MARSHALL'S PHYSIOLOGY OF REPRODUCTION

EDITED BY

A. S. PARKES, M.A., D.Sc., Sc.D., F.R.S.
National Institute for Medical Research, London

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CHAPTER 20

LACTATION

By S. J. Folley

The possession of mammary glands is an essentially mammalian character. The function of the mammary gland, which forms a necessary part of the sexual apparatus of mammals, is to secrete milk, a fluid elaborated from precursors furnished by the blood, and which constitutes the sole source of nourishment for the newly-born young, since in most species the latter can only obtain food by the act of suckling. The complex of phenomena comprising the synthesis of milk, its passage through the alveolar cell membrane, and its expulsion from the mammary gland, is called lactation. In the case of the cow and to a lesser degree the goat and the sheep, lactation has assumed great economic importance on account of the wide use of milk and milk products in human nutrition, and this has led to the development, by selective breeding for milk production, of breeds of cattle and goats the capabilities of which as regards lactation are far above those of the species in the wild state.

Mammary glands are present in both sexes though they are usually functional in the female only. That the male gland is equipotential with that of the female, at any rate in many species, is shown by the occurrence from time to time of cases of spontaneous lactation in the male (*see*, for example, Shannon, 1932) and by the fact that the hormonal induction of mammary growth and lactation in males of certain species of laboratory mammals, made possible by modern developments, is now a commonplace procedure.

The number and position of the glands varies greatly among different species, the number varying from one pair as in most species of Primates to eleven pairs as in *Centetes*. In a general sense the average number of mammary glands characteristic of a species is related to the average litter size and the needs of the young. Thus in the guinea-pig, where the normal litter size is small and the young are born in a comparatively advanced state of development so that if necessary they can fend for themselves, there are only two mammae, while in the rabbit, in which the normal litter size is larger and the newly-born young are naked and helpless and the gestation period is far shorter, there are usually eight mammae.

In species in which the mammary glands are few in number they are sometimes confined to the thoracic region (Primates—excepting some lemurs, Cheiroptera, Sirenia, elephants, sloths) while in other species (most Ungulata, Cetacea) they are restricted to the inguinal region. When the mammae are more numerous they are usually situated in two nearly parallel rows along the ventral aspect of the thorax and abdomen. In all orders of mammals except the Monotremata, each mammary gland is provided with a teat or nipple through which milk is withdrawn

from the gland during suckling. In the cow and most other Ungulata the mammae are contained within a definite milk-bag or udder, which is surrounded by a fibrous envelope and is suspended below the abdomen ; each gland (four in the cow and two in the sheep and goat) is provided with a milk cistern or galactophorous sinus, communicating with the teat, in which milk accumulates between milkings.

In monotremes alone there are no teats, the orifices of the mammary glands being mere scattered pores in the skin, the exuded milk probably passing along the hairs, which in this region are arranged in bunches.

I. THE NORMAL DEVELOPMENT OF THE MAMMARY GLAND

The available information on the development and comparative anatomy of the mammary gland in various species has been comprehensively summarised by Turner (1939a).

It is convenient to consider the development of the mammary gland in the female as it proceeds during each of the following epochs in the sexual life of the animal, namely, embryonic and foetal, birth to puberty, during recurrent sexual cycles, pregnancy, lactation and finally, involution (*see also* reviews by Nelson, 1936, and Turner, 1939b).

Embryonic and Foetal Development

Many excellent studies of the development of the mammary gland during the embryonic and foetal period in various species have been made, among which may be cited those of Lustig (1915) on man, Hammond (1927) and Turner (1930, 1931) on the cow, Turner and Gomez (1936) on the goat, Rein (1882a) on the rabbit, Myers (1917a, b) on the rat, Turner and Gomez (1933a) on the mouse, O'Donoghue (1912) on the marsupial cat *Dasyurus viverrinus*, and Bresslau (1920) on various monotremes and marsupials. The following account, which mainly refers to the bovine mammary gland, is based on the afore-mentioned descriptions given by Turner.

The mammae belong to one of the three types of skin gland which have their origin as invaginations of the ectoderm. The location of the future mammary glands first clearly appears in the young embryo when first the formation of milk streaks and then mammary lines begins on either side of the ventral mid-line. The mammary lines consist of thickenings of the epidermis due to proliferation of the Malpighian layer. The appearance of centres of proliferation along the mammary lines defines the number and position of future teats, both normal and supernumerary. In many species the occurrence of supernumerary teats (polythelia) and sometimes mammae (polymastia) is not uncommon. At each such centre of proliferation a mammary bud or hillock gradually develops, very little of which projects above the surface, but which grows downwards, forcing the basement membrane into the mesenchyme tissue. Turner (1930) described sex differences in the size and shape of the mammary bud in the bovine at an early stage of development. According to him the male buds are spherical in shape and larger than in the case of the female, in which the shape is ovoid. The formation of the embryonic teat is marked by a gradual raising of the proximal

end of the mammary bud above the surrounding epithelium due to proliferation of the mesenchyme cells encompassing it. As the teat develops, the ovoid shape of the mammary bud is gradually obliterated by the pull of the epidermis and it is finally transformed into a funnel-shaped crater filled with cornified cells forming a kind of plug at the tip of the teat. The next significant stage in mammary development is the formation of the primary sprout which begins as an invagination at the proximal end of the funnel-shaped residue of the mammary bud. As development of the primary sprout proceeds, secondary sprouts form and each grows out at an angle from the end of the primary sprout. Later, tertiary sprouts develop and form the " anlagen " of the future arborescent duct system of the udder. The extent of development of the secondary and tertiary sprouts during the foetal period is, however, relatively slight.

Early in its development a lumen forms in the primary sprout, gradually proceeding from the proximal to the distal end. The distal end of this lumen is called the streak canal; nearer the gland it forms the teat cistern, while in the gland itself it forms the gland cistern. The streak canal, which forms the entrance to the teat and the lumen of which always remains narrow, is thus the last to open. In the cow each teat is provided with a single such excretory duct. On the other hand, in some species, including man, there are a number of excretory ducts in each teat which have developed from multiple primary sprouts, each such duct being now regarded (*see*, for example, Rein, 1882b; Bresslau, 1920; Hammond, 1927; and Turner, 1930), in contradistinction to the once widely accepted views of Gegenbauer (1876) and Klaatsch (1884), as a structure homologous with the single primary sprout in the cow. After complete differentiation of these structures the extent of the streak canal is defined by the continuation of the many-layered epithelium characteristic of the skin, in contrast to the two- or three-layered epithelium lining the teat and gland cisterns.

As the foetus grows, development of the teat gradually proceeds. Even shortly before birth, however, the development of the sphincter muscle which later surrounds the streak canal has not advanced beyond the stage where circularly disposed elastic connective tissue fibres are present. At this stage, differentiation of smooth muscle cells has hardly begun. The vascular zone of the teat is well developed prior to birth. The type of teat found in the bovine and in most other placental mammals may be called a " proliferation teat."

In the rat and the mouse the teats develop as a result of the epithelium surrounding the mammary bud growing downwards into the mesenchyme tissue. When the cornified " epithelial hood " (Myers, 1916) is shed there results a shallow depression or sulcus surrounding a somewhat deeply embedded teat (*see* Turner and Gomez, 1933a, for the mouse). Turner and Gomez call this an " epithelial ingrowth " teat. A third type of teat (" eversion teat ") is found in many marsupials. The primary sprouts develop at the base of a pocket which, during lactation, becomes everted like the finger of a glove thus forming the teat (*see* Bresslau, 1920).

The formation of the udder begins quite early in the female bovine foetus, its structure at first consisting almost solely of mesenchyme cells which subsequently differentiate to fibrous tissue cells. Later, whorls of connective tissue cells and adipose tissue cells develop and gradually the embryonic udder

structure comes to consist largely of adipose tissue divided by septa of connective tissue. The early development of the septum which longitudinally divides the udder into two halves is worthy of note. Udder development is entirely lacking in the male foetus since the mammary primordia are situated on or just anterior to the scrotum, and the underlying mesenchyme tissue, which in the female gives rise to the pad of fatty-connective tissue which later provides room for the growth of the secretory tissue of the udder, is therefore very restricted.

At birth the female calf possesses an udder equipped with gland and teat cisterns of mature form, the development of secretory tissue being, however, confined to regions round the teats. Duct development has progressed very little beyond that apparent in the male at this stage.

Development During the Period from Birth to Puberty

There is some variation among different species as regards the extent of mammary development which occurs during this phase. In many species this period is characterised by a gradual increase in the extent of the mammary duct system, while in other species the duct growth before puberty is comparatively slight. In general, no alveolar development occurs in the prepubertal period. In species in which the mammary glands assume a definite compact form, as the bovine udder, there is a gradual growth of the latter due to an increase in the fatty and connective tissue.

The available information on the prepubertal development of the mammary gland in primates is relatively scanty but it seems clear that in the rhesus monkey the mammary duct system undergoes considerable extension between birth and puberty. Folley, Guthkelch and Zuckerman (1939) studied glands from two immature rhesus monkeys which had never menstruated. In each case the duct system extended well beyond the base of the nipple and showed a moderate degree of arborescence ; no alveolar tissue was present. Turner and Allen (1933) and Allen, Gardner and Diddle (1935) also reported extensive duct growth in glands taken from prepubertal rhesus monkeys. In man, according to Turner (1939a), the mammary duct system undergoes slow and gradual development during this period but again no alveolar tissue forms. In well-nourished girls the breasts may increase in size before puberty owing to increase in the fatty-connective tissue.

Hammond (1927) obtained evidence of a progressive increase with age in the size of the udder in the prepubertal calf. There appeared to be some growth of the duct system accompanied by end bud formation, but no development of alveolar tissue. Similarly in the female goat (Turner and Gomez, 1936), the udder growth from birth to puberty is due, as in the case of the calf, to accumulation of fatty-connective tissue. Meanwhile, there proceeds a gradual increase in the length and ramification of the ducts, which are lined with two-layered epithelium. In the male, duct growth is much slower.

Turner and Gomez (1933a) have described the development of the mammary gland of the prepubertal mouse (*see also* Cole, 1933). Soon after birth, secondary sprouts become canalised and tertiary and quarternary sprouts form. Thereafter, in the female, the ducts increase in length and number due to dichotomous branching. According to Turner and Gomez the end buds which are observed at this

stage are formed of solid masses of epithelial cells and should not be mistaken for aveolar tissue.

Similar studies were made on the rat by Myers (1916, 1917b). The extent and degree of arborescence of the duct system gradually increases from the fifth to the ninth week from birth, but during the ninth and tenth weeks, just prior to the initiation of the oestrous cycles, the increase in duct development is more marked. At puberty, glandular development is so extensive that in some cases left and right members of pairs of thoracic or inguinal glands have met at the ventral mid-line.

In contrast to the examples just considered, mammary duct growth during the prepubertal period in the guinea-pig is comparatively slight. Some extension of the duct system does occur, however, in both male and female during this period, but more noticeable is the increase in gland size in the female due to the growth of the fatty-connective stroma (Turner and Gomez, 1933b).

In the rabbit (Ancel and Bouin, 1911), cat (Turner and De Moss, 1934), dog (Turner and Gomez, 1934b), hedgehog (Deanesly, 1934), grey squirrel (Deanesly and Parkes, 1933), fox (Rowlands and Parkes, 1935), and common shrew (Brambell, 1935) there is little mammary duct growth during the prepubertal period. In these species the prepubertal gland usually consists of a few primary ducts hardly extending beyond the base of the teat.

Development in the Virgin Female During the Oestrous (or Menstrual) Cycle and in Pseudo-pregnancy

There are significant differences between various species in the extent to which mammary development occurs in nulliparous females exhibiting cyclic ovarian activity. In general the extent and type of mammary development in the sexually mature virgin female is related to the type of cycle characteristic of the species in question. In many species the ovarian cycles are accompanied by cycles of mammary growth and regression upon which is often superimposed a progressive, if gradual, development of the mammary structures with successive cycles. Since some of the species (dog, fox, opossum, *Dasyurus*) to be considered exhibit cycles characterised by a prolonged luteal phase (pseudo-pregnancy), it will be convenient to deal in this section also with the mammary changes accompanying pseudo-pregnancy in those forms (rat, mouse, rabbit, ferret) in which it is not a natural feature of the cycle but can be induced by procedures such as sterile copulation or cervical stimulation. Species exhibiting regular cycles which do not normally include a pseudo-pregnant phase will first be considered. The rat and mouse will be included under this heading although their cycles are short as compared with those of, for example, the primates and the bovine, since if the duration of the cycle is considered in relation to the gestation period and the normal life-span the difference vanishes.

In contrast to the situation in most of the common laboratory animals, the primates are noteworthy in that mammary lobule-alveolar tissue appears to be developed as a result of successive menstrual cycles. Folley *et al.* (1939) in a study of mammary development in the nulliparous rhesus monkey, in which animal the mammary gland is a relatively flat structure, observed a gradual increase in the area of the mammary gland with age (*see also* Aberle, 1934). In

a series of non-pregnant monkeys, many of which were parous, the rate of increase of mammary gland area was found to be allometric as compared with general body growth (for terminology of relative growth, *see* Huxley and Teissier, 1936). The first menstrual cycles in this form are usually anovulatory and in cyclic monkeys which had never come under the influence of a corpus luteum, no alveolar tissue was found. Alveoli were, however, present in the mammary gland of monkeys which had ovulated. The development of alveolar tissue in the mammary gland of the rhesus monkey during recurrent menstrual cycles was also reported by Turner and Allen (1933). Cyclic changes in the mammary gland of the monkey during the menstrual cycle were described by Speert (1941).

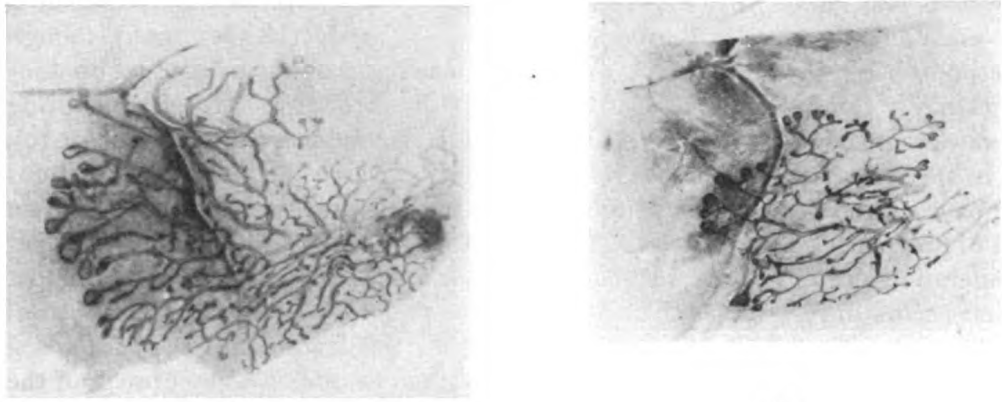
In woman, according to Rosenburg (1922), the ovarian cycle is accompanied by cyclic changes in the mammary parenchyma, lobule-alveolar growth occurring during the luteal phase, followed by complete regression after menstruation. Evidence of cyclic alternations of growth and, to a greater or lesser degree, regression, was also reported by Lewis and Geschickter (1924), Polano (1924), Ernst (1925) and Luchsinger y Centeno (1927), though the validity of Rosenburg's conclusions was questioned by Dieckmann (1925) whose material indicated that during the early years of puberty the duct system is gradually laid down, after which there is gradual growth of lobules. The significance of the above-mentioned studies is rather difficult to evaluate since in most cases the material, obtained at post-mortem, was rather heterogeneous as regards age and parity of the subjects. Gershon-Cohen and Strickler (1938) in an X-ray study of the normal human breast could find no evidence of cyclic changes in the parenchyma during the menstrual cycle but they confirm Dieckmann's finding of oedema of the stroma just before menstruation.

The histology of the udder in the maiden heifer was studied by Lenfers (1907) who found that the epithelium of the finer ducts consisted of a single layer of cells. Such ducts should therefore be classified as alveolar ducts. Evidence of secretory activity in the duct epithelium was obtained by Hammond (1927) who also observed cyclic changes during the oestrous cycle. At oestrus the ducts are large and filled with secretion and the epithelial cells cuboidal; after oestrus the ducts shrink and the epithelium becomes columnar. With successive cycles the size of the udder increases as more and more fatty-connective tissue is laid down, and presumably there is progressive development of the ducts and secretory elements since Hammond demonstrated the presence of alveolar ducts in the udders of virgin heifers. It is, however, not clear from his description and figures whether or not true alveoli lined with single-layered epithelium were present.

The mammary gland of the guinea-pig at various stages of the oestrous cycle was studied by Loeb and Hesselberg (1917a, b) who reported cyclic changes in the structure of the gland associated with the ovarian cycle. Their conclusions have, however, been rightly criticised by Turner (1939a) on the ground that their material was not confined to glands from animals undergoing their first few cycles but even included glands from parous guinea-pigs. Turner and Gomez (1933b) observed growth of the mammary duct system in virgin females at oestrus, at which time a number of buds form along the ducts and the latter become filled with secretion, presumably originating from the cells of the duct epithelium.

During the luteal phase the ducts appeared shrunken. These changes were particularly marked during the initial cycles. No convincing evidence of the occurrence of alveolar development in glands taken from virgin females was obtained. Turner and Gomez conclude that in the guinea-pig the luteal phase of the cycle stimulates little or no mammary alveolar growth.

In the mouse and rat, which exhibit recurrent oestrous cycles of short duration, measured on the human time scale, most observers agree that the oestrous cycle is accompanied by cyclic changes in the mammary duct system (*see* Fig. *20*. 1). Detailed studies of the changes in the mammary gland of the mouse in various phases of the life cycle have been made by Turner and Gomez (1933a), Cole (1933), Gardner and Strong (1935) and Fekete (1938). According to Cole, considerable growth of the existing duct system coincides with the first oestrus while each subsequent oestrus is accompanied by a further slight burst of growth. During pro-oestrus



(*a*) (*b*)

Fig. *20*. 1—Whole mounts of second thoracic mammary gland of virgin mouse. (*a*) At first oestrus. (*b*) At first di-oestrus. Note the elongation of the end buds and dilation of the ducts at oestrus. Alveoli are completely absent. × 6.

buds form at the ends of the ducts, while in early oestrus the ducts dilate and the buds elongate. Regression was observed before the end of oestrus, and by the end of met-oestrus the duct system had returned to a condition characteristic of the previous di-oestrus. Histologically, no cyclic changes could be detected during the oestrous cycle, indicating that the oestrous proliferation was due rather to changes in existing epithelial cells at the duct endings, than to their multiplication. Observations which on the whole agree with the foregoing were made by Turner and Gomez, while Cogswell (1929), Bradbury (1932a), Gardner and Strong (1935) and Fekete (1938) also observed cyclic changes in the mammary gland during the oestrous cycle in the mouse. Bradbury's observations indicated that a succession of oestrous cycles causes a gradual cumulative growth of the mammary duct system in this species, though the observations of Gardner and Strong (1935) on five strains of mice suggest that full virginal development must be attained soon after the initiation of oestrous cycles, since they found that the duct system increases up to about the 70th day of age but that from the 70th to the 100th day little further development occurs. No alveoli were found in the glands of virgin mice provided they had never been pseudo-pregnant.

Cyclic changes in the mammary duct system of the female rat correlated with the ovarian cycle were observed by Sutter (1921) and Astwood, Geschickter and Rausch (1937). At oestrus, sprouting of the duct buds and the formation of fresh buds were observed ; at the succeeding met-oestrus regression had set in. Turner and Schultze (1931) observed some lobule-alveolar formation in the mammary glands of adult virgin rats which suggests that successive cycles exert a cumulative effect on mammary growth in the rat.

In the dog, the marsupials *Dasyurus viverrinus* and the opossum, and probably the fox (Rowlands and Parkes, 1935) in which the luteal phase of the oestrous cycle is so prolonged as to be comparable with true pregnancy in duration (complete pseudo-pregnancy), development of the mammary alveoli to an extent similar to that characteristic of pregnancy (*see* below) occurs during the prolonged luteal phase (*see* O'Donoghue, 1912, for *Dasyurus* ; Marshall and Halnan, 1917, and Turner and Gomez, 1934b, for the bitch ; Hartman, 1923, for the opossum ; and Rowlands and Parkes, 1935 for the fox). In the bitch, copious milk secretion is not uncommonly seen at the end of pseudo-pregnancy (Heape, 1906), though it is improbable that this occurs in virgin bitches at the end of the first pseudo-pregnancy. Similar mammary alveolar proliferation occurs in the ferret during pseudo-pregnancy induced by sterile copulation (Hammond and Marshall, 1930).

In species such as the mouse, rat and rabbit, in which the duration of pseudo-pregnancy induced by sterile copulation or cervical stimulation is much less than that of pregnancy (incomplete pseudo-pregnancy), the degree of mammary alveolar proliferation during the former condition is more or less comparable with that which occurs during the equivalent interval of pregnancy. In the rabbit (Ancel and Bouin, 1911 ; Hammond and Marshall, 1914 ; Hammond, 1917 ; Parkes, 1929 ; Fauvet, 1939), initiation of pseudo-pregnancy is followed by growth of the mammary ducts until adjacent glands meet, then by alveolar growth up to about the sixteenth day (*see* Fig. *20*. 2), after which regression sets in. Similarly in the mouse (Turner and Gomez, 1933a ; Cole, 1933), rat (Freyer and Evans, 1923 ; Schultze and Turner, 1933 ; Weichert, Boyd and Cohen, 1934) and golden hamster (Deanesly, 1938), mammary development during pseudo-pregnancy runs more or less parallel with that occurring during the first half of pregnancy, though Weichert *et al.* regard pseudo-pregnancy in the rat as resembling a shortened pregnancy in that they observed a secretory phase towards the end of pseudo-pregnancy.

In the rabbit and the ferret, which do not ovulate spontaneously, and thus, in the absence of copulation or other stimuli to ovulation, remain in continuous oestrus for a considerable time, the mammary glands in the mature virgin do not normally come under the influence of a corpus luteum. Ancel and Bouin (1911), who studied the changes in the rabbit mammary gland during oestrus, found that the restricted duct system previously present ramifies considerably at this time. In the mature virgin rabbit, in her first oestrus, not only was extensive duct development found, but also some alveoli lined with single-layered epithelium, which, if the animals had indeed never ovulated, would indicate that in the rabbit, some degree of alveolar development can occur in the absence of luteal influence. In the ferret, on the other hand, Hammond and Marshall (1930) report that no significant mammary development occurs during oestrus, which is somewhat surprising in view of the long duration of oestrus in the unmated ferret.

The outstanding implication of the results of the studies considered in this section is the close relationship of the corpus luteum to the growth of the mammary alveolar system. In species in which the cycle does not include a prolonged luteal phase there is little unequivocal evidence that growth of true alveoli,

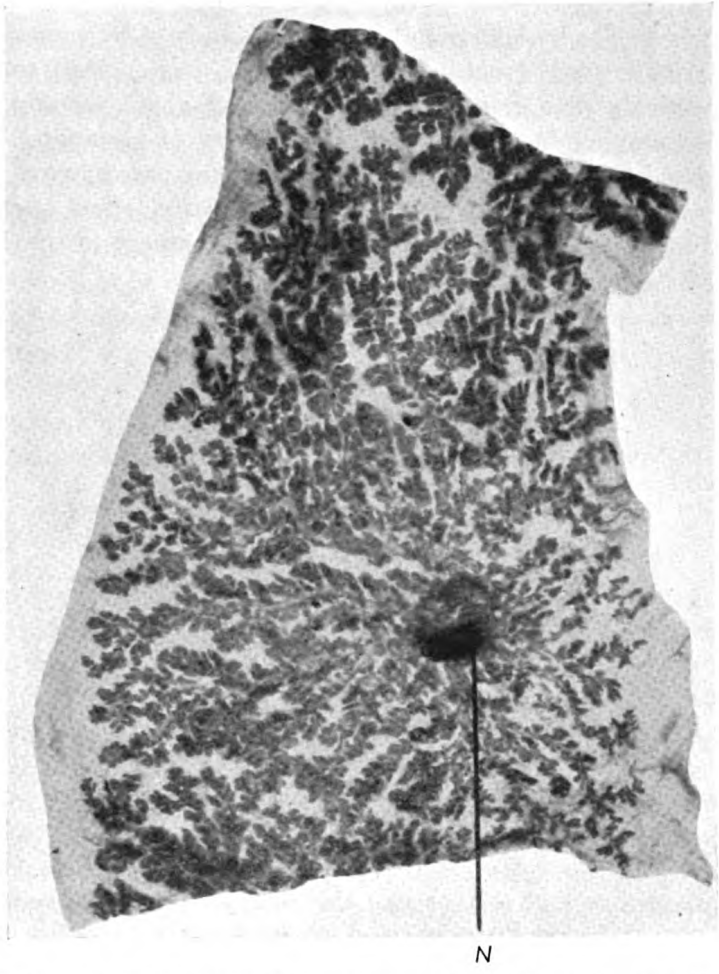


Fig. 20. 2—Whole mount of mammary gland of rabbit 12 days pseudo-pregnant. N. denotes nipple. × 1·1. (From Parkes, 1929.)

characterised by a single-layered epithelium, occurs as a result of a succession of cycles, except in the primates.

Development During Pregnancy

Two main phases of mammary development can be distinguished during pregnancy, namely, the growth phase and the secretory phase. The essential difference between the *hyperplasia* of the mammary parenchyma, which is characteristic of the first half or two-thirds of pregnancy and the *hypertrophy* of the mammary gland, due to distension of the epithelial cells and alveolar lumina with secretion, which occurs during the later stages of this condition, is thus

emphasised. It should be understood, however, that there is no sharp division between these two phases of mammary gland development, inasmuch as there is evidence that glandular growth may proceed side by side with the gradual development of the secretory process. The above distinction has been emphasised, notably by O'Donoghue (1912), for *Dasyurus*, Turner and Gomez for the mouse (1933a), guinea-pig (1933b), dog (1934b) and goat (1936), Ancel and Bouin (1911) for the rabbit, as well as by Hammond (1927) in a study of the cow, while Roberts (1921) and Weatherford (1929) have presented histological and cytological evidence respectively that in the rat the mammary gland secretory cells reach their maximum number by about the thirteenth day of gestation. Cole (1933), on the other hand, observed growth of the mammary parenchyma of the albino mouse proceeding into lactation (*see* below). Similarly Jeffers (1935a) reported that mitotic, but not amitotic nuclear division was frequent in the rat mammary

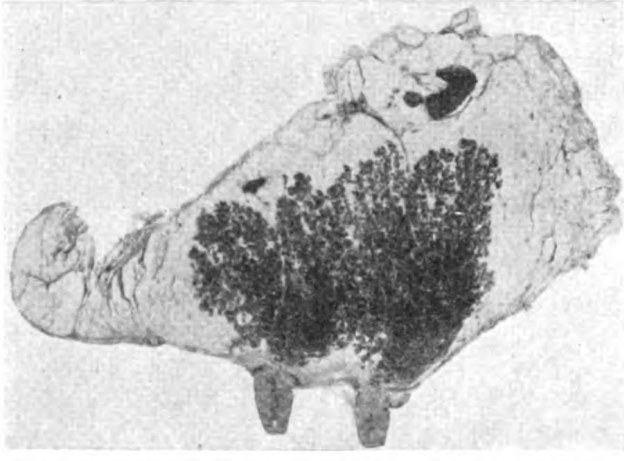


Fig. 20. 3—Section of udder of heifer 8 months pregnant.
(From Hammond, 1927.)

gland in late pregnancy and further that the average number of cells per alveolus increased steadily during the latter half of pregnancy.

Additional evidence that secretory activity of the mammary gland begins in mid-pregnancy is afforded by the fact that a secretion containing milk constituents can be drawn at this stage from the udders of heifers pregnant for the first time (*see* page 596), and also by the work of Cutler and Lewis (1933), who were able to detect casein in the udders of nulliparous pregnant heifers after the twenty-fourth week of pregnancy but never before the twentieth week. Moreover, it has been shown that if pregnancy is experimentally terminated by abortion, milk secretion only develops if the operation is performed late in pregnancy (*see* Loeb and Hesselberg, 1917b, for the guinea-pig and Drummond-Robinson and Asdell, 1926, for the goat).

A good description of mammary development during pregnancy in the heifer is given by Hammond (1927). During the first six months the main ducts sprout to form the alveolar ducts and the lobules themselves form. At the fifth month the lobules have become definitely formed but are still small, while by the sixth month they have greatly increased in size because of distension by secretion.

From this stage until parturition the gradual accumulation of secretion causes the whole gland to swell (*see* Fig. *20*. 3).

The growth phase of the mammary gland during pregnancy in the albino mouse has been well described by Turner and Gomez (1933a). Following conception, formation of buds occurs along the walls of the ducts and particularly at their ends. The end buds representing the " anlagen " of the future lobes increase in length to form intralobar ducts, secondary buds later forming along the walls and at the ends of these. Gradually these elongate to interlobular ducts at the ends of which more buds form which eventually develop into alveoli. According to these authors, hyperplasia of the gland parenchyma is complete at the twelfth day after conception. Cole (1933), studying the mammary gland of the same animal claims, as indicated above, that only just before parturition does the glandular tissue become equal in amount to the stroma, while according to him the proportion of the former is greatest at twelve days *post-partum*.

Apart from the detailed studies already considered, papers in which sections or whole mounts of the mammary gland during pregnancy in various species are figured include those of Keynes (1923) and Dabelow (1934) on man, Wahl (1915) on the rabbit, Deanesly and Parkes (1933) on the grey squirrel, Deanesly (1934) on the hedgehog, Deanesly (1944) on the weasel, and Brambell (1935) on the common shrew.

Changes During Lactation

Following parturition, the mammary alveoli and lobules increase in size, due to increase in secretory activity, for a period which varies with the species (*see*, for example, Turner and Gomez, 1933a, and Cole, 1933, for the albino mouse). According to the latter, the amount of glandular tissue in the mammary gland of the mouse reaches its maximum at about the twelfth day *post-partum*, though it was not quite clear whether this was due to cellular hypertrophy or to increase in the number of cells. However, most observers (*see*, for example, Maeder, 1922, and Weatherford, 1929, for the rat) agree that mitosis is rarely seen in the mammary gland during full lactation, and since Cole found no mitoses after seven days *post-partum* it is likely that the former alternative is true. It may be noted that Jeffers (1935a) observed occasional mitoses in the rat mammary gland during lactation and estimated that the average number of epithelial cells per alveolus continued to increase throughout the suckling period. These results may indicate the replacement of the cells which have degenerated rather than continued growth of the parenchyma in the ordinary sense.

In the mammary gland of the lactating guinea-pig, Kuramitsu and Loeb (1921) observed mitotic cells in the alveoli shortly after parturition. The glands reached their maximum size two days after parturition, at which stage mitosis was no longer observed, though amitotic figures were frequent. At three weeks *post-partum*, diminution of secretory activity was apparent, and after four weeks the lobules and alveoli were visibly shrunken and lymphocytes had begun to appear in the alveoli. Turner and Gomez (1933b) made observations which, on the whole, agree with these.

The cytological changes in the mammary gland during lactation will not be considered here, as an account of the cytology of lactation is given in a later

section. The histological picture of the fully-lactating gland is illustrated in Fig. *20*. 4.

Post-lactational Involution

Involutional changes in the lactating mammary gland (Figs. *20*. 5 and 6) quickly follow failure to withdraw the secretion, whether due to removal of the young at weaning or to the gradual self-weaning of the young as they acquire other sources of food. In animals such as the cow and goat which are milked regularly, involution is more gradual, but does inevitably occur as is evidenced by the slow decline in milk yield. The studies of Lenfers (1907) on the cow indicate that this decline may be due, at least in part, to gradual reduction of the amount of tissue capable of active secretion as involution slowly spreads through the gland. Likewise in the rabbit after weaning, Wahl (1915) observed that portions of the mammary gland may undergo involution while lactation continues elsewhere in the same gland.

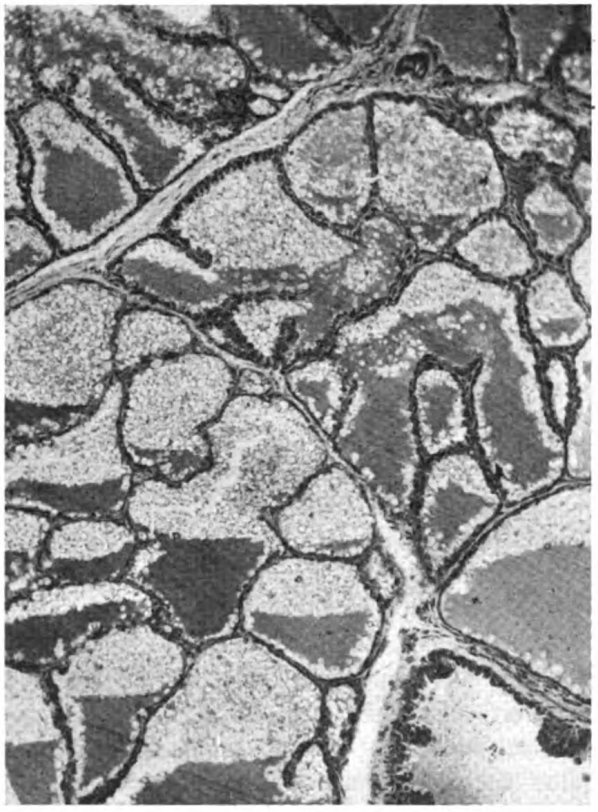


Fig. *20*. 4—Section of lactating mammary gland of cat. The fat has been dissolved out leaving numerous vacuoles in the milk. A creaming effect may be seen in many of the alveoli. The protein coagulum (grey) is emphasised. × 121.

Among studies of the post-lactational involution of the mammary gland may be mentioned those of Turner and Gomez (1933a), Cole (1933), Fekete (1938), and Williams (1942) in the mouse, Myers and Myers (1921), Kuramitsu and Loeb (1921) and Maeder (1922) in the rat, Turner and Gomez (1934b) in the dog and Turner and Reineke (1936) in the goat. Involution of the rabbit mammary gland following pseudo-pregnancy was described by Ancel and Bouin (1911). In general, cessation of suckling or milking is followed by a longer or shorter period of secretory engorgement during which resorption of the stagnant milk begins ; the alveoli collapse and disintegrate and the lobules gradually disappear. Frequently, vacuolisation, pyknosis and karyolysis are observed. In the albino mouse, Cole (1933) noted the formation of epithelial masses due to fatty degeneration of epithelial cells. According to Williams (1942), the rapidly growing adipose stroma plays an important role in removing necrotic parenchymal elements. Williams studied mammary involution in mice after removal of the young at birth, at the height of secretion (10th day) and at the usual weaning time (21st day). When no suckling was allowed, secretion ceased within

24 hours and involution was complete in five days. If the young were removed when secretion was at its height, secretion continued for two days and engorgement

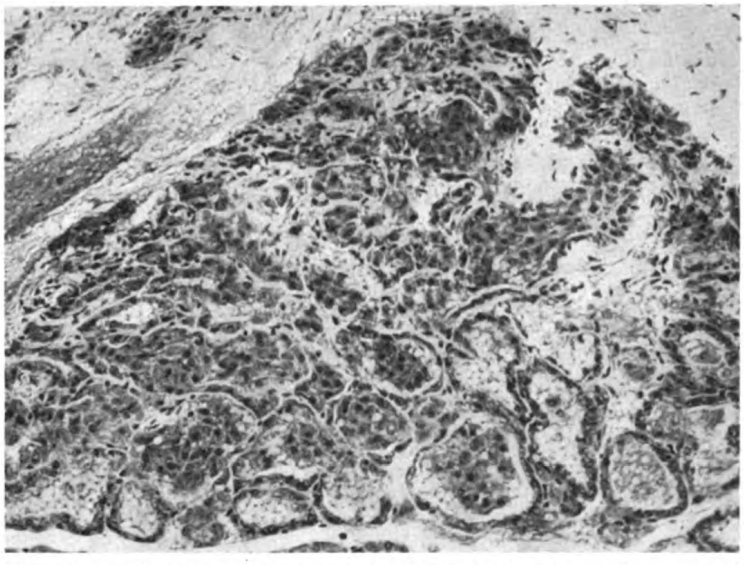


Fig. 20. 5—Section of mammary gland of rat showing early phase of involution in a lobule. Many of the alveoli have collapsed and in some the epithelium is forming central masses as it disintegrates. × 174.

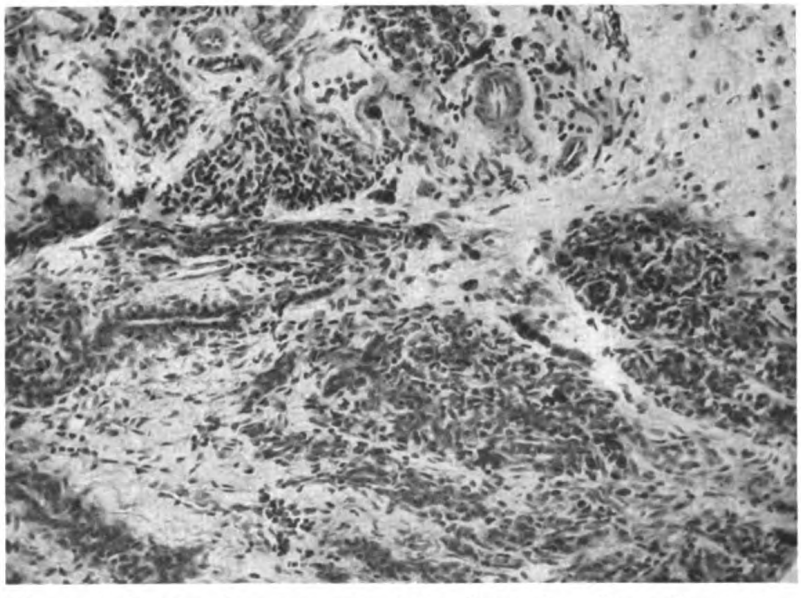


Fig. 20. 6—Section of mammary gland of rat illustrating advanced involution. Lactating alveoli are no longer recognisable and the lobular areas are reduced to disordered masses of epithelium combined with macrophages and other connective tissue cells. × 176.

for as long again, the mice showing a tendency to develop mastitis. Fifteen days were required for complete involution under these conditions. When the young were weaned as usual at the 21st day, mammary congestion lasted only for about 24 hours and extensive regression occurred during the next two days.

The resting condition assumed by the mammary gland at the end of the period of involution has sometimes been described as resembling the condition of the mammary gland of the virgin (*see* Turner and Reineke, 1936, for the goat), though often the duct system is more ramified than that of the virgin gland (*see* Cole, 1933, for the mouse).

The Male Mammary Gland

In many species of mammal, the mammary gland in the male consists of a restricted duct system hardly extending beyond the base of a rudimentary teat (*see* Turner and Gomez, 1933a, for the mouse ; Turner and De Moss, 1934, for the cat ; Turner and Gomez, 1934b, for the dog ; and Turner and Gomez, 1936,

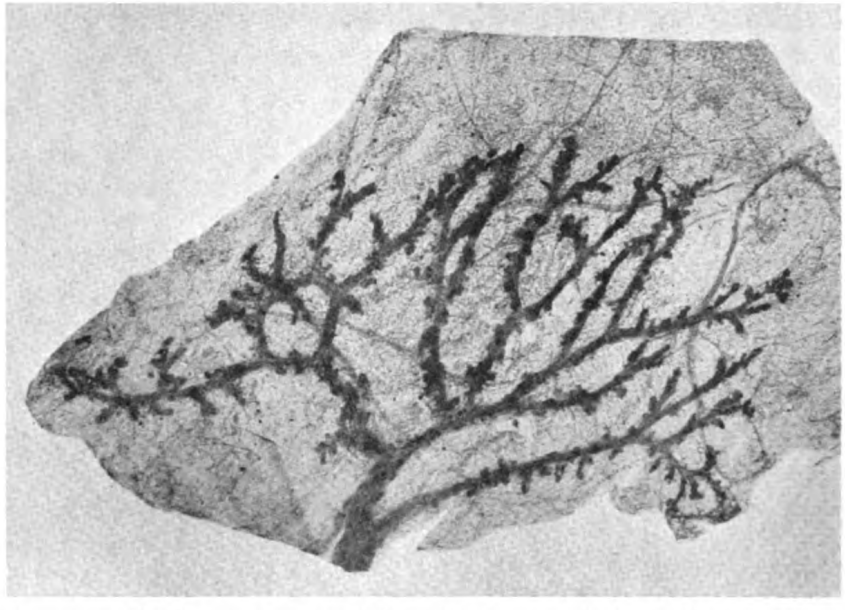


Fig. *20*. 7—Whole mount of second thoracic mammary gland of male rat at 30 days illustrating the relatively extensive duct growth which occurs during the prepubertal period. × 10·5.

for the goat). The observations of Turner and Allen (1933), Van Wagenen (1935), and Folley *et al.* (1939) on the whole indicate that in the rhesus monkey mammary development in the male is likewise normally very restricted though a moderate degree of individual variation in mammary gland area was indicated by the findings of Gardner and Van Wagenen (1938). In man, on the other hand, the mammary gland appears to undergo a certain amount of development at puberty (Andrews and Kampmeier, 1927 ; Jung and Shafton, 1935).

In the rat (Myers, 1917b) and guinea-pig (Turner and Gomez, 1933b) there is a certain amount of mammary duct growth in the period between birth and puberty so that the duct system in the pubertal male gland is relatively extensive (Fig. *20*. 7). Further development seems to occur in the male rat at or after puberty since lobules of alveoli (Fig. *20*. 8) are found in the mammary glands of mature male rats (Turner and Schultze, 1931 ; McEuen, Selye and Collip, 1936 ; Astwood *et al.*, 1937). Turner and Schultze (1931) for the rat, and Smelser (1933) for the guinea-pig reported that the male mammary gland does not regress after

castration. Contrary results for the rat were, however, reported by McEuen *et al.* (1936), and Astwood *et al.* (1937) state that acini do not develop in the mammae of castrated male rats.

In monotremes (*see* Bresslau, 1920, for *Tachyglossus* (*Echidna*) and *Ornithorhynchus*) the mammary glands of the male are as well developed as those of the female.

Abnormal mammary development (gynaecomastia) in the male, sometimes accompanied by milk secretion, occasionally occurs in some species. Numerous authors, for example Seifert (1920), Kriss (1930) and Menville (1933), the last two of whom give good bibliographies, have described cases of gynaecomastia in man ; it appears that this condition is often associated with hyperthyroidism,

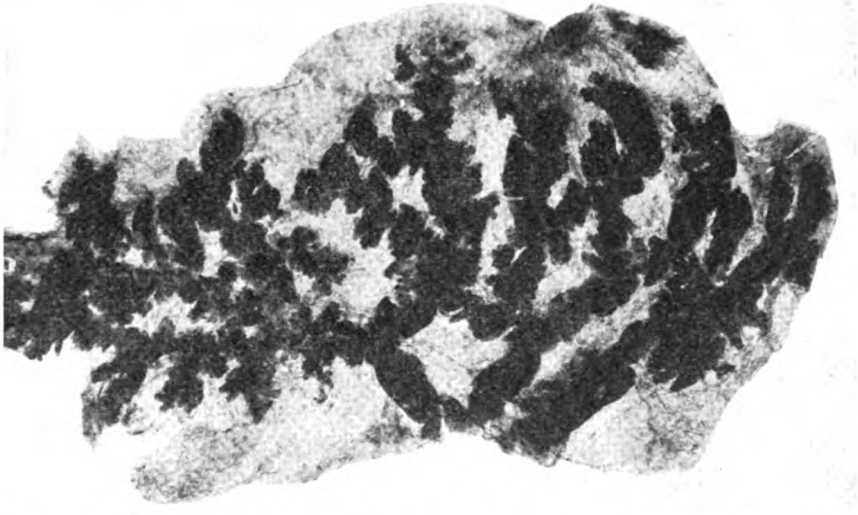


Fig. 20. 8—Whole mount of second thoracic mammary gland of male rat at 100 days. Note the dense clusters of alveoli associated with a duct system which shows relatively little arborescence as compared with a virgin female of the same age. × 6·4.

tumours of the adrenal cortex or chorionepithelioma. Cases which undoubtedly should be classified as gynaecomastia were described by Folley *et al.* (1939) in the rhesus monkey and in *Macaca nemestrina*. Such cases appear to occur with especial frequency in the goat (*see* Shannon, 1932 ; and Spann, 1933), though according to Turner and Gomez (1936) mammary development in the male goat is normally very restricted.

II. Experimental Analysis of the Endocrine Factors concerned in Mammary Growth

Experimental evidence has long been in existence which indicates that the development and secretory activity of the mammary gland is controlled by hormonal rather than neural mechanisms, but as will be seen in a later section, recent work suggests that nervous influences may be involved in the secretion of pituitary hormones concerned in lactation and particularly in the ejection of milk from the mammary gland.

Experiments involving severance of nervous pathways to the mammary gland, which indicated that mammary growth and lactation were largely independent of the central nervous system, were reported by Eckhard (1858), Mironow (1894), Goltz and Ewald (1896) and Pfister (1901), while Routh (1898) described the case of a woman with paraplegia below the " sixth dorsal vertebra " who lactated successfully.

Though, as is well known, the mammary glands regress after bilateral ovariectomy, this is not due to section of nervous connections, since Grigoriew (1897), Halban (1900) and Knauer (1900) were each able successfully to transplant ovarian tissue thus removed to other parts of the body, with the result that normal mammary development continued. Mammary development following the grafting of ovaries into males was reported by Steinach (1912), Athias (1915), Sand (1919), Moore (1921), Lipschütz and Krause (1923), and Gardner (1935). These experiments naturally gave rise to the belief that the ovary was concerned in mammary growth.

Lane-Claypon and Starling (1906) appear to have been the pioneers in attempts to induce mammary development by the use of organic extracts supposedly containing the relevant hormones. Their studies with aqueous extracts of foetus and placenta were followed by many others, but progress in this field was slow until the use of organic solvents for the extraction of sex hormones was introduced by Iscovesco (1912). The subsequent isolation and chemical characterisation of the sex hormones and the resulting advances in our knowledge of their physiology, described in other chapters of this book, have inevitably led to a much clearer insight into the nature of the endocrine factors involved in mammary development and lactation.

A priori consideration of the kind and degree of mammary development associated with various types of oestrous cycle, with pseudo-pregnancy and with pregnancy, would indicate that in general, the oestrogenic hormones probably stimulate duct development and that the extensive alveolar proliferation of early pregnancy and pseudo-pregnancy needs also the participation of the corpus luteum hormone, as was suggested by Ancel and Bouin (1911), Hammond and Marshall (1930), Asdell and Salisbury (1933) and others. The experimental studies now to be considered have shown that these ideas are, in broad outline, correct, though there appear to be differences between species as regards the nature of the response of the rudimentary mammary gland to oestrogens.

Oestrogen

Many species respond to oestrogen at moderate (more or less physiological) dosage levels by duct growth, often, especially when the treatment is prolonged, accompanied by a limited amount of alveolar proliferation. In other species there is evidence, much of it indirect, that oestrogen alone will evoke extensive, if not complete, alveolar development.

In the mouse, growth of the mammary ducts in response to oestrogen was observed by Turner, Frank, Gardner, Schultze and Gomez (1932), Bradbury (1932a, b), Turner and Gomez (1934a), Gardner, Diddle, Allen and Strong (1934), and Lewis and Turner (1941a). Data for the relative mammary duct growth promoting potencies of various oestrogens in mice are given by Lewis and Turner (1941b). Alveolar development, of an abnormal type, since it was localised in

certain limited areas (nodules), occurs after prolonged oestrogen administration in some strains of mice, both susceptible and resistant to spontaneous mammary cancer (Gardner *et al.* 1934 ; Gardner, 1935 ; Bonser, 1936 ; Gardner, 1941a). Prolonged treatment with large doses of an esterified oestrogen evoked extensive alveolar development resembling that of pregnancy, but again the response was abnormal in that the ducts were stunted (Gardner, Smith and Strong, 1935). Burrows (1935, 1936) has also reported alveolar development (probably localised) in the mouse following prolonged percutaneous application of certain oestrogens among which equilin seemed most effective in developing alveoli and oestrone least so. More recently, Mixner and Turner (1942b ; 1943) have observed minimal lobule-alveolar development in mice treated simultaneously with oestrone and high doses of diethylstilboestrol. Burrows' results provide some indication that various oestrogens may differ to some extent in ability to evoke mammary alveolar growth ; further investigation of the action of synthetic oestrogens from this point of view would be of interest. There seems little doubt that complete development of a normal mammary alveolar system in the mouse cannot be induced by oestrogen alone ; such alveolar growth as can be induced is of an abnormal, often precancerous, type and requires treatment with unphysiological doses for periods which amount to an appreciable fraction of the normal life-span.

Fig. 20. 9—Whole mount of part of mammary gland of male rabbit subcutaneously injected with 240 i.u. oestrone 5 days each week for 5 weeks. An extensive duct system has been developed but alveolar development is absent. × 1·5. (From Scharf and Lyons, 1941.)

In the rat, Laqueur, de Jongh and Tausk (1927) reported mammary growth in response to oestrogen but gave no morphological descriptions. Oestrogen-induced growth of the mammary duct system was reported by Turner and Schultze (1931), Astwood *et al.* (1937) and Lewis and Turner (1941a). Turner and Schultze, however, observed slight lobule formation with the higher dosage levels used and evidence of more extensive alveolar formation was reported by Halpern and D'Amour (1934) and Nelson (1935a). Alveoli in a state of secretion were observed by Lewis and Turner (1941c) in rats treated with diethylstilboestrol but these animals were mature multiparae and the glands might possibly have contained pre-existing alveoli.

A clear description of mammary duct proliferation in the rabbit, experimentally induced by injection of follicular fluid, was given by Vintemberger (1925). More recent studies in which purified extracts or pure oestrogens were used (*see* Fig. 20. 9), on the whole indicate that in the rabbit oestrogen induces only duct growth (Parkes, 1930 ; Turner and Frank, 1930, 1932 ; Turner *et al.* 1932 ; Anselmino, Herold and Hoffmann, 1935 ; Macdonald, 1936 ; Pallot, 1936 ; Fredrikson, 1939 ; Lyons and McGinty, 1941 ; Scharf and Lyons, 1941). The findings of Frazier and Mu (1935) and Frazier and Hu (1941) who observed lactation in male rabbits following oestrogen treatment might, however, be taken as indicative of alveolar formation, though some authorities (e.g. Mixner and Turner, 1943) believe that such results may be explicable on the basis of secretion by the duct

epithelium. However, alveolar formation has been reported in rabbits treated with natural oestrogen (Lyons, 1936) and diethylstilboestrol (Lewis and Turner, 1941a). There is no evidence that oestrogen alone is capable of causing *complete* mammary development in the rabbit.

In the cat (Turner and De Moss, 1934) and dog (Turner and Gomez, 1934b), oestrogen treatment seems to evoke mammary duct growth but no alveolar development. Indirect evidence (e.g. nipple growth, palpable enlargement of the mammae, secretion of milk) of mammary development in oestrogen-treated dogs was noted by Laqueur, Borchardt, Dingemanse and de Jongh (1928), Kunde, D'Amour, Carlson and Gustavson (1930) and Houssay (1935b). Gardner (1941a) observed nipple growth in young female dogs treated with oestrogen but there was little if any glandular proliferation.

With the guinea-pig we pass on to consideration of species in which there is evidence that oestrogen will cause extensive alveolar development in addition to duct growth. A number of authors (Steinach, Dohrn, Schoeller, Hohlweg and Faure, 1928; Laqueur *et al.* 1928; de Jongh and Laqueur, 1930; de Jongh and Dingemanse, 1931) have shown that in the guinea-pig, oestrogen will cause nipple and mammary gland development, lactation beginning when the treatment is stopped or the dosage reduced, thus providing indirect evidence that oestrogen will cause complete mammary development in this species. Direct morphological evidence that oestrogen will cause extensive alveolar development has been presented by Turner and Gomez (1934a) and Nelson (1937) using natural oestrogens, and Lewis and Turner (1942b) with diethylstilboestrol. In the guinea-pig the mammary glands of both sexes appear to possess equal potentialities for experimental development.

In ruminants, there is much evidence, mainly indirect, that oestrogen will evoke development of alveoli in addition to the duct system. In the virgin female goat (Fig. *20*. 10) treatment with oestrogen alone will cause the development of an udder capable of copious lactation (Hogreve, 1936; de Fremery, 1938; Folley, Scott Watson and Bottomley, 1940, 1941b; Lewis and Turner, 1940, 1941a, 1942a, d; Folley, Malpress and Young, 1945a; Mixner, Meites and Turner, 1944). In many of these experiments, which incidentally were mostly performed on intact goats so that the possible influence of ovarian progesterone cannot be excluded, the milk yields were so large as to justify the conclusion that extensive, though not necessarily complete, alveolar development had occurred. And indeed, histological studies of oestrogen-treated goats, including a spayed virgin, by Lewis and Turner (1941a, 1942d) and Mixner and Turner (1943) gave direct evidence of extensive lobule-alveolar development, though it could not be described as complete or normal in character. In many cases the alveolar lumen was abnormally large and the epithelium showed papillomatous outgrowths. On the other hand, it seems to be generally agreed that in the male goat oestrogen treatment causes very little udder growth (Hogreve, 1936; de Fremery, 1938; Folley *et al.* 1941b; Lewis and Turner, 1942d). Experimentally developed glands in the male goat were studied histologically by Folley *et al.* (1941b) who observed restricted alveolar formation (Fig. *20*. 11). Synthetic oestrogens were used in the great majority of the above-mentioned experiments, but since copious yields were obtained by de Fremery (1938) from goats treated with oestradiol monobenzoate the results cannot be ascribed to a special property of synthetic oestrogens.

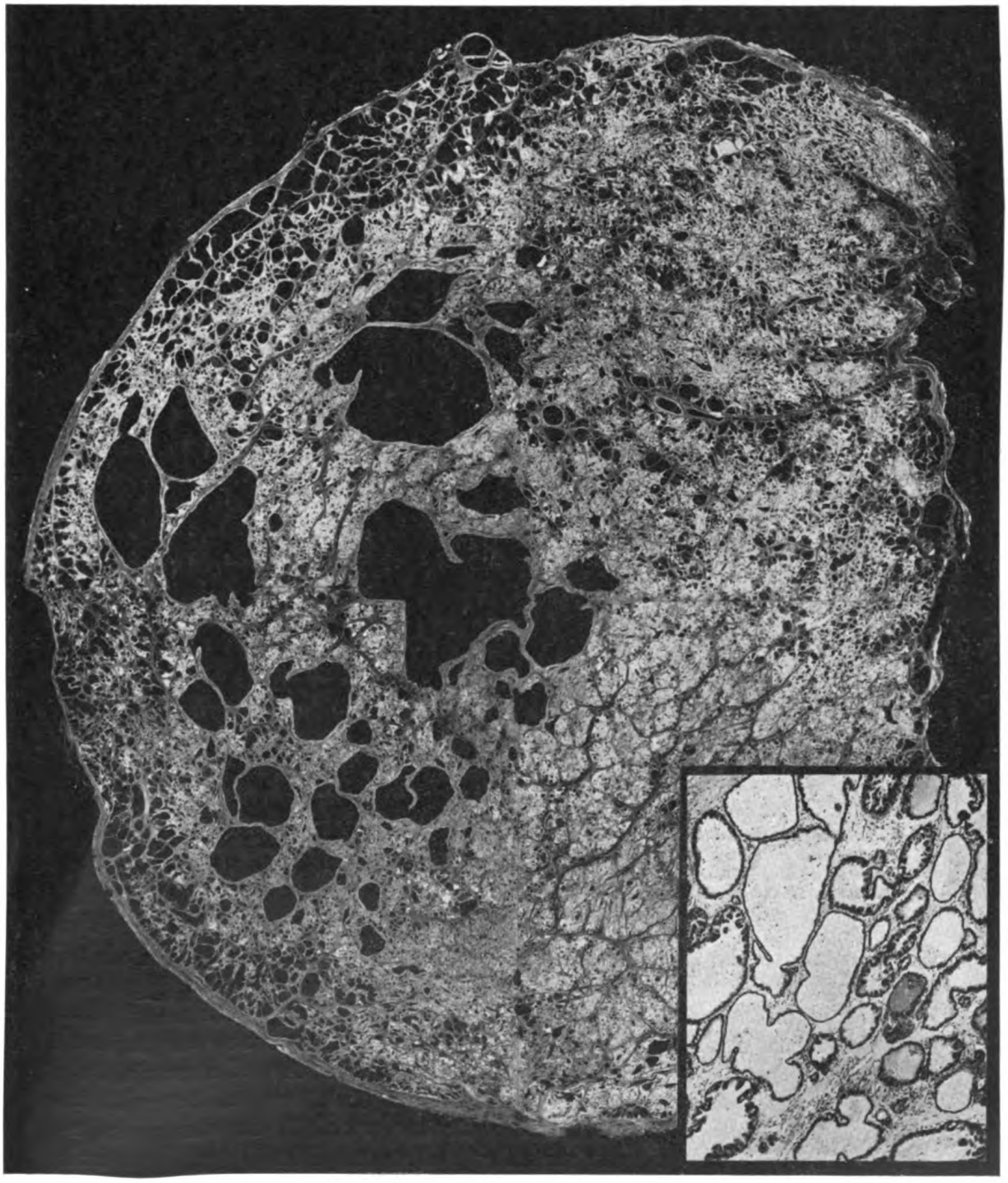


Fig. 20. 10—Portion of complete section (100μ) of one mammary gland of a virgin goat which had received prolonged treatment with hexoestrol. The glandular tissue, which has been stained with haematoxylin, is shown white on a black ground. Note the extensive alveolar development. × 1·6. The inset shows a high-power view of a portion of the alveolar epithelium. × 33.

Even more spectacular results along similar lines have been obtained in bovines (virgin heifers—in at least one case spayed, dry cows and freemartins) by Walker and Stanley (1940, 1941), Folley, Scott Watson and Bottomley (1941c), Reece (1943), Folley and Malpress (1944a, b), Folley, Stewart and Young (1944), Hammond, Jnr., and Day (1944), Parkes and Glover (1944) and Folley *et al.* (1945a), synthetic oestrogens being used exclusively in these investigations. The milk yields obtained, in some cases exceeding three gallons daily, could only be produced by abundant alveolar tissue and macroscopic examination of whole mounts of udder slices from oestrogen-treated heifers (Folley and Malpress, 1944a, b; Hammond, Jnr., and Day, 1944) showed that considerable lobule-alveolar growth had occurred. Successful induction of lactation in a spayed heifer (Walker and Stanley, 1940, 1941) and in a freemartin (Folley and Malpress, 1944a) indicates that in bovines, oestrogen will promote alveolar development in the absence of ovarian progesterone. In the bovine, as in the goat, the male mammary gland does not appear to be equipotential with that of the female since Folley and Malpress (unpublished observations) obtained no visible mammary enlargement, as a result of prolonged oestrogen treatment, in two bullocks, though there was some growth of the teats and the secretion of a small quantity of milk.

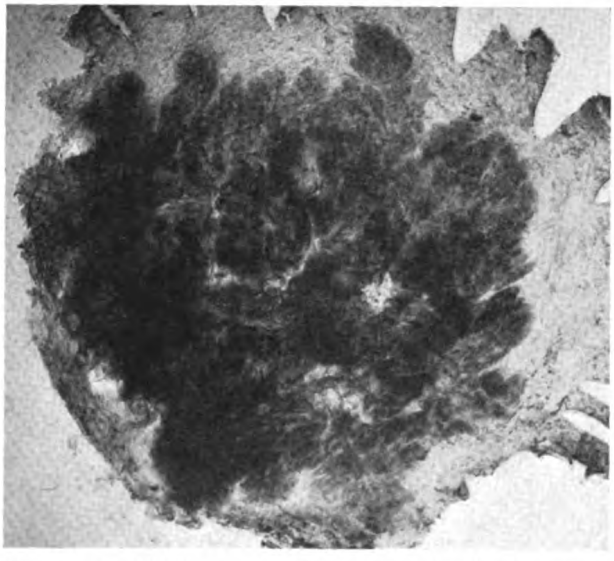


Fig. 20. 11—Whole mount of mammary gland of immature male goat which had received prolonged treatment with diethylstilboestrol. The duct system is very limited in extent and confined to a thin layer; lobules of alveoli are present. Compare with Fig. 20. 10. × 2. (From Folley, Scott Watson and Bottomley, 1941b.)

Many authorities believe that the mammary gland of the rhesus monkey may be stimulated to complete development by oestrogen. Changes in the mammary glands of monkeys in response to the administration of oestrogenic extracts were reported by Allen (1927) and duct and alveolar growth in response to oestrogen may perhaps be inferred from the results of Allen *et al.* (1935). Mammary duct growth in male monkeys resulting from treatment with oestrone was reported by Van Wagenen (1935), but the figures shown do not allow of a certain decision as to whether or not alveoli were formed. Brief reports, but with no experimental details, of the induction of alveolar growth in the monkey mammary gland by oestrogen were made by Turner and Allen (1933) (one male monkey) and Nelson (1936) (one male and two immature females). In a more extensive series of animals Gardner and Van Wagenen (1938) observed only duct growth in males treated for periods varying from 15 days to nine weeks, but in one male given oestradiol monobenzoate for 22 weeks, extensive alveolar development was observed. Gardner (1941a) later reported alveolar development in one additional male monkey which was given regular injections of oestradiol

monobenzoate over 31 weeks. In considering the significance of these observations it should be remembered that Folley *et al.* (1939) described well-developed glands containing alveoli in one of five untreated male monkeys. The results of Folley *et al.* (1939) with male monkeys are not in agreement with those just discussed, for they observed duct growth in most, but alveolar growth in only one (and that not one of the longest treated), of twelve monkeys given oestrone for periods varying from 6 to 484 days. Here again the significance of the exceptional result cannot be great and it must be concluded that further evidence is necessary before the claim that the mammary gland of the male monkey can be completely developed by oestrogen alone can be accepted. The mammary gland of the female monkey seems to be much more responsive to oestrogen stimulation, alveolar development being obtained even after relatively short periods of treatment in spayed immature monkeys (Gardner and Van Wagenen, 1938; Folley *et al.* 1939) as well as in intact females (Gardner, 1941a).

Certain general considerations regarding the growth response of the mammary gland to oestrogen are worthy of note. It seems clear that for optimum growth of the mammary duct system the oestrogen dosage must be kept within fairly narrow limits. Evidence that high doses of oestrogen develop stunted glands was first reported for the mouse by Gardner *et al.* (1935) and confirmed by Gardner *et al.* (1936), van Heuverswyn, Folley and Gardner (1939) and Gardner (1941a). Similar findings have been reported for the rat (Astwood *et al.* 1937), rabbit (Scharf and Lyons, 1941) and monkey (Gardner, 1941a). The latter author was inclined to ascribe his failure experimentally to induce mammary growth in bitches to overdosage with oestrogen. Optimal dosages for mammary duct growth have been given as less than 10 μg. oestrone daily for the rat (Astwood *et al.* 1937), 12 μg. oestrone daily for the rabbit (Scharf and Lyons, 1941) and 200–400 μg. oestradiol monobenzoate weekly for the monkey (Gardner, 1941a).

The response also appears to depend on the nutritional state of the test animal. Astwood *et al.* (1937) found a decreased response in undernourished rats and Trentin and Turner (1941) found that in mice the threshold for a minimal duct growth response to oestrogen was decreased in a state of inanition.

It is possible that the rudimentary mammary gland is not equally responsive to oestrogen at all stages of the life cycle, for Astwood *et al.* (1937) found the rudimentary mammae in the rat to be unresponsive to oestrogen during the first two weeks of life.

Progesterone

Observations on mammary development in pseudo-pregnant animals, which were reported in an earlier section, have indicated a relation between mammary growth, particularly of the alveolar tissue, and the function of the corpus luteum, and have led some workers (e.g. Ancel and Bouin, 1911; O'Donoghue, 1912; Hammond and Marshall, 1914, 1930; Hammond, 1917; Loeb and Hesselberg, 1917b) to suggest that the corpus luteum exerts an important influence on mammary development.

Early experiments (e.g. those of Loeb and Hesselberg, 1917b and Bencan, Champy and Keller, 1927), in which attempts were made experimentally to produce mammary development by the use of corpus luteum extracts, failed. In any case the extracts probably contained little or no progesterone. On the other hand, some

later experiments (e.g. those of Nelson and Pfiffner, 1931) with active extracts may have given misleading results (*see* Nelson, 1937) because the extracts were contaminated with unsuspected oestrogen.

However, experiments in which the effects of oestrogen alone and combined with active corpus luteum extracts (or later, crystalline progesterone) have been compared in species in which oestrogen by itself will produce little or no alveolar growth (*see* Figs. *20*. 9 and 12), have shown that the combined treatment will evoke development of duct and alveolar systems, often to a degree characteristic of mid-pregnancy (*see* Turner and Gomez, 1934a, for the mouse ; Turner and Schultze, 1931, Freud and de Jongh, 1935, and Nelson, 1935, for the rat ; Turner and Frank, 1932, Asdell and Seidenstein, 1935, Macdonald, 1936, Pallot, 1936, and Fredrikson, 1939, for the rabbit ; and Turner and De Moss, 1934, for the cat). Some of these workers held that the experimental development of an extensive lobule-alveolar system requires first the application of oestrogen (presumably to develop and sensitise the mammary duct system) followed by a period of combined treatment with oestrogen and progesterone. In view of later developments to be discussed below it is doubtful whether or not this hormone sequence has any real significance.

Fig. *20*. 12—Whole mount of part of mammary gland of male rabbit injected with 240 i.u. oestrone and 1 i.u. progesterone 5 days each week for 5 weeks. Note the extensive alveolar development. Compare with Fig. *20*. 9. × 1·5. (From Scharf and Lyons, 1941.)

As might be expected, the synergistic action of oestrogen and progesterone is less striking in species in which oestrogen causes extensive alveolar growth. In the guinea-pig, Nelson (1937) found that oestrogen and progesterone caused no more mammary growth than oestrogen alone, while Folley *et al.* (1939) observed no alveolar growth in immature male monkeys given short courses of treatment with oestrogen and progesterone in various ratios. In the virgin goat, however, Mixner and Turner (1943) found that combined treatment with oestrogen and progesterone caused mammary growth similar to that of mid-pregnancy, while the alveolar growth resulting from treatment with oestrogen (diethylstilboestrol) alone was variable in extent and histologically abnormal.

For optimal synergism between oestrogen and progesterone in promoting growth of the mammary alveoli, the dosage ratio in which the two types of hormone are applied appears to be of crucial importance. The work of Scharf and Lyons (1941) and Lyons and McGinty (1941) has shown that optimal prolactational mammary growth in the rabbit requires the preservation of a delicate balance (in the neighbourhood of 240 i.u. oestrone/1 i.u. progesterone) between the amounts of oestrone and progesterone administered. An excess of progesterone in relation to oestrogen inhibited alveolar growth. Studying the synergism between oestrone and progesterone in causing minimal lobule-alveolar growth in female mice, Mixner and Turner (1942a, 1943) obtained the best results with total doses of 40–133 i.u. oestrone/1 i.u. progesterone. It seems possible that

the failure of Folley *et al.* (1939) and Selye, Browne and Collip (1936a) to produce alveolar growth in male monkeys and rats respectively by simultaneous administration of oestrogen and progesterone may have been due to the use of unfavourable ratios.

As regards the mammary growth-promoting action of progesterone alone, earlier studies (*see* Turner and Schultze, 1931, Selye, Browne and Collip, 1936b, Astwood *et al.* 1937, for the rat; Corner, 1930; Fredrikson, 1939, for the rabbit; Turner and Gomez, 1934a, Nelson, 1937, for the guinea-pig) indicated that in the absence of oestrogen, progesterone was inactive. The first report to the contrary was made by Gardner and Hill (1936) who found that high doses of an oestrogen-free preparation of progesterone induced mammary duct growth in mice. More recently it has been confirmed in more than one species that provided the dosage is sufficiently great (of the order of 15 mg. daily in the rat) pure progesterone, without previous or simultaneous treatment with oestrogen, will promote growth of the mammary ducts and alveoli (*see* Selye, 1940a, b, Reece and Bivins, 1942, Selye, Borduas and Masson, 1942, Chamorro, 1944, for the rat; Mixner and Turner, 1942a, 1943, Chamorro, 1944, for the mouse; Hartman and Speert, 1941, for the monkey). It seems clear from these studies that the simultaneous presence of oestrogen considerably reduces the amount of progesterone required for the induction of mammary growth. And indeed studies by Mixner and Turner (1941b, 1942a, 1943) of the relative potencies of progesterone and its orally active derivative, pregneninolone, in causing minima llobule-alveolar proliferation in mice indicate that about six times as much progesterone or pregneninolone is needed for unit response in the absence of oestrogen as when oestrogen is also given. Mixner and Turner (1942a, 1943) believe, and Petersen (1944) apparently concurs, that the explanation of this " progesterone sparing " action of oestrogen may be sought in the idea that oestrogen causes an increased vascularity and hyperaemia of the mammary stroma associated with an increased vascular permeability, all of which favour easy access to the gland tissues of mammary growth-stimulating hormones and also metabolites necessary for tissues undergoing rapid growth.

The isolation of progesterone from the adrenal cortex (Beall and Reichstein, 1938) has indicated a possible extra-ovarian source of progesterone, and it is clear that apparent species differences in the extent to which oestrogen alone will promote alveolar development in ovariectomised test animals may merely reflect differences in the extent to which the adrenal cortex is capable of producing progesterone.

Androgen

Evidence suggesting a relation between the testes and the mammary gland, at any rate in the rat (McEuen *et al.* 1936; Astwood *et al.* 1937), has been considered in a previous section. The probability of such a relationship has been confirmed by experiments on the administration of pure androgens which have shown that among the gynaecogenic properties known to be possessed in varying degrees by androgenic steroids may be numbered the power to develop the mammary gland. In most of the experiments the naturally occurring androgens, testosterone (either free or esterified), *cis*-androsterone and dehydro*iso*androsterone have been used.

There appear to be considerable species differences in the type of mammary

growth obtained in response to testosterone. In the male mouse, testosterone caused mammary duct development (van Heuverswyn *et al.* 1939) but even in doses totalling 10 mg. evoked no alveolar development when administered to spayed females together with oestrone (Mixner and Turner, 1942b, 1943). Lewis and Turner (1939), however, observed very little duct growth in male mice with testosterone even after relatively lengthy treatment, so the possibility of strain differences in response must be considered, but their total dose was lower than that of van Heuverswyn *et al.* In the rat, on the other hand, Selye, McEuen and Collip (1936) found that testosterone caused acinar development and secretory changes in gonadectomised animals of both sexes, but it is not clear from their report whether or not duct development had occurred. Astwood *et al.* (1937) state that testosterone, even in large doses, causes no duct development in the rat but that in gonadectomised immature males and females it promotes the development of glands of restricted area with dense clusters of alveoli along the sides and at the ends of the ducts, a type of gland characteristic of the normal, mature, male rat. In mature females in which the duct system is more extensive, testosterone treatment also produces dense alveolar glands of male type but of larger area. Reece and Mixner (1939) also noted acinar development with secretory changes in the mammae of spayed adult rats treated with testosterone. In addition, mammary proliferation in the rat in response to testosterone has been reported by Nelson and Merckel (1937) and Noble (1939b) but without adequate morphological details. In the male guinea-pig, testosterone (2 mg. daily) caused some duct proliferation but no formation of alveoli (Bottomley and Folley, 1938). Interesting effects of testosterone, reminiscent in some respects of results with the rat, were reported for the male monkey by Folley *et al.* (1939). Very little, if any, macroscopically visible duct extension was caused, but microscopic study revealed a characteristic papillomatous heaping of the duct epithelium. Well-developed alveoli were present in the glands of one monkey treated for a considerable period. Somewhat similar results were reported by Van Wagenen and Folley (1939) in the ovariectomised immature female, with the difference that new alveoli were formed, in one case extensively, only in glands possessing alveoli at the outset. Again no extension of the duct system was observed, but the ducts were dilated, probably by secretion, and papillomatous outgrowths of the glandular epithelium were again observed.

Like testosterone, dehydro*iso*androsterone evoked duct growth in the mouse (van Heuverswyn *et al.* 1939; Lewis and Turner, 1939) but differed in that lobule-alveolar growth occurred in the presence of oestrone (Mixner and Turner, 1942b, 1943). Negative results were obtained in the spayed female rat (receiving 1·0 mg. daily) by Nelson and Merckel (1937) and male guinea-pig (receiving 2·0 mg. daily) by Bottomley and Folley (1938). In one monkey studied by Van Wagenen and Folley (1939) dehydro*iso*androsterone caused secretory changes in the duct epithelium resulting in dilation of the ducts.

By contrast, all investigators who have tested *cis*-androsterone agree that this substance, at any rate in the doses hitherto tried, produces little or no mammary growth [*see* van Heuverswyn *et al.* 1939, for the mouse (total dose of 8·0 mg.); Nelson and Gallagher, 1936 (1·0 mg. daily), Nelson and Merckel, 1937 (1·5 mg. daily) and Reece, 1941 (200 μg, daily) for the rat; and Van Wagenen and Folley, 1939, (1350 mg. over 60 days) for the monkey].

The action of a number of semi-synthetic androgens on the mammary gland has been studied with rather conflicting results, though in view of the results obtained with pure progesterone, discussed in the preceding section, it is obvious that negative results obtained with androgens may have been due to inadequate dosage. 17-methyl testosterone, in the presence of oestrone, promotes alveolar growth in the mouse (Mixner and Turner, 1942b, 1943) and mammary duct growth in the male guinea-pig (Bottomley and Folley, 1938). The latter investigators found another testosterone derivative, dihydrotestosterone, inactive in the male guinea-pig. Little or no duct growth was obtained in the mouse with Δ_5-*trans*-androstenediol (van Heuverswyn *et al.* 1939) but this substance evoked extensive duct and sparse alveolar development in the male guinea-pig (Bottomley and Folley, 1938). In the rat, androstanediol caused alveolar growth and secretory changes (Nelson and Gallagher, 1936) though it was inactive in the male guinea-pig (Bottomley and Folley, 1938). Δ_4-androstenedione caused extensive duct growth in the male mouse (van Heuverswyn *et al.* 1939), alveolar development in the rat (Nelson and Gallagher, 1936), but was inactive in the male guinea-pig (Bottomley and Folley, 1938). Androstanedione caused mammary proliferation in the rat (Nelson and Merckel, 1937).

The above results may have an important bearing on the aetiology of gynaecomastia, which is often ascribed to the production of oestrogen in the male body for which there is abundant evidence. In the light of the above findings it seems equally reasonable to suppose that this condition may in some cases be due to the abnormal production of primarily androgenic steroids.

Adrenal Cortex

The frequent association of gynaecomastia with tumours of the adrenal cortex, mentioned in an earlier section, serves as an indication that the adrenal cortex may, under some circumstances, be concerned in mammary growth, though it does not necessarily follow that specific cortical hormones are involved. The first direct evidence of the possibility of such a relationship, involving specific cortical hormones, was provided by van Heuverswyn *et al.* (1939), who showed that 11-deoxycorticosterone, an apparently specific steroid which has been isolated from cortical extracts, will promote mammary duct growth in male mice. Later, in confirmation, it has been shown that deoxycorticosterone will evoke mammary alveolar growth in the rhesus monkey (Speert, 1940a) and guinea-pig (Nelson, Gaunt and Schweizer, 1943) and, in the presence of oestrone, in the spayed female mouse (Mixner and Turner, 1942b, 1943). Also of some significance in the present connection is a case, described by Edwards, Shimkin and Shaver (1938), of gynaecomastia, following treatment with adrenal cortex extract, in a male patient suffering from Addison's disease. At the time of writing no studies of the effects of C_{11}-oxygenated cortical steroids on mammary development have been made.

Results in keeping with the foregoing were briefly reported by Nelson (1941b) who found that administration of adrenocorticotrophin evoked mammary growth in gonadectomised or hypophysectomised rats but not in adrenalectomised-ovariectomised rats, while Cowie and Folley (1944, 1947c) also observed decreased mammogenic effects of crude saline extracts of anterior pituitary in adrenalectomised-gonadectomised rats, as compared with gonadectomised controls. Nelson,

however, interpreted his results as evidence of the production of sex hormones by the adrenal.

Studies on the effect of adrenalectomy on normal mammary development have been rather conflicting. Butcher (1939) reported faster mammary development in underfed adrenalectomised rats (with or without gonads) than in controls ; at autopsy the glands of the adrenalectomised animals were larger than those of the controls and showed a greater degree of arborescence. Reeder and Leonard (1944) similarly found that adrenalectomy in the rat was followed by an increase in the number of lateral duct buds but could observe no significant changes in mammary gland area. In many cases, oestrone had more effect on the glands of adrenalectomised animals than on those of intact controls. The results of Cowie and Folley (1944, 1947c), who in contrast to previous workers studied all the glands of each rat, were, however, more in harmony with the experiments on the administration of deoxycorticosterone. In quantitative studies of changes in the structure of the mammary gland they found that though adrenalectomy was not invariably followed by morphological changes, when they did occur they were always in the direction of regression.

Thyroid

There is no evidence that the thyroid hormone is capable of exerting a direct growth-promoting effect on either mammary ducts or alveolar tissue. Thus thyroid feeding caused no mammary growth in ovariectomised rats (Weichert *et al.*, 1934) and thyroxine was equally without effect in hypophysectomised rats and guinea-pigs (Gomez and Turner, 1937c).

Further, it seems clear that the mammary gland is capable of development in the absence of the thyroid, as may be inferred from the results of Bodansky and Cooke (1937) and Nelson and Tobin (1937) in the rat, Dragstedt, Sudan and Phillips (1924) in the bitch and Spielman, Petersen and Fitch (1944) in the cow, all of whom found that thyroidectomised females could become pregnant and would eventually lactate. It is improbable, however, that mammary growth proceeds to a normal extent in thyroidectomised females, for though Nelson and Hickman (1937) reported extensive experimental mammary growth in thyroidectomised rats under the influence of oestrone, Mixner and Turner (1942c, 1943) found that thyroidectomy considerably reduced the mammary lobule-alveolar growth-promoting effect of oestrone and progesterone in mice, and in the thyroidectomised cow Petersen, Knodt, Ludwick and Pomeroy (1944) found that oestrogen caused no udder growth until symptoms of myxoedema were relieved by suitable treatment. Recent morphological studies of mammary development in thyroidectomised rats, while in some respects difficult to reconcile with the results of Mixner and Turner on mice, nevertheless support the general conclusion that the absence of the thyroid profoundly modifies the morphological characteristics of the growing mammary gland. Leonard and Reece (1941), using moderately mature female rats in which the duct system at the outset was fairly extensive, found that thyroidectomy was followed by an increase in the numer of lateral duct buds. Further analysis of this phenomenon (Smithcors and Leonard, 1942) indicated that thyroidectomy inhibited normal or experimentally produced duct growth but caused an increased development of alveoli. Thus, in thyroidectomised rats, oestradiol no longer showed its typical

duct growth-promoting action but resembled testosterone in causing the growth of glands with short, wide ducts but containing dense clusters of alveoli.

Additional evidence that the thyroid, as might be expected from its rôle as a general regulator of body metabolism, exerts an indirect influence on mammary development has been provided by experiments involving the administration of thyroid hormone. Weichert and Boyd (1934) reported an acceleration of the rate of mammary development and of the onset of the secretory phase in rats fed thyroid during pregnancy. Cohen (1935) found that thyroid feeding increased the degree of organisation of the mammary gland structure in the rat. In the intact mouse, Gardner (1942) observed that thyroid feeding promoted mammary duct growth, but the effect did not occur in castrated mice, which emphasises its indirect nature. Enhancement of the ability of oestrone and progesterone to promote lobule-alveolar growth in mice by administration of thyroxine was reported by Mixner and Turner (1942c, 1943) whose results suggested the existence of a broad optimum zone of thyroxine dosage for the enhancement effect. In view of this, the failure of Folley and Malpress (unpublished observations) to detect any enhancement by thyroxine treatment of the mammary growth-promoting action of hexoestrol in virgin goats may have been due to incorrect dosage.

Placenta

Comparatively little attention has been given to the possible function of the placenta as an endocrine organ concerned in mammary development or secretion during pregnancy. However, the small amount of evidence available, which exclusively refers to the rat and mouse, strongly suggests the existence of an endocrine relationship between the placenta and the mammary gland, but it is far from certain whether the growth or the secretory phase (*see* earlier section on mammary growth during pregnancy) is principally concerned.

Selye, Collip and Thomson (1935b) reported that pregnant ovariectomised rats whose uteri at autopsy contained placental tissue had well-developed mammary glands contrasting with the involuted glands of rats with empty uteri. Analogous results in ovariectomised pregnant mice which underwent foetal destruction at the twelfth day of pregnancy were obtained by Newton and Lits (1938). If the placentae were retained until autopsy on the eighteenth day, the mammae resembled those of normal mice at term, but in mice in which the placentae were aborted, the mammae had undergone unmistakable involution. Later, Newton and Beck (1939) in similar experiments showed that this action of the placenta was independent of the presence of the pituitary, but their figures hardly lend conclusive support to their contention that in many cases the presence of the placenta had caused true growth (as distinct from the hypertrophy due to secretion) beyond the condition characteristic of the twelfth day of pregnancy. That mammary regression does not follow hypophysectomy in pregnant rats and mice is shown by the occurrence of transient lactation following parturition (*see* page 557). Lyons (1944) briefly reported experiments in which oestrone and progesterone, singly or in combination, were administered to rats hypophysectomised early in pregnancy. Mammary growth only occurred in animals in which foetuses or placentae were carried to term ; where resorption of the placentae had occurred the glands consisted only of ducts with a few alveolar buds. Lyons concluded that the placenta secretes a hormone which synergises with ovarian hormones in causing

mammary alveolar growth and initiates secretion. Leonard (1945) also contends that the placenta secretes a mammogenic hormone which acts synergistically with the pituitary or ovaries in causing mammary growth during the second half of pregnancy. He studied the effects on mammary development in pregnant rats of the removal at the thirteenth day of the pituitary, ovaries, foetuses and placentae, singly and in all possible combinations. The placenta was unique among the factors studied in that it was the only one of them which by itself would prevent regression of the mammary gland. In the presence of either the pituitary or the ovaries in addition to the placenta the glands at autopsy resembled those characteristic of the nineteenth day of pregnancy.

Considering all these results in the light of the generally accepted view that the phase of true mammary growth is mainly confined to the first half of pregnancy, a consideration which has largely been neglected by the aforementioned workers, it would seem that while there is some suggestion that the placenta may be involved in normal mammary growth, its chief rôle may be concerned with the initiation of the secretory phase in the latter half of pregnancy.

Anterior Pituitary

Mammary growth in hypophysectomised animals.—The studies on experimental mammary development, considered in the foregoing sections, were concerned with animals with intact pituitaries. The still controversial question whether or not ovarian hormones are effective in evoking mammary growth in hypophysectomised animals has received considerable attention in recent years. This question is important since it involves the possibility that mammary growth is due to the action of the pituitary rather than to the direct effect of ovarian hormones.

Those who first studied the effects of oestrogen alone or combined with progesterone on the mammary gland of the hypophysectomised animal, mostly agreed that sex hormones would promote mammary growth in the absence of the hypophysis (*see* Ruinen, 1932, Freud and de Jongh, 1935, and Nelson, 1935a, for the rat; Nelson, 1935b, for the guinea-pig; and Houssay, 1935b, for the dog). These results, if valid, would thus indicate that the pituitary plays no direct part in mammary development.

However, opposing reports soon followed. Already in 1935 Selye, Collip and Thomson (1935a) stated that oestrogen did not prevent mammary involution following hypophysectomy in the rat but their evidence as stated is hardly convincing. Many other workers have since reported failure to evoke mammary development in hypophysectomised animals by procedures known to succeed in the presence of the hypophysis. In the hypophysectomised mouse oestrogen was reported to be inactive by Gomez, Turner, Gardner and Hill (1937), Gomez and Turner (1937c) and Lacassagne and Chamorro (1939). Similar negative results with deoxycorticosterone were reported by Chamorro (1940) and Smithcors and Leonard (1943). In the hypophysectomised rat, negative results with oestrogen were reported by Reece, Turner and Hill (1936), Gomez and Turner (1937c), Astwood *et al.* (1937), Nathanson, Shaw and Franseen (1939), Reece and Leonard (1941), Samuels, Reinecke and Petersen (1941), Leonard and Reece (1942) and Reece and Leathem (1945); with oestrogen and progesterone by Gomez and Turner (1937c); with androgen by McEuen, Selye and Collip (1937), Noble

(1939b), Reece and Leonard (1942) and Leonard and Reece (1942); and with deoxycorticosterone by Leonard and Reece (1942). Essentially similar results with oestrogen were obtained in the hypophysectomised guinea-pig by Gomez and Turner (1936a) and Lyons and Pencharz (1936) and with oestrogen and progesterone by Gomez and Turner (1937c). The latter workers extended their studies to include rabbits, cats and ground squirrels.

In incompletely hypophysectomised animals, even when only minute pituitary remnants remain, sex hormones apparently cause marked mammary stimulation (Gomez and Turner, 1937c; Gomez, Turner, Gardner and Hill, 1937; Reece and Leonard, 1941; Leonard and Reece, 1942), so it appears that the onus of demonstrating the completeness of hypophysectomy is on any who claim that ovarian hormones will evoke mammary growth in the absence of the pituitary. The earlier claims referred to above are open to the criticism that convincing evidence of the completeness of hypophysectomy was not given, but this does not apply to the mostly more recent studies, now to be considered, in which positive results have been obtained, since these studies were based on animals in which completeness of pituitary removal has been proved by histological examination.

Growth of mammary ducts and alveoli under the influence of oestrogen and progesterone has been reported in completely hypophysectomised rabbits by Asdell and Seidenstein (1935) (*see also* Asdell, Brooks, Salisbury and Seidenstein, 1936) and Fredrikson (1939). Gardner (1940) has reported slight growth of the mammary rudiments of completely hypophysectomised male mice with oestradiol, progesterone or deoxycorticosterone, but more extensive growth with oestradiol combined with either of the other two steroids. Essentially similar results were also reported by Gardner (1941b) and Gardner and White (1942). In completely hypophysectomised rats, Leonard (1943) obtained growth of end buds with oestradiol and slight hyperplasia of duct epithelium with testosterone, while Smithcors and Leonard (1943) evoked limited mammary growth with oestrogen and progesterone, singly or in combination, only if the treatment began immediately after hypophysectomy.

There is thus some conflict of evidence as to whether or not ovarian hormones and other steroids will promote mammary growth in the absence of the hypophysis. However, in the studies in which positive results have been obtained in completely hypophysectomised animals, with the exception of that of Asdell and Seidenstein (1935), it is doubtful whether the response was as great as would be obtained in animals with intact pituitaries. The experiments of Leonard (1943) suggest that the age of the experimental animals may be a factor which affects the response.

Further evidence on this subject comes from experiments showing that in pregnant rodents, in which hypophysectomy at mid-pregnancy does not usually cause abortion, mammary growth proceeds, or at any rate the mammary glands do not regress, in the absence of the hypophysis (*see* Newton and Beck, 1939, Newton and Richardson, 1941, Gardner and Allen, 1942, for the mouse; Pencharz and Long, 1933, Jeffers, 1935b, for the rat; Desclin, 1939, for the guinea-pig; but *see also* Pencharz and Lyons, 1934, who noted reduced mammary development in three hypophysectomised guinea-pigs which carried young to term). The conclusion to be drawn from these studies is that the hypophysis is not necessary for mammary growth, or at least for the maintenance of an already

existing mammary structure (*see* page 551), so long as placental tissue is present, and it has been suggested by some who hold that the pituitary is involved in mammary development that the placenta can assume the function of the pituitary in this respect. This contention, however, requires more direct proof.

It has been suggested by Astwood *et al.* (1937) that the failure of mammary development in response to ovarian hormones, in the absence of the pituitary, may be due not to the lack of anterior pituitary hormones which directly cause mammary growth, but to a lowered state of nutrition consequent upon the operation, since they found that oestrogen caused no mammary growth in intact rats whose food intake was restricted. On the other hand, Samuels *et al.* (1941) failed to evoke mammary growth with oestrogen in force-fed hypophysectomised rats. Support to the idea that absence of mammary growth responses in hypophysectomised animals may be due, partially at any rate, to generalised metabolic effects, comes from experiments in which mammary growth has been evoked by combined treatment with oestrogen and anterior-pituitary growth hormone (Nathanson *et al.* 1939 ; Reece and Leonard, 1941). In general the best responses occurred in animals which gained most weight or lost least during the treatment. Against these experiments it may be objected that the growth hormone preparations were not pure and must have contained other anterior-pituitary factors, conceivably some acting directly on the mammary gland, but the responses to the growth preparation alone were either nil or very slight. Finally, we cannot escape the significance of the finding of Trentin and Turner (1941) that the threshold of response of the rudimentary mammary gland of the mouse to oestrogen was increased by inanition.

Mammogenic action of anterior-pituitary extracts.—The first direct and unequivocal demonstration of mammary growth in ovariectomised animals (rabbits) in response to anterior-pituitary extracts was provided by Corner (1930). Similar results were later reported by Asdell *et al.* (1936). Lyons and Catchpole (1933b) reported that anterior-pituitary extracts containing the anterior pituitary-lactogenic hormone, prolactin (*see* page 558), caused mammary hyperplasia in addition to secretion. However, prolactin extracts used by Gardner and Turner (1933) and Riddle, Bates and Dykshorn (1933) exhibited no mammary stimulating activity. Later, Lyons (1942) claimed to have observed local hyperplasia of the mammary epithelium in response to intra-mammary duct injections of purified prolactin in the rabbit.

A few years later, failure to evoke mammary growth with ovarian hormones in completely hypophysectomised animals led Turner and his school to postulate, as the agent directly responsible for mammary development, a hitherto unrecognised anterior-pituitary hormone (or hormone complex)—mammogenic hormone—secreted in response to stimulation by ovarian hormones. Direct evidence in support of this theory was provided by Gomez and Turner (1937c) and Gomez, Turner and Reece (1937) who produced mammary development in hypophysectomised guinea-pigs by repeated implantation of pituitaries from oestrogenised rats, pituitaries from untreated rats being ineffective. Later, extracts of anterior-lobe tissue from pregnant cows, in which the mammogenic factor would be operative, were shown to stimulate mammary growth in gonadectomised rats and rabbits while material from non-pregnant cattle was without effect (Gomez and Turner, 1938a). A method of assay of anterior-pituitary mammogenic hormone, based on

the stimulation of mammary duct growth in male mice, was developed by Lewis, Turner and Gomez (1939) (*see also* Lewis and Turner, 1939), and the mammogenic potency of pituitary tissue from various types of cattle evaluated (Lewis and Turner, 1939). In support of the mammogenic hypothesis, pituitary tissue from dairy cows was found to contain more mammogen than tissue from beef cattle, the highest content being found in tissue from lactating dairy cows. Some evidence was also provided that in accordance with the mammogen theory oestrogen treatment will increase the mammogenic potency of rabbit anterior-lobe tissue (Gomez and Turner, 1938b ; Lewis and Turner, 1939).

Lewis and Turner (1938, 1939) claimed that the mammogenic duct growth factor, unlike other known anterior-pituitary hormones, was soluble in organic solvents, which immediately suggests that the results might have been due to oestrogen present in the blood contained in the pituitary tissue. Parallel determinations of the oestrogenic and mammogenic activities of a number of anterior-pituitary extracts indicated, however, that the oestrogen content of the extracts was not sufficient to account for their mammogenic activities (Lewis and Turner, 1939). Further positive results with lipoid extracts of anterior pituitary were claimed by Lewis, Gomez and Turner (1942) and Gomez (1942), but Greep and Stavely (1941) were unable to confirm the ether-soluble nature of anterior pituitary mammogen. Turner and his school (Trentin, Lewis, Bergman and Turner, 1943) have, however, more recently abandoned the claim that ether-alcohol extracts of pituitary tissue will cause mammary development ; they now believe that this property is associated with the protein fraction.

Lewis and Turner (1939) believed that some of their results suggested the presence in anterior-pituitary extracts of two mammogenic hormones, one secreted in response to oestrogenic stimulation and responsible for mammary duct growth, the other capable of evoking alveolar growth and needing for its production the additional stimulus of progesterone. Later, a more definite claim was made to have obtained evidence of the existence of a mammogenic hormone responsible for lobule-alveolar development (Mixner, Lewis and Turner, 1940). Assay of the progesterone content of these extracts indicated that the former was not present in sufficient amounts to account for the observed effects (Trentin, Mixner, Lewis and Turner, 1941). A method of assay of this factor employing ovariectomised female mice was developed (Mixner and Turner, 1941c) and since it was later shown (Mixner and Turner, 1942a) that simultaneous treatment of the test animals with oestrogen enhanced the lobule-alveolar growth-promoting potency of anterior-pituitary extracts, the method was later modified by specifying the use of oestrogenised ovariectomised mice (Mixner and Turner, 1943). This method is of course applicable to the assay of the lobule-alveolar growth-promoting activity of substances other than anterior-pituitary extracts, for example, of sex steroids. Since the original claim to have effected a partial separation of the two mammogens was based on the supposed lipoid solubility of the duct growth factor, the separate existence of a second factor must remain somewhat dubious until purified preparations of both hormones have been obtained. Mixner, Bergman and Turner (1942), however, believe that the lobule-alveolar growth-promoting mammogen is distinct from prolactin, thyrotrophin and the gonadotrophins.

Mammary growth in hypophysectomised and gonadectomised animals under the influence of crude aqueous extracts of anterior pituitary has been reported by

other workers, e.g. Greep and Stavely (1941) and Cowie and Folley (1944, 1947c), but such results do not necessarily prove that specific mammogens are involved. And indeed the mammogen theory has not found universal acceptance since not only have some workers, as we have seen, succeeded in producing some mammary growth with steroids in completely hypophysectomised animals, but others have failed to confirm the claim that pituitaries from oestrogen-treated donors are particularly rich in mammogenic activity (Nelson, 1938, 1939a ; Reece and Leonard, 1939). Further, it has been repeatedly shown in a number of species that mammary growth may be produced by local, percutaneous application of oestrogen and that, if the dose be low enough, growth preferentially occurs in the treated gland, the neighbouring glands either being unaffected or showing much less growth (*see* MacBryde, 1939, for women ; Lyons and Sako, 1940, for the rabbit ; Nelson, 1941a, for the guinea-pig ; Gardner and Chamberlin, 1941, for the mouse ; Speert, 1940b, and Chamberlin, Gardner and Allen, 1941, for the monkey). This phenomenon of localised mammary growth under the stimulus of doses of oestrogen incapable of exerting systemic effects (which should not be confused, as Petersen, 1944, has done, with generalised mammary growth in farm animals due to percutaneous application of supra-threshold doses) has been cited (e.g. by Lyons and Sako, 1940) as conclusive evidence against the mammogen theory, since if oestrogens act by way of the pituitary (i.e. to all intents and purposes systemically) growth would be expected in all glands alike. Mixner and Turner (1943), however, contend that the phenomenon is explicable on the supposition that such oestrogen treatment causes local hyperaemia, thereby increasing the supply, to the treated gland, of circulating mammogen already present in the blood in amounts ordinarily insufficient to cause mammary growth (*see also* Mixner and Turner, 1942a). But attempts to produce mammary growth by the local application of an irritant such as turpentine, either alone or combined with systemic injection of oestrogen, hardly produced convincing evidence in support of this explanation (Mixner and Turner, 1941a ; Lewis and Turner, 1942c). That the co-operation of a pituitary factor, not necessarily mammogen, may be necessary for the localised mammary responses to oestrogen is indicated by the failure of Leonard and Reece (1942) to secure such responses in hypophysectomised animals.

From the foregoing discussion it is clear that further evidence is required before the question of the role of anterior-pituitary hormones in mammary growth can be regarded as clarified. One general conclusion seems to emerge. Notwithstanding the fact that a number of workers have successfully stimulated some degree of mammary growth in the absence of the hypophysis, the co-operation of pituitary hormones seems necessary for the experimental production of normal growth such as occurs during pregnancy. In the present state of knowledge, however, it seems best to reserve judgment regarding the existence of specific mammogenic hormones, particularly since there is some indication that certain of the already well-characterised anterior-pituitary hormones may in co-operation with ovarian hormones produce marked growth of the mammary glands in the absence of the hypophysis. Thus there is a growing body of evidence that purified preparations of prolactin, in combination with ovarian hormones, will promote mammary development in completely hypophysectomised animals perhaps to a degree unrealisable by use of the ovarian hormones by themselves (*see* Gardner and White, 1941, 1942 ; Gomez, 1942 ; and Lyons, 1943). The possibility, suggested by Mixner

and Turner (1943) that these results are due to the presence of mammogens in the prolactin preparations used must be considered in the light of the fact that prolactin can now be obtained in a state of considerable purity (*see* page 563); it is a point that, like many others arising in connection with the mammogen hypothesis, requires further study. Gardner and White (1941) suggest in explanation of their results that prolactin sensitises the mammary gland to the growth-promoting action of oestrogen, which is considered to be direct, a diametrically opposite view to that adopted by Mixner and Turner (1943) whose views may be epitomised thus: oestrogen causes the secretion, by the anterior lobe, of the mammogen responsible for duct growth, at the same time producing favourable conditions for its action by increasing the vascularity and permeability of the capillaries and perhaps sensitising the gland to mammogen stimulation; progesterone evokes the secretion of the mammogen which directly stimulates lobule-alveolar growth, oestrogen exerting a synergistic effect by continuing to influence the vascularity and growth of the stroma.

Evidence which suggests the possibility that, in addition to prolactin, anterior-pituitary adrenotrophic hormone may also play some rôle, not yet elucidated, in mammary growth has been presented by Nelson (1941b), Gardner and White (1942) and Cowie and Folley (1944, 1947).

III. Endocrine Factors in Lactation

Anterior Pituitary

Effect of hypophysectomy on lactation.—Numerous experiments on the effect of hypophysectomy on lactation have provided evidence of the important rôle of the hypophysis in milk secretion. In various species it has been shown that hypophysectomy during pregnancy will prevent normal lactation even though live young are born. Failure of lactation after hypophysectomy during pregnancy was reported in the rat by Pencharz and Long (1933) and Bergman (1934), in the guinea-pig by Nelson (1935b), in the ferret by McPhail (1935a), in the cat by Allan and Wiles (1932) and McPhail (1935b) and in the dog by Houssay (1935a). Transient mammary secretion following parturition in hypophysectomised rodents has been observed (*see* Selye, Collip and Thomson, 1933c, 1934, Newton and Beck, 1939, Newton and Richardson, 1941, Gardner and Allen, 1942, for the mouse; Selye, Collip and Thomson, 1933b, 1934, for the rat; and Pencharz and Lyons, 1934, for the guinea-pig). It has been suggested in explanation of such results that the placenta may take over the function of the pituitary as regards the initiation of milk secretion (*see also* section on the rôle of the placenta in mammary growth, page 551).

Hypophysectomy during lactation invariably causes rapid and, as far as is known, complete cessation of secretion; observations in the mouse (Selye *et al.*, 1933c, 1934), rat (Selye *et al.*, 1934), guinea-pig (Nelson, 1935b), ferret (McPhail, 1935a), cat (McPhail, 1935b) and dog (Houssay, 1935a) are in complete agreement on this point.

Further, certain experimental procedures which normally cause lactation in the presence of the hypophysis do not do so in its absence. Thus neither removal of all the foetuses from the rat in late pregnancy (Collip, Selye and Thomson,

1933) nor removal from the non-pregnant rat of ovaries intensely luteinised by injections of chorionic gonadotrophin (Selye, Collip and Thomson, 1933a) results in lactation if the pituitary is simultaneously removed. Similarly, in the guinea-pig, Nelson (1935b) found that removal of functional ovarian grafts from males which had carried them for some time, or cessation of oestrogen treatment in animals of either sex, was not followed by lactation if the hypophysis was removed at the same time.

Initiation of lactation by anterior-pituitary extracts.—The experiments just considered point to the existence of a hormone (or hormone complex) of the pituitary essential for the initiation and maintenance of lactation. The first direct evidence of the existence of such a hormone was provided by Stricker and Grueter (1928) who found that injection of aqueous anterior-pituitary extracts into ovariectomised pseudo-pregnant rabbits initiated lactation. Soon afterwards the same authors reported the re-initiation of secretion in bitches which had gone dry and the stimulation of lactation in the sow and cow with anterior-pituitary extracts (Grüter and Stricker, 1929).

The ability of anterior-pituitary extracts to initiate lactation in adequately developed glands was quickly confirmed in experiments on a variety of species including the monkey (Riddle *et al.* 1933 ; Allen *et al.* 1935), virgin heifer (Catchpole, Lyons and Regan, 1933), goat (Evans, 1933), sow (Gardner and Turner, 1933), dog (Gardner and Turner, 1933 ; Lyons, Chaikoff and Reichert, 1933 ; Houssay, 1935b), rabbit (Parkes, 1929 ; Corner, 1930 ; Turner and Gardner, 1931 ; Gardner and Turner, 1933 ; Lyons and Catchpole, 1933b ; Anselmino *et al.* 1935 ; Asdell *et al.* 1936), guinea-pig (Nelson and Pfiffner, 1930 ; Lyons and Catchpole, 1933a). In many of these experiments the amounts of milk obtained were not great, certainly less than would have been secreted under conditions of normal lactation, but the response would of course depend on the extent of mammary development existing at the time of treatment, unless the extracts themselves were capable of causing mammary proliferation (*see* discussion in a preceding section).

Some of the earlier workers were of the opinion that their anterior-pituitary extracts contained a specific lactation-stimulating hormone, and the idea that a new hormone was involved was strengthened as evidence became available (Riddle *et al.* 1933 ; Gardner and Turner, 1933 ; Catchpole and Lyons, 1933) that lactogenesis was not associated with the anterior-pituitary hormones (growth, gonadotrophic and thyrotrophic hormones) comparatively well characterised at that time. Finally, Riddle, Bates and Dykshorn (1932, 1933) provided evidence that associated with the new hormone was the property of causing enlargement of and secretory activity in the pigeon crop-gland, a property of anterior-pituitary extracts first described by Riddle and Braucher (1931). This hormone now enjoys the status of a well-characterised anterior-pituitary protein hormone which is generally recognised as being concerned in the initiation and probably the maintenance of lactation (*see* Fig. 20. 13). When first discovered it was variously named *prolactin* (Riddle), *galactin* (Turner) and *mammotropin* (Lyons). Recent American practice tends to favour the terms *lactogen* or *lactogenic hormone* ; the name *prolactin* is adopted in this chapter in accordance with English usage.

Assay of prolactin.—The majority of methods used for the bioassay of prolactin involve the pigeon crop-gland response. There are many respects in which this

response is convenient for assay purposes (*see* e.g. Bates, 1937 ; Meites, Bergman and Turner, 1941a) and its discovery has undoubtedly facilitated the isolation of prolactin as a pure protein. Nevertheless, from the standpoint of the rôle of prolactin in lactation, assay methods based on lactation responses in mammals are obviously preferable for theoretical reasons, and thus the development of a suitable mammalian method is desirable if only to provide an assessment of crop-gland results. Some attempts have been made to devise mammalian assay methods, but as yet without conspicuous success. The crop-gland methods fall into three

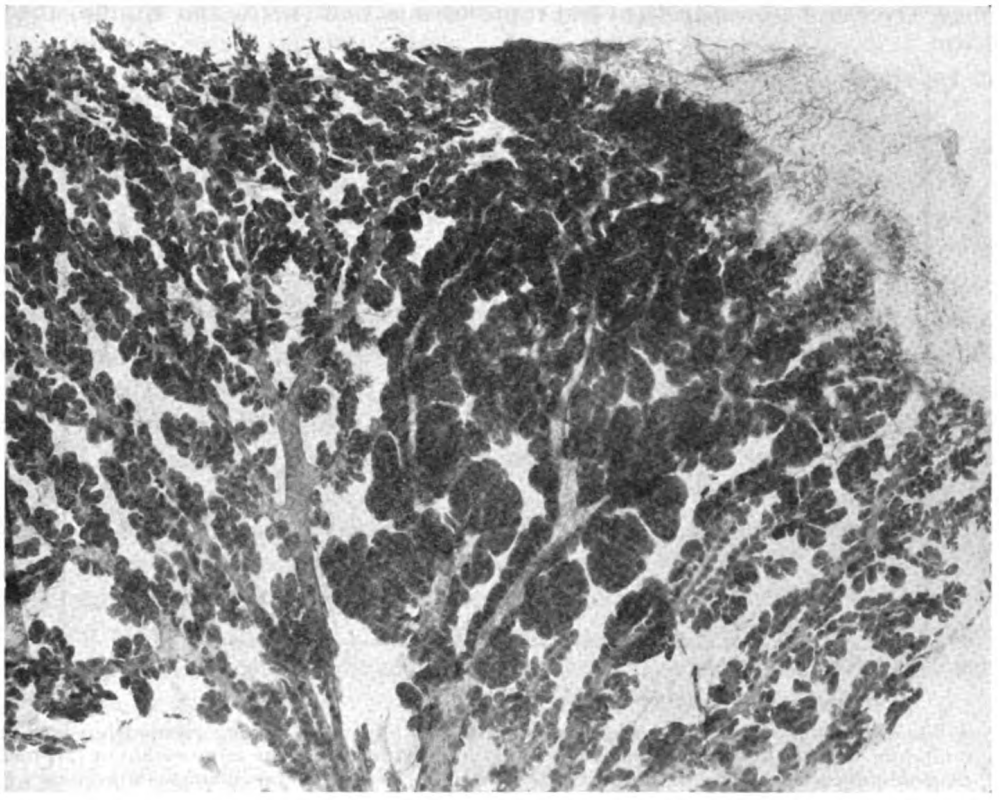


Fig. 20. 13—Portion of whole mount of mammary gland of female rabbit into one galactophore of which prolactin has been injected. Note the secretory hypertrophy in response to prolactin confined to the treated sector. × 3. (From Lyons, 1942.)

main classes : " systemic " methods entailing determination of crop weights ; " systemic " methods involving minimal stimulation ; and " local " or micro methods also involving minimal stimulation. Review articles on the assay of prolactin have been published by Bates (1937), Lyons (1937b) and Riddle and Bates (1939).

Systemic methods involving intramuscular or subcutaneous injections and weighing of the stimulated crop-glands have been described by de Fremery (1936), Riddle *et al.* (1933), Dyer (1936) and Rowlands (1937), whose papers should be consulted for details. For a statistical evaluation of a typical systemic crop-weight method and a discussion of the necessary precautions, see Folley, Dyer and Coward (1940). The relationship between crop weight and the logarithm of the dose is linear or approximately so over a useful range (Riddle *et al.* 1933 ; Dyer,

1936; Evans, 1937; Wolff, 1937; Folley, Dyer and Coward, 1940). Hall (1944a) has shown that data in the lower ranges of response are fitted by a curve of the form

$$by = \log (ax + 1)$$

where y represents the dose, x the crop weight and a and b are constants, while Emmens (1940) has pointed out that the sigmoid dose-response curve (*see* Fig. 20. 14) may be well fitted over the whole range by a logistic equation. Many factors which affect the response have been uncovered. These include age of bird (Riddle *et al.* 1933), race (Bates, Riddle and Lahr, 1936; 1939a), environmental temperature (Folley, Dyer and Coward, 1940) and route of injection (Bates and Riddle, 1936;

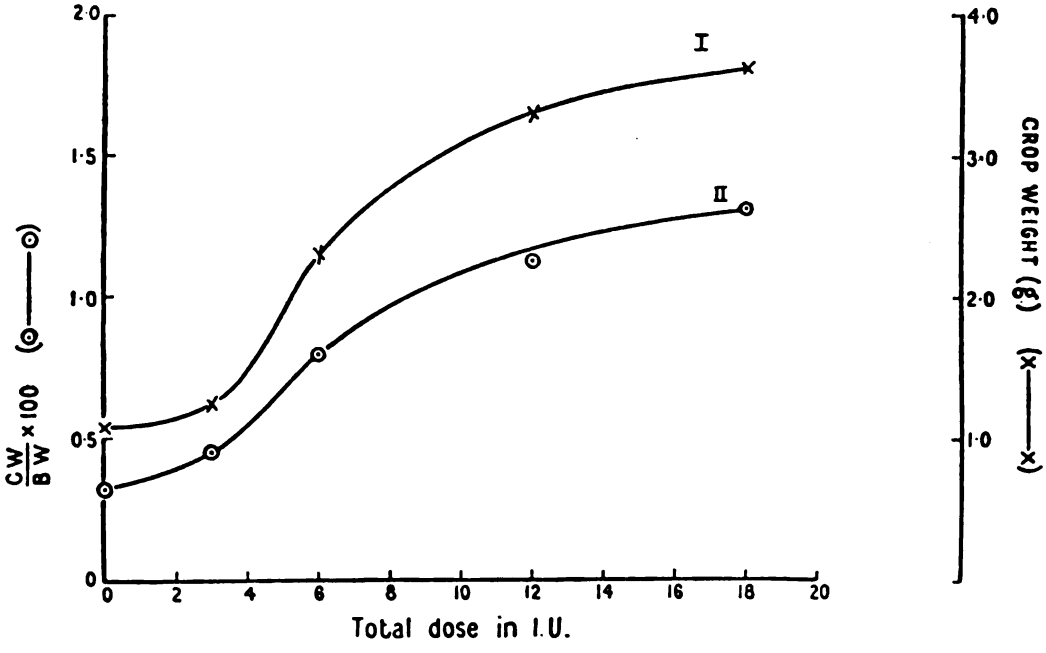


Fig. 20. 14—Response of pigeon crop-gland to prolactin. Curves showing relationship between total dose of prolactin (International Standard) and (I) mean absolute crop-weight or (II) mean crop-weight expressed as a percentage of body weight. Six daily subcutaneous injections were given followed by autopsy on the seventh day. (From Folley, Dyer and Coward, 1940.)

Lahr, Elwell and Riddle, 1941; Hall, 1944a). Birds kept in darkness during the test give the same response as birds continuously exposed to artificial light (Folley, Dyer and Coward, 1940). Seasonal variations in response to a given preparation have been reported by Bates and Riddle (1941) and confirmed by Hall (1944a). The response may be affected by the presence of inert substances (e.g. muscle extract) in the test preparations (Friedman and Hall, 1941; Hall, 1944a). There appears to be no appreciable sex difference in response to prolactin injected intramuscularly (Bates, Riddle and Lahr, 1939b; Hall, 1944a) but there are indications that males give a greater response than females, particularly if the birds are immature, when the hormone is injected by the subcutaneous route (Folley and White, 1937; Bates *et al.* 1939b; Hall, 1944a). The response is diminished by hypophysectomy (Gomez and Turner, 1936c; Schooley, Riddle and Bates, 1937) and by simultaneous treatment of the birds with oestrogen (Folley and White, 1937; Folley, 1939; Bates *et al.* 1939b). Since the latter workers observed a decreased response in

fasting pigeons it is possible that these last-named effects may be partly due to reduction in food intake.

McShan and Turner (1936) described an assay method involving systemic injection of the prolactin, but in which the end-point, which was of necessity estimated subjectively, was the occurrence of threshold stimulation of the crop as determined by visual examination. The prolactin unit for this purpose was defined as the amount of hormone which, under the specified conditions, would evoke a threshold response in 50 ± 11 per cent. of a group of 20 birds. Using this method, Meites *et al.* (1941a) found that, as in the case of crop-weight methods, a smaller amount of hormone is needed per (bird) unit by the subcutaneous route than when injected intramuscularly. This type of method, since it involves subjective judgments is probably less accurate than methods involving crop weights.

An extremely sensitive method of detection of prolactin, which has been adapted to the quantitative assay of smaller amounts of the hormone than can be assayed by the methods already described, was introduced by Lyons and Page (1935). In this method small amounts of hormone are injected intradermally over the crop-sac, the end-point being the production of threshold stimulation of a small area adjacent to the injection site. In addition to its great sensitivity, this method has the additional advantage that two preparations may be compared in one bird, since the pigeon has two crop-sacs, thus eliminating differences in sensitivity among individual birds. Like the systemic minimal stimulation method the local intradermal method may be criticised on the ground that it involves a subjective judgment ; however, it seems possible that, as suggested by Leblond and Allen (1937), the colchicine technique might be used to give an objective response—perhaps as soon as 9 hours after injection.

The micro method, originally used for the assay of prolactin present in urine, has been modified by Reece and Turner (1937b) for the determination of prolactin in the rat pituitary, the response being subjectively rated according to the size of the area of stimulation.

According to Bates and Riddle (1940) the response to a given dose of intradermally injected prolactin is dependent on the volume of fluid injected, so that this factor must be rigidly standardised. This observation was confirmed by Hall (1944b) but Meites *et al.* (1941a) reported that the response was independent of the injection volume over a wide range. Lahr, Bates and Riddle (1943) observed that various procedures such as plucking feathers, intradermal injection of saline as well as of solutions of various non-specific irritants, increased locally the mitosis rate of the crop epithelium and pointed out that such factors might introduce a disturbing influence into micro prolactin assays. Hall (1944b), however, considers that such artifacts are unlikely to be of much importance. Another factor which needs standardisation would appear to be the location of the injection in relation to the underlying crop-sac, since Hall (1944b) found that different parts of the crop-gland were not equally sensitive. As will be seen later, in assaying the prolactin content of pituitary tissue by the intradermal method, it is important to remember that, according to Hall (1944b), extracts give appreciably higher results than tissue suspensions.

Various estimates have been given of the magnitude of the micro (bird) unit in relation to the amounts of hormone necessary to give threshold responses by

systemic routes. The (bird) units (i.e. total amounts of prolactin required to elicit the chosen response), both micro and systemic, described by different authors obviously depend to a considerable extent on such details of technique as duration of the test, subdivision of the dose, route of the systemic injection, etc., and for such details the original papers should be consulted. Ratios of micro to " systemic " units have been given as $\frac{1}{100}$ (Lyons and Page, 1935 ; Bates and Riddle, 1936), $\frac{1}{100}-\frac{1}{150}$ (Chasin, 1936), $\frac{1}{1000}$ (Lyons, 1937a), $\frac{1}{10.000}$ (Lyons, 1937b) and $\frac{1}{160}$ (Meites *et al.* 1941a). A table of comparisons between types of pigeon unit has been given by Bergman, Meites and Turner (1940). In the present connection it is interesting to note that Hall (1944b) reported that subcutaneous injections over the crop-gland gave results little different from those given by intradermal administration ; on the other hand, Bates and Riddle (1936) state (somewhat ambiguously) that the threshold dose for a single subcutaneous injection over the crop-gland is practically the same as when four daily injections are given in a region remote from the crop.

There are two main difficulties in devising mammalian methods for the assay of prolactin. One is the difficulty of choosing a suitable end-point ; the other problem is to select a type of experimental animal which is reasonably uniform in respect of the mammary tissue both as regards morphology and physiological reactivity. In most of the methods dealt with below an " all-or-none " end-point has been chosen, namely, the ability of the operator to express milk from the nipples. Rabbits and guinea-pigs have been used exclusively ; rats and mice have proved relatively unresponsive to prolactin (e.g. *see* Gardner and Turner, 1933 ; Riddle *et al.* 1933).

Gardner and Turner (1933) described a method involving the use of pseudo-pregnant rabbits. In their method an attempt was made to obtain a quantitative measure of the response by grading the glands according to an arbitrary and subjective scale. This method has also been used with some success by Nelson (1934b). Lyons and Catchpole (1933b) suggest that the non-ovulated, non-lactating virgin rabbit, ovariectomised just prior to testing, may be superior to the pseudo-pregnant rabbit for assay purposes.

The use of the guinea-pig ovariectomised at oestrus was proposed by Lyons and Catchpole (1933a), the end-point being the ability to withdraw milk by manipulation. Nelson (1934b) criticised this method on the grounds that adult female guinea-pigs frequently lactate spontaneously after ovariectomy while immature ones are unsuitable because of insufficient mammary development. Instead, he recommended the use of pregnant guinea-pigs hysterectomised late in pregnancy. Lyons (1941) has more recently carried out prolactin tests on guinea-pigs 6–18 months old and weighing at least 650 g. If the injections were made during the first few days of the oestrous cycle approximate comparisons of the potencies of different extracts could be made. Some animals which have proved to react satisfactorily could be used repeatedly.

Comparative assays of prolactin preparations by mammalian and pigeon methods have been reported by Bergman *et al.* (1940), Meites *et al.* (1941a) and Lyons (1941). As might be expected, the minimal amounts of hormone necessary for lactation responses in guinea-pigs and rabbits are of higher order than are necessary for minimal crop-gland responses in pigeons.

It cannot be said that any assay method, based on the initiation of lactation

in mammals, of accuracy comparable to the pigeon crop-weight method or of sensitivity anywhere approaching the intradermal crop test has yet been developed. Lyons (1942) has been able to induce localised secretion in rabbit mammary glands by injections of small amounts of prolactin into single galactophores and it should be possible to develop a more satisfactory and sensitive assay method on this basis.

Purification of prolactin.—Prolactin is a protein which can be readily brought into aqueous solution but is relatively insoluble in an isoelectric region centring around *p*H 5·5 approx. The fact that prolactin activity is associated with an iso-insoluble substance, first recorded by Riddle *et al.* (1932), was quickly taken advantage of to prepare partially purified preparations (Riddle *et al.* 1933 ; Lyons and Catchpole, 1933a ; Gardner and Turner, 1933) and has since proved an important factor in the preparation of the pure hormone, the first protein hormone of the anterior lobe to be isolated.

Prolactin may be extracted in acid or alkaline solution from fresh, undissected whole pituitaries or anterior lobes, or from acetone-desiccated glands. Bergman and Turner (1937), comparing four extraction procedures, found that in their hands extraction with alkaline 60–70 per cent. alcohol (Bates and Riddle, 1935) was superior to extraction with aqueous acid (Riddle *et al.* 1932), aqueous alkali (Riddle *et al.* 1933), acid acetone (Lyons and Catchpole, 1933a) or glacial acetic acid (McShan and Turner, 1935). Later (Bergman and Turner, 1942) they studied the extraction of acetone-dried gland with alkaline aqueous alcohol of various concentrations and found that 80 per cent. alcohol at *p*H 10–11 gave the most active product, assaying 20 i.u. per mg.

Bates and Riddle (1935) described a method of purification which has been widely used. Fresh or desiccated gland is extracted at *p*H 9–10 with 60–70 per cent. ethanol followed by precipitation at *p*H 6 in presence of an increased concentration of ethanol. The product is then re-dissolved and separated from gonadotrophin (F.S.H.) and thyrotrophin by precipitation at *p*H 3–4 in the presence of $S\bar{O}_4^-$. Further purification is effected by precipitation of the product from 70 per cent. ethanol at *p*H 6 in the presence of NaCl. The authors state that about 70 per cent. of the original hormone is obtained uncontaminated with F.S.H. or thyrotrophin. Young (1938) replaces the last stage of this method by a series of isoelectric precipitations from aqueous solutions at *p*H 5·5.

Lyons (1937a, b) produced a modified version of his original procedure (Lyons and Catchpole, 1933a) involving extraction of undissected sheep pituitaries with acid-acetone followed by precipitation of the prolactin by raising the acetone concentration. This method not only gives a preparation free from gonadotrophin and thyrotrophin but also is capable of removing most of the adrenocorticotrophin ; the latter is precipitated from aqueous solution at *p*H 6·5 and the prolactin at *p*H 5·5. An essentially similar method was subsequently used by Li, Lyons and Evans (1940a, b, 1941a) to prepare prolactin assaying 20–30 i.u. per mg. which was found to exhibit a high degree of molecular homogeneity. Li, Simpson and Evans (1942) have recently devised another method for obtaining what is probably pure prolactin, since it is a protein exhibiting molecular homogeneity with respect to solubility, electrophoretic mobility and diffusion, from the crude powder obtained in the first stage of the Lyons acid-acetone extraction procedure. In this method prolactin is separated from its most troublesome biologically

active contaminant—adrenocorticotrophin—by alternate precipitations at *p*H 3 in presence of NaCl and at *p*H 5·6.

A novel and interesting method giving preparations assaying approximately 30 i.u. per mg. (sheep gland) has been developed by Schwenk, Fleischer and Tolksdorf (1943). This method is based on the discovery by Fleischer (1943) that prolactin is soluble in absolute methanol. Undissected pituitaries are extracted with water at *p*H 8–9 and the prolactin isoelectrically precipitated in the presence of chloroform whereupon a chloroform gel is formed containing the prolactin. The latter is extracted from the dried gel with acid methanol and then precipitated with ether at *p*H 3. Further purification is effected by precipitation with NaCl from aqueous solution at *p*H 2·5 followed by solution in methanol and precipitation with ether. The yield is 2 g. per kg. of fresh sheep gland, but cattle and hog glands give lower yields of a product not quite so potent. The degree of contamination with other hormones is, at the time of writing, unknown.

The first claim to have prepared crystalline prolactin was made by White, Catchpole and Long (1937), the amorphous starting material being made by the acid-acetone method of Lyons (1937a). The crystals exhibited an activity of the same order as that given by Lyons (1937a) for his purified amorphous preparations and from this fact and other subsequent chemical evidence it seems that Lyons had obtained practically pure prolactin as early as 1936–37. Later, White, Bonsnes and Long (1942) (*see also* White, 1943) described two procedures for obtaining crystalline prolactin in low yield, starting from an acid-acetone preparation which had been carefully separated from adrenocorticotrophin. Crystallisation of the hormone was achieved by an acetic acid-pyridine procedure, as previously described by White *et al.* (1937), or by slow precipitation from saturated aqueous solutions by addition of acetone. The crop-stimulating activity of the crystalline protein agreed well with those of the most active amorphous preparations (30–35 i.u. per mg.) and physico-chemical studies indicated that the preparation was molecularly homogeneous.

Chemistry of prolactin.—Prolactin (pigeon crop-stimulating) activity is associated with a protein which has been prepared in amorphous and crystalline forms which are molecularly homogenous by all physico-chemical criteria at present available. At the present time there is no reason to believe that the actual hormone is an impurity inseparable from the protein. Neither is there any evidence that the biological activity is due to the presence of a prosthetic group since no such group has been detected spectroscopically (*see* White and Lavin, 1940) or by any other means.

The protein nature of prolactin was long ago suspected on account of its solubility relations (*see* Bates, Riddle and Lahr, 1934 ; Riddle and Bates, 1939). It is soluble in water except in the isoelectric region (*p*H 5–6). On the basis of electrophoretic studies the following values for the isoelectric point have been given ; for crystalline prolactin, *p*H 5·6 (Shipley, Stern and White, 1939), *p*H 5·65–5·70 (White *et al.* 1942) ; for pure amorphous prolactin, *p*H 5·7 (Li *et al.* 1940a) ; *p*H 5·73 (Li *et al.* 1940b). The latter workers found identical values for cattle and sheep hormones. At *p*H values acid to the isoelectric point the hormone is precipitated by the usual protein precipitants (*see* Riddle and Bates, 1939), and at neutrality by the hydroxides of Zn, Cu, Al, and Fe (Bates and Riddle, 1938).

Figures for the elementary analysis of various crystalline and amorphous preparations from three laboratories are tabulated by White *et al.* (1942); with the exception of the figures for sulphur the various analyses show good agreement. The following percentage figures for crystalline prolactin, from the Table given by White *et al.*, are typical: C = 51·81, H = 6·81, N = 16·49, S = 2·03, ash = 0·50. Tests for phosphorus and carbohydrate were negative. Sulphydryl groups appear to be absent (Li, Lyons and Evans, 1941b; White *et al.* 1942; Fraenkel-Conrat, Simpson and Evans, 1942; Fraenkel-Conrat, 1942) but appear on prolonged reduction with NaCN (White *et al.* 1942) or on treatment with thiol compounds (Fraenkel-Conrat, 1942).

Crystalline and purified amorphous preparations give a protein biuret colour and the usual colour reactions for amino acids (White *et al.* 1942). A broad band in the ultra-violet spectrum of prolactin was attributed by White and Lavin (1940) to the combined absorption of tyrosine, phenylalanine and tryptophane. The tyrosine content of bovine prolactin has been given as 5·73 per cent. (Li, Lyons and Evans, 1940c), 5·42 per cent. (Li, Lyons and Evans, 1941c) and 5·51 per cent. (White *et al.* 1942); somewhat lower values, 4·53 per cent. and 4·7 per cent., have been found for sheep prolactin (Li *et al.* 1940c, 1941c). The following values for the tryptophane content have been given: bovine hormone 1·31 per cent., sheep hormone 1·19 per cent. (Li *et al.* 1940c); bovine hormone, 1·30 per cent. (White *et al.* 1942). Later, Li *et al* (1941c), using a method considered superior to the one they used formerly, obtained a value of 2·5 per cent for hormone from both sources. The arginine content of sheep and bovine hormones has been given as 8·31 per cent. (Li *et al.* 1941c) and the cystine content as 3·0 per cent. (Fraenkel-Conrat, 1942) and 3·11 per cent. (Li, 1943). No cysteine has been found in prolactin (Li *et al.* 1941b; Fraenkel-Conrat, 1942). Methionine is present to the extent of 4·3 per cent. (Li, 1943), this together with cystine accounting for the total sulphur.

The molecular homogeneity of highly active amorphous or crystalline preparations has been demonstrated by electrophoretic (Li *et al.* 1940a; White *et al.* 1942), solubility (Li *et al.* 1941a; White *et al.* 1942) and ultracentrifugal (White *et al.* 1942) studies. Li *et al.* (1941a) observed differences in the solubilities of sheep and bovine hormones which pointed to species specificity. As already mentioned, however, no such species differences in electrophoretic mobility were observed, nor are there any as regards specific rotation (Li, 1942) while Bischoff and Lyons (1939) found that the hormones from the two sources were immunologically indistinguishable.

At the time of writing the molecular weight of prolactin is not known with certainty. From osmotic pressure determinations Li *et al.* (1941c) obtained a mean value of 26,500 in good agreement with the mean value, 25,000, estimated from the percentages of tryptophane, tyrosine, cystine, arginine and sulphur. On the other hand, determination of the diffusion constant and sedimentation velocity in the ultracentrifuge indicated a molecular weight of 32,000–35,000 on the assumption of a spherical molecule (White *et al.* 1942). Li (1942), however, on the basis of viscosity data, contends that the prolactin molecule is not spherical (dissymmetry constant = 1·29) and from the latter and the diffusion constant estimates the molecular weight as 22,000.

The effect of heat on the biological activity of prolactin depends on conditions

such as *p*H, the presence or absence of salts and so forth, as has been emphasised by Riddle and Bates (1939). At *p*H 8 it withstands boiling for one hour except in the presence of salts but is rapidly inactivated by heat at acid or alkaline *p*H values (Riddle and Bates, 1939). Relatively crude preparations studied by McShan and French (1937) were fairly stable to heat between *p*H 3·2 and *p*H 9·7. White *et al.* (1942) reported that pure prolactin in dilute solution undergoes some inactivation after 30 minutes on a boiling water-bath at all *p*H values between *p*H 1 and *p*H 13. The assay technique used (the intradermal minimal response method) was, however, hardly adequate to provide a true quantitative picture of the effects involved, but it is probably justifiable to conclude that pure prolactin is a heat-labile protein. The activity is also destroyed on hydrolysis with acid (White *et al.* 1942), pepsin (McShan and French, 1937 ; White *et al.* 1942) and trypsin (Bates *et al.* 1934 ; McShan and French, 1937 ; White *et al.* 1942). The results of White *et al.* (1942) indicate that the biological activity is destroyed before the disappearance of all protein fragments which can be precipitated by trichloracetic acid. The presence of free amino groups (probably the ε-amino groups of lysine) appears to be necessary for biological activity, since the latter is completely or largely destroyed by treatment with ketene (Li, Simpson and Evans, 1939), nitrous acid (Li, Lyons, Simpson and Evans, 1939) and phenyl *iso*cyanate (Bottomley and Folley, 1940). The biological activity also seems to be bound up with the tyrosine residues since Li *et al.* (1941b) have shown that iodination of prolactin, under conditions in which the iodine appears to combine only with the tyrosine molecules, also decreases its activity. Treatment with a large excess of cysteine (or thioglycollic acid) also causes inactivation of the hormone (Fraenkel-Conrat *et al.* 1942) accompanied, according to Fraenkel-Conrat (1942), by the appearance of SH and other reducing groups. Small proportions of cysteine render the hormone insoluble without destroying its activity (Fraenkel-Conrat *et al.* 1942).

The prolactin molecule appears to be relatively stable since biological activity is retained after treatment with urea (Li *et al.* 1941b) and precipitation with trichloracetic acid (Li *et al.* 1941c), both of which denature proteins. Osmotic pressure determinations indicate that this molecule, unlike those of many proteins, does not fragment in presence of urea (Li *et al.* 1941c). It is of course possible that the hormone may be reversibly denatured by urea without undergoing a change in molecular weight.

It is clear that considerable information has already been accumulated with regard to the chemical, and particularly the physico-chemical, properties of the prolactin molecule. Moreover, a beginning has been made with the correlation of chemical structure with biological activity. It must be emphasised, however, that in many of the inactivation experiments discussed above, the assay methods, which in some cases amounted to little more than qualitative tests and subjective ones at that, have not been adequate for quantitative studies. Further work with amounts of material allowing accurate assay of reaction products in terms of international units, preferably by means of the objective crop-weight method, may be expected to yield additional information on quantitative aspects.

Physiological responses to prolactin.—In addition to the pigeon crop-gland response, already dealt with, other physiological mechanisms not primarily concerned with lactation seem to involve prolactin (for review *see* Riddle, 1937). The rôle of this hormone in lactation, undoubtedly its best-known physiological

function, has already been considered and will be further discussed in later sections, but the presence of prolactin in non-mammalian forms indicates that lactation cannot be the only, or necessarily the most important, physiological function in which it is involved.

There is now reason for the belief that prolactin must be considered to be a member of the anterior-pituitary gonadotrophic complex. An antigonadotrophic action of prolactin was first described by Riddle and Bates (1933) who found that prolactin decreases the testis weight in male pigeons. Later (Bates, Lahr and Riddle, 1935), it was found that the ovary (and secondarily the oviduct) of the domestic hen responded similarly. Such effects were thought to be due to interference with the secretion or release of F.S.H. by the pituitary since simultaneous treatment with F.S.H. abolished the effect of prolactin on the pigeon testis (Bates, Riddle and Lahr, 1937). Prolactin is also capable of influencing, directly or indirectly, the ovaries of mammals. Thus treatment with prolactin interrupts oestrous cycles in the mouse (Dresel, 1935) and rat (Lahr and Riddle, 1936 ; Lyons, Simpson and Evans, 1941), but as yet has not been found to affect the length of the menstrual cycle in the monkey (Hisaw, 1944) and woman (Kupperman, Fried and Hair, 1944). Lyons *et al.* (1941) contended that since prolactin did not delay vaginal opening in the rat it probably does not directly suppress the output of F.S.H. Persisting vaginal mucification and development of mammary lobule-alveolar tissue in rats in prolactin anoestrus, pointed to the continued secretion of progesterone by the persistent corpora lutea. In their experiments Lahr and Riddle (1936) had also obtained histological evidence of continued luteal function under the influence of prolactin.

Subsequently, further evidence of the luteotrophic action of prolactin has accumulated. Astwood (1941) postulated the existence of a new pituitary gonadotrophin, luteotrophin, responsible for the control of luteal function and present in extracts prepared by methods similar to those used for prolactin. Using the formation of traumatic uterine placentomata as an index of luteal function, Evans, Simpson and Lyons (1941) and Evans, Simpson, Lyons and Turpeinen (1941) have shown that prolactin is probably the only pituitary hormone which regularly maintains luteal function in hypophysectomised rats. Further evidence, based on the deciduoma response, of the luteotrophic action of prolactin has been presented by Lyon (1942), Fluhman and Laqueur (1943) and Sydnor (1945), while Cutuly (1941a, b ; 1942) has interpreted his observations that prolactin tends to favour implantation of ova and to some extent maintain pregnancy in rats hypophysectomised soon after mating, as evidence that prolactin exerts a luteotrophic action. Additional evidence comes from the work of Nelson and Pichette (1943) who found that prolactin will prevent the return of oestrus which ordinarily follows hypophysectomy in rats in which pseudo-pregnancy has been induced and maintained by oestrogen, and of Everett (1944) who showed that besides progesterone, prolactin (in the presence of luteal tissue) would interrupt continuous oestrus in rats of a strain prone to exhibit this condition under constant illumination. Moreover, it should not be forgotten, that Tobin (1942) has found that certain prolactin preparations will, like progesterone itself, prolong the survival of adrenalectomised rats.

Riddle and Bates (1939) have pointed out that prolactin is particularly concerned in responses related to the care of the young. It has been said to induce

maternal behaviour in rats (Riddle, Lahr and Bates, 1935), under certain conditions broodiness in hens (Riddle, Bates and Lahr, 1935), and nesting behaviour in fish (Noble, Kumpf and Billings, 1936).

Various metabolic effects of prolactin have been described but it seems doubtful whether these are specific and primary responses to prolactin. According to Bates, Riddle, Lahr and Schooley (1937) prolactin treatment increases the weights of the liver and intestine (and also the length of the latter) in the hypophysectomised pigeon. Prolactin has also been said to stimulate general body growth in the normal and hypophysectomised pigeon (*see* Riddle and Bates, 1939) and, in conjunction with thyrotrophin, in genetically dwarf mice (Bates, Laanes and Riddle, 1935). A calorigenic action of prolactin has been reported in the dove in the absence of the adrenals (Riddle and Smith, 1940) and the thyroid (Riddle, Smith, Bates, Moran and Lahr, 1936). Evidence about the effects of prolactin on carbohydrate metabolism has been rather conflicting. Preparations used by Nelson, Turner and Overholser (1935) had no effect on the blood sugar of mammals but Riddle, Dotti and Smith (1937) reported hyperglycaemia in response to prolactin in pigeons. Equally conflicting are reports of the effect of prolactin on the insulin content of the pancreas. According to Funk, Chamelin, Wagreich and Harrow (1941) prolactin decreases the pancreatic insulin ; the reverse is claimed by Fraenkel-Conrat, Herring, Simpson and Evans (1942, 1944). According to Gaebler and Robinson (1941, 1942) prolactin, in contradistinction to other anterior-pituitary fractions, caused nitrogen storage in depancreatised bitches kept on a constant insulin dosage. Under these conditions the calorigenic and diabetogenic effects of the hormone were relatively mild.

Occurrence of prolactin.—Prolactin has been found in anterior-pituitary tissue from all mammals so far examined, including man. The available evidence points to the eosinophiles as the site of synthesis of the hormone (Schooley and Riddle, 1938 ; Azimov and Altman, 1938 ; Friedman and Hall, 1941). Pituitaries from the sheep, the ox, and to a lesser extent, the pig, provide the principal sources for large-scale preparation. According to Lyons (1937b) posterior lobes also contain considerable amounts of prolactin, probably because of *post-mortem* diffusion. As regards sub-mammalian classes, positive responses by the local crop test have been obtained with pituitaries of birds (Burrows and Byerly, 1936 ; Leblond and Noble, 1937), reptiles, amphibia and fish (Leblond and Noble, 1937). The prolactin content of the mammalian pituitary under various conditions will be considered in the next section.

Positive crop reactions have also been obtained with liver tissue (or extracts thereof) from various species (Leblond and Noble, 1937 ; Keller, 1939 ; Lessmann, 1939 ; Rabald and Voss, 1939 ; Cunningham, Bickell and Tanner, 1940), with placental (particularly unripe) tissue (Ehrhardt, 1936 ; Keller, 1939 ; Turner and Meites, 1941a) and mammary gland (Geschickter and Lewis, 1936). Positive intradermal crop tests must at best be considered as tenuous evidence of the occurrence of prolactin in tissues other than mammalian anterior lobe in view of the non-specific crop responses described by Lahr *et al.* (1943) (*see also* Leblond and Noble, 1937), but it seems possible that the liver responses may be actually due to prolactin since Rabald and Voss (1939) obtained an active liver extract, by essentially the method of Bates and Riddle (1935), which was assayed by a crop-weight method. The presence of a crop-stimulating substance in adrenal cortex

extracts was claimed by Hartman, Lockwood and Brownell (1933). Later, the active substance, called cortilactin, was separated by isoelectric precipitation at *p*H 5·8 but no claim as to its identity with prolactin was made (Spoor, Hartman and Brownell, 1941). Hurst, Meites and Turner (1942), on the other hand, failed to detect crop-stimulating material in adrenal cortex extracts.

Crop-stimulating substances have been detected in blood from the turtle (Cunningham *et al.* 1940), the rabbit (Meites and Turner, 1942a), the lactating cow and mare (Leblond, 1937) and woman (Tesauro, 1936 ; Ehrhardt and Voller, 1939). The latter workers reported peak titres at ovulation and menstruation.

The first detection of prolactin in *post-partum* human urine was achieved by Lyons and Page (1935) and later, assays were carried out by Tesauro (1936), Hoffman (1936), Lessmann (1939), Ehrhardt and Voller (1939) and Meites and Turner (1941). Prolactin has also been detected in the urine of men (Lyons, 1937b) and in urine of new-born infants of both sexes (Lyons, 1937c). Hoffmann (1936) found no prolactin in human urine before parturition and reduced excretion in cases of hypogalactia (*see also* Meites and Turner, 1941). Hurst, Meites and Turner (1941) reported positive responses with urine from lactating goats. Positive mammalian lactogenic responses with human urine extracts were reported by Langecker and Schenk (1936) and Liard (1937).

In general comment on the foregoing results it may be well to re-emphasise that as regards tissues other than mammalian pituitary tissue it is not safe to deduce the presence of prolactin on the sole evidence of positive crop tests. The only sure criterion would be the isolation of a pure protein and its chemical and biological characterisation. Further attempts to isolate pure prolactin from mammalian liver would therefore be of interest. The occurrence of prolactin in the blood and urine of mammals, at any rate during lactation, is *a priori* to be expected and it seems likely that the crop-stimulating reactions obtained with these body fluids are in fact due to prolactin.

Prolactin content of the pituitary gland.—Species differences in the prolactin content of the pituitary gland were reported by Reece and Turner (1937b) who, using a modification of the intradermal crop test, arranged the pituitaries from females of various species in descending order of potency as follows : ox (dairy breed), guinea-pig, rat, rabbit, mouse, cat. The results for males were in harmony with these. Later, Holst and Turner (1939a) obtained a similar ranking for pituitaries of female guinea-pigs, rats and rabbits. Chance, Rowlands and Young (1939) assayed, by a crop-weight method, partially purified extracts of batches of glands and gave the following descending order of potency for sources which might be useful for large-scale preparation : sheep, ox, man, pig, horse. Bates and Riddle (1935) had already found that pig glands were much less potent than those of the sheep or ox.

The existence of strain or breed differences within a species, particularly as between cattle of dairy and beef breeds respectively, might be expected and indeed Reece and Turner (1937b) claim that glands from dairy cows contain more hormone than glands from beef cows. Hall (1944b), however, criticises the method of assay used by Reece and Turner (1937b), which involved the injection of material in suspension, since he found that extracts gave higher results than suspensions. With Hall's technique there was no difference in prolactin content between pituitaries from dairy and beef cattle (*see also* Hall and Nicolet, 1942). The

work of Hall raises the important issue of the validity of many of the rather fine differences in the prolactin content of the pituitaries of small animals, to be discussed below, since the assays involved the intradermal injection of tissue suspensions.

As regards sex differences, Bates, Riddle and Lahr (1935) could find no marked differences between the potencies of anterior-lobe tissue from non-pregnant cows and bulls (or steers) while, on the other hand, among a variety of species Reece and Turner (1937b) and Holst and Turner (1939a) generally found female pituitaries more potent than those from males. It may be noted that Bates *et al.* assayed partially purified extracts by a crop-weight method while Turner *et al.* used the local crop test.

Considerable differences in the prolactin content of the pituitary, in both sexes at different stages of maturity and in different reproductive states, have been reported. Of seven classes of cattle pituitaries assayed by Bates *et al.* (1935) foetal pituitaries were the most potent; by contrast, Reece and Turner (1937b) found less prolactin in foetal pituitaries than in any other type of cattle pituitary examined. In the bull, Reece and Turner (1937b) reported an increase, with advancing maturity, in the total prolactin per gland, mainly due to increasing pituitary size. An increase in the pituitary prolactin in the male guinea-pig at puberty was noted by Holst and Turner (1939a). The results of Bates *et al.* (1935) and Reece and Turner (1937b) were once more in conflict, this time about the effect of castration on the prolactin content of the bull pituitary, the results of the former indicating that castration has little effect while the latter reported a significant decrease following the operation. In the male rat, however, Reece and Turner (1937b) found no change in pituitary prolactin after castration or experimentally induced cryptorchidism.

According to Turner and his colleagues, sexual maturity in the female is accompanied by an increase in the prolactin content of pituitary tissue (Reece and Turner, 1937b; Holst and Turner, 1939a). Increased values in the rat have been reported at oestrus and conversely, decreased values following ovariectomy (Reece and Turner, 1937b). In the mouse, however, ovariectomy did not effect the pituitary prolactin (Hurst and Turner, 1942). Some workers have reported increased values during pregnancy (*see* Bates *et al.* 1935 and Reece and Turner, 1937b, for the cow; Wiegand, 1937, for the rat; Hall and Nicolet, 1942, for the rabbit; Hurst and Turner, 1942, for the mouse), but others report no increase during pregnancy in the rat (Reece and Turner, 1937b), guinea-pig (Holst and Turner, 1939b) and rabbit (Turner and Meites, 1941a; Holst and Turner, 1939b). Similarly conflicting results have been reported concerning the effect of pseudo-pregnancy in the rabbit; Hall and Nicolet (1942) reported increased assays during pseudo-pregnancy while Meites and Turner (1942b) could find no such effect.

Turner and his school have put forward considerable evidence that parturition is followed by a rise in pituitary prolactin potency which rise they associate with the initiation of lactation (*see* later). Data have been obtained on the mouse (Hurst and Turner, 1942), rat (Reece and Turner, 1937b), guinea-pig (Reece and Turner, 1937b; Reece, 1939) and rabbit (Reece and Turner, 1937b; Holst and Turner, 1939b; Meites and Turner, 1942f). Confirmatory data for the rat were given by Wiegand (1937). Assays of the prolactin content of the pituitary at different stages of lactation have been made by Hurst and Turner (1942) in the

mouse, Reece, Hathaway and Davis (1939) in the rat and Meites and Turner (1942f) in the rabbit. In general, the values show a progressive decline but it is thought that the maximum attained does not coincide with the peak of lactation.

In a later section evidence will be considered which indicates that suckling, probably through a nervous reflex evoking the discharge of hormones by the anterior pituitary, may affect the function of the mammary glands. It is therefore of some interest to note that Reece and Turner (1936b, 1937a, b) found that, in the rat, the suckling stimulus following a period of non-suckling caused a decrease in the pituitary prolactin content (*see also* Holst and Turner, 1939b, for similar results in the guinea-pig and rabbit). Later, Meites and Turner (1942c, f) presented evidence that regular application of the suckling stimulus is capable of exerting a long-term effect on the prolactin content of the pituitary ; they found less prolactin in the pituitaries of parturient rats and rabbits prevented from suckling than in normally nursing controls. It should be noted, however, that Meites, Bergman and Turner (1941b) found that in the rabbit the size of the litter suckled had no effect on the pituitary prolactin.

The effect of oestrogen on the prolactin content of the pituitary is of interest in view of current ideas about the rôle of oestrogen in lactogenesis and galactopoiesis (*see* later). All the available evidence indicates that by contrast with the known suppressive effects of oestrogen on the secretion of F.S.H. and probably also growth hormone by the pituitary, the secretion of prolactin is increased (for cytological evidence of hypersecretion by the anterior pituitary under oestrogen influence, *see* Zeckwer, 1944). Reece and Turner (1936a) first reported an increase in the pituitary prolactin in the male rat under the influence of oestrogen. This has since been repeatedly confirmed in rats, rabbits and guinea-pigs (Reece and Turner, 1937b ; Reece, 1938 ; Lewis and Turner, 1941c ; Meites and Turner, 1942a, c) and according to Meites and Turner (1942c) occurs in lactating rats despite the fact that oestrogen tends to inhibit established lactation. Wiegand (1937), however, found less prolactin in the pituitaries of parturient rats given sufficient oestrogen to inhibit the growth of the young than in those of controls. It is of course possible that if the observed increases in pituitary prolactin content following oestrogen administration are real, they merely signify an inhibition of release rather than increased secretion. Meites and Turner (1942a), however, using the intradermal crop test, reported an increase in the blood prolactin of oestrogenised rabbits coinciding with the increased potency of the pituitary, which would indicate an actual increase in prolactin production. The data, however, are hardly extensive enough to prove such an important point beyond doubt.

Turner and his colleagues have found that progesterone by itself does not increase the prolactin content of the pituitary (Reece and Turner, 1937b ; Meites and Turner, 1942d) but given in combination with oestrogen tends to inhibit the effect of the latter (Meites and Turner, 1942d). Hence they believe that the corpus luteum prevents a rise in the pituitary prolactin during pregnancy. In keeping with this view they reported an increase in the prolactin content of the pituitary of the pregnant rabbit following hysterectomy (Meites and Turner, 1942e). Reece and Bivins (1942), however, found that high doses of progesterone (15 mg. daily) increased the prolactin content of the pituitary in the spayed female rat. Testosterone has been reported to increase the pituitary prolactin (Reece and

Mixner, 1939; Meites and Turner, 1942c) but androsterone (200 μg. daily) was inactive (Reece, 1941). Meites, Trentin and Turner (1942) reported that the pituitary prolactin in the rat was reduced by adrenalectomy, though the *post-partum* increase still occurred. Deoxycorticosterone, however, did not affect the pituitary of the mouse (Turner and Meites, 1941b).

McQueen-Williams (1935), who introduced the widely used technique for the assay of the prolactin content of the rat pituitary, reported decreased values in thyroidectomised rats. Reineke, Bergman and Turner (1941), on the other hand, found no such effect in young male goats; neither was the prolactin content of the mouse pituitary altered by the administration of thyroxine (Hurst and Turner, 1942).

Lactogenic action of anterior-pituitary hormones in hypophysectomised animals.— It is now commonly accepted that hypophysectomised test animals should be used for the investigation of primary responses to anterior-lobe hormones in order to eliminate possible disturbing factors due to the action of endogenous pituitary hormones. If lactogenic hormones are defined as anterior-pituitary hormones which initiate lactation in *hypophysectomised* animals, the generally accepted theory (*see* for example Bergman and Turner, 1940; Lyons, 1941) of the existence of a single lactogenic hormone, prolactin, cannot be maintained, since it has generally been found that purified prolactin will not initiate lactation in hypophysectomised animals possessing mammary glands which might be expected to respond in presence of the pituitary (Gomez and Turner, 1936b, 1937c; Nelson and Gaunt, 1936, 1937a; Nelson *et al.* 1943). The work of Fredrikson (1939) provides an exception since he reported initiation of lactation in hypophysectomised rabbits with purified prolactin. Unfractionated anterior-pituitary preparations, on the other hand, have been generally found to initiate lactation in hypophysectomised animals (*see* McPhail, 1935a, b, for the ferret and cat; Houssay, 1935b, for the dog; Gomez and Turner, 1936b, 1937c and Nelson and Gaunt, 1936, 1937b, for the guinea pig). From these results it may be concluded that if, as appears probable on the basis of experiments with intact animals, prolactin is concerned with lactogenesis, it is not the only anterior-pituitary hormone so concerned.

There is considerable evidence that adrenocorticotrophin is also involved in lactogenesis since lactation can be initiated in hypophysectomised guinea pigs by treatment with purified prolactin (and sometimes glucose) together with adrenal cortex extract (Gomez and Turner, 1936d, 1937c; Nelson and Gaunt, 1936, 1937b), certain cortical steroids (Nelson *et al.* 1943) or adrenotrophin (Gomez and Turner, 1937a; Nelson and Gaunt, 1937a). Gomez and Turner (1937b, c) could obtain no evidence of the importance of thyrotrophin in this connection, since combinations of prolactin and thyroxine were ineffective.

Folley and Young (1941a), in a discussion of the status of prolactin as a lactogenic hormone, pointed out that if these results are to be relied upon—and admittedly in view of Fredrikson's (1939) positive results in rabbits the existing rather sparse data on lactogenesis in the absence of the hypophysis should be extended by experiments on a variety of species with the purified pituitary hormones now available—adrenotrophin must be conceded an equal claim with prolactin to be regarded as a lactogenic hormone since either may be considered capable of initiating lactation in the presence of the other. That the two hormones may play quite distinct

rôles in lactogenesis, prolactin perhaps being necessary for the expression of the synthetic activities of the alveolar cells and adrenotrophin probably influencing through the adrenal cortex the supply of milk precursors, does not affect the point at issue. Thus, in place of the conventional single lactogenic hormone theory, Folley and Young (1941a) were led to the conception of a lactogenic complex of anterior-pituitary hormones, two members of which, prolactin and adrenotrophin, seem already to have been identified. Others probably remain to be discovered since the purified preparations used in the aforementioned experiments doubtless contained other unidentified hormones.

Bergman *et al.* (1940) have affirmed their belief in the existence of a single anterior-pituitary lactogenic hormone, prolactin, since they assayed a prolactin preparation at different stages of purification by methods depending on the pigeon crop-gland and mammalian lactogenic responses and found that purification did not alter the ratio of the potencies of the extract expressed in the two types of unit. This might merely mean, however, that components of a lactogenic complex were concentrated in the same proportions. As further evidence in favour of the single lactogenic hormone theory, Bergman and Turner (1940) have cited the ability of the prolactin moiety of a fractionated anterior-pituitary extract regularly to initiate lactation in suitably prepared rabbits as against the comparative failure to do so of the remaining fraction containing certain other hormones but little prolactin. Among other criticisms of this paper of Bergman and Turner, Folley and Young (1941a) pointed out that since rabbits with intact pituitaries were used, their results, which undoubtedly indicate that prolactin can be a limiting and essential factor in lactogenesis, are hardly relevant to the question of the existence or otherwise of a single lactogenic hormone. More recently, Turner (*see* Hurst and Turner, 1942) appears to have accepted the view that more than one hormone is concerned with the initiation of lactation since in the mouse the prolactin content of the pituitary, a rise in which is regarded by Turner and his school (*see* Meites and Turner, 1942d) as a decisive factor in the initiation of lactation, increases considerably during pregnancy though no milk secretion occurs.

The action of prolactin on the mammary gland has generally been assumed to be direct and this assumption has been regarded as in accord with the theory that prolactin is the specific lactogenic hormone. Thus Bergman *et al.* (1940) apparently envisaged the action of prolactin on the mammary gland as in the nature of a " trigger " effect, since lactation was assumed to follow the " activation " of the alveolar cells by prolactin. It is only recently, however, that any proof of the direct action of prolactin has been provided. Lyons (1942) essayed to obtain such proof by elegant experiments in which minute amounts of prolactin were introduced into individual galactophores of the rabbit mammary gland. Lactation (and, according to Lyons, epithelial hyperplasia) was induced in those gland sectors communicating with the injected galactophores but not in adjacent sectors (*see* Fig. *20.* 13.). But the rabbits were not hypophysectomised, and while these results point to the possibility of a direct effect on the mammary epithelium they do not demonstrate that prolactin is the only hormone concerned in lactogenesis, since the co-operative action of endogenous pituitary hormones was not excluded.

Galactopoietic action of anterior-pituitary extracts.—In the present state of knowledge it is desirable, for clear discussion, to adopt different terms in respect of the initiation of lactation and its stimulation once established. Folley and Young

(1940) suggested that as long as it should be thought necessary to draw a distinction between these two processes they should be respectively designated by the terms *lactogenesis* and *galactopoiesis*. Bergman and Turner (1940) subsequently and independently suggested a similar terminology.

Following the discovery of the lactogenic properties of anterior-pituitary extracts, galactopoietic effects of the crude extracts then available were soon demonstrated in farm animals (*see* Grüter and Stricker, 1929, for the cow and sow; Asdell, 1932, for the goat; Kabak and Kisilstein, 1934, and Kabak and Margulis, 1935, for the sheep). With the discovery of prolactin and its characterisation as a hormone concerned in lactogenesis arose a belief that prolactin would possess outstanding galactopoietic properties which might find application both in

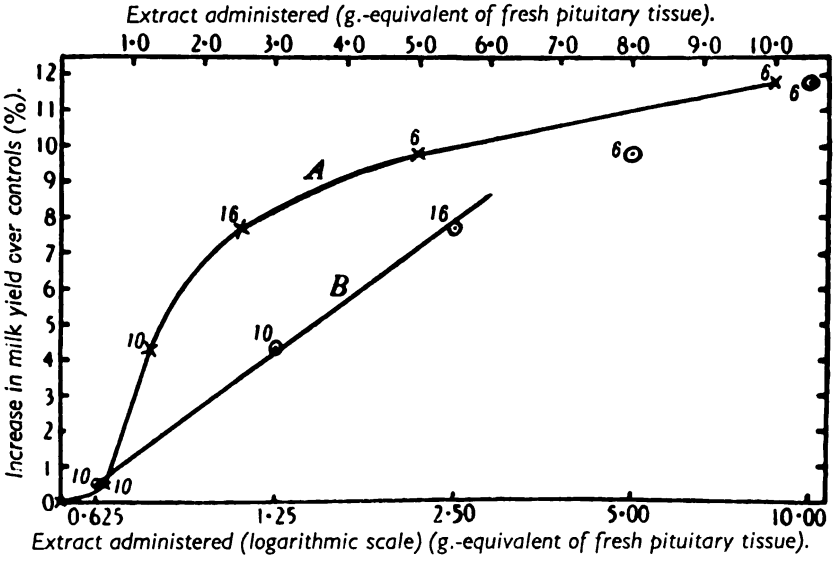


Fig. 20. 15—The influence of a single injection of ox anterior-pituitary extract on the milk yield of groups of cows in declining lactation. The figures attached to the points give the number of cows in the group. Curve A shows the mean daily increase in milk yield over the two days following the injection plotted against the dose of extract given. Curve B is a plot of the responses against the logarithm of the dose of extract given. (From Folley and Young, 1945.)

medicine and in the dairy industry. This idea has, however, not been substantiated by subsequent work. It was first reported by Azimov and Krouze (1937) and later shown more conclusively by Folley and Young (1938) that on the basis of equal prolactin content, determined by pigeon crop methods, unfractionated anterior-pituitary extracts exhibit much more galactopoietic activity, assayed in lactating cows, than purified prolactin. Both groups of workers reported substantial, temporary increases in milk yield following single injections of crude ox anterior-pituitary extracts into lactating cows (*see* Fig. 20. 15), but whereas Azimov and Krouze (1937), whose paper should be consulted for references to earlier papers on the subject by Azimov and his colleagues, found the treatment to be most effective during the first four months of lactation during which time the yield could be increased slightly above the natural peak value, Folley and Young (1938, 1940, 1945) obtained good responses during the declining phase, and subsequently showed that their crude saline extract was ineffective at the time of peak milk yield (Fawns, Folley and Young, 1945). Similarly in goats

Asdell *et al.* (1936) found that anterior-pituitary extracts were effective during the declining phase but not at the peak of lactation. Folley and Young (1945) obtained a sigmoid dose-response curve for single injections of crude ox anterior-pituitary extract in cows, and later showed that horse gland extracts were even more active than extracts of ox pituitary while material from the sheep and pig possessed little activity (Folley *et al.* 1945b).

In contrast to the relative ineffectiveness of single injections of purified prolactin, repeated injections of relatively high doses of prolactin preparations produced substantial increases in milk yield (Folley and Young, 1938, 1939, 1940). In some experiments the initial stimulus soon disappeared despite continuance of the treatment, but this did not appear to be due to the formation of anti-prolactin (Folley and Young, 1940).

As with single injections, the galactopoietic effect of repeated injections of crude ox pituitary extracts was, on the basis of equal prolactin content, considerably greater that that of prolactin fractions (Folley and Young, 1940), but repeated injections of crude extracts of sheep and pig gland actually decreased the milk yields of lactating cows and here it seems possible that the formation of anti-hormones to heterologous glandular material was involved (Folley *et al.* 1945b). Various technical factors connected with the practical use of crude extracts of ox pituitary for increasing the milk yield of cows were studied by Folley and Young (1945).

Thyrotrophic preparations from ox anterior lobe, containing little or no prolactin, also exhibited galactopoietic activity in cows (Folley and Young, 1938, 1939) but it is not certain that these responses were mediated by the thyroid since no rise in milk fat content, a characteristic result of thyroxine treatment (*see* page 584), occurred.

Changes in milk composition in response to pituitary treatment seem in general to be slight (Azimov and Krouze, 1937; Folley and Young, 1938), but marked increases in lactose (Folley and Young, 1938; Sykes, Gould, Duncan and Huffman, 1944) and fat (Folley and Young, 1939; Sykes, Mueleman and Huffman, 1942) contents have been observed in response to certain extracts.

Folley and Young (1938, 1939, 1940) investigated the relation between galactopoietic activity and certain other biological properties of a number of anterior-pituitary preparations. Galactopoietic activity bore little relation to prolactin content but was closely paralleled by glycotropic (anti-insulin) activity, save that an extract rich in glycotropin but free from prolactin showed no galactopoietic activity (Folley and Young, 1940). Similarly, the galactopoietic activities of anterior-pituitary extracts from four species (Folley *et al.* 1945b) bore no relation to their relative prolactin contents (Chance *et al.* 1939). It was concluded that, like lactogenesis, galactopoiesis must be ascribed to the action, not of a single anterior-pituitary hormone, but rather of a hormone complex of which prolactin and glycotropin are members. A claim to have identified glycotropic action with adrenotrophin has been made by Jensen and Grattan (1940). Folley and Young (1941a) pointed out that if this idea should prove to be correct, the necessity for drawing a distinction between lactogenic and galactopoietic activities, as far as anterior-pituitary extracts are concerned, would be open to doubt since the same combination of hormones would have been found to play an important rôle in both processes.

Purified prolactin preparations have been used for the treatment of deficient lactation in women with conflicting and sometimes disappointing results (Kurzrok, Bates, Riddle and Miller, 1934; Ehrhardt, 1936; Hoffmann, 1936; Ross, 1938; Lessmann, 1939; Stewart and Pratt, 1939; Werner, 1939). The most successful results seem to have been obtained by Kenny and King (1939) and Winson (1943). Folley and Young (1941a) point out that lack of agreement about the clinical efficiency of prolactin is not surprising since the extracts used have been assayed only for prolactin content on the tacit assumption that this is a measure of galactopoietic power. The lactating woman is surely more analogous, from the physiological point of view, to the lactating cow than to the pigeon and it would therefore seem desirable to use for clinical purposes, preparations which have been assayed for galactopoietic potency in lactating mammals.

Oestrogen

Inhibitory effect of oestrogen.—It has been generally believed for many years that oestrogens exert an inhibitory effect on lactation. The first direct evidence of such an effect was provided by Parkes and Bellerby (1927) who showed that administration of oestrogenic extracts to lactating mice depressed the growth of their young. With the advent of crystalline and synthetic oestrogens confirmatory evidence has been provided in the mouse (de Jongh, 1933a; Robson, 1935), rat (de Jongh, 1933b; Wade and Doisy, 1935; Anselmino and Hoffmann, 1936; Folley and Kon, 1937; Folley and Scott Watson, 1938; Noble, 1939a; Reece, Bartlett, Hathaway and Davis, 1940; Edelmann and Gaunt, 1941), guinea-pig (Nelson, 1934a; Sardi, 1935), rabbit (Smith and Smith, 1933), goat (Mixner *et al.* 1944; Folley *et al.* 1945a) and cow (Folley, 1936; Waterman, Freud and Vos-de Jongh, 1936; Folley, Scott Watson and Bottomley, 1941a; Stanley and Owen, 1941).

In accord with this conception, while not providing decisive proof of it, are the following facts: (a) administration of chorionic gonadotrophin or pregnant mares' serum has been generally found to inhibit lactation in the presence of the ovary (Enzmann and Pincus, 1933; de Jongh, 1933b; Selye *et al.* 1934; Connon, 1937; but *see also* Hathaway, Davis, Reece and Bartlett, 1939, and Reece *et al.* 1940, whose results were rather indecisive) but not in its absence (de Jongh and van der Woerd, 1939; Edelmann and Gaunt, 1941), which indicates that ovarian hormones, probably but not certainly oestrogen (*see* Edelmann and Gaunt, 1941), are involved; (b) in guinea-pigs complete mammary growth may be induced by oestrogen treatment and lactation sets in when the dose is suddenly decreased (for references *see* page 542); (c) grafting or retention of placental tissue inhibits lactation (Frankl, 1923; Smith and Smith, 1933; but *see* Selye *et al.* 1934, for opposite results).

Oestrogens have been widely used by clinicians for inhibiting lactation when this is desirable. Clinical reports are too numerous to be quoted; for a recent, well-controlled study see Walsh and Stromme (1944).

Some workers have questioned the reality of the inhibiting action of oestrogen on lactation. Anselmino and Hoffmann (1936), for instance, claimed that the effect could not be demonstrated in the absence of the ovary and contended that the agent directly responsible for lactational inhibition was produced by the corpus luteum in response to oestrogen. Progesterone by itself did not appear to

be implicated, however, since purified luteal extracts were ineffective. Folley (1942) has subsequently shown that 15 mg. progesterone daily causes not the slightest lactational inhibition in the rat. Moreover, inhibition by oestrogen in the absence of the ovary has been demonstrated in the mouse (de Jongh, 1933a; Robson, 1935) and rat (Folley and Kon, 1937; Edelmann and Gaunt, 1941), significantly higher doses being required in ovariectomised rats than in intact ones. The reality of the inhibitory effect has also been questioned by Reece and Turner (1937b) and Meites and Turner (1942c), by the latter principally because they observed not a decrease but an increase in the prolactin content of the pituitary of the lactating rat in response to high doses of oestrogen. Yet Meites and Turner (1942c) did in fact note evidence of decreased milk secretion in oestrogenised lactating rats which they attributed to suppression of the secretion of anterior-pituitary galactopoietic hormones other than prolactin. They apparently regarded an experimentally produced depression of lactation as a true inhibition only when associated with a decrease in the prolactin content of the pituitary. More recently, however, Turner and his school have accepted the reality of the oestrogen inhibition (Mixner *et al.* 1944) and have speculated on the biochemical mechanisms involved. Scepticism as to the usual interpretation of the effects of oestrogen in puerperal women has been expressed by Abarbanel and Goodfriend (1940). They point out that in women the situation is normally complicated by the absence or removal of the milking stimulus, and they believe that the beneficial effects of oestrogen are entirely due to the prevention of engorgement, a condition due to lymphatic and venous stasis.

It is true that much of the evidence in favour of the inhibition theory was obtained on small animals in which lactational performance can only be indirectly inferred from the growth of the young. Admittedly such experiments should be interpreted with caution since it has been claimed that oestrogen administered to lactating animals may appear in the milk in sufficient amounts directly to affect the growth and well-being of the sucklings (Weichert and Kerrigan, 1942). Nevertheless, the balance of the evidence obtained on farm animals, in which milk yield can be directly measured, would appear to be decisive. Admittedly the dosages necessary to inhibit lactation are almost certainly unphysiological and the importance of oestrogen as a factor preventing lactation in the pregnant animal is thus open to question.

Lactogenic and galactopoietic effects of oestrogen.—Even while the belief was widely held that the effects of oestrogen upon lactation were solely inhibitory, evidence was coming forward which indicated that quite opposite effects were possible under some circumstances. Thus, in the course of studies of experimental mammary growth, some workers had observed evidence of secretion in the experimentally grown glands. As perhaps the most striking instance, may be quoted the results of Frazier and Mu (1935), whose male oestrogen-treated rabbits eventually came into milk and in some cases suckled young.

Results pointing in the same direction were soon afterwards reported by Folley (1936), who observed prolonged increases in the fat and non-fatty solids content of the milk of cows given single injections of oestrogen. This " enrichment effect " represents a true galactopoiesis because in favourable cases there is no more than a transient decrease in yield, and the daily output of milk solids is increased over a considerable period (Folley *et al.* 1941a). Moreover, since the

milk nitrogen partition is unchanged (Folley, 1936) and the lactose content increases (Folley and Scott Watson, 1938; Spielman, Ludwick and Petersen, 1941), there is no question of a change to colostrum such as had been reported by de Fremery (1938) in oestrogen-treated lactating goats.

The most striking evidence of the ability of oestrogens to induce lactation under suitable conditions has been obtained on farm animals. Hormonal induction of lactation in goats, under circumstances in accord with classical views, was long ago achieved by de Fremery (1936, 1938). Udder growth was induced by percutaneous application of an ointment containing oestradiol monobenzoate, but lactation only set in when the oestrogen treatment was replaced by prolactin injections. A revision of classical concepts became necessary, however, when Folley, Scott, Watson and Bottomley (1940, 1941b) showed in the goat that not only mammary growth but also copious and prolonged lactation resulted from treatment (by inunction) with diethylstilboestrol alone. Two points of importance emerged from this work; first, that lactation was initiated in the absence of prolactin treatment and second, that secretion began, and the yield steadily rose to its peak, during the period of oestrogen administration. Thus there was no doubt that a galactopoietic effect of oestrogen, probably mediated by the anterior pituitary, was involved. After the oestrogen treatment was stopped the goats continued to milk for long periods, the lactation curves being similar to those for normal lactation. These results in the goat were generally confirmed by Lewis and Turner (1940, 1941a, 1942a).

Similar experiments by the inunction method in nulliparous heifers were less successful (Folley *et al.* 1941c), only small quantities of colostral fluid being secreted, but meanwhile Walker and Stanley (1940, 1941) had obtained promising yields from nulliparous heifers by repeated injections of diethylstilboestrol dipropionate, sometimes in conjunction with androgen. Hammond, Jnr., and Day (1944) successfully applied the tablet implantation method to the practical solution of the problem and the extensive investigations on dry cows, nulliparous heifers and freemartins with this technique also include those of Folley and Malpress (1944a), Folley *et al.* (1944) and Spriggs (1945). Oral administration has so far proved less effective (Folley and Malpress, 1944b) but results of some promise were obtained by Parkes and Glover (1944) with a convenient technique involving one injection of a mixture of diethylstilboestrol esters. Consideration of the extensive results so far obtained by the tablet implantation method indicates that some 50 per cent. of the treated animals may be expected to give an economic yield of milk. In a few favourable cases peak yields of over 30 lb. daily and lactation yields of nearly 1000 gallons have been obtained (Fig. *20*. 16). A striking feature of the results, and a drawback from the point of view of practical application, is the large and hitherto unexplained individual variation in response.

It seems possible that more uniform responses might be obtained by combined treatment with oestrogen and progesterone since Mixner and Turner (1943) found that in the goat the glandular development induced by such combined treatment was more normal histologically than when only oestrogen was given. Unpublished experiments by Folley and Malpress, in which the effect of treatment of virgin goats with oestrogen and progesterone in various ratios was compared with that of treatment with oestrogen alone, however, provide no evidence of the superiority of the former treatment. Animals with intact ovaries were used in the

majority of the experiments quoted above, but in most cases no functional corpora lutea could have been present in the ovaries during the period of treatment since prolonged oestrogen treatment appears to render the bovine ovary hypoplastic (Folley and Malpress, 1944a ; Folley *et al.* 1944 ; Hammond, Jnr., and Day, 1944).

The chemical composition of the artificially induced secretions has been investigated in the goat by Folley *et al.* (1941b, 1945a), and in the bovine by Folley and Malpress (1944c). At first the secretions resemble colostrum in composition but slowly change to normal milk. In general, a daily yield of 500 ml. in the goat and 5 lb. in the bovine is indicative of normality in composition.

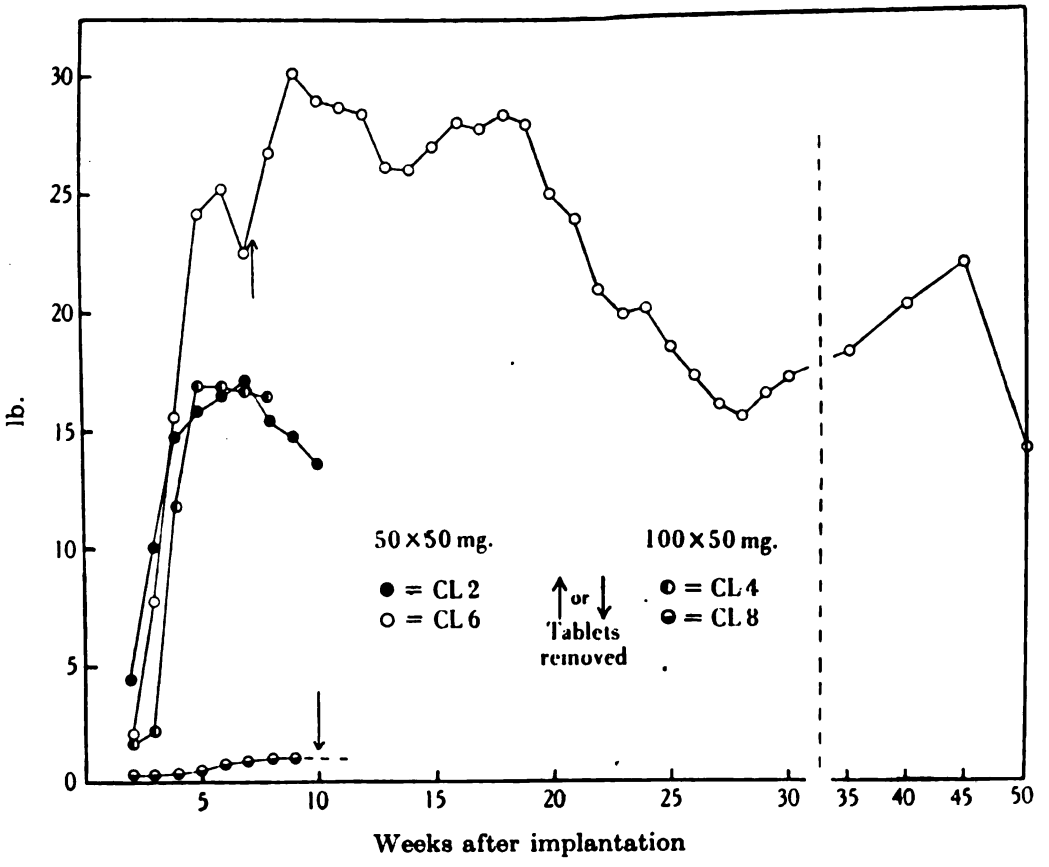


Fig. 20. 16—Lactation curves for dry cows in which lactation had been initiated by subcutaneous implantation of the stated number of tablets of diethylstilboestrol. Mean daily yields over 7-day periods are plotted. CL6 gave 7,400 lb. of milk in 365 days. (From Folley and Malpress, 1944a.)

Combined treatment with oestrogen and crude ox anterior-pituitary extract induces lactation more quickly than treatment with oestrogen alone (Folley *et al.* 1945a). There is evidence that some animals which fail to respond to oestrogen may be then brought into secretion by anterior-pituitary treatment (Folley and Young, 1941b ; Folley *et al.* 1945a) ; moreover, animals in artificially induced lactation respond to the galactopoietic action of anterior-pituitary extracts (Lewis and Turner, 1942a) even at the peak of lactation (Folley and Young, 1941b ; Folley *et al.* 1945a). Subsequent administration of oestrogen to animals previously brought artificially into lactation has in some cases resulted in further increases in milk yield (Walker and Stanley, 1941).

As regards the mechanism of the interesting effects of oestrogen discussed in this section, it is virtually certain that they are mediated by changes in the secretion by the anterior pituitary of hormones concerned in the initiation and maintenance of lactation, though it remains possible that the inhibitory effect of high doses of oestrogen may be partly due, as Nelson (1936) contended, to a direct action on the mammary gland. It seems likely, as suggested by Folley (1941), that the threshold for pituitary inhibition is higher than for stimulation, so that the nature of the effect of oestrogen treatment on mammary function would largely depend on the level of oestrogen attained in the body fluids and the duration of treatment. In general, high levels are likely to produce inhibition and lower levels, stimulation. Various phenomena encountered in the course of studies on the artificial induction of lactation, such as the rise in milk yield which often follows cessation of oestrogen treatment (Folley and Malpress, 1944a, b; Folley *et al.* 1945a; Day and Hammond, Jnr., 1945), are in general accord with this concept, as are the results of Stanley and Owen (1941) and Mixner *et al.* (1944). It is not clear at present whether the synthetic oestrogens differ from the natural oestrogens, as Lewis and Turner (1940) affirm, in possessing the property of initiating lactation. The results of Frazier and Mu (1935), who used natural oestrogens, indicate that they do not.

Androgen

Testosterone inhibits lactation in mice (Robson, 1937) and rats (Folley and Kon, 1937; Edelmann and Gaunt, 1941; Huffman, 1941), whether the ovary is present or not (Edelmann and Gaunt, 1941). Androsterone in equivalent doses has little or no effect on lactation in either species (Robson, 1937; Folley and Kon, 1937). As we have seen previously, these two steroids show similar differences as regards mammogenic properties. This, together with their findings regarding the effects on lactation of oestrogen and progesterone respectively, led Folley and Kon (1937) to the generalisation that substances which promote mammary growth also inhibit lactation. When later it was found that deoxycorticosterone, pregneninolone and, at high dose levels, progesterone, stimulated mammary growth, an opportunity of testing this theory arose. Nelson (1941c) and Nelson *et al.* (1943) reported that, in accord with the theory, deoxycorticosterone would inhibit lactation in the guinea-pig. In the lactating rat, however, Folley (1942) found no evidence of inhibition with doses of progesterone of the order of those necessary for mammary growth, nor with 10 mg. deoxycorticosterone acetate daily, but pregneninolone exerted a slight inhibitory effect (*see also* Cohen and Stein, 1940). This generalisation would therefore appear to be untenable unless it proves that the thresholds for lactation inhibition are in general higher than those necessary for mammary growth.

Androgens have been used clinically for the inhibition of lactation in women (*see*, for example, Kurzrok and O'Connell, 1938).

Adrenal Cortex

Early studies of the physiology of the adrenal cortex showed that the physiological disturbances resulting from adrenalectomy include failure to lactate normally (Carr, 1931a; Swingle and Pfiffner, 1932; Brownell, Lockwood and Hartman, 1933; Gaunt, 1933; Britton and Kline, 1936). Later studies on the rat have

shown that the lactational failure resulting from adrenalectomy, though always serious, is not complete (Fig. *20.* 17), though complete abolition may be approached in exceptional cases (Levenstein, 1937 ; Gaunt, 1941 ; Gaunt, Eversole and Kendall, 1942 ; Folley and Cowie, 1944 ; Cowie and Folley, 1947a). Effects of varying severity have been reported from different laboratories (compare Gaunt *et al.* 1942 with Folley and Cowie, 1944) but Cowie and Folley (1947a) have shown that they are not constant even in one rat colony. These workers observed variations in the intensity of lactation in their normal rats from time to time, and the degree of lactational inhibition due to adrenalectomy was inversely related to the lactational performance of simultaneously studied normal controls.

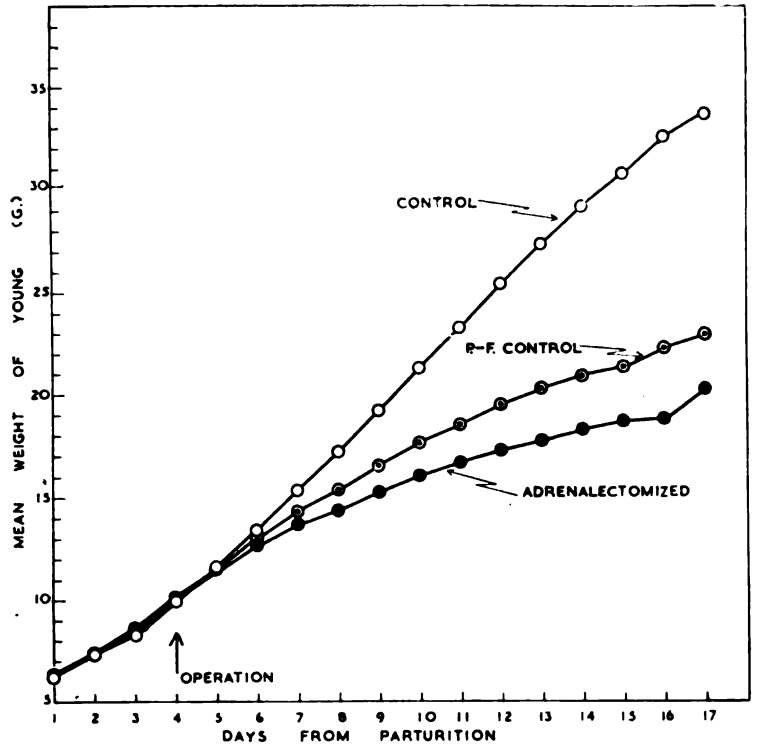


Fig. *20.* 17—Effect of adrenalectomy on lactation as shown by the mean growth curves of litters of groups of sham-operated rats (upper curve), adrenalectomised rats (lower curve) and sham-operated rats pair-fed with the adrenalectomised rats (middle curve).

The first reports on the ability of cortical extracts to support lactation in adrenalectomised animals were conflicting. Life-maintaining extracts tested by Carr (1931b) and Brownell *et al.* (1933) were without effect, but successful replacement therapy was reported by Swingle and Pfiffner (1932) and Britton and Kline (1936). Gaunt and Tobin (1936) did much to clarify the position when they showed that considerably more extract was necessary to support lactation than is required for maintenance of life. At this time the best-known function of the adrenal cortex was the regulation of fluid and electrolyte exchanges, and Gaunt and Tobin (1936) pointed out that the known facts regarding the relation of the adrenal cortex to lactation could be interpreted on this basis since lactation involves considerable loss of fluids and electrolytes from the body. In support of this theory Gaunt and Tobin (1936) also showed that administration of salt

improves lactation in adrenalectomised rats (*see also* Levenstein, 1937; Folley and Cowie, 1944) and that the effectiveness of cortical extract in supporting lactation was increased if salt were given as well. In subsequent studies Gaunt *et al.* (1942) have confirmed the ability of cortical extracts to support normal lactation in adrenalectomised rats, but Folley and Cowie (1944) could only obtain partial success (confirmed by Cowie and Folley, 1947b).

The recent separation and characterisation of a number of crystalline steroids from adrenal cortex extracts has stimulated further analysis of the rôle of the adrenals in lactation. Gaunt (1941) reported that deoxycorticosterone caused no improvement in lactation in adrenalectomised rats, but later Gaunt *et al.* (1942) found partial restoration of lactation in a majority of their treated animals. The results were irregular, however, and bore no relation to the dose administered. On the other hand, Cowie and Folley (1947a) have shown that in their rats deoxycorticosterone will afford considerable, though usually incomplete, protection against the effects of adrenalectomy on lactation (*see also* Folley and Cowie, 1944). They demonstrated a linear relation between log. dose and response and found that the latter varies directly with the severity of the lactational failure due to adrenalectomy. In an experiment in which the effects of adrenalectomy were particularly marked, 3·0 mg. deoxycorticosterone acetate daily gave complete restoration of lactation (Cowie and Folley, 1947a). In contrast to their results with deoxycorticosterone, Gaunt *et al.* (1942) obtained complete restoration of lactation with 17-hydroxy-11-dehydrocorticosterone and they thus conclude that while the restoration of normal electrolyte metabolism is helpful for maintenance of normal lactation, the limiting factor for maximal secretion is a sufficiency of those cortical hormones, the 11-oxygenated steroids, which are concerned in carbohydrate metabolism, particularly in gluconeogenesis. Incidentally, the 11-oxygenated cortical steroids appear also to be more important than deoxycorticosterone as regards the initiation of lactation in the hypophysectomised guinea-pig (Nelson *et al.* 1943). Folley and Cowie (1944) have, however, found 11-oxygenated cortical steroids inferior to deoxycorticosterone for maintenance of lactation in their adrenalectomised rats, even when the latter receive a diet containing 50 per cent. protein, which should provide conditions particularly favourable for the action of agents which promote gluconeogenesis (Cowie and Folley, 1947b).

The existence of a specific lactation hormone of the adrenal cortex, cortilactin, has been postulated by Brownell *et al.* (1933) (*see also* Hartman *et al.* 1933). They claim that life-maintaining cortical extracts will only support lactation in the presence of a fraction which is removed by chilling at −12° C. Recently, Spoor *et al.* (1941) reported that cortilactin is a pigeon crop-stimulating hormone which can be separated from cortical extracts by isoelectric precipitation at *p*H 5·5. They claim that this substance, which does not promote gluconeogenesis and which they consider to be different from prolactin, supports lactation in adrenalectomised rats maintained with cortical extracts. However, since Hurst *et al.* (1942) failed to detect crop-stimulating activity in cortical extracts, and in view of claims to have effected complete restoration of lactation in adrenalectomised rats by administration of crystalline steroids (Gaunt *et al.* 1942; Cowie and Folley, 1947a), further evidence will be necessary before the existence of a specific lactation hormone of the adrenal cortex can be taken as established.

It can hardly be said that much progress has yet been made with the elucidation of the mechanism of the lactational failure resulting from adrenalectomy. It seems that failure of the pituitary to secrete prolactin is not involved since Gaunt and Tobin (1936) found that prolactin did not improve lactation in adrenalectomised rats maintained with cortical extract and since Meites *et al.* (1942) observed a *post-partum* rise in pituitary prolactin in adrenalectomised rats.

The replacement studies discussed above suggest that the crucial factor in some circumstances may be an upset in carbohydrate metabolism, in others a disturbance of some function more particularly related to the action of deoxycorticosterone. Tissue enzyme studies by Folley and Greenbaum (1946) on adrenalectomised lactating rats receiving treatment with various adrenal steroids provide some indication that the adrenal cortex may affect lactation directly through the mammary gland arginase and indirectly through the liver arginase.

Thyroid

Evidence as to the effect of thyroidectomy on lactation is somewhat conflicting. As regards earlier work, observations (hardly critical, however, regarding the point at issue) which indicate that thyroidectomised animals lactate more or less normally have been made on the mouse (Davenport and Swingle, 1927), rabbit (Richon and Jeandelize, 1904), and dog (Dragstedt *et al.* 1924), while marked interference with lactation was noted in the rat (Hammett, 1922; Rickey, 1925) and goat (Zietzschmann, 1907; Grimmer, 1918; Trautmann, 1919; von Fellenberg and Grüter, 1932). Recent studies have scarcely clarified the situation. In the rat, Nelson and Tobin (1937) observed normal lactation after thyroidectomy (confirmed by Nelson, 1939b), while serious depression but not complete abolition of lactation was reported by Folley (1938), Folley, Scott Watson and Amoroso (1942) and Karnofsky (1942). Preheim (1940) reported lactational disturbances which were relatively slight. Temporary and relatively slight effects on milk yield following thyroidectomy in the goat were observed by Ralston, Cowsert, Ragsdale, Herman and Turner (1940). In the cow, Graham Jnr. (1934a) reported a decrease in milk production following thyroidectomy, but it is significant that the effects of a control operation were almost as great. On the other hand, the experiments of Spielman *et al.* (1944) indicate that thyroidectomy lowers the milk yield and shortens the lactation period of the cow.

It is not clear how far the post-thyroidectomy effects on milk yield, which many have observed in farm animals, were due to operative disturbances. Marked temporary effects can almost certainly be ascribed to this cause. This possibility was certainly considered by Grimmer (1918) and the importance of operative disturbances is also evident from the report of Graham Jnr. (1934a). Spielman *et al.* (1944), however, obtained evidence of sub-normal lactation in thyroidectomised cows long after operative disturbances must have subsided. There is the further possibility that negative results might be due to incomplete thyroid removal or to the presence of accessory thyroid tissue; the histological studies of Folley *et al.* (1942) and Karnofsky (1942) indicate that complete thyroid removal is very difficult, if not impossible, in the rat. A further complication is the concomitant removal of the parathyroid tissue which itself may adversely affect lactation. This is particularly important in the rat in which there are only two parathyroids,

each embedded in a thyroid lobe, and it may be noted that Folley *et al.* (1942) found that the lactational deficiences resulting from thyroidectomy in their rats could be partly alleviated by parathyroid extract.

On the whole, the available results indicate that lactation is possible in the absence of the thyroids but only at a somewhat reduced level. The thyroid hormone does not seem to be essential for the initiation of lactation since Houssay (1935b) induced milk secretion in thyroidectomised bitches by treatment with anterior-pituitary extract. Here there could be no question of foetal thyroids substituting for the maternal glands.

There is much more agreement about the galactopoietic effects of thyroid hormone administration. Many years ago it was claimed (Hertoghe, 1896) that the mild yield of the lactating cow could be increased by thyroid feeding. What was perhaps the first unequivocal demonstration of galactopoiesis resulting from thyroid feeding was given by Graham Jnr. (1934a) who observed considerable increases in milk production and even greater increases in milk fat production by cows fed dried thyroid gland. Injection of thyroxine gave similar results (Graham Jnr., 1934b). These results were extended by Folley and White (1936) who not only obtained striking confirmation of the increase in milk yield and fat percentage due to thyroxine injections but also showed that the non-fatty solids content of the milk increased during the treatment. Further extensive investigation of the galactopoietic effects in cows has left no doubt of their essential reproducibility under suitable conditions (Herman, Graham Jnr. and Turner, 1938 ; Hurst, Reece and Bartlett, 1940 ; Ralston *et al.* 1940 ; Smith and Dastur, 1940), while more recently, essentially similar results have been obtained in extensive studies involving the feeding of iodinated proteins (notably iodocasein) which exhibit biological activity characteristic of the thyroid hormone (Reineke and Turner, 1942a, b ; Blaxter, 1943, 1945a, b, 1946 ; Reece, 1944 ; Van Landingham, Henderson and Weakley Jnr., 1944). The galactopoietic effects of anterior-pituitary thyrotrophin preparations noted by Folley and Young (1938, 1939) are, on the face of it, in accord with the above results but it is not certain that the responses were mediated by the thyroid since the relative galactopoietic activities of the thyrotrophic extracts bore no relation to their relative thyrotrophic potencies (measured by a method which, however, may not give a satisfactory indication of ability to promote the secretion of thyroid hormone [*see* Heyl and Laqueur, 1935]) and since the characteristic increase in milk fat content was not observed.

The galactopoietic effect of thyroid treatment in cows is most noticeable during the declining phase of lactation, the effect in early lactation being relatively insignificant (Graham Jnr., 1934a ; Herman *et al.* 1938 ; Ralston *et al.* 1940). The response decreases absolutely, but increases relatively to the initial milk yield as lactation advances and the milk yield falls (Blaxter, 1945b). Thyroxine treatment does not prevent the normal decline in lactation but temporarily shifts the declining lactation curve upwards (Folley and White, 1936). The fat metabolism of the mammary gland is more sensitive to stimulation by thyroid hormone than the mechanisms for the synthesis of protein and carbohydrate since the increase in the milk non-fatty solids content is relatively small (but nevertheless unmistakable when investigated by proper methods [Folley and White, 1936 ; Blaxter, 1945a]) while the fat percentage may, under favourable conditions, increase to such an extent that the total daily fat production increases by as much as 50 per

cent. (Folley and White, 1936). Overdosage with thyroxine causes a decrease in milk yield (Graham Jnr., 1934a ; de Fremery, 1936) which may explain clinical reports of lactational inhibition following thyroid administration.

The mechanism of the galactopoietic effect of the thyroid hormone is still a matter of conjecture. Various possibilities may be considered. In the first place it is reasonable to suppose that an increase in the metabolic rate of the mammary alveolar cells would result in an increased rate of milk synthesis, so that the galactopoietic action of the thyroid hormone would represent a specialised aspect of its well-known function as a stimulant of body metabolism. This might well apply particularly to the effects on milk fat, the secretion of which, as we have seen, is particularly susceptible to stimulation by thyroid treatment, since there is some evidence (for citations *see* Graham Jnr., 1934a) that, in the cow, milk fat production increases in circumstances in which an increase in B.M.R. might be expected and vice versa. Another possibility is that thyroid treatment may increase the supply of milk precursors to the mammary gland. This could be brought about by an increase in the rate of blood flow through the gland and also by virtue of an elevation of the level of such precursors in the blood. A considerable acceleration of heart rate accompanying the galactopoietic response to thyroid treatment was observed by Folley and White (1936) and most subsequent workers, which would imply an increase in the mammary circulation rate, and it is noteworthy that Fuller (1928) observed a positive correlation between pulse rate and milk yield in the cow. If the supply of milk precursors is a limiting factor in the declining phase of lactation it seems possible that an increased supply resulting from one or both of the above-mentioned causes might enhance the rate of milk secretion. Equally, if an increase in the metabolic activities of the milk-producing tissue led to an increase in the rate of milk synthesis an increased supply of milk precursors would be needed. Finally, there is the probability that galactopoiesis might result from interaction of the thyroid hormone with the endocrine system, probably by way of the anterior pituitary. For example, it is conceivable that a moderate degree of hyper-metabolism resulting from thyroid treatment might be accompanied by increased secretion of the galactopoietic complex by the anterior pituitary. What is virtually certain is that the galactopoietic effect under discussion is the result of a very complex readjustment of intermediary metabolic processes in which alterations in the activity of more than one endocrine gland are involved.

Parathyroids

Lactation involves a heavy drain of calcium and phosphorus from the body and it is to be expected that the integrity of the parathyroids would be essential for normal lactation. However, the relation of the parathyroids to lactation has been very little studied.

Such investigations as those of Dragstedt *et al.* (1924) and Kozelka, Hart and Bohstedt (1933) showed that thyroparathyroidectomised bitches would rear puppies if tetany were prevented by suitable methods (administration of calcium lactate or vitamin D), but since the situation was complicated by loss of the thyroids the results tell us little about the relationship between the parathyroids and lactation except, perhaps, that some milk can be secreted in the absence of the parathyroid hormone.

A little more has recently been learnt from the rat. The experiments of Folley *et al.* (1942) strongly suggested that the impairment of lactation observed by them and other workers in thyroparathyroidectomised rats might be due to loss of the parathyroids, since there was an indication that the lactational failure was less severe in the minority of rats possessing accessory parathyroid remnants, but more particularly because lactation could be partially restored by parathyroid hormone. These findings added significance to the earlier observations of Chandler (1932) that the young of parathyroidectomised rats were often abnormally small. Direct and conclusive evidence of the effect on lactation of parathyroid removal has recently been obtained by Cowie and Folley (1945) whose quantitative studies show that lactation is severely impaired but not completely abolished by parathyroidectomy. This would suggest that those workers who found lactation to be unaffected by thyroparathyroidectomy may have used a strain of rat in which accessory parathyroids were the rule rather than the exception.

Such evidence as is available then suggests that the parathyroid hormone has no direct action on the mammary gland but is concerned with lactation only indirectly and in so far as optimal secretion requires the maintenance of a normal calcium and phosphorus metabolism. In this connection it is a curious fact that Dragstedt's (1927) contention that lactation is a particularly effective stimulus for the conversion of a latent tetany into an acute condition does not seem to apply to the rat. Tetany is rarely observed during lactation in thyroparathyroidectomised or parathyroidectomised rats (Nelson and Tobin, 1937 ; Folley *et al.*, 1942 ; Cowie and Folley, 1945) except when parathyroid hormone has been given for a time and then withheld (Folley *et al.* 1942). The parathyroidectomised bitch seemingly reacts to the crisis of lactation by going into tetany, the rat by refusal to lactate.

The Endocrine Pancreas

Present knowledge indicates that insulin may influence the mammary gland in two ways ; indirectly by virtue of its effect on general intermediary metabolism, by which the supply of milk precursors may be governed, and directly through its rôle in the carbohydrate metabolism of the mammary gland itself. Thus large doses of insulin decrease the milk yield of the cow (Gowen and Tobey, 1931b ; Brown, Petersen and Gortner, 1936a), presumably mainly because the resulting hypoglycaemia is incompatible with the satisfaction of the considerable glucose requirements of the lactating udder. Evidence (*see* page 612) indicating a ***direct*** participation of insulin in the synthesis of lactose in the mammary gland has recently been reported by Knodt and Petersen (1946b).

Observations incidental to studies of insulin requirements of depancreatised bitches during pregnancy and lactation indicate that lactation may be initiated and puppies in many cases reared, provided sufficient insulin is given (Markowitz and Simpson, 1925 ; Cuthbert, Ivy, Isaacs and Gray, 1936 ; but see contrary observations by Markowitz and Soskin, 1927). Two investigations on the hormonal initiation of lactation in depancreatised bitches receiving insulin have given contradictory results. Chaikoff and Lyons (1933) failed in five cases out of six to initiate lactation with larger doses of prolactin than were used successfully in intact bitches. Rejecting the remote possibility that the pancreas might secrete a hormone, other than insulin, directly affecting the mammary glands,

these authors suggested that their negative results might be due to metabolic disturbances occurring despite the administration of insulin. But it seems possible that these failures might have been due to lack of mammary development since Nelson, Himwich and Fazekas (1936) were regularly able to initiate lactation with prolactin in depancreatised bitches receiving insulin provided that they were pseudo-pregnant and thus possessed well-developed mammary glands. Bitches in other phases of the oestrous cycle possessed poorly-developed mammary glands and failed to lactate in response to prolactin. Folley and Greenbaum (unpublished work) have initiated lactation by oestrogen treatment in a virgin goat rendered diabetic with alloxan and maintained with insulin.

Posterior Pituitary

Ott and Scott (1911) were the first to show that injection of posterior-pituitary extracts produces a rapid but evanescent action on the lactating mammary gland. They found that a single intravenous injection into a lactating goat with a cannulated teat, gave an almost immediate increase in the flow of milk through the cannula. The effect was transitory, however, lasting in their experiments for a few seconds only, and a repeat dose gave a diminished effect. This discovery was quickly confirmed in the cat by Schäfer and Mackenzie (1911) and Mackenzie (1911). Subsequent experiments on the goat (Hammond, 1913; Hill and Simpson, 1914; Maxwell and Rothera, 1915; Gaines, 1915) and cow (Turner and Slaughter, 1930) showed that posterior-pituitary extract injected into an animal which had been milked out would enable an additional quantity of milk to be obtained, or if injected into an animal just prior to milking would increase the yield at that milking above the expected amount. Again, a feature of the results was the evanescent nature of the responses and there was some indication (Hammond, 1913; Hill and Simpson, 1914; Turner and Slaughter, 1930) that extra milk obtained in response to posterior-pituitary extract at or following a milking was compensated for by a decreased yield at the next milking. There was little agreement as to whether or not the compensation was exact but in any event experiments on cows by Gavin (1913) indicated that repeated injections of posterior-lobe extract gave no increase in yield over a long period, a conclusion supported by modern experiments (Shaw, 1942a).

The effect of posterior-pituitary extract on the lactating mammary gland was at first regarded as a true galactopoietic effect, i.e. it was tacitly assumed that the extract temporarily stimulated the secretory activities of the alveolar cells, but since such extracts were known to cause contraction of smooth muscle tissue the alternative possibility that the effect merely represented the squeezing of otherwise unavailable preformed milk from the alveoli and finest ducts by virtue of the contraction of muscle tissue present in the mammary gland, soon came under consideration. At this time the question as to whether muscle fibres existed in close association with the mammary alveolar elements was under dispute (*see* Turner, 1939a, for discussion), but work by Swanson and Turner (1941) would appear to indicate that myo-epithelial cells, which might be capable of contraction under the influence of posterior-lobe hormones, are indeed present. The second of these alternative theories of the mode of action of posterior-pituitary extracts on the mammary gland was considered by Simpson and Hill (1915), but rejected because they were unable to simulate the effects of posterior-pituitary

extract by injection of $BaCl_2$, a muscle stimulant. Others to consider this hypothesis only to reject it for one reason or another were Maxwell and Rothera (1915) and Hammond (1913). The first clearly to enunciate the currently accepted view that posterior-lobe extract has no direct effect on the secretory activities of the alveolar cells but causes discharge of milk from the gland by virtue of its ability to evoke contraction of contractile tissue surrounding the alveoli, appears to have been Heaney (1913) who, by means of a special apparatus, demonstrated a decrease in breast volume in women following the injection of posterior-lobe extract. Schäfer (1915), who gave reasons for discounting the $BaCl_2$ experiment of Simpson and Hill (1915) also, apparently independently, advanced the same theory, mainly because a second injection of posterior-lobe extract soon after the emptying of the gland due to a previous treatment did not produce any more milk. Others to adopt the same view were Gaines (1915) and Turner and Slaughter (1930). Most of the well-established facts already discussed and others, such as the rise in the internal udder pressure following an injection of posterior-lobe extract (Gaines, 1915 ; Maxwell and Rothera, 1915), point to such an interpretation, and there is little doubt that it is correct. In keeping with this view are the experiments of Smith (1932) in the rat and Houssay (1935a) in the dog which indicate that milk secretion is possible in the absence of the posterior hypophysis.

Despite the fact that the mode of action of posterior-lobe extracts on the mammary gland has been understood in its essentials for thirty years, the theory that the posterior lobe may be concerned in the normal discharge of milk from the mammary gland during nursing or milking has only recently been enunciated, though Gaines (1915) in a remarkable paper came within an ace of doing so. Gaines demonstrated a striking parallelism between the effect on the mammary gland of treatment with posterior-pituitary extract and of suckling, in that both, after a short latent period, produced a rise in udder pressure associated with the possibility of sucklings obtaining milk ; i.e. both induced what is called the " let-down " of milk. He showed that suckling failed to stimulate the " let-down " in anaesthetised animals but that following an injection of posterior-lobe extract the " let-down " occurred and sucklings were able to obtain milk. But though he produced strong evidence that posterior-pituitary hormones may be concerned in the " let-down " of milk under normal conditions, Gaines omitted specifically to postulate this.

Ely and Petersen (1941) in experiments on cows showed that fright or injection of adrenalin just before milking resulted in a reduced yield due to failure of the subjects to " let-down " their milk. The " let-down " could, however, be subsequently induced by injection of posterior-lobe extract and the udder completely emptied. Preparations of the oxytocic hormone seemed to be more effective than extracts rich in pressor hormone. On the basis of these results Ely and Petersen advanced the theory that the ejection of milk from the mammary gland may be governed by a balance between circulating adrenalin and oxytocin, the secretion of the latter at milking time being due to a nervous reflex normally actuated by tactile stimulation of sensory nerve endings in the teat, but capable of being conditioned to respond to such stimuli as washing the udder or other events regularly associated with milking time. Further evidence that the normal " let-down " of milk is due to the secretion of oxytocin into the blood was provided by Petersen and Ludwick (1942) who, in udder perfusion experiments, showed

that addition of blood from a cow which had been stimulated in the normal way to " let-down " its milk caused an immediate flow of milk from the perfused udder. Blood from cows not so stimulated had no effect. In other perfusion experiments Petersen (1942) tested a number of drugs and found that acetylcholine in addition to oxytocin caused ejection of milk. Turner and Cooper (1941) have attempted to devise a method for assaying posterior-pituitary extracts for their power of causing contraction in the tissues of the mammary gland of the rabbit. Among three types of extract the relationship between " mammary gland contracting " and oxytocic potencies was not very close and the authors suggested the possibility that the mammary gland effects might be due to the action of a third factor present in both oxytocic and pressor preparations.

What part the particular function of the posterior pituitary under discussion may play indirectly in the normal secretory activities of the mammary gland is at present an intriguing question. Miller and Petersen (1941) found that the " let-down " in cows was incomplete if there was undue delay between the application of the stimulus for the " let-down " and the beginning of milking, possibly because of dissipation of the circulating oxytocin. It thus seems probable that repeated failure to remove all the available milk, either because of faulty technique on the part of the milker or innate deficiency at some point of the neuro-hormonal discharge mechanism, might lead to an accelerated involution of the mammary gland and a shortened period of lactation. If this be the explanation of some cases of lack of persistence of lactation in cows and perhaps partial failure of lactation in women it is still not certain whether the neural or hormonal component of the mechanism is the more likely to be at fault. It should be noted, however, that Knodt and Petersen (1944) were able to decrease for a time the rate of decline of lactation in cows showing unexpected lack of persistence by regularly emptying the udder after each milking by means of oxytocin. Other experiments indicating the possible indirect importance of the posterior pituitary in the maintenance of normal lactation are those of Gomez (1939, 1940) who found that hypophysectomised lactating rats receiving replacement therapy were able to maintain their litters longer if the treatment was supplemented with regular posterior-pituitary injections.

The Initiation of Lactation

Though secretory changes in the mammary parenchyma may begin about mid-way through pregnancy, copious lactation as ordinarily understood does not normally set in until shortly after parturition. The mechanism responsible for the onset of lactation and its timing has long been the subject of speculation.

Prior to the discovery of the lactogenic function of the anterior pituitary it was customary to regard the initiation of copious lactation as an event of passive nature which followed the removal at parturition of stimuli causing the gestational growth of the mammary glands or, more particularly, of some inhibitory influence only operative during pregnancy. The idea that the onset of lactation was due to the abolition of an inhibitory influence appears to have been first formulated by Hildebrandt (1904) who suggested that the products of conception exert an influence whereby the mammary gland cells are protected from autolytic disintegrative processes supposed to occur during active secretion. Halban (1905)

held that lactation develops after the removal of an inhibitor of placental origin, a theory which in its essentials is not in conflict with some more modern views.

Later, the corpus luteum was suggested as the source of an inhibitor and the work of Hammond (1917) and Drummond-Robinson and Asdell (1926) appeared to support this idea. The latter workers showed that, in the pregnant goat, ablation of the corpora lutea sufficiently late in pregnancy for the mammary alveolar system to have developed, resulted in lactation. Anselmino and Hoffmann (1936) also suggested, on hardly adequate grounds as we have seen (page 576), that the corpus luteum is responsible for the inhibition of lactation during pregnancy but were forced to conclude that progesterone could not be the responsible agent.

A new possibility arose when evidence was presented that oestrogens possess the ability to inhibit lactation (*see* page 576) and attention naturally became focused on oestrogen as the agent responsible for the suppression of lactation during pregnancy, particularly since studies of the excretion of oestrogen in the urine of pregnancy indicated a high production of oestrogen during the later stages of this condition. The discovery of the positive lactogenic stimulus emanating from the anterior pituitary, however, introduced a new factor into the situation of which it was necessary to take account in any theory seeking to explain the mechanism of the initiation of lactation.

The first theory in which an acceptable rôle was assigned to the hypophysis was that of Nelson who in 1936 gave a good account of a series of his earlier papers covering the development of his hypothesis (Nelson, 1936). Moore and Price (1932) had attempted to solve what is in many ways an analogous problem, namely, the mechanism controlling the oestrous cycle, by postulating a reciprocal relationship between hypophysis and ovary. This concept was utilised by Nelson, according to whose theory oestrogens, probably of placental origin, suppress lactation during pregnancy (*a*) by inhibiting the secretion of prolactin by the hypophysis and (*b*) by an inhibitory action exerted directly on the mammary gland. The removal of this inhibition consequent upon the decrease in the circulating oestrogen at parturition, for which there is ample evidence, permits the release of prolactin by the anterior hypophysis and thus the initiation of lactation. Nelson's (1936) review and his earlier papers cited therein should be consulted for the details of the considerable amount of experimental evidence he amassed in support of this theory and of concordant facts established by other workers. It only remains to say that the theory was successful in harmonising most of the facts known at that time.

It is clear that the main tenet by which Nelson's theory must stand or fall is the postulate that the production of prolactin by the anterior pituitary is suppressed by oestrogen. This contention has recently been called in question by Meites and Turner (1942c) mainly because Turner and his collaborators have regularly found (*see* page 571) that oestrogen, even in huge doses, far from decreasing, actually increases the prolactin potency of the pituitary. They also cast doubt on the reliability of much of the evidence suggesting that oestrogens inhibit lactation (*see* discussion on page 577) and in this connection cite certain species in which pregnancy and lactation commonly proceed together. In regard to this last point, it is well known that in cows which are both pregnant and lactating, there is a rapid decline in milk yield after the fifth month of pregnancy which

Gaines and Davidson (1926) attributed to the effect of a then unknown inhibiting hormone.

In a series of four papers Meites and Turner (1942c, d, e, f) advance a new theory of the initiation of lactation according to which oestrogen stimulates the secretion of prolactin by the anterior lobe but is prevented from doing so during pregnancy by the simultaneous presence of progesterone, which they find (Meites and Turner, 1942d) will antagonise the evocation by oestrogen of an increase in the prolactin potency of the pituitary if the progesterone/oestrone ratio is high enough. The theory holds that during pregnancy the prolactin output of the pituitary is thus insufficient to induce lactation, but at parturition the overriding luteal influence is removed leaving the circulating oestrogen free to stimulate prolactin secretion. It will be seen that this is essentially a modern version of the old theory which held that lactation is suppressed by the corpus luteum of pregnancy, and it may be noted that Selye (1940b) has also recently suggested that the initiation of lactation at parturition is a progesterone-withdrawal phenomenon. He observed not only mammary growth but also initiation of secretion in female rats receiving oestradiol, but in rats receiving progesterone as well, the mammae were better developed but secretory changes were entirely absent.

The interesting and ingenious theory of Meites and Turner is open to certain serious objections which hamper its unqualified acceptance. First, the theory clearly assigns the key rôle in the initiation of lactation to prolactin and overlooks the possibility that other anterior-pituitary hormones may be involved to an equal extent. It has been shown above (*see* page 572) that the status of prolactin as the sole, specific lactogenic hormone is, to say the least, questionable, and Turner himself in the face of his observation that the pituitary prolactin potency of the pregnant yet non-lactating mouse is higher than that of the virgin animal (Hurst and Turner, 1942) has admitted that other hormones must be involved in the initiation of lactation. Second, the validity of the theory depends on the reality of changes observed under various conditions in the prolactin potency of the pituitary in small animals and on the further assumption that these changes, if real, reflect actual alterations in prolactin secretion and are not merely storage effects. As regards the first point, Hall (1944b) has questioned whether the assay method used by Turner and his collaborators, involving the intradermal injection over the pigeon crop-gland of suspensions of pituitary tissue, is capable of giving even true relative values as between one gland and another, while the available evidence suggesting that the observed increases in pituitary prolactin content are accompanied by an increased secretion of prolactin into the blood (Meites and Turner, 1942a) is hardly convincing and needs further confirmation. Third, there is no evidence that at parturition the decrease in circulating progesterone precedes the decrease in oestrogen as the theory demands. Fourth, Meites and Turner (1942d) found that gravimetric progesterone/oestrone ratios of the order of 1000 : 1 were necessary for the former to suppress the effects of the latter on the prolactin potency of the pituitary. We have no idea what ratios actually obtain during pregnancy, but judging from the experimentally determined ratios for optimal mammary alveolar growth (*see* page 546), which is a typical pregnancy phenomenon, they may well be considerably lower than this—perhaps of the order of 40 : 1. Fifth, the theory takes no account of the possible rôle of the placenta

in the initiation of the secretory phase in late pregnancy, for which a certain amount of evidence exists (*see* page 552).

If the relevancy of apparent changes in the pituitary prolactin potency is regarded as needing further proof, the main facts regarding the initiation of copious lactation at parturition could be equally well explained by a modification of the original theory of Nelson incorporating the concept (Folley, 1941) that low levels of circulating oestrogen stimulate the secretion by the anterior pituitary of hormones concerned in lactogenesis and galactopoiesis, while high levels, on the other hand, inhibit such secretion. At about the time of parturition, the level of circulating oestrogen, previously above the threshold value for pituitary inhibition, will fall, and in passing through the range between the two thresholds will cause functional activation of the anterior pituitary with respect to the production of lactogenic hormones, thus initiating full lactation.

Yet another theory, at present in disfavour, pointed to the intervention of a uterine factor in the chain of events leading to normal lactation. Selye *et al.* (1934) showed that surgical removal of uterine contents from pregnant rats resulted in lactation but not if the uterus were immediately distended with paraffin. These authors urged that mechanical distension of the uterus must be considered as a factor causing suppression of lactation during gestation and pointed out that the distension of the uterine walls decreases just before parturition despite the continued growth of the foetuses. Neither Bradbury (1941) nor Greene (1941) was, however, able to confirm these observations. The results of Freud and Wijsenbeek (1938) also seem to exclude uterine distension as a factor inhibiting lactation during pregnancy. They transferred rat foetuses to the abdomen but noted that no lactation occurred until the foetuses were artificially delivered.

This discussion of the mechanism of the initiation of *post-partum* lactation may be rounded off by a brief consideration of the novel views of Petersen (1944) who emphasises the fact that the secretory phase begins well before parturition. He believes that the copious flow of milk following parturition is due to the beginning of the ejection of the alveolar contents under the influence of oxytocin secreted during labour. The milk flow thus initiated is believed to be maintained by the subsequent stimuli of nursing or milking which cause an outpouring of prolactin by the anterior pituitary (*see* page 593).

IV. Neural Factors in Lactation

There is no evidence of the existence of secretory nerves directly controlling the permeability or synthetic activities of the alveolar epithelium ; conclusive evidence that mammary secretion is under humoral rather than neural control comes from experiments such as those of Ribbert (1898) and Stricker (1929), who showed that lactation can occur in transplanted mammae. But it should not be thought that the normal function of the mammary gland is entirely independent of the nervous system ; the contrary is the case as we shall now see.

Sympathectomy and lactation.—Recently there has been a revival of interest in the possible rôle of the sympathetic nervous system in lactation. Basch (1906), who incidentally should be consulted for references to early papers on the innervation of the mammary gland, had found that extirpation of the coeliac sympathetic

ganglion had little effect on milk secretion except perhaps when performed soon after parturition. A more modern paper by Cannon and Bright (1931) reported a delayed impairment of lactation following sympathectomy in one cat and one bitch (the latter in two successive lactations). Actually these results tell us very little, since so few animals were involved and the lactational observations were hardly critical. Bacq (1932) reported that in the rat, removal of both abdominal and one of the thoracic sympathetic chains occasionally interfered with lactation; but this happened in only two cases and critical examination of his data leads to the conclusion that his results may well have been coincidental. The more recent results of Simeone and Ross (1938) were no more decisive. Gross impairment of lactation in the cat following various sympathectomy operations was the exception rather than the rule, and in only one animal of seven could involutionary changes in the mammary gland be demonstrated histologically after section of its sympathetic nerve supply. It would thus appear that there are no grounds whatever for concluding that sympathectomy interferes with lactation, since in none of the above-mentioned experiments were the experimental conditions such as to allow of any lactational phenomena being ascribed with certainty to the effects of the operation. This accords with the findings of Ely and Petersen (1941) who, in the cow, sectioned the centrifugal sympathetic fibres to one half of the udder and found that during the subsequent lactation the capacity of the denervated glands to secrete and eject milk was unimpaired.

The suckling stimulus and milk secretion.—We now proceed to consider the possibility that neural influences may be indirectly concerned in the control of lactation in so far as the secretion of hormones involved in the maintenance of milk secretion may be affected by nervous stimuli. Selye (1934) has shown that in lactating rats which are continuously suckled, but in which the main galactophores have been ligated, thus preventing the escape of milk, the characteristically rapid mammary involution and cessation of secretion, such as results when the young are removed from intact rats, does not occur. He also found that if some nipples were suckled, whether the corresponding galactophores had been ligated or not, secretion was maintained for some considerable time in the remaining glands where suckling had been prevented by excision of the nipples. Selye and his collaborators (for full discussion see Selye *et al.*, 1934) interpreted their results as indicating that the suckling stimulus excites a nervous reflex which elicits the secretion of prolactin by the anterior pituitary, thus maintaining the secretory activities of the mammary gland, and they suggest that the mammary involution which follows weaning of the young is due to removal of the suckling stimulus rather than to accumulation of milk in the gland. The effects of non-removal of milk had previously been emphasised by the work of Kuramitsu and Loeb (1921) in the guinea-pig and Hammond (Hammond and Marshall, 1925) in the rabbit, who observed rapid involution in glands, suckling of which had been prevented by covering the nipples with collodion, even though other glands in the same animal were suckled. Selye and McKeown (1934a) later pointed out that the effects of the accumulation of milk in ligated glands eventually cause mammary involution despite the continuation of suckling. It thus seems probable that in actual fact both causes contribute to the mammary regression which follows weaning of the young in experimental or domestic animals or the gradual self-weaning of the young under natural conditions. An additional and as yet unknown cause must be postulated

to account for the gradual decline in milk yield which is observed in farm animals despite the regular application of the milking stimulus and subsequent withdrawal of milk. Similarly, Selye and McKeown (1934a) found that in mice, though lactation may be prolonged for upwards of two months by the continual provision of actively suckling litters, mammary involution does eventually occur though histologically it is of an abnormal type.

Williams (1941) has confirmed in mice the effect of the nursing stimulus in retarding mammary involution, but his results appear to differ somewhat from those of Selye (1934) in that he found less diminution of secretory activity in suckled glands from which escape of milk was prevented than in unsuckled glands from the same animal. The effects of the suckling stimulus could be reproduced in essentials by irritation of the nipples by the application of turpentine (Hooker and Williams, 1940). Here again, when some only of the nipples were treated the effects were manifest to varying degrees in all mammary glands.

Certain other results are in harmony with the theory that suckling reflexly causes discharge of prolactin from the anterior pituitary. Administration of prolactin retards mammary involution and to some extent maintains secretion in mice from which the litters have been removed (Hooker and Williams, 1941 ; Williams, 1945). Further, on the assumption that changes in the pituitary prolactin potency in small animals, as at present determined, reflect changes in prolactin secretion, the observations of Meites and Turner (1942c, f) who found greater pituitary prolactin potencies in rats and rabbits suckling litters than in animals prevented from suckling, could also be cited in support of the theory. Also significant in this regard may be the finding of Selye and McKeown (1934b) that application of the suckling stimulus to adult, cyclic rats and mice caused mammary development, the oestrous cycles being interrupted by long dioestrous periods. This condition, which since it showed many features in common with pseudo-pregnancy resulting from sterile copulation was called " suckling pseudo-pregnancy," and which could only be induced in the presence of the ovary (Selye and McKeown, 1934a), is explicable on the basis of the luteotrophic action of prolactin (*see* page 567) secreted in response to the nursing stimulus.

Since there is considerable evidence that a complex of anterior-pituitary hormones, rather than prolactin alone, is concerned with the maintenance of lactation, it seems probable that if lactation is normally maintained by a reflex neuro-hormonal mechanism of the kind discussed, the reflex must call forth the discharge of other anterior-lobe hormones in addition to prolactin.

The discharge of milk from the mammary gland.—The involvement of nervous mechanisms in the discharge of milk from the mammary gland has been mentioned briefly in connection with the relation of the posterior pituitary to lactation (*see* page 587, *et seq.*). The phenomenon of the so-called " let-down " of milk has naturally been studied mostly in connection with the cow, but it is likely that similar considerations are generally applicable. The salient facts are briefly as follows. By cannulation of the teat, and in the absence of tactile stimulation of this or other teats in the same animal, only the milk present in the gland cistern and larger ducts can be drawn off ; the full yield of milk consisting, in addition, of much of the milk present in the alveoli and finer ducts can only be obtained when the teat is subjected to tactile stimulation, i.e. when the milking or nursing stimulus is

applied. Manometer readings of the milk pressure show a steady rise between one milking and the next (Tgetgel, 1926), but shortly after the beginning of milking there is a sudden further rise in pressure (Gaines, 1915 ; Maxwell and Rothera, 1915 ; Tgetgel, 1926) with which is associated the possibility of obtaining the full yield of milk. This phenomenon, which is known as the " let-down," may be identified with the active forcing of milk under pressure from the lumina of the alveoli and finer ducts against the forces of capillary attraction which would otherwise prevent its egress.

Largely because of failure to distinguish clearly between two distinct processes, the secretion of milk (which itself may be said to consist of two phases, the synthesis of milk and its passage from the cells into the alveolar lumen) and its discharge from the gland, it was once widely held that the rise in milk pressure in response to the milking stimulus was due to reflex secretion, during the actual milking process, of much of the milk then obtained. Hammond (1936) has collected much evidence, existing in the literature, which effectively disposes of this fallacy, the inherent improbability of which renders consideration of the refuting evidence unnecessary here. Those interested are referred to Hammond's article. Hammond (1936) further, after considering many lines of evidence for which his paper should be consulted, put forward the view that the " let-down " of milk is an active process due to a nervous reflex excited by stimulation of the teat. He envisaged a purely nervous arc which brought about the contraction of smooth muscle fibres running in conjunction with the venous system of the udder, thereby occluding the venous vessels and engorging the udder tissues with blood. This in turn was supposed to force the milk under pressure from the alveoli and fine ducts. It seems surprising that Hammond should have apparently overlooked the significance of the important paper by Gaines (1915) which described many interesting experiments on the mechanism of the discharge of milk, the most striking of which was the demonstration that though anaesthesia inhibited the " let-down " in a bitch, the puppies could obtain milk after the injection of posterior-pituitary extract. This work, together with the experiments of Heaney (1913) and Schäfer (1915) among others (pages 587 *et seq.*), indicated a mechanism for causing contraction of the mammary tissues for which, as suggested by Folley (1940), there was evidence ready to hand, thus obviating the need for postulating the existence of a complex mechanism involving the induction of a state of " erection " in the udder tissues for which the evidence can only be described as circumstantial and tenuous. It remained for Ely and Petersen (1941) to formulate what seems at the present time to be the most acceptable theory of the mechanism of milk discharge, a theory postulating a neuro-hormonal arc the centripetal portion of which is nervous and the centrifugal portion mainly hormonal. Stimulation of the teat (or, in the case of cows, other conditioned stimuli habitually associated with preparation for milking) is supposed to cause reflex secretion of oxytocin by the posterior pituitary which in turn is directly responsible for the " let-down."

The remarks of Hammond (1936) regarding the inhibition of his postulated reflex by impulses from the brain equally well apply to the neuro-hormonal mechanism under discussion. It is well known that the " let-down " in cows can be inhibited by factors such as fright, the approach of an unfamiliar milker, etc. Ely and Petersen (1941) believe that such stimuli may cause the reflex secretion of adrenalin which, they found, interferes with the " let-down."

There is thus evidence for the existence in connection with lactation of two neuro-hormonal mechanisms both actuated by stimulation of the teat. That responsible for the discharge of milk presents no difficulty from the anatomical point of view since the innervation of the posterior lobe with fibres passing from the hypothalamus through the pituitary stalk is well-established. The intervention of nervous impulses in the discharge of hormones by the anterior lobe is more problematical, but in this connection should be recalled the secretion of gonadotrophins responsible for ovulation in the rabbit in response to nervous stimuli arising during coitus.

It is clear that failure of the mechanism governing milk discharge will amount to failure of lactation even though secretory hormones are being secreted in adequate amounts by the anterior lobe. For this reason it is possible that the apparent failure of lactation observed by Herold (1939) and Desclin (1940) in rats after section of the hypophyseal stalk (incidentally not confirmed by Dempsey and Uotila, 1940) may have been due to failure of the discharge mechanism rather than to obliteration of the mechanism postulated by Selye *et al.* (1934), though against this interpretation may be cited the observations of Smith (1932) and Houssay (1935a) who found that lactation was possible after removal of the posterior lobe. Equally, this explanation might apply to the interesting experiments of Ingelbrecht (1935) who found that if in rats the spinal cord was resected at a level appropriate for the sensory denervation of the six posterior mammae, the young died, in spite of vigorous suckling, when the six anterior nipples were shielded. If, however, suckling was permitted at two of the thoracic glands milk could be obtained from the abdominal and inguinal glands also. It would appear from these experiments that the centripetal portion of the particular mechanism concerned, whichever of the two it may be, involves a spinal pathway.

V. THE LACTATION CURVE

Variations in milk yield.—Variations in the rate of milk secretion throughout lactation have naturally been most closely studied in farm animals ; it is probable that similar phenomena could be observed in other mammalian species. In accordance with histological studies in various species indicating that mammary secretion begins before parturition, appreciable amounts of secretion can often be withdrawn from the udders of heifers during pregnancy and the yield appears to increase with regular milking (Woodman and Hammond, 1923 ; Asdell, 1925). Indeed, small amounts of a " serous " secretion which Woodman and Hammond (1922) have shown to be relatively rich in globulin and albumin (particularly the former) and to contain small quantities of casein, lactose and fat can often be obtained from the udders of virgin heifers. In this connection it may be noted that the occurrence of lactation in the virgin goat is not at all uncommon judging from the cases recorded by Asdell (1925) and from the author's own experience (*see* Folley *et al.* 1941b).

Woodman and Hammond (1923) and Asdell (1925) studied the composition of the *ante-partum* secretions obtained from the udders of heifers pregnant for the first time. In early pregnancy the secretion, which is usually only obtainable in small amounts, is a " serous " liquid similar in composition to that obtainable from the udders of maiden heifers. At about the twentieth week of pregnancy it becomes

a viscous honey-like secretion containing large amounts of globulin. If this secretion is withdrawn at intervals from the udder the amount increases towards the end of pregnancy, particularly in the last week, and approaches normal milk in composition (Asdell, 1925). The composition of *ante-partum* mammary secretions in the human has been studied by Widdows, Lowenfeld, Bond, Shiskin and Taylor (1935).

Woodman and Hammond and also Asdell concluded that colostrum is a mixture of the honey-like secretion with normal milk. The characteristic colostral composition (*see* below) of the mammary secretion normally obtained just after parturition appears to be due to the fact that the *ante-partum* mammary

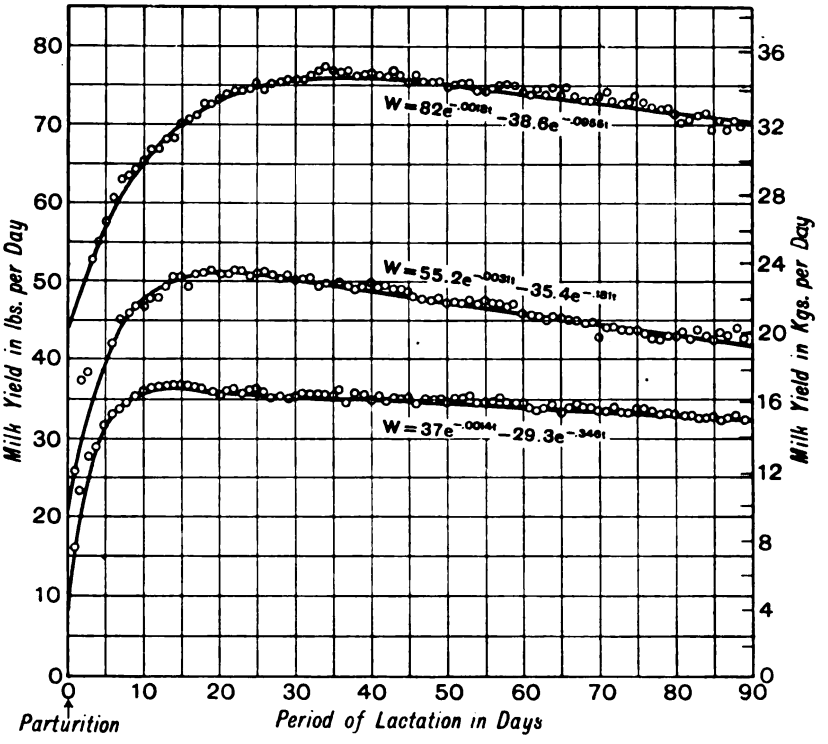


Fig. 20. 18—Mean lactation curves for groups of Holstein-Friesian cows. (After Brody, Turner and Ragsdale, 1924.)

secretions are not withdrawn from the gland. When, as in the experiments quoted above, secretion is regularly withdrawn from the mammary gland during pregnancy the fluid obtained at parturition is, to all intents and purposes, normal milk (Asdell, 1925). Petersen (1944) states that colostrum can be produced during lactation by suspending milking, the retained fluid tending to come into equilibrium with the blood (*see also* Petersen and Rigor, 1932).

Parturition is immediately followed by a striking increase in mammary secretion, the rate of which (and hence the daily milk yield) rises to a maximum, usually at two to four weeks *post-partum*. Thereafter a slow decline in milk yield sets in, which under constant conditions continues uninterruptedly over the remainder of the lactation period. Brody, Turner and Ragsdale (1924) studying the milk yields of groups of cows over the whole lactation period showed that the normal lactation curve can be fitted by the equation $M = Ae^{-k_1 t} - Be^{-k_2 t}$, where M is the milk yield at time t and A, B, k_1 and k_2 are constants (Fig. 20. 18).

Pregnancy has the effect of accelerating the normal decline in lactation in the cow, the effect according to Hammond and Sanders (1923) becoming appreciable at about the twentieth week from conception. The lactation yield of a pregnant cow is therefore less than it would be under similar conditions if she were allowed to remain barren. It seems more likely that the depressing effect of pregnancy on lactation is due to inhibitory hormones, as suggested by Gaines and Davidson (1926), than to the competition of the rapidly growing foetus for the nutrients of the maternal blood as Brody, Ragsdale and Turner (1923) were inclined to believe.

The lactation milk yield in the cow varies with age. Bartlett (1934b), analysing the results obtained over a large number of lactations, showed that the lactation yield on the average increases with each lactation to the seventh and thereafter declines rather sharply.

Variations in milk solids.—Bartlett (1929) has derived the normal lactation curve for milk fat percentage using data relating to large numbers of Dairy Shorthorn and Guernsey cows. The milk fat percentage falls slightly after parturition, reaching a minimum at a point roughly corresponding to the point of maximum milk yield, and then slowly increases during the period of declining lactation. It should be noted that Bartlett's figures related to cows that in most cases became pregnant during the lactation period. With successive lactations the mean lactation fat percentage appears slowly to decrease (Bartlett, 1934b).

The normal lactation curve for the non-fatty solids content of cows' milk as established by Bartlett (1934a) shows the following features. After parturition there is a slow fall in non-fatty solids content until about the eighth week *post-partum*. The mean values thereafter remain practically constant until the end of the lactation period is approached when in the case of pregnant cows there is a slight rise. The values for barren cows, on the other hand, show a further slight fall at the end of lactation. The mean lactation values for non-fatty solids content slowly decrease with increasing age of the cow (Bartlett, 1934b).

VI. THE NATURE AND CHEMICAL COMPOSITION OF MILK

Milk is an opaque white fluid which essentially consists of an emulsion of fat globules dispersed in an aqueous phase containing protein in colloidal solution and crystalloids in true solution. Milk contains three major constituents which are peculiar to it, namely, casein, lactose and milk fat. The first two substances are not found elsewhere in nature, while the particular mixtures of glycerides which constitute the milk fats of various species appear to be characteristic of milk. In addition to the major milk constituents, numerous other substances occur in milk in relatively small amounts.

For convenience the solids of milk can be considered as belonging to two classes, the fatty and non-fatty solids. Bovine milk fat is a complex mixture of glycerides, the fatty acids occurring there in largest amounts being oleic, myristic, palmitic and stearic acids, with smaller amounts of many others including butyric, capric, lauric and linoleic acids. Appreciable proportions of short-chain fatty acids (butyric and hexanoic) occur not only in bovine milk fat but also in the milk fat of other ruminants (Hilditch and Jasperson, 1944) but apparently not in that of non-ruminants such as man (Hilditch and Meara, 1944) and the horse (Hilditch and

Jasperson, 1944). With the fatty solids of milk should also be included the small amounts of the fat-soluble vitamins and carotenoid pigments which normally occur in milk. The non-fatty solids consist of the phosphoprotein casein, the disaccharide lactose (4-galactosido-glucose), the proteins lactalbumin and lactoglobulin, various non-protein nitrogenous compounds, water-soluble vitamins and pigments, enzymes, and mineral salts such as calcium phosphate and sodium chloride. For further information on the chemistry of milk the reader is referred to Rogers (Associates of) (1935) and Davies (1939).

The average composition of milks of various species shows considerable variations which no doubt depend to a large extent on the varying needs of the young. Thus the milk of carnivores is in general characterised by high protein and low sugar content, while the milk of aquatic mammals such as the whale and porpoise contains much fat which is of high calorific value. The average composition of the milk of various species is given in Table I.

Colostrum.—Colostrum is the name given to the fluid secreted by the mammary gland during a short period following parturition when, as is normally the case, secretion has not been regularly removed from the gland during pregnancy. In composition, colostrum differs in important respects from milk, perhaps most strikingly in its high content of nitrogenous substances, chiefly consisting of globulin (serologically identical with blood serum globulin according to Crowther and Raistrick, 1916), and its low lactose content. Engel and Schlag (1925) studying changes in the composition of the fluid secreted by the bovine mammary gland during the colostral period, i.e. the period of transition from colostrum to milk, which in the cow lasts about 6–12 days, found that the specific gravity and the concentration of total solids, protein, chlorides and ash, the values for which at parturition are considerably higher than those characteristic of normal milk, gradually fall, while the lactose content which at parturition is low, gradually rises.

The presence of formed bodies in colostrum was first described over a century ago. These bodies, which are sometimes globular in form and sometimes of irregular shape and which often contain granules and even nuclei, are known as colostrum corpuscles. During the period of transition from colostrum to milk, the corpuscles gradually disappear from the mammary secretion. Most of the earlier workers on the subject regarded colostrum corpuscles as transformed leucocytes and this view of their origin has been upheld by more recent workers such as Emmel, Weatherford and Streicher (1926). During lactation, when milk is regularly removed from the mammary gland, leucocytes passing into the alveolar tissue do not accumulate there but are withdrawn regularly in the milk. In the period prior to parturition, however, when the mammary gland is engorged with secretion which is not withdrawn, the leucocytes accumulate in the alveoli where, by ingestion of fat and other milk constituents, they become transformed into corpuscles ; the same happens when nursing is suspended.

The physiological function of colostrum is of considerable interest. It was once believed that colostrum possessed laxative properties which were of value in enabling the newborn young to excrete faecal matter. Howe (1921), however, considered that though colostrum does not tend to delay defaecation in the newborn young as does the feeding of milk, it is not strictly a laxative. There is now considerable evidence that in many species ingestion of colostrum is of great

TABLE I

Percentage composition of milk of various species of mammals

(Adapted from Davies, 1939)

	Water.	Fat.	Sugar.	Casein.	Other protein.	Ash.
Man	88·50	3·30	6·80	0·90	0·40	0·20
Rabbit	—	16·71	1·98	8·17	2·21	—
Rat	68·3	14·8	2·8	9·2	2·6	1·5
Guinea-pig	—	7·31	2·31	4·60	0·49	—
Dog	75·44	9·57	3·09	6·10	5·05	0·73
Cat	81·63	3·33	4·91	3·12	5·96	0·58
Porpoise	41·11	48·50	1·33	11·19		0·57
Whale	48·67	43·67	—	7·11	—	0·46
Elephant	67·85	19·57	8·84	3·09		0·65
Ass	89·88	1·50	6·09	0·73	1·31	0·49
Mule	91·50	1·59	4·80	1·64		0·38
Horse	90·68	1·17	5·77	1·27	0·75	0·36
Zebra	—	4·80	5·34	3·03		—
Pig	84·04	4·55	3·13	7·23		1·05
Hippopotamus	—	4·51	—	—	—	—
Camel	86·57	3·07	5·59	4·00		0·77
Llama	86·55	3·15	5·60	3·00	0·90	0·80
Reindeer	68·20	17·10	2·08	8·40	2·00	1·50
Ox	87·32	3·75	4·75	3·00	0·40	0·75
Goat	82·34	7·57	4·96	3·62	0·60	0·84
Buffalo	86·04	4·63	4·22	3·49	0·86	0·76
Sheep	79·46	8·63	4·28	5·23	1·45	0·97

importance for the protection of the newborn animal against disease. In species such as the cow, horse and goat in which the placenta is relatively impermeable to antibodies, colostrum is of great importance as a means whereby maternal antibodies can be transmitted from the mother to her offspring during the short period after parturition when they can be absorbed from the alimentary tract

without undergoing destruction. In some other species, notably the human, colostrum seems to be of less importance in this respect since the transfer of immune bodies from mother to offspring appears to be mainly across the placenta. It is of interest to note that cows' colostrum contains very much more vitamin A and carotene than normal milk (Drummond, Coward and Watson, 1921 ; Dann, 1933), so that in the cow and possibly other species, ingestion of colostrum enables the young to build up a reserve of vitamin A soon after birth. Human colostrum, on the other hand, does not appear to differ very markedly from human milk as regards vitamin A content (Dann, 1936).

VII. The Mode of Formation of Milk

Cytological Studies

As a result of cytological studies various theories have been advanced as to the mode of secretion of milk. For a detailed review of the historical aspect of this subject the reader is recommended to consult Turner (1939a) on whose account the following is, to some extent, based. It was at one time believed (e.g. Virchow, 1871) that milk was formed as a result of fatty degeneration of the alveolar epithelium. Heidenhain (1883) pointed out, however, that if this theory were true the alveolar epithelium would need very frequent renewal (perhaps five times daily) during milk secretion. Evidence of the active cell proliferation postulated by this theory has never been obtained ; Maeder (1922), for instance, emphasised the scarcity of mitotic and amitotic division in the epithelial cells of the lactating mammary gland of the rat. This theory of the origin of milk has long been abandoned and milk formation is now generally regarded as a process of true secretion.

Some workers have put forward theories which may be classed as " cell decapitation " theories. The essential element in theories of this type is the idea that during lactation portions of the alveolar epithelial cells come to project into the lumina (" cupola formation "). These " cupolas " become detached and fall with their contained secretory droplets into the alveolar lumina, the secretion being set free on degeneration of the detached fragment of cell cytoplasm. This type of theory was advanced by Paartsch (1880) and Heidenhain (1883) who described two types of cells which they observed in actively secreting epithelium, namely, tall columnar cells with well-defined cell walls and containing fat droplets, and flat cells with ill-defined cell walls. The latter cells were supposed to arise from the former by loss of cytoplasm containing secretory droplets. A somewhat similar theory, involving a three-stage secretory cycle, in which the height of the epithelial cells was thought to indicate their secretory activity was put forward by Limon (1902). More recent workers who have observed evidence of " cell decapitation " are Weatherford (1929) and Jeffers (1935a), while Beams' (1927) observation of the presence of Golgi material in the mammary alveoli during lactation would seem to support the view that some cellular cytoplasm is lost during secretion. Da Fano (1922) in an earlier study, however, could find no evidence of the loss of Golgi material with the secretion from the epithelial cells of the mammary gland.

Theories of milk secretion which postulated " cell decapitation " were, however, not acceptable to all workers. Benda (1894), Michaelis (1898), Arnold (1905) and Bertkau (1907) took the view that secretory droplets were normally extruded into the lumina from cells which remained intact. The latter considered that all evidences of loss of cellular cytoplasm during secretion were artifacts of histological fixation. A more recent study by Maeder (1922) of the mammary gland of the albino mouse led him to conclude that " cell decapitation " does not normally occur and that milk arises as a result of a purely secretory process of the alveolar

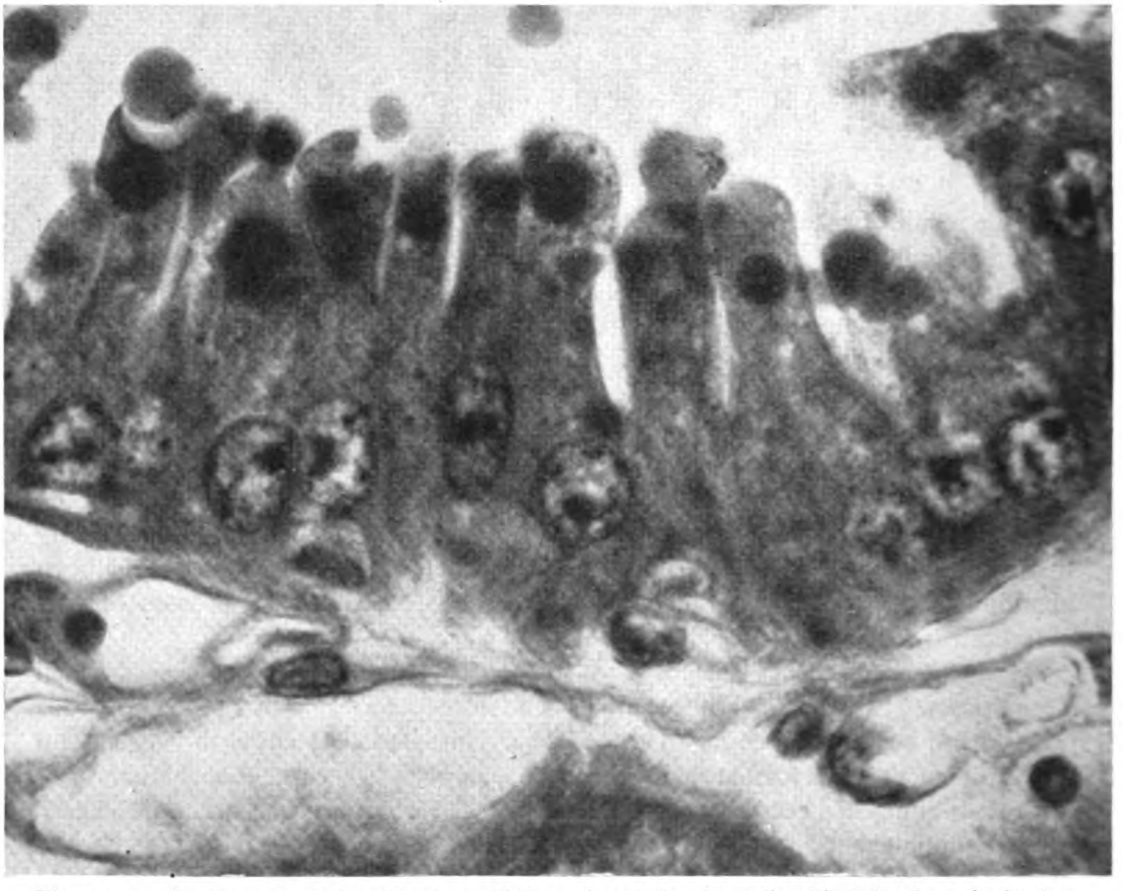


Fig. 20. 19—Section of lactating alveolar epithelium (goat) showing tall epithelial cells at the beginning of the secretory cycle. Fat droplets are in process of release from the cytoplasm. × 1,760.

epithelium. The observations of Weatherford (1929) on the rat seem to favour a combination of both types of theory, since they suggest that though loss of cytoplasm may occur during extrusion of secretion from the epithelial cells, the occurrence of this process is the exception rather than the rule.

Weatherford (1929) has carried out an extensive cytological study of the mammary gland of the albino rat in the several stages of development and activity. In the gland of the virgin animal the alveolar epithelium consists of low columnar or cuboidal cells, the ovoid nuclei being usually situated near the basal membranes. The Golgi apparatus consists of a granular network near the nucleus and often surrounding it. In primiparous animals during the first half of pregnancy, the alveoli increase in size and number, mitotic but not amitotic figures being frequent

in preparations from this stage. Weatherford observed very little epithelial cell proliferation during the second half of pregnancy, which agrees with the observations of Roberts (1921) who showed that though the alveoli continue to increase in size during late pregnancy, this is due to hypertrophy of the cells and increase in the size of the lumina owing to gradual accumulation of secretion. During pregnancy the epithelial cells lengthen and vacuoles of secretion begin to appear in the region of the Golgi apparatus, while at the same time the amount of Golgi material undergoes an increase. Just before parturition the epithelial cells are

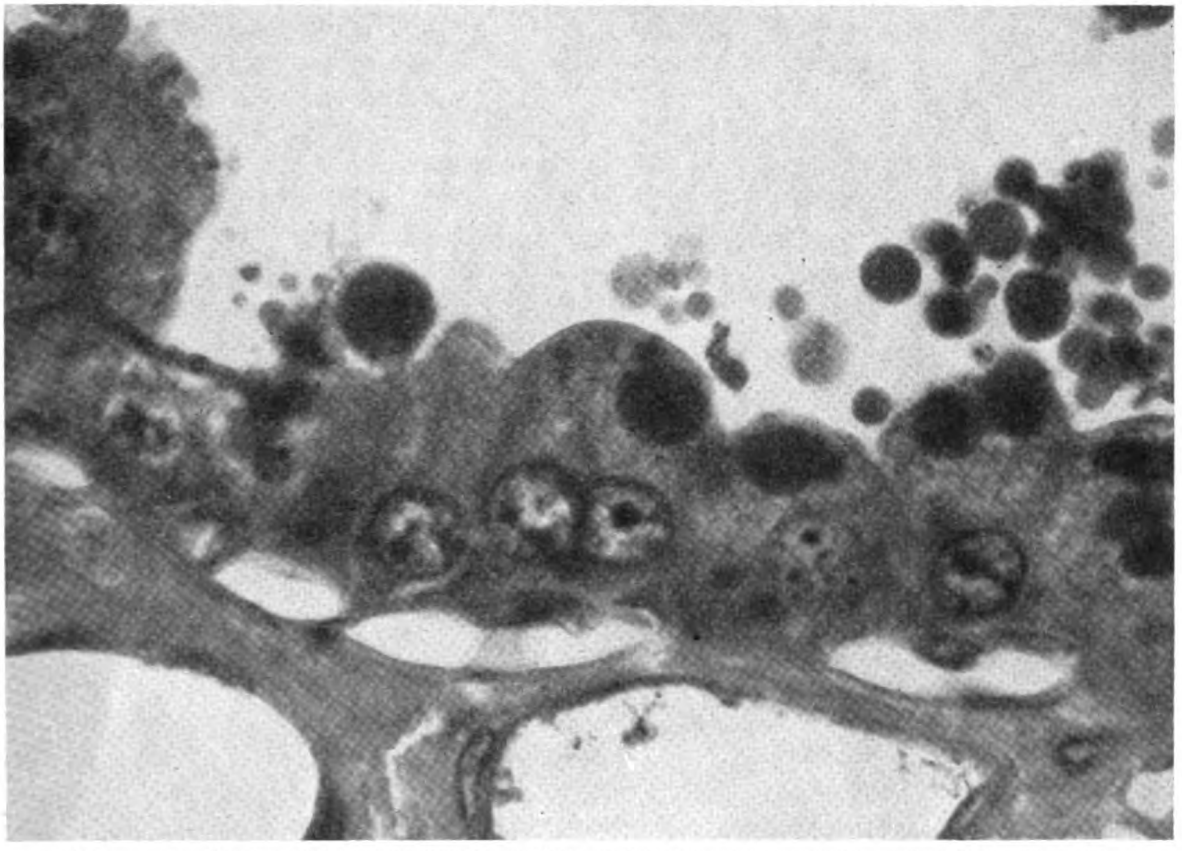


Fig. *20*. 20—Section of lactating alveolar epithelium (goat) at an intermediate stage of the secretory cycle. The alveolus is distended and the epithelial cells are becoming shorter and about to flatten. Fat is still being secreted. The capillary blood vessels are well shown beneath the epithelium. × 1,760.

distended with secretion and the Golgi apparatus, which is now greatly hypertrophied, often appears to surround the vacuoles. Distortion of the cells with secretory droplets at this stage, often results in the nucleus being pushed towards the basement cell membrane.

During lactation there is a great increase in the size of the alveoli, and accumulation of secretion in the lumina may cause flattening of the epithelial cells. In preparations from lactating glands Weatherford could detect cells in which the membranes bordering the lumina showed irregular outlines presumably caused by rupture during discharge of secretion. At a later stage, the membranes are reconstituted and their contours become smooth. Three stages in the secretory cycle exhibited by mammary epithelial cells are illustrated in Figs. *20*. 19, 20 and

21. Hypertrophy of the Golgi apparatus persists during lactation, Weatherford confirming Da Fano (1922) in this, and the results of the former indicate that secretory droplets arise within the meshes of the Golgi apparatus and move thence through the cell towards the lumen. As lactation declines, the proportion of low cuboidal cells in the epithelium increases. In the period of post-lactational involution the alveolar lumina become smaller and the Golgi material fragmented. Da Fano (1922) also noticed that involution was accompanied by the fragmentation of the Golgi material into small rounded bodies.

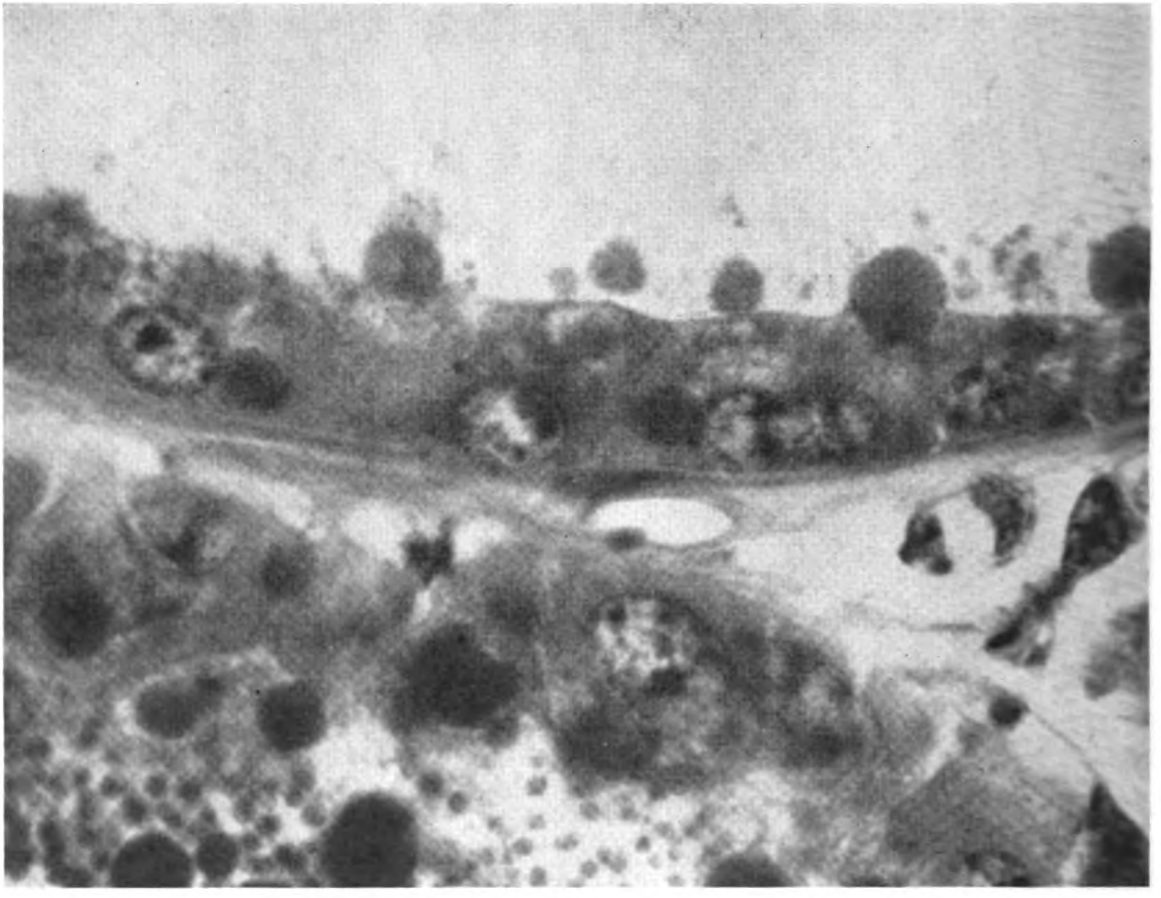


Fig. 20. 21—Section of lactating alveolar epithelium (goat) showing alveolus at maximum distension. The epithelium is flattened and the surface cell membranes are more or less intact. × 1,760.

The rôle of cell structures in lactation.—The fact that the nucleus is rich in phosphorus suggested to Nissen (1886) that it might play a part in the synthesis of casein. Observations of bi- and tri-nucleate cells in sections of lactating mammary tissue have been reported by Nissen and others, but other workers (e.g. Arnold, 1905) denied the frequent occurrence of bi-nucleate cells in mammary gland preparations. If the casein (and perhaps other constituents) of milk did arise from nuclear material as a result of nuclear degeneration, the occurrence of frequent mitosis during lactation would be expected, but as has been indicated above, most modern workers deny this.

Da Fano (1922) who studied the Golgi apparatus in the mammary epithelium of the rat, mouse and other mammals, observed hypertrophy of the Golgi material

during pregnancy and lactation and interpreted his observations as indicating that the Golgi apparatus was in some way involved in milk secretion. Later, Beams (1927) observed in the epithelial cells of lactating glands Golgi material surrounding fat droplets. The observations of Weatherford (1929) strongly suggest that the Golgi apparatus is concerned in the secretory process. Very noteworthy in this connection was the fact that the amount of Golgi material in the mammary epithelial cells of his rats varied with the intensity of their secretory activity, and that when the rate of secretion was experimentally retarded by suspension of nursing, the amount of Golgi material decreased. Judged by the amount of Golgi material in the alveolar epithelial cells, lactation in the rat appeared to reach its maximum rate at 8–10 days *post-partum* with a secondary rise at about the fourteenth day.

The presence of fuchsinophilic granules and filaments (mitochondria) was probably first observed in mammary gland cells by Altmann (1889). Later they were studied by Steinhaus (1892), Arnold (1905), Hoven (1911, 1912) and others. Weatherford (1929) included observations of mitochondria in his study of the mammary gland of the rat. His observations, on the whole, confirmed those of earlier workers in showing that during the resting state the mitochondria are chiefly granular and that during pregnancy and lactation there is a progressive appearance of filamentous mitochondria which are often orientated parallel to a proximo-distal axis of the cell. The filaments decrease in number as lactation declines. Some of the earlier workers believed that the mitochondria themselves became directly transformed into secretion, though Cowdry (1918) inclined to the view that the mitochondria play a rôle in the elaboration of cellular products more analogous to that of a catalytic surface. Weatherford, however, could not observe any of the intermediate stages of the transformation of mitochondria into secretory droplets, but his results do emphasise the close correlation between the secretory activity of the alveolar epithelium and the number and appearance of the mitochondria in the cells. Jeffers (1935a) observed chondriosomes in rat mammary tissue during pregnancy, lactation and involution.

Biochemical Studies

The mammary gland is an organ of particular interest from the point of view of intermediary metabolism, since, apart from vascular and nervous connections, it is a self-contained organ, exterior to the body, which during lactation is the site of very active synthesis of protein, fat and carbohydrate. Since the three major constituents of milk are peculiar to this fluid it is clear that they must be synthesised from precursors transported to the gland in the blood. Any of the reactions and transformations characteristic of anabolism or catabolism in the body as a whole must be considered as possibilities in connection with mammary gland metabolism. The further possibility that the mode of synthesis of a given milk constituent may vary under different conditions in a given species, and under comparable conditions between one species and another, should not be ignored.

Investigation of the biochemistry of milk synthesis has hardly progressed beyond the stage of attempted identification of the blood precursors of milk. Study of the intermediate stages of mammary gland metabolism is as yet in its infancy and an adequate attack upon this problem will scarcely be feasible until the nature of the milk precursors is known with greater certainty.

Most of the techniques used in metabolic studies of other organs and tissues have been applied to the mammary gland with varying degrees of success. The technique hitherto most widely used for mammary gland studies is based on the comparison of the concentration of suspected metabolites in the blood entering and leaving the gland. This, the so-called arterio-venous technique, has so far given most information and seems peculiarly suited to mammary gland studies since in farm animals the necessary blood samples can be taken without operative preparation. The perfusion technique has also been used to some extent (*see* Petersen, Shaw and Visscher, 1941, for a description of a modern apparatus) and Petersen and Shaw (1939) claim that it offers the advantage that general disturbance to the animal, which may affect arterio-venous (A–V) differences, is obviated. A certain amount of information has accrued from *in vitro* studies mostly involving attempted synthesis of milk constituents in the presence of mammary gland slices, pulp or extracts and also from studies on enzymes present in the latter. Experiments involving experimental alterations in the level of suspected milk precursors in the blood have given relatively little useful information. Tracer isotopes have so far been used in one or two studies only, but undoubtedly much progress in mammary gland biochemistry may be expected from the extended use of this technique in the future.

The arterio-venous technique as applied to the mammary gland.—Since this is easily the most important technique at present in use for the investigation of mammary gland metabolism, some consideration of it as applied to this purpose is necessary. The principle was first used in a study of mammary metabolism by Kaufmann and Magne (1906). Samples of mammary venous blood can readily be obtained in the cow and goat since one of the principal veins from the udder, the abdominal subcutaneous vein, is superficially placed on the abdomen ; but the work of Kaufmann and Magne was carried out before the more difficult problem of obtaining arterial blood had been solved. Accordingly, these workers, wishing to determine the uptake of blood sugar by the udder of the lactating cow, compared the blood sugar content of mammary venous blood with that of blood from the jugular vein. On the assumption that the sugar requirements of the tissues of the head and neck for energy production were about equal to those of the lactating udder, they considered that the jugular blood sugar could be taken as representing that of arterial blood less the sugar used by the udder for the production of energy. They found that in the lactating cow, the mammary venous blood contained less sugar than the jugular blood and considered that the difference represented sugar used by the udder for purposes other than energy production. The original Kaufmann-Magne technique involving jugular-mammary venous comparisons has since been used by Meigs, Blatherwick and Cary (1919), Cary (1920), Nikitin (1935) and McCay and Maynard (1935).

Blackwood and Stirling (1932a) (*see also* Blackwood and Wishart, 1936) showed that the Kaufmann-Magne method may give misleading results because blood traversing the head and neck undergoes concentration due to loss of water in the saliva, so that the composition of jugular blood may, in important respects, be very different from that of arterial blood. As Lintzel (1934) has pointed out, evaporation from the naso-pharynx will doubtless contribute to this effect. Nevertheless, as recently as 1938, Maynard, McCay, Ellis, Hodson and Davis (1938) were of the opinion that the Kaufmann-Magne technique may be useful (presumably

for qualitative studies only) where large jugular-mammary venous differences are involved, since they consider that jugular blood samples can be taken with less disturbance to the cow than can arterial samples by the methods now to be enumerated.

Blackwood and Stirling (1932a, b) were the first to carry out true arterio-venous studies on the udder of the cow, the arterial blood being obtained from the radial artery. Other and more convenient methods of obtaining arterial blood for arterio-venous studies in cows have since been introduced by Graham Jnr., Kay and McIntosh (1936) who puncture the internal iliac artery through the rectal wall, Maynard *et al.* (1938) who puncture the internal pudic artery *per vaginam* and Shaw and Petersen (1938a, 1940) who puncture the pre-pudic artery *per rectum*. For arterio-venous studies in the goat Lintzel (1934) obtained arterial blood by cardiac puncture and Graham Jnr., Turner and Gomez (1937) from an exteriorised carotid artery.

Results obtained by application of the arterio-venous technique to the mammary gland must be evaluated in relation to certain important considerations that arise respecting its use. These are cited below as illustrating technical difficulties involved in the method and as exemplifying objections that may be raised against it on theoretical grounds, and they must be kept in mind in assessing the significance of the results obtained by its use.

First, the two blood samples must be taken as nearly simultaneously as possible because arterial blood composition appears to vary from time to time (Shaw and Petersen, 1939), the venous puncture being made first, so that the effect of any disturbances connected with the arterial sampling which might temporarily cause a cessation of milk secretion (*see* Graham Jnr., Kay and McIntosh, 1936) will be minimised, the venous sample having already been taken. Further, according to Graham Jnr., Kay and McIntosh (1936) the sampling should be completed with a minimum of delay otherwise blood volume changes occur, leading to untenable results.

Second, there is ample evidence that the blood samples must be taken with a minimum of disturbance to the subject if misleading results are to be avoided. Graham Jnr., Kay and McIntosh (1936) reported that disturbance in the course of bleeding causes milk secretion temporarily to cease with resulting diminution in and sometimes reversal of A–V differences for certain blood constituents. Shaw and Petersen (1939) found that, regardless of the rapidity with which the samples are taken, such disturbances are accompanied by fluid shifts (detected by changes in blood haemoglobin content) between the blood and udder tissues which particularly affect A–V differences in non-diffusible blood constituents such as fat and protein. Correction of the A–V differences for haemoconcentration or haemodilution produced results which were obviously untenable and it appears that results corrected for blood concentration changes, such as were reported by Graham Jnr., Peterson, Houchin and Turner (1938), may be of doubtful validity. In the absence of visible disturbance it has been generally found that the passage of water from blood to milk causes no appreciable concentration of the blood passing through the udder and it would seem that only experiments in which no detectable blood volume changes have occurred should be considered as valid (*see* Shaw and Petersen, 1939). Reineke, Williamson and Turner (1941a) who found that the rates of milk secretion and blood sugar utilisation in the goat were unaffected under nembutal anaesthesia,

have proposed the use of the nembutalised goat for arterio-venous studies since blood samples can be taken under anaesthesia without disturbance.

Third, it may be objected that blood from the abdominal subcutaneous vein is not necessarily representative of the total venous blood of the mammary gland since this vein is not the sole venous outlet from the udder. Despite the fact that the existence of venous anastomoses in the udder tends to equalize the composition of the blood in the different veins this objection may be a serious one and it would be of interest to compare the composition of blood from the abdominal subcutaneous vein with that of the blood from an alternative exit such as the external pudic vein. An attempt at overcoming this difficulty was made by Graham Jnr. (1937) and Graham Jnr., Houchin and Turner (1937) who used unilaterally mastectomised goats in which all major veins from the remaining gland other than the abdominal subcutaneous vein were ligated. An even more serious objection, and one which it has not yet been possible to overcome, is the fact that a proportion of the blood constituents absorbed by the mammary gland from the blood must undoubtedly leave the gland in the lymph. Virtually nothing quantitative is known of the lymph drainage from the udder, but it is evident that the loss of metabolites in the lymph makes it impossible to conduct strictly accurate balance experiments on the mammary gland and will to an unknown extent reduce the significance of quantitative conclusions drawn from A–V differences.

Fourth, the apparent absorption of a blood constituent by the lactating udder, even if it can be shown that there is no uptake by the non-lactating gland, does not prove that the substance in question is a milk precursor. Quite apart from the possible passage of the substance into the lymph it may be used by the glandular tissues for purposes other than milk synthesis, such as energy production. Relatively little that is certain and quantitative is known about the energy requirements of the mammary gland. Shaw and Petersen (1938a, 1940) held that the gland obtains energy by the partial oxidation of fatty acids and, later, Shaw (1942b) obtained results consistent with the hypothesis that about 37 per cent. of the oxygen utilised by the lactating udder was used for the oxidation of β-hydroxybutyric acid (*see also* Shaw, Powell Jnr. and Knodt, 1942 ; Shaw and Petersen, 1943). In cows suffering from ketosis the udder is said to turn from the oxidation of higher fatty acids to the sole oxidation of β-hydroxybutyric acid (Shaw, 1942b), but this interpretation of the evidence may need revision in view of the fact that Knodt and Petersen (1946a) have reported the partial conversion of β-hydroxybutyric acid into acetone and acetoacetic acid (which are not utilised by the udder of the intact cow (Shaw, 1942b)) by mammary gland slices *in vitro*. Graham Jnr., Houchin, Peterson and Turner (1938) on the basis of the dubious assumption that the gland obtains its energy solely from the oxidation of carbohydrate, and using a formula that as printed appears to be in error, calculated that only about 10 per cent. of the energy taken from the blood is used in transforming blood constituents to milk constituents.

Fifth, in order to determine whether the rate of absorption of a suspected milk precursor by the udder is sufficient to account for the observed rate of synthesis of the corresponding milk constituent, the ratio of blood flow to milk secreted must be known. Average values for this ratio over a given period have been estimated for the goat by Graham Jnr., Peterson, Houchin and Turner (1938) from direct blood flow measurements as 150–250 : 1 (*see also* Graham Jnr., Houchin,

Peterson and Turner, 1938), values which are slightly higher than the ratios reported by Jung (1933). It should be noted that these measurements were made on the abdominal subcutaneous vein in goats operatively prepared as mentioned above and since there was some remaining peripheral drainage the results are probably somewhat low. Alternatively, the average blood/milk volume ratio can be indirectly calculated from A–V concentration differences for an element such as calcium which occurs both in blood and milk, provided the concentration of the element in the milk is also known. Ratios for the cow determined in this way from calcium uptake have been given as 387 : 1 (Shaw and Petersen, 1938a), 410 : 1 (Shaw and Petersen, 1940) and 488–650 : 1 (Shaw *et al.* 1942). Phosphorus uptake determinations have given ratios varying from 331–511 : 1 (Shaw *et al.* 1942). It seems likely that this method somewhat underestimates the true ratios since there is almost certain to be considerable passage of calcium and phosphorus into the lymph. Blood volume/milk volume ratios calculated from experimentally observed A–V differences for suspected milk precursors have been given by Lintzel (1934), Graham Jnr., Jones and Kay (1936) and Shaw and Petersen (1938a). Such calculations have no meaning except in so far as they are used for the purpose of comparison with an independent estimate of the blood/milk volume ratio as a test of a precursor hypothesis. To discuss them as independent estimates in their own right, as was done by the afore-mentioned authors as well as Petersen (1944) and Kay (1945), involves an obvious logical fallacy. Quite apart from difficulties associated with the determination of the blood/milk volume ratio, balance calculations of the type discussed above are subject to error due to the fact that single A–V determinations give approximately instantaneous values for the uptake of the blood constituent in question, while the milk data used in the calculations correspond to the milk secreted over a considerable period, during which the rate of milk secretion and precursor uptake (e.g. *see* Shaw and Petersen, 1940, for blood fat and calcium uptake) are certainly not constant.

In sum, the above considerations make it clear that there are considerable technical difficulties associated with the arterio-venous method as applied to the mammary gland and, further, that the results even of experiments which, in the light of present knowledge, are unobjectionable from the technical point of view are still open to a considerable degree of uncertainty as regards quantitative interpretation.

Carbohydrate metabolism of the mammary gland.—Bert (1884) amputated the udders of goats which he afterwards caused to become pregnant and bear young. For a short time immediately following parturition, he was able to detect a reducing substance in the urine, assumed by him to be glucose which in the intact lactating animal would be used for the synthesis of lactose. Similar observations were later made by Porcher (1909) with goats which had undergone mastectomy during pregnancy or lactation. Porcher claimed to have proved that the reducing substance appearing temporarily in the urine was glucose and not lactose and that the temporary glycosuria was accompanied by hyperglycaemia. Neither Bert, Porcher, nor Marshall and Kirkness (1907) were able to obtain similar results with the guinea-pig, and contradictory results with the sheep were reported by Foà (1912) and with the goat by Moore and Parker (1901). These discrepancies were discussed by Meigs (1922).

Kaufmann and Magne (1906) observed that the blood from the mammary vein in lactating cows contained less sugar than jugular blood, while in a dry cow the sugar contents of the two types of blood were nearly equal. These results were subsequently interpreted (Meigs, 1922) as evidence that blood sugar is the precursor of lactose.

Foà (1912) used the perfusion technique in an attempt to determine the precursor of lactose. He found that when a sheep's udder was perfused with a mixture of blood and Ringer's solution, milk containing lactose was secreted, while the sugar content of the perfusate decreased. Addition of dextrose to the perfusion fluid increased the lactose content of the secretion. When the gland was perfused with Ringer containing dextrose, an aqueous fluid containing lactose was secreted, but no lactose was secreted when the gland was perfused with Ringer containing galactose or with Ringer alone.

Modern work on the precursors of lactose dates from the arterio-venous studies of Blackwood and Stirling (1932b), who observed A–V differences in true blood sugar amounting to 5–15 mg./100 ml. in lactating cows and smaller differences in dry cows. In subsequent arterio-venous studies A–V blood sugar differences, on the whole of the same order, have been observed in lactating cows (Graham Jnr., Jones and Kay, 1936; Shaw, Boyd and Petersen, 1938; Maynard *et al.* 1938; Shaw *et al.* 1942) even in cases of hypoglycaemia associated with ketosis (Shaw, 1943), and in goats both normal (Lintzel, 1934; Graham Jnr., 1937) and under the influence of nembutal (Reineke, Williamson and Turner, 1941a). Graham Jnr., Jones and Kay (1936) reported that the A–V blood sugar difference was positively correlated both with the arterial blood sugar level and with the milk yield but these correlations were not confirmed by Shaw *et al.* (1938).

Qualitatively, these findings support earlier ideas regarding the formation of lactose from blood glucose, but the possibility that a proportion of the absorbed glucose may be oxidised or perhaps converted into milk fat must not be overlooked. Graham Jnr.'s (1937) quantitative arterio-venous studies in the goat, involving blood flow measurements, indicated that the glucose uptake was quite insufficient to account for the observed rate of lactose synthesis. The observation that considerable quantities of lactic acid were also utilised by the gland in these experiments suggested that this substance might also be used for lactose synthesis, though even then there was a deficit of about 15 per cent. of the lactose synthesis unaccounted for. Graham also observed a definite loss of amino-acids from the blood traversing the udder and he suggested that further lactose precursors might arise from deamination of amino-acids, presumably the anti-ketogenic moiety. The possibility that the mammary gland is capable of deaminising amino-acids is supported by the observations that the lactating udder in the goat produces urea (Graham Jnr., Houchin and Turner, 1937; Graham Jnr., Houchin, Peterson and Turner, 1938; Graham Jnr., Peterson, Houchin and Turner, 1938) and that actively lactating mammary tissue contains appreciable amounts of arginase (Shaw and Petersen, 1938b; Folley and Greenbaum, 1946, 1947). The uptake of glucose, lactic acid and amino-acids (calculated as alanine) determined by Graham Jnr. (1937) was together sufficient to account for the lactose synthesised, but Graham appears to have neglected the fact that part of the amino-acids, if indeed deamination does occur in the udder, would give rise to ketone bodies. Results confirming the uptake of lactic acid by the bovine mammary gland were obtained

by Shaw *et al.* (1938) who, however, found that the combined uptake of glucose and lactic acid was sufficient to account for the observed lactose synthesis. The position has since been complicated by the findings of Powell Jnr. and Shaw (1942) whose results indicated that lactic acid is absorbed by the udder from the blood only in cases of disturbance associated with the blood sampling. Later, Shaw *et al.* (1942) reported A–V blood sugar differences, in undisturbed cows, of sufficient magnitude by themselves to account for the observed rate of lactose synthesis provided of course that none of the sugar was used for energy production or conversion to fat. In this latter connection an additional source of carbohydrate for the mammary gland was indicated by Reineke, Williamson and Turner (1941b) who observed a definite uptake of glycoprotein, presumably globulin, by the udder of the anaesthetised lactating goat.

Studies of lactose synthesis *in vitro* have, on the whole, given results which are in harmony with the view that glucose is one of the main precursors of lactose. Early efforts to effect synthesis *in vitro* in the presence of mammary gland preparations were hardly convincing. Perhaps the first report of *in vitro* lactose synthesis to carry any degree of conviction was that of Michlin and Lewitow (1934) who used a mixture of glucose and galactose as substrate, but the first apparently unequivocal synthesis was achieved by Grant (1935) who obtained lactose synthesis by mammary gland slices from glucose but not from fructose, mannose or galactose. A mixture of glucose and galactose gave no more synthesis than glucose alone (Grant, 1936). Petersen and Shaw (1937) isolated lactose from a mixture of glucose and lactate incubated with macerated udder tissue, but in view of Grant's successful results with glucose alone this cannot be taken as evidence that lactic acid is an essential precursor of lactose. Weinbach (1936) claimed to have demonstrated the synthesis of a substance believed, perhaps on rather inadequate grounds, to be lactose, from glucose in the presence of a non-living preparation of mammary gland tissue. More recently Knodt and Petersen (1945), using mammary gland slices, have reported lactose formation in the presence of glucose, glucose and lactic acid, maltose and glycogen.

Additional evidence pointing to a relationship between blood sugar and lactose is provided by studies involving experimental alterations in the blood sugar level. Temporary lowering of the milk lactose content accompanies hypoglycaemia due to inanition (Nitzescu, 1925 ; Gowen and Tobey, 1931a), treatment with insulin (Nitzescu and Nicolau, 1924 ; Gowen and Tobey, 1931b ; Brown *et al.* 1936a) or phlorizin (Paton and Cathcart, 1911 ; Gowen and Tobey, 1931b). Equally temporary and usually rather small increases in milk lactose content have been correlated with alimentary hyperglycaemia in cows (Whitnah, Riddell and Hodgson, 1933) and with prolonged hyperglycaemia caused by subcutaneous implantation of adrenalin tablets in cows and goats (Bottomley, Folley, Walker and Scott Watson, 1939). On the other hand, hyperglycaemia produced in cows by infusion of glucose solutions into the udder (Brown *et al.*, 1936b) or into the external pudic artery (Petersen and Boyd, 1937) had no effect on the milk lactose.

The only firm conclusion that emerges from the sum total of the foregoing investigations is that glucose is almost certainly concerned in the synthesis of lactose and is probably its principal precursor. The afore-mentioned uncertainties connected with the quantitative aspects of arterio-venous studies together

with the paucity of well-attested knowledge of the oxidation processes occurring in mammary tissue and the fact that the ability of the udder to convert carbohydrate into fat is still controversial (*see* page 614) renders a decision as to whether other precursors are involved impossible at the present time.

Our knowledge of the intermediate stages in lactose synthesis is equally scanty. The possibility that glycogen is an intermediate, suggested by the presence of small quantities of glycogen in the mammary gland (Barrenscheen and Alders, 1932 ; Petersen and Shaw, 1938), has been strengthened by the finding (Knodt and Petersen, 1945) that perfusion of the isolated bovine udder with blood containing glucose markedly increases the glycogen content of the tissue. It is further significant that incubation of such glycogen-enriched udder tissue results in the disappearance of glycogen accompanied by the formation of lactose. As a result of perfusion experiments in which addition of insulin to the perfusion fluid increased the udder glycogen stores and decreased the lactose content of the milk secreted, Knodt and Petersen (1946b) postulated that lactose arises from the breakdown of glycogen. Phosphorylation processes as intermediate stages in lactose synthesis were postulated by Barrenscheen and Alders (1932) who, together with Borst (1932) and Brenner (1932), demonstrated the presence of phosphoric esters in the mammary gland. Grant (1936), however, found no evidence of increased lactose synthesis by mammary gland slices in the presence of hexose phosphates or phosphoglycerate. Lactic, pyruvic and citric acids—other possible intermediates—were found to have no effect on the ability of mammary gland slices to synthesise lactose (Knodt and Petersen, 1945). On incubation, mammary gland tissue produces lactic acid (Svanberg, 1930 ; Barrenscheen and Alders, 1932 ; Knodt and Petersen, 1945) and citric acid (Knodt and Petersen, 1946a) ; the production of the latter being increased in the presence of added glucose, lactic acid, pyruvic acid, maltose and glycogen.

Fat metabolism of the mammary gland.—In considering possible precursors of milk fat it seems *a priori* most likely that one fraction or a combination of fractions of the blood lipins—glycerides, phospholipins or cholesterol esters—is involved. The possibility that milk fat may, in part at any rate, be formed from carbohydrate, protein or amino-acids absorbed from the blood must, however, not be neglected.

Meigs *et al.* (1919), using the original Kaufmann-Magne method, found more lipin phosphorus in the jugular blood of lactating cows than in mammary venous blood, and accordingly advanced the theory that milk fat originated from the blood phosphatides. They considered that if this theory were true, the mammary gland would absorb more than sufficient lipin phosphorus to provide the phosphorus of the milk, and the return of the excess to the blood as inorganic phosphate would be expected. The results of Meigs *et al.* were apparently in accord with their theory in this respect since the mammary venous blood contained more inorganic phosphate than the jugular blood. This theory of the origin of the milk fat, which harmonised with prevailing views as to the prominent part played by phosphatides in intermediary fat metabolism was later upheld by Nikitin (1935) who also used the Kaufmann-Magne technique. Subsequent re-investigation of this question by workers using the more exact arterio-venous technique has, however, uniformly failed to demonstrate any uptake of blood phosphatide by the udder of the lactating cow (Blackwood, 1934 ; Graham Jnr., Jones and Kay, 1936 ; Maynard

et al. 1938) or goat (Lintzel, 1934). Moreover, far from the inorganic phosphorus content of the blood increasing as it passes through the lactating udder as demanded by the theory of Meigs *et al.*, workers using the arterio-venous technique have found that the active gland absorbs considerable amounts of inorganic phosphate from the blood (Blackwood, 1934 ; Lintzel, 1934 ; Graham Jnr., Jones and Kay, 1936). Lintzel (1934) and Graham Jnr., Jones and Kay (1936) were led by their results to the view that most of the milk phosphorus arises from the blood inorganic phosphate, a conclusion which has since received strong support from the results obtained by Aten Jnr. and Hevesy (1938) in a study involving the use of radioactive phosphorus. It may be concluded that at the present time there is no evidence for the participation of blood phospholipin in the formation of milk fat.

The currently accepted view that blood triglycerides are the precursors of milk fat was first advanced many years ago by Foà (1912) who perfused a sheep's udder with an emulsion of olive oil or triolein and observed the secretion of an aqueous fluid containing globules of fat, the iodine number of which was lower than that of the fat in the perfusion fluid. These results were regarded with scepticism by Meigs (1922) because the gland became oedematous during the perfusion. Later perfusion experiments by Petersen, Palmer and Eckles (1929) indicated that the mammary gland could absorb triglycerides, because after perfusion of an udder with an emulsion of corn oil stained with Sudan III, dye was found in the glandular tissues though none appeared in the secretion. It may be noted that a theory which postulates that the mammary alveolar cells are permeable to neutral fat does not conflict with modern views on the mechanism of fat absorption from the intestine (*see* Frazer, 1946).

Arterio-venous studies have on the whole given results which are consistent with the idea that the blood triglycerides are the main if not the sole precursors of milk fat. Lintzel (1934) in the goat, and Maynard *et al.* (1938) in the cow, obtained indirect evidence that the lactating udder selectively utilised the triglyceride fraction of the total blood lipins ; they observed a decrease in the total lipin of the blood passing through the gland but no evidence of any change in the phospholipin or cholesterol ester fractions. Similar results for the cow were reported by Graham Jnr., Jones and Kay (1936) except that these workers did not determine the cholesterol esters and thus did not exclude the possibility that fatty acids combined with cholesterol were also utilised by the gland. Subsequently, Voris, Ellis and Maynard (1940) devised a method for determining the blood triglycerides directly, depending on the estimation of glycerol after saponification of the acetone-soluble blood lipins, and were thus able to demonstrate the uptake of neutral fat by the lactating mammary gland more conclusively than had previously been possible.

In the cow, Shaw and Petersen (1938a, 1940) carried out arterio-venous studies in which blood fat was determined by a method which included cholesterol and cholesterol esters but not phosphatides and in which the blood/milk volume ratio was estimated from calcium uptake. Their results indicated that the blood fat uptake by the udder was more than sufficient to provide for the observed rate of milk fat formation and they therefore postulated that some blood fat was oxidised by the glandular tissues. The actual blood fat uptake would be even greater if, as Shaw and Petersen (1940) state, the udder produces a considerable flow of lymph containing much calcium but very little fat. Later, Shaw *et al.*

(1942) obtained results indicating the absorption of sufficient blood fat by the lactating bovine udder to provide the fat secreted in the milk. Shaw and Petersen (1940) reported that, contrary to what might be expected, there was no fat uptake by the udder immediately after milking; the uptake gradually rose during a few hours following milking and then remained sensibly constant for some time. The alveolar cells were thought to be permeable to blood fat only when the alveoli were distended with milk.

The possibility that a portion of the milk fat is synthesised from carbohydrate was suggested by Graham Jnr., Houchin, Peterson and Turner (1938) because they obtained a mean respiratory quotient (R.Q.) greater than unity for the udder of the lactating goat. This finding was confirmed for the goat by Reineke, Stonecipher and Turner (1941) who obtained a mean value of 1·18 for the R.Q. of the udder of the anaesthetised goat, after application of a correction for carbon dioxide supposedly used for the synthesis of urea by the udder. This correction was made on the assumption that the urea is formed from carbon dioxide and ammonia arising from the deamination of amino-acids as suggested by Graham Jnr., Houchin and Turner (1937) and not from the decomposition of arginine absorbed from the blood by the mammary gland. Bottomley and Folley (unpublished observations) in a series of determinations of the mammary gland R.Q. in lactating cows found no such variability as was reported by Shaw (1939) and Petersen and Shaw (1942) in cows and Reineke, Stonecipher and Turner (1941) in unanaesthetised goats. Their value for the mean R.Q. of the lactating bovine udder in cows, in experiments in which there was no haemoconcentration, was $1 \cdot 25 \pm 0 \cdot 12$ (5 per cent. fiducial points); in experiments in which haemoconcentration occurred the R.Q. was, however, less than unity. Shaw (1942b) obtained an R.Q. of 1·15 for the udder of the cow in ketosis. Mammary gland R.Q. values below unity have been reported for perfused bovine glands by Shaw (1939), for fasted lactating and non-pregnant dry goats by Reineke, Stonecipher and Turner (1941) and for fasted lactating cows by Shaw *et al.* (1942). Reineke, Stonecipher and Turner (1941), however, question whether values obtained on perfused glands are relevant to the intact gland *in vivo*. The consensus of evidence undoubtedly points to an R.Q. greater than unity for the lactating udder in the intact animal, but it must be conceded that the R.Q. may be a misleading index of reactions occurring even in a single organ so that further independent evidence would seem to be required before the formation of fat from carbohydrate in the mammary gland can be taken as established (*see* page 615).

The origin of the short-chain fatty acids which are a striking feature of the milk fat in the ruminant (*see* page 598) is an intriguing problem which has not yet been satisfactorily solved. The main possibilities as to their origin may be summarised thus: (*a*) they may be formed in the gland from long-chain fatty acids by a process of oxidation followed by reduction; (*b*) carbohydrate absorbed from the blood by the gland may be there transformed into short-chain fatty acids; (*c*) they may be formed in the gland by the transformation of, or synthesis from, lower fatty acids arising in the course of metabolism and transported to the gland by the blood. These possibilities will now be considered in turn.

(*a*) The possibility that the short-chain fatty acids of milk fat are formed from oleo-glycerides by oxidation in such a manner that the carboxyl groups are not involved and that one molecule of oleic acid gives rise to one molecule of short-

chain fatty acid was suggested by Hilditch and his colleagues (Hilditch and Sleightholme, 1931; Hilditch and Paul, 1936; Hilditch and Thompson, 1936) in consequence of regular relationships which emerged in a study of the fatty acids of the component glycerides of bovine milk and depot fats. This theory was supported by the fact that in the lowest unsaturated fatty acids found in milk fat the double bond is in the same position relative to the carboxyl group as in oleic acid (Hilditch and Paul, 1936) suggesting that these acids represent intermediate stages in the degradation of the carbon chain of oleic acid and by the observation that the feeding of cod-liver oil results in a reduction of the proportion of short-chain acids and an increase in the proportion of oleic acid in the milk fat glycerides (Hilditch and Thompson, 1936). This, it was believed, was due to the poisoning by highly unsaturated acids present in cod-liver oil of an oxidising enzyme system responsible for the degradation of the oleic acid chain. Hilditch (1937) in summarising the evidence for his theory points out that it is in accord with modern views on the mechanism of oxidation of long carbon chains exemplified by the alternate multiple oxidation theory of Jowett and Quastel (1935) and the ω oxidation theory of Verkade and van der Lee (1934). The results of Smith and Dastur (1938), who found that during inanition the short-chain fatty acids of bovine milk fat decrease concomitantly with an increase in the proportion of oleic acid, are also in harmony with this theory. Shaw and Petersen (1938a) who, as we have seen, reported that the lactating bovine udder absorbs more than sufficient fat to provide for the observed rate of fat synthesis, also postulated that fatty acids were partially oxidised in the mammary gland, thus giving rise to the short-chain fatty acids of milk. In accordance with this view, Shaw (1939) obtained an R.Q. of 0·8 for perfused lactating bovine udders which was taken to be indicative of fat oxidation. However, it seems probable that the R.Q. for the intact udder is greater than unity and the R.Q. in any case cannot be relied on as an indicator of the reactions occurring in an organ with so complicated a metabolism as that of the mammary gland.

(*b*) The evidence in favour of the possibility that the milk short-chain fatty acids are synthesised from carbohydrate consists in the observation that the R.Q. of the intact lactating gland is greater than unity and declines below unity during inanition (*see* page 614). Since inanition also lowers the proportion of short-chain acids in the milk triglycerides (e.g. *see* Smith and Dastur, 1938) it seems possible that if the high R.Q. of the normal gland does denote the synthesis of some portion of the milk fat from carbohydrate, then this portion may consist particularly of the short-chain fatty acids as suggested by Reineke, Stonecipher and Turner (1941). Smith and Dastur (1938) suggested that their results, already mentioned under (*a*) above, would be in accord with this hypothesis if it be assumed that oleic acid is synthesised in the mammary gland from carbohydrate, the short-chain acids representing by-products of this process. The decline in the latter during inanition, they say, could then be ascribed to the greater facility with which oleic acid synthesis would go to completion in conditions such as those of inanition, in which the total production of milk fat declines. On this view, however, the mammary gland R.Q. during inanition should be greater than unity, since oleic acid is still being synthesised from carbohydrate, whereas Shaw *et al.* (1942) found values less than unity under these conditions. Moreover, Hilditch, Paul, Gunde and Maddison (1940) believe that the glyceride

structure characteristic of milk fat is such as would be unlikely to result from a process of fat formation from carbohydrate.

(*c*) The possibility that the short-chain fatty acids of milk fat pre-exist in the blood has no evidence to support it. Such a mixture of fatty acids has never been detected among the blood lipins (*see* Smith and Dastur, 1938). However, the observation of Shaw and Knodt (1941a) that the lactating bovine udder absorbs considerable amounts of β-hydroxybutyric acid from the blood led them to suggest that the short-chain fatty acids of milk might be elaborated from this blood constituent. The observed uptake was sufficient to account for the milk lower fatty acids up to those with chains of fourteen carbon atoms. Such a synthesis would, of course, tend to increase the R.Q. and its occurrence would thus be consistent with experimental findings. Further support to this theory of the origin of the lower fatty acids of milk came from further observations of Shaw and Knodt (1941b) who reported a decrease in the mammary gland R.Q. as well as in the milk short-chain fatty acids in cows fasted or fed cod-liver oil. However, since the short-chain acids in the milk fat undergo a decrease in cows suffering from severe ketosis (Shaw, 1941 ; Shaw *et al.* 1942) while the mammary gland absorption of β-hydroxybutyric acid is markedly increased (Shaw, 1942b), it seems more likely that this intermediate of fat metabolism is oxidised in the udder as suggested by Shaw (1942b), Shaw *et al.* (1942) and Shaw and Petersen (1943).

There remains another and most interesting possibility which at present is purely theoretical.* It must surely be regarded as significant that short-chain fatty acids are, as far as present knowledge goes, only found in any quantity in the milk of ruminants (*see* page 598). It has recently been shown that in ruminants considerable amounts of lower fatty acids, particularly acetic acid, are produced as a result of the degradation of carbohydrate by the micro-organisms of the rumen and are absorbed into the blood (*see* Elsden and Phillipson, 1948). Moreover, Rittenberg and Bloch (1945) using acetic acid marked with heavy carbon have demonstrated the synthesis of carbon chains from acetic acid in the body. It therefore seems possible that the lower fatty acids of milk fat may be synthesised in the udder from acetic acid absorbed from the rumen and transported to the udder by the blood. Such a reaction might explain the high R.Q. of the intact lactating gland since it involves the synthesis of oxygen-poor compounds from a compound relatively rich in oxygen.

Nitrogen metabolism of the mammary gland.—Relatively little is known about the nitrogen metabolism of the mammary gland. It is clear that some fraction or combination of fractions of the nitrogenous compounds of the blood must provide the precursors of the milk protein, the most likely *a priori* possibilities in this connection being the amino-acids and some fraction of the blood proteins.

Cary (1920) using the Kaufmann-Magne technique concluded that the blood amino nitrogen suffered a diminution in passing through the udder of the lactating but not the dry cow. He was thus led to the view that the milk proteins are synthesised from the amino-acids of the blood. This theory seemed to be supported by subsequent arterio-venous studies which have uniformly demonstrated a small but definite uptake (in most cases less than 1 mg. amino N/100 ml.) of

* Since this chapter was completed in 1945 evidence has come both from *in vitro* experiments on mammary gland slices (Folley and French, 1950), and from an *in vivo* experiment on a lactating goat (Popják, French and Folley, 1950) that acetate is utilised for milk fatty acid synthesis by the mammary gland. This and other aspects of the biochemistry of lactation have been reviewed by Folley (1949).

amino-acids by the lactating udder of the cow (Blackwood, 1932; Shaw and Petersen, 1938a, c; Bottomley and Folley, unpublished observations) and goat (Lintzel, 1934; Graham Jnr., 1937; Graham Jnr., Peterson, Houchin and Turner, 1938; Reineke, Peterson, Houchin and Turner, 1939), but not by the non-lactating bovine udder (Blackwood, 1932). Quantitative arterio-venous studies have, however, indicated that the uptake of amino-acids from the blood is quite insufficient to provide the proteins of milk (Graham Jnr., 1937; Shaw and Petersen, 1938a, c; Reineke *et al.* 1939), the observed A–V differences corresponding to only about 40 per cent. of the milk nitrogen. The conclusion therefore seems inescapable that if the blood amino-acids are concerned in the formation of milk proteins, their rôle is inconsiderable. Further evidence against Cary's theory was cited by Reineke, Williamson and Turner (1941b) who could detect no uptake of amino nitrogen by the udder of the fasted goat even though milk secretion continued.

Graham Jnr., Peterson, Houchin and Turner (1938) suggested that blood globulin may be utilised by the mammary gland for transformation into milk protein. They found that the udder of the lactating goat absorbed from the blood, in addition to amino-acids, appreciable quantities of other non-protein nitrogenous substances and of a fraction of the blood protein identified as globulin, while returning albumin to the blood. They suggested that the globulin molecule might undergo partial degradation in the mammary gland into still relatively large molecular fragments some of which might be used, together with non-protein nitrogenous material from the blood, for the synthesis of milk protein, the residues being returned to the blood stream. Later, rather irregular changes in various fractions of the blood nitrogen passing through the mammary gland were reported by Reineke *et al.* (1939). These results seem hardly susceptible of a simple interpretation, but the authors suggest that they are consistent with the idea that plasma protein fractions undergo reversible shifts among themselves in the mammary gland involving partial breakdown of the molecules as suggested by Graham Jnr., Peterson, Houchin and Turner (1938). The possibility that the mammary gland may at some periods in the lactation cycle store nitrogenous substances which later may be partially utilised for the synthesis of milk protein may be offered towards an explanation of such results which are otherwise difficult to interpret. Further evidence that blood globulin may be a precursor of milk protein has been provided by Reineke, Williamson and Turner (1941b) who observed the uptake of glycoprotein, believed to be globulin, by the lactating udder of the goat.

It is not certain whether all or part of the blood amino-acids absorbed by the lactating mammary gland are incorporated into the milk protein or whether some proportion is deaminised. An appreciable output of urea by the goat udder has been reported (Graham Jnr., Houchin and Turner, 1937; Graham Jnr., Peterson, Houchin and Turner, 1938; Reineke *et al.* 1939) and it has been claimed that in early lactation the ouput of urea nitrogen exceeds the intake of amino nitrogen (Graham Jnr., Peterson, Houchin and Turner, 1938). It seems possible that some of the urea could arise from the decomposition of arginine by the action of the arginase known to be present in mammary tissue (Shaw and Petersen, 1938b; Folley and Greenbaum, 1946, 1947).

The unidentified portion of the plasma non-protein nitrogen which, according to Reineke *et al.* (1939) is absorbed by the lactating goat udder may also be concerned in the elaboration of milk protein. The studies of Shaw and Petersen (1938c),

however, indicate that uric acid, creatine and creatinine are not involved since these blood constituents were not utilised in significant amounts by the udder of the cow.

The phosphorus of casein appears to come from the inorganic phosphate of the blood (Aten Jnr. and Hevesy, 1938).

Enzymes of the mammary gland.—The enzymes of the mammary gland have been relatively little studied, despite the interest of the subject as affording an insight into the chemistry of milk synthesis.

The most closely studied enzyme of mammary tissue is the alkaline phosphatase first detected in the mammary gland by Kay (1925). In the same year Tateyama (1925) obtained evidence of the presence in mammary gland extracts of an enzyme capable of dephosphorylating nucleic acid and hexose phosphates. Later, Borst (1932) confirmed the presence of a phosphatase in mammary gland tissue. Folley and Kay (1935) examined many of the properties of the alkaline phosphatase (optimum *p*H 9–10) of the guinea-pig mammary gland and showed that in all respects studied it was identical with the phosphatase of the kidney and therefore to be classed as a phosphomonoesterase A1 in the classification of phosphatases proposed by them (Folley and Kay, 1936). Caputto and Marsal (1941) have described a method of purification of mammary gland phosphatase and on the basis of studies of its inhibition by various ions (Caputto and Marsal, 1944a), believe it to be identical with the alkaline phosphatase of bone, intestinal mucosa and blood plasma, but different from that of the kidney. Spectographic examination of purified preparations (Caputto and Marsal, 1944b) indicate the presence of a protein rich in aromatic amino-acids. Folley and Kay (1935) found that the phosphatase potency of lactating mammary tissue was of the same order as that of kidney tissue so that the mammary gland is one of the three or four richest known sources of the enzyme. More extensive studies in the lactating rat by Folley and Greenbaum (1946, 1947) indicate that in this species mammary tissue exhibits about 33 per cent. of the phosphatase activity of the kidney. Neither adrenalectomy nor treatment with adrenal cortex steroids significantly altered the phosphatase levels of the rat mammary gland.

Arginase was detected in lactating bovine udder tissue by Shaw and Petersen (1938b) who could find none in non-lactating tissue. The presence of arginase in the lactating mammary gland of the rat has recently been reported by Folley and Greenbaum (1946) who find the arginase potency of mammary tissue to be about 2·5 per cent. of that of the liver. The former may thus be reckoned as the second most potent source of arginase in the body. A study of the mammary gland arginase during pregnancy, lactation and involution in the rat showed that the mammary gland arginase content increased only slightly during pregnancy and during early lactation but underwent a dramatic increase in mid-lactation. By contrast the alkaline phosphatase increased during the mammary growth phase and remained at a high but constant level throughout lactation (Folley and Greenbaum, 1947). The arginase content of the mammary gland decreased significantly after adrenalectomy (Folley and Greenbaum, 1946) but was not restored to normal levels by treatment with adrenal cortex steroids.

Other enzymes which have been reported as present in the mammary gland tissue include lipases (Tateyama, 1925; Virtanen, 1924; Kelly, 1938), amylases (Grimmer, 1913; Tateyama, 1925), maltase (Kleiner and Tauber, 1932) and purine oxidases (Michlin and Ryżowa, 1934).

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