTATION

Recently the galactopoietic (lactation-stimulating) effect of growth hormone has been attracting attention. This activity was first demonstrated by Dr. Folley, Dr. F. G. Young and their colleagues who found that the galactopoietic effect of unfractioned extracts of anterior pituitary tissue was related to their diabetogenic (blood sugar increasing) effect. Of the two anterior pituitary hormones known to be diabetogenic, ACTH (adrenocorticotrophin) was found to inhibit and growth hormone to stimulate lactation in cows when given in single doses (Cotes, Crichton, Folley and Young 1949). Work with single doses of growth hormone has since then been done by other workers (Donker and Petersen, 1951) and with repeated doses [Chung, Shaw & Gill, 1953, and in N.Z. by Brumby & Hancock (1955)]. These workers all obtained increased milk yields, some of the increases being spectacular, of the order of fifty per cent in the experiments of Chung et al and Hancock and Brumby. No explanation for the galactopoietic effect of growth hormone has yet been published but it is interesting to note here the comment of Professor F. G. Young, that growth and lactation are similar in that both involve the preservation of metabolites from oxidation (Young 1947).

Because there were contradictory reports about the effect of ACTH on lactation and adrenalectomy in rats was known to depress milk yield (Folley 1952; Cowie 1952; Flux 1955, Flux, Folley and Rowland (1954) treated cows with ACTH suspended in a medium which prolonged its action. There was no doubt about the depressions in milk yield resulting from the use of this hormone either in single doses or smaller doses repeated several times. Detailed analyses of the milk from the treated and control cows were carried out in the hope that changes in milk composition might give an indication as to where the increased levels of steroid hormones from the adrenal cortices released in response to the ACTH were acting. However, the only changes in milk composition seen were similar to those which take place more slowly in cows drying off at he end of lactation.

THYROXINE AND TRIIODOTHYRONINE

With the synthesis and commercial manufacture of l-thyroxine the use of iodinated protein in attempts to stimulate milk yields has declined. Use of the pure hormone has great advantages over iodinated protein in that no flavour is given to the feed in which it is mixed and there is no need to have it bioassayed. Recently another active agent from the thyroid has been isolated. This is triiodothyronine, which, in some experiments, has been found to be more active than thyroxine. It will stimulate milk yields in cows but only if given by injection as it appears to be destroyed in the rumen (Bartlett, Burt, Folley & Rowland 1954). From a practical point of view it seems that thyroxine may be useful for altering the shape of the lactation curve rather than for increasing total production (Leech & Bailey 1953).

INDUCED LACTATION IN RUMINANTS

Despite a few spectacular successes in which individual cows brought into lactation by means of hormones have given more than eight gallons of milk a day (Meites, Reineke & Huffman 1950) it must be said that progress in this work has been disappointing. Progesterone, which has become available in adequate amounts and at reasonably low cost, has been used with oestrogen in a number of experiments. Its use has helped to suppress some of the undesirable oestrous symptoms seen in cows given oestrogen alone and has resulted in the development of mammary gland tissue more nearly normal than that resulting from the use of oestrogen alone (Cowie, Folley, Malpress & Richardson 1952), but has not brought much improvement as far as milk yields are concerned. Responses in milk yield range from about zero to several gallons a day, as they did in the earlier experiments in which oestrogen was used alone (Folley & Malpress 1944). Hancock, Brumby & Turner (1952) at Ruakura have shown that in heifers which were presumably normal, milk yield obtained in an induced lactation depended on the inherent productive capacity of the animals used. One difficulty in this type of work is that the animals in which one desires to induce lactations in practice are usually those which cannot be got in calf and this group will contain those which are grossly abnormal in the functioning of their endocrine glands. It seems at present that further substantial progress in this work will depend on the further development and application of methods for measuring the levels of hormones in the circulation. Great progress has already been made in methods for steroid hormones and we are fortunate in having in New Zealand Dr. Edgar and Dr. Raeside who are both interested in this type of work, particularly in relation to ovarian hormones..

In the early experiments on induced lactation in ruminants the labour for treating animals was reduced by the use of tablets of steroid hormones implanted under the skin. More recently the use of suspensions of crystals of the hormones has done away with the necessity for even the small operations necessary to implant and remove tablets. The dose rate and duration can be regulated by adjusting the range of crystal sizes and choosing substances of suitable solubility (Folley & Flux 1953, unpublished). Even after progesterone has been used with oestrogen some cows have suffered broken pelvic bones (Folley & Flux 1953, Flux 1954 unpublished). These mishaps may perhaps be eliminated by omitting the high doses of oestrogen which have been given in the last weeks of treatment before milking started to stimulate secretory activity in the udder. In an experiment with spayed goats in which mammary development was successfully induced by means of oestrogen and progesterone injections doses of oestrogen known to have been "lactogenic" in other experiments were given to half the goats when milking was started. The other goats were milked but given no oestrogen. There was no significant difference between the rates at which the goats in the two groups came into milk which suggests that the oestrogen added little or nothing to the lactation initiating or stimulating effects of milking (Benson, Cox and Flux 1953, unpublished). There is still some element of doubt about this, however, as there was considerable variance within the group of goats. Unfortunately we had no monozygous twins of this species.

CONCLUSION

In conclusion it is interesting to compare the knowledge of the role of the endocrine glands in mammary gland growth and function at the present time, far from complete though it is, with that existing twenty years or so ago. As with the study of endocrinology in general there have been tremendous advances. Then it was known that mammary development was related to reproduction and that the ovaries and pituitary glands were concerned in it, but most of the experimental work was done with crude tissue extracts. Now, by use of a

few specific (or, in the case of the anterior pituitary hormones, supposedly specific) compounds experimentalists can cause mammary glands to grow and begin to secrete in gonadectomised, hypophysectomised animals in which only the barest rudiments of mammary tissue existed to start with. Much of the progress has been made possible by the efforts of the chemists who have isolated protein, peptide and steroid hormones, gone far towards purifying the former and synthesised the latter. When it is possible to measure levels of hormones in the general circulation, and this will probably be before very long, by use of these materials and objective means of measuring changes in mammary gland structure, it will very likely be possible to develop more uniformly successful methods for inducing lactation in ruminants, and perhaps for improving the productive capacity of some of those which calve naturally.

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Discussion

Dr. HENDERSON: Was there any effect on hair growth cycles, moulting etc.?

Dr. FLUX: Effects on these were not observed. An androgen—17-vinyltestosterone—did seem to cause a toughening of the skin, but despite considerable efforts we were not able to measure differences in skin strength quantitatively.

Dr. EDGAR: Agreed that further progress in induced lactation and similar work depended on measurement of hormones in the blood; the levels of natural hormones vary greatly and target organs may also vary in sensitivity.

Dr. HENDERSON: Are there any breed differences?

Dr. FLUX: Differences between strains of mice in the sensitivity of the uteri, mammae and vaginal epithelium to oestrogen are known. Little work has been done on differences between breeds in sensitivity to hormones, but such differences are suspected to exist. For example the milking goat seems to be relatively less sensitive to hormone therapy than the cow.

Dr. EDGAR: Breeds of sheep differ in their sensitivity to P.M.S. Suffolks react to smaller doses than Romneys. The ovaries may vary in sensitivity.