THE POST-NATAL DEVELOPMENT OF THE RAT SUBMAXILLARY GLAND

By F. JACOBY and C. R. LEESON*

Department of Anatomy, University College, Cardiff

The rat submaxillary gland has received considerable attention in recent years as an exocrine gland profoundly influenced by hormones, especially that of the thyroid. From the study of the relevant literature three surprising facts emerge: (1) the complex structure of the fully differentiated gland of the adult rat is often not fully appreciated nor, indeed, understood; (2) a confusing nomenclature exists concerning the different parts or segments which compose the glandular unit, as it repeats itself within a lobule; (3) the post-natal development of this gland does not seem to have been properly studied or described. Yet a glance at Figs. 1 and 2 of Pl. 1 is sufficient to make one realize what strides in development and differentiation the gland must obviously have made during the period which—so to speak—separates these two pictures. It is also obvious that an accurate and precise knowledge of the state of development of the gland is of particular importance in endocrinological studies, for which often very young animals are used. Moreover, certain histomorphological problems connected with the adult gland, but as yet unsolved, could well be clarified by a study of the post-natal developmental stages.

The present paper, therefore, deals in the main with the post-natal development of the rat submaxillary gland. But in order to make this account intelligible it is essential that the two other points mentioned above are considered first. Also any reference to the literature will be facilitated by an exposition given first of the structure of the gland as seen in the adult animal.

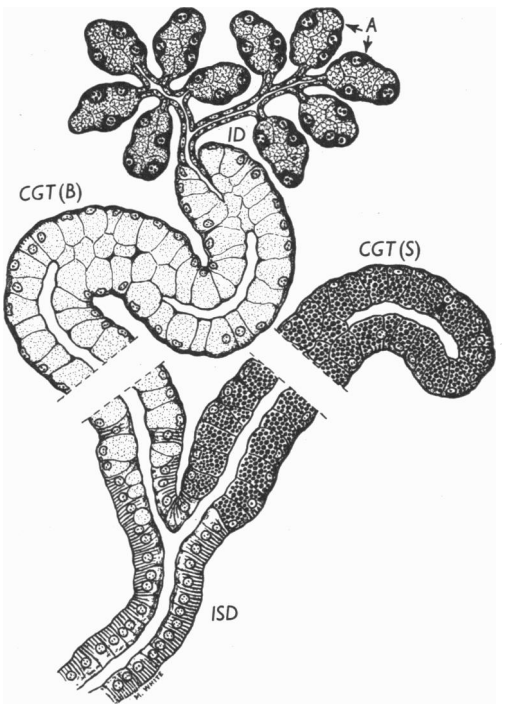
HISTOLOGY OF THE SUBMAXILLARY GLAND OF THE ADULT RAT

A lobule of such a gland appears as a compact structure containing various epithelial elements. Sorted out, there are four main parts which make up what one could call a glandular unit proceeding from end-pieces to an interlobular duct: (1) acini; (2) intercalated ducts; (3) convoluted tubules; and (4) intralobular 'striated' ducts (Text-fig. 1).

The acini are composed of pyramidal cells with a foamy basophil cytoplasm, the basophilia being strongest near the base of the cell, where it cushions the nucleus. There are no distinct secretion granules anywhere in the cytoplasm. The cells do not stain appreciably with mucicarmine or alcian blue. They give, especially after Susa or Orth fixation, a positive PAS reaction, which is, however, much less strong than that of the mucous cells of the neighbouring major sublingual gland. Fine intercellular canals (Sekretkapillaren) can be detected. The units are so compact that they deserve the name 'acini', and should not be called alveoli, although they

* Present address: Department of Anatomy, Dalhousie University, Halifax, N.S., Canada.

are not always spheres but frequently elongated ovoids. Myo-epithelial cells (basket cells) are present embracing the acinar cells; they are not conspicuous in ordinary preparations, but can be clearly demonstrated by means of the alkaline phosphatase reaction (Pl. 1, fig. 3) (Leeson, 1956; Leeson & Jacoby, 1957). Several such acini are connected to a branching intercalated duct, a narrow tube lined by flat epithelial cells. Here, too, occasional basket cells occur, though they are less complex in their ramifications than those of the acini. These intercalated ducts are not very easily seen in the adult gland, as they are lying compressed between adjacent bulging structures, around which they often curve (Pl. 1, fig. 2; Pl. 4, fig. 25). A single intercalated duct becomes continuous with the next element, the convoluted



Text-fig. 1. Diagrammatic representation of the different epithelial portions of a glandular unit of the adult rat submaxillary gland. *A* = acini, *ID* = intercalated duct, *CGT(B)* = convoluted granular tubule after alcoholic Bouin fixation, *CGT(S)* = convoluted granular tubule after Susa fixation, *ISD* = intralobular striated duct. Myo-epithelial cells are not shown. The *CGT(S)* is also continuous with an intercalated duct; but this has been omitted from the diagram.

tubule. The transition from the low epithelium to the high columnar type of the convoluted tubule is fairly abrupt, but the point of entrance (junction) is rarely seen clearly. In fact, Loewenthal (1908), who gave the first detailed microscopic description of this gland, was unable to convince himself of this junction, and also Tupa (1926) in his otherwise exquisite account experienced difficulties about this point and mentions a lateral as well as an end-to-end junction. In our material, we have seen only the latter and cannot confirm the existence of the former. There are, however, variations of this junction which will be discussed later on. In this paper,

we shall, not only for convenience but also for reasons which will become clear later, refer to this junction throughout as the intercalated duct-striated duct junction, although in the adult it is, strictly speaking, an intercalated duct-convoluted tubule junction.

The convoluted tubule, which, in the adult gland, is perhaps the most striking epithelial component, is part of a widely branching tubular system. It is characterized by tall columnar epithelial cells with their nuclei lying near the base of the cell; below the nuclei short acidophil basal 'striations' can sometimes be discerned. The appearance of the cells above the nucleus depends on type of fixation and staining (see Text-fig. 1). For instance, after alcoholic Bouin fixation and staining with haematoxylin and eosin they will appear rather empty, almost vacuolated (e.g. Pl. 1, fig. 2; Pl. 3, fig. 16); and only high magnification will reveal the presence of dispersed fine, almost unstained granules (Pl. 3, fig. 17). On the other hand, after fixation with Susa, Zenker or Orth, they are more or less packed full with large distinct granules of unequal size, which stain intensely with, amongst others, Altmann's acid fuchsin and orange G (Pl. 3, fig. 18; Pl. 4, fig. 19). They give only a very weak PAS reaction and do not stain with either mucicarmine or alcian blue. These granules have been interpreted as secretion granules, and they can be found also within the lumen, presumably after discharge from the cells. Hence, these convoluted tubules are also referred to as 'serous' tubules or 'granular' tubules; and it is these segments which are so profoundly affected by hormones.

Such a branching convoluted 'granular' tubule eventually becomes continuous with the fourth segment, the 'striated' intralobular duct, well known in its appearance. It has high columnar cells with more or less centrally or even apically placed nuclei and a pronounced basal striation ('Streifenstücke' of the German authors). The junction between the two segments is sometimes abrupt, and the overall diameter of the tubule suddenly narrows down; or else the transition is more gradual, and over a certain stretch cells typical of either segment are intermingled (see Text-fig. 1 and Pl. 3, fig. 18).

These are the main four epithelial elements forming the bulk of a lobule. Adding to the complexity of the gland, there are found, not infrequently, a few scattered, probably aberrant, typical mucous acini. These are more often present in lobules adjacent to the neighbouring major sublingual gland than elsewhere and are identical with the acini of the latter. They were already noticed by Loewenthal (1908).

Finally, in some of the glands, complexes of narrow acinar and/or tubular formations, quite unlike anything else in the lobule, were observed. They form, as it were, little islands, yet connected to the general duct system, and their nature is quite obscure. These last two epithelial components are mentioned for the sake of completeness only and will not be referred to further.

Coming now to point (2), viz. the confusing nomenclature existing in the literature pertaining to the different segments of the gland—not only in the rat, but also in other species whose submaxillary gland has a corresponding composition, e.g. mouse, shrew, golden hamster—one has to consider the confusion in terminology both as to form and as to presumed function.

Thus, the acini are referred to as glandular tubules by Stormont (1932) and Duthie (1934), as lobes by Kurtz (1954), as tubulo-alveolar end-pieces by

Boerner-Patzelt (1955–56); and the convoluted granular tubules are referred to as acini by Honda (1927), as terminal tubules by Fekete (1941) and as serous alveoli by Pease (1956). This slackness in nomenclature is not only confusing, but often indicates a complete misinterpretation of the architecture of the gland. Probably thanks to Tupa's paper (1926), confusion as to form hardly exists in the French literature.

Regarding the functional interpretation of the same two main elements, the majority of workers assigns to the acini the term 'serous'. Grad & Leblond (1949) and Leblond (1950), however, declare them as 'mucous' and 'atypical mucous', respectively, in view of the fact that, after formol-bichromate fixation, they give a positive PAS reaction and stain metachromatically with toluidine blue. Gautier & Diomede-Fresa (1953) also call these cells 'mucous'. Schaffer (1908) had named them 'sero-mucinous', and Boerner-Patzelt (1955–56) concludes that they are more mucous than serous. Stormont (1932) introduced the term 'special serous' (see below). Other workers do not commit themselves (Tupa, 1926; Hillarp, 1949).

A decision as to the nature of the acinar cells is, indeed, difficult to make, but there is something to be said for considering them to be 'mucoid' rather than serous; there is, however, hardly any evidence for calling the heavily granulated cells of the convoluted tubules 'mucous'. Yet they are referred to as such by Kurtz (1954) and by Rutenburg *et al.* (1958) and, with some reservation, by Burkl (1953) and as 'mucoid' by Burstone (1956) and by Glenner & Lillie (1957).

Stormont (1932) in an attempt to classify the non-mucous cells of salivary and other glands introduced the term 'special serous' as distinct from sero-zymogenic. The latter term, characterizing cells with distinct zymogenic granules, basal chromidial substance and intercellular secretory capillaries, is, according to Stormont, applicable only to a very few distinguished members of this class of cells, e.g. the acinar cells of the pancreas, peptic cells of the stomach and some selected crescent cells of certain salivary glands. The unfortunate result is that such heterogenous types of cell as the acinar cells of the rat submaxillary gland and the 'granulated' cells of its convoluted tubules are both referred to as 'special serous'.

In view of this confusing and unsatisfactory state of affairs we shall, for the purpose of the present paper, follow Tupa's example and call the two elements *acinar cells* and *cells of the granular tubules*, respectively.

Not only has the functional nature of the granular tubules been subject to speculation, but even their precise position within the gland, their anatomical and developmental nature, has not been clearly recognized or established. Loewenthal (1908), who was probably the first to pose the question whether these tubules were a special gland somehow connected with the duct system or, in fact, transformed intralobular segments of this system, was unable—from his study of the adult gland—to answer it. Tupa (1926) did not discuss the origin of the tubules but merely described them as part of the duct system. Gabe (1950*a*) refers to the concept of the tubular granulated segments representing a specially differentiated portion of the excretory canal as a hypothesis. Shafer & Muhler (1956) say vaguely that the granular tubules *appear* to lie between the acini and the intralobular ducts. Vaguer still, inaccurate and incomplete is the account and diagram given by Bixler *et al.* (1958). Only Screebny *et al.* (1955) state that 'the cells of the intralobular ducts... undergo a slow transformation into secretory cells'.

Another point, which may be clarified by the study of the post-natal development of the gland, is the nature of the intercalated duct-striated duct junction, which up to date has puzzled most investigators and has remained somewhat obscure.

THE POST-NATAL DEVELOPMENT OF THE GLAND

During the study of the extensive literature relating to this gland we could find only one paper concerned with the post-natal development of the gland in rats. Screebny *et al.* (1955), though primarily interested in the proteolytic activity of extracts of the submaxillary gland of young male and female rats, give also a brief morphological account of the gland at five stages between 15 and 150 days, but this account is very general, superficial and in parts inaccurate. They begin with the curious statement that 'the general morphological pattern remains the same in all stages'. Later they do, however, refer rightly to the acinar formation running ahead of that of the convoluted tubules up to day 60. Further comments on this paper are best postponed until our own findings have been given.

MATERIAL AND METHODS

Seventy-six male and female rats aged from 1 day to 6 months were used. Up to 12 weeks the series includes glands differing in age by a week, thereafter by a month. For each stage at least three glands were examined, but for the more important stages, between 1 week and 8 weeks, up to ten glands were used. The animals were killed by a blow on the head, and the submaxillary gland or glands removed together with the major sublingual gland (retrolingual of Ranvier, 1886), which incidentally served as a convenient control for mucin stains.

The following fixatives were employed: 80 % alcohol, alcoholic Bouin, Susa and Orth. Serial sections from the paraffin-embedded material were cut at 6 μ. Some sections from individual glands were mounted separately; in most instances, a series of sections from glands of different ages were mounted in chronological order on the same slide. This was done not only to facilitate histological comparison of the various stages, but also to insure similar staining conditions throughout a series in order to put the assessment of differences in staining properties on a firm basis. The following staining techniques were used: haematoxylin and chromotrope, haematoxylin and eosin, azan stain, Altmann's acid fuchsin, Mayer's mucicarmine, alcian blue, the PAS reaction, Best's carmine (before and after saliva digestion) and metachromatic stains, such as toluidine blue, methylene blue and azure A.

RESULTS

We shall use the terms 'proximal' and 'distal' with reference to the flow of the secretion; hence the opening of the major excretory duct in the mouth cavity is the most distal point. But for the designation of the glandular end-pieces as formed during foetal life we shall use the expression 'terminal tubules'. These are not found as such in the adult gland, but are—as will be seen—transitory structures.

0–2 *days post-natal* (Pl. 1, figs. 1, 4, 5). Compared with the adult gland the organ is still in a relatively rudimentary state. Lobulation is just recognizable, but a fair amount of loose mucoid connective tissue still pervades the lobule. The larger inter-

lobular ducts with wide lumina are often seen as isolated units surrounded by more connective tissue. They have a columnar epithelium with a definite basal striation. The intralobular ducts have narrower lumina, a low columnar or cubical epithelium, the cells of which are so small that their nuclei appear crowded together; only in some cells is a low basal striation discernible. When cut longitudinally, these ducts can be seen to be continuous proximally with somewhat narrower ducts (Pl. 1, fig. 5), whose epithelium is lower still and whose equally 'crowded' nuclei are often elongated parallel to the direction of the duct. These narrow ducts, in turn are continuous with branching terminal tubules. The columnar or narrow-pyramidal cells of these terminal tubules are characterized by strongly eosinophil, PAS-positive (Pl. 1, fig. 4), rather fine granulations and by round basally situated nuclei. The extent of the granulation is so great that, in haematoxylin-chromotrope or haematoxylin-eosin preparations, a basophilia cannot be discerned, but in sections stained with neutral red or toluidine blue there appears to be some chromidial substance around the nucleus. The lumen of these terminal tubules can often be seen clearly. At some places at the periphery of the tubules one or two paler epithelial cells are 'budded' out (Pl. 1, fig. 5); these, too, are fundamentally eosinophil and often also show granulation. In an azan-stained section (after Susa fixation) the granules of the terminal tubules and also of some of the cell-buds stain in varying shades of blue, but within any cell the intensity of the stain is uniform, and the discreteness of the granules is brought out particularly well. Many mitoses are present in the terminal tubules (Pl. 1, fig. 1), including buds, and in all segments of the duct system.

Interpretation. The place of narrowing of the intralobular duct is, in our opinion, the site of the future intercalated duct-striated duct junction, and hence these two segments are, at this stage, already determined and mitotic proliferation takes care of their future growth. The nature of the 'budded' cells is more difficult to interpret. They have obviously arisen either by a process of differentiating mitosis or simply by movement from the cells of the terminal tubule, with which they share some features, such as eosinophilia and granulation, though the latter is often reduced. We believe that they are the immediate forerunners of the definitive acinar cells.

1 *week* (Pl. 1, fig. 6; Pl. 2, fig. 7). The gland appears somewhat more compact, but there is still plenty of loose mucoid connective tissue separating lobules and also the glandular units within the lobule. Basal striations in the cells of the inter- and intralobular ducts are more distinct and more widespread. Intercalated ducts are more clearly suggested by narrowing of the most proximal part of the intralobular duct system and by the arrangement of their nuclei. The fine granules of the terminal tubules are again found to be eosinophil and, in azan preparations, aniline blue-positive, but variation in staining intensity from cell to cell is more marked. The same holds for the PAS reaction (Pl. 2, fig. 7). Individual cells budded out from the terminal tubules are again seen. They are pale and may or may not contain granules with staining reactions similar to those of the terminal tubules. Numerous mitoses are present in the terminal tubules; occasional mitoses are seen in all segments of the duct system.

2 *weeks* (Pl. 2, fig. 8). Increased compactness of the lobules is now very definite. There is only a moderate intensity of staining of the granules of the terminal tubules both with eosin or chromotrope and with aniline blue. Around the terminal

tubules cellular buds are now seen frequently; they appear either as individual cells or even as small crescents; though pale, they have now a distinct general basophilia and no longer contain granules. In some specimens the formation of crescents is so advanced as to make the terminal tubules appear as 'centres', and there is no doubt that the crescents represent the future definitive acini. Definite intercalated ducts are present and are clearly continuous with the striated intralobular ducts, which, at places, show convolutions. Mitoses are fairly frequent in buds and terminal tubules, but are seen only occasionally in ducts.

3 *and* 4 *weeks* (Pl. 2, figs. 9, 10). Acini and terminal tubules with crescents now dominate the picture outnumbering by far the striated ducts; acini, crescents and buds, in turn, outnumber the remnants of the terminal tubules or 'centres'. The acinar cells have a somewhat foamy appearance with a well-marked basophilia. The eosinophilia of the granules of the terminal tubules and 'centres' is increased, especially so at 4 weeks, but the staining of the granules with aniline blue (after Susa fixation) is very intense at both periods, which makes these structures stand out most strikingly. Secretory material with similar staining reactions is also seen in the lumina of terminal tubules and intercalated ducts. In some places, the cells comprising the centres are reduced in height and their nuclei flattened at the bases; and here and there where these centres link up with intercalated ducts, 'centro-tubular' nuclei are seen (Pl. 2, fig. 11*b*). Convolutions of the striated ducts, noted already at 2 weeks, are now more advanced. Mitoses are most frequent in acini, buds and crescents, but are also seen in terminal tubules and intercalated ducts, more rarely in other ducts.

5 *and* 6 *weeks* (Pl. 2, figs. 11*a*, 12; Pl. 3, figs. 13, 14). Acini predominate by far over ducts. Centres are much reduced, both in number and size; in some specimens they are no longer present. In others they can be seen to be flattened, still containing granules staining deeply blue with aniline blue, and now forming the most proximal branching system (2nd order) of intercalated ducts. Each little branch is linked to an acinus which developed from an individual crescent. Of particular interest is the junction between an intercalated duct (1st order) and the proximal end of the now convoluted striated duct. It should be emphasized that, in contrast to the state of affairs in the adult gland, this junction is easy to see in these earlier developmental stages. The intercalated duct enters, as it were, the pole, sometimes with a few of its flat epithelial cells (nuclei) lying right in the interior of the striated duct, forming a kind of 'intussusception' (Pl. 4, figs. 22–25). Very often the most distal part of the intercalated duct curves round the first bend of the convoluted striated duct. The striated ducts show, in some of the specimens, the earliest signs of change into 'granular' tubules; i.e. beginning apical vacuolization (after alcoholic Bouin fixation) of scattered cells (Pl. 2, fig. 12), or accumulation of coarse yellow or orange granules after Susa fixation and azan staining. This change is confined to the more proximal segments of the intralobular striated duct system. The acini still show considerable mitotic activity; mitoses are less frequently seen in 'centres' and all ducts.

7, 8, 9, 10 *weeks* (Pl. 3, figs. 15, 16). The convolutions of the branching system of the intralobular striated ducts is now well displayed, although the space they occupy is still less than that occupied by acini. Centres with aniline blue-staining granules are

not present any more, i.e. they all have by now been transformed into the 2nd order intercalated ducts. The intercalated duct-striated duct junctions show frequently the 'intussusception' described above. From the seventh week on vacuolization or granulation, respectively, is definitely under way in the proximal portions of the convoluted ducts and, by the ninth week, has become fairly extensive. The distribution is at first erratic, and changed cells alternate with others as yet unchanged. Later whole continuous rows of changed cells are encountered. Some cells appear pale and 'empty' apically, others have already produced definite granules which stain yellowish-orange with the azan stain, and which vary in size from fine ones to very coarse ones (up to 3 μ or more) (Pl. 4, fig. 19 shows this in a mature tubule); many cells are stuffed full with these granules, and then their height and width are increased. In addition (in azan preparations) many of these cells have scattered, *red*-staining granules, also of varying sizes. Their occurrence is constant, but the significance of the difference in staining reaction is not understood. With the accumulation of granules in these cells their nuclei tend to lie in the basal third of the cell, sometimes flattened; and the basal striation is very much reduced or no longer discernible. This change, though predominantly localized in the proximal portions of the convoluted ducts, seldom involves, at these stages, their very 'tips' (intercalated duct-striated duct junction). Mitoses are rare, but when seen, are more frequent in ducts than in acini (Pl. 4, fig. 20).

3–6 *months* (Pl. 1, fig. 2; Pl. 3, figs. 17, 18; Pl. 4, fig. 19). The appearance of the gland is now very similar to that in the adult animal, already described. The large, highly convoluted, heavily granulated tubules dominate the picture. The granulation commonly extends right down to where the duct leaves the lobule, and often the change from the wide granulated portion to the somewhat narrower striated portion is fairly abrupt. Now, even some cells of the 'tips' are more or less filled with granules (Pl. 4, fig. 25); and an 'intussusceptional' junction is rarely found, as if with enlargement of the tubular cells the invaginated flat cells of the intercalated duct had been pushed out. This enlargement of the cells at the 'tip' of the convoluted tubules is often unequal or one-sided with the result that the point of junction appears to lie somewhat eccentrically to the very pole (Pl. 4, fig. 25). Such an arrangement could have caused Tupa (1926) to talk of a lateral connexion. But it should have become clear from the description of the various developmental stages that fundamentally the junction is an end-to-end one. Various factors combine to make its analysis difficult: (*a*) the convolution of the granular tubule, (*b*) the branching, in a Y- or T-shaped fashion, of the intercalated duct which frequently occurs close to the junction, and (*c*) the curved deviation of the intercalated duct from a straight course (Pl. 4, figs. 21–25). Sometimes instead of an abrupt change from flat epithelial cells to high-columnar ones there is a gradual increase in height involving three or four cells.

At 4 months duct mitoses were still observed.

The more important staining reactions of the various structures of the gland, both during its post-natal development and in its adult form, are summarized in Table 1, in which for comparison, a column for the acini of the major sublingual gland is included.

The table shows clearly the distinct difference between the granules of the terminal tubules and those of the granular tubules. The granules of the terminal tubules give a positive PAS reaction; this reaction is even stronger in the definitive acini, which might indicate that the secretion, in granular form, of the terminal tubules is akin to the material formed later in the acini. This adds a cytochemical relationship to the developmental one between these two elements.

Table 1

	Submaxillary gland				*Sublingual gland*
	Granules of terminal tubules and 'centres'	Acini*	Contents of intercalated ducts	Granules of granular tubules	Acini
Eosin or chromotrope	++	−	−	+	−
Aniline blue†	+++++	(+)	+	−	(±)
Orange G†	−	−	−	+++	−
PAS‡	+	++	(+)	(±)	+++
Best carmine (saliva-resistant)	+	−		−	−
Mucicarmine	−	(∓)	−	−	+++
Alcian blue	−	(∓)	−	−	+++
Metachromasia	−	(±)	−	−	(+)

* These do not include the aberrant mucous acini, which give the same staining reactions as do those of the sublingual gland.
† As used in the composite azan stain.
‡ Especially after Susa or Orth fixation.

It should be noted that only mast cells—always present in these sections—were found to give an alcohol-resistant metachromasia, but the much weaker, though definite metachromasia given by the acini of both the submaxillary and the sublingual glands was quickly removed, more or less completely, by alcohol. Staining of the acini with mucicarmine or alcian blue was negligible. Thus they do not qualify for the term 'mucous' nor, in view of the absence of secretory granules, for the term 'sero-zymogenic', though they are rich in chromidial substance. Further cyto- and biochemical work will be necessary to decide the nature of the secretion both of the acini and of the granular tubules.

DISCUSSION

The most striking points brought out by this investigation are: (*a*) the absence of acini at birth; (*b*) the part played in the development by the terminal tubules and (*c*) the relatively late differentiation of the granular tubules. Another interesting point, already noted by Screebny *et al.* (1955), is the considerable length of time required for the gland to reach full maturity; this is usually not attained before the third or fourth month, that is well past the pubertal stage of the animal.

This post-natal developmental period clearly falls into two phases, the first one from birth to approximately 6 weeks and the second thereafter. During the first phase acinar formation, clearly under way at 2 weeks, is in the foreground. Definitive acinar cells arise in the form of buds from the terminal tubules. This, together with mitotic proliferation of the 'budded' cells, results in the formation first of multiple crescents and eventually of acini. In this process the terminal tubules become first the 'centres' which, as previously described, are a quite remarkable

feature of the gland from 3 to 5 weeks, and then, by reduction in size of cells and loss of granules, become intercalated ducts of a 2nd order which form the twig-like links between the emancipated acini and the original intercalated ducts of the 1st order. Mitotic activity is present during this first phase also in the whole of the duct system and accounts for lengthening of its units and convolution of parts of this system. Screebny *et al.* (1955) do not seem to have recognized what we have called 'terminal tubules' and later 'centres'. This may be partly due to the fact that the earliest stages they studied were 15 days old; though it is surprising that they failed to see the centres at the 30 days' stage when they are most impressive. They state that the acini arise from those intralobular ducts which later transform into the granular tubules, which is obviously not so. Intercalated ducts are entirely omitted from their account.

During the second phase acinar growth gradually subsides and growth and differentiation of the intralobular striated ducts become more and more marked.

Some quantitative studies were made to supplement the descriptive histological observations. We measured in thirty-four specimens (covering the period from day 1 to week 24) diameters of the intralobular striated ducts and, in twenty-one specimens, from 6 weeks on, in addition, diameters of the granular tubules and also of the acini, which only then have achieved distinct separate existence. The mean values are given in a condensed form in Table 2 with the ranges in brackets.

Table 2. *Diameters in* μ

	Intralobular striated ducts	Granular tubules	Acini
1 day	25·3 (20–30)	—	—
1–3 weeks	27·2 (23–35)	—	—
4–5 weeks	30·5 (25–38)	—	—
6–7 weeks	30·2 (25–35)	33·1 (28–40)	26·4
8–10 weeks	31·7 (25–40)	36·9 (30–45)	26·3
12–14 weeks	35·4 (28–40)	43·4 (35–55)	26·8
4–6 months	35·6 (28–40)	44·5 (38–50)	26·4

They show that the diameter of the intralobular striated ducts increases steadily from birth to the third month. As the size of the lumen increases only by 2–3 μ, this growth is mainly due to increase in height of the constituent cells. The granular tubules, only measurable from the time they become recognizable as such, that is 6 weeks at the earliest, have greater diameters which increase to a greater extent, also up to the third month. Accumulation of granules within the cytoplasm naturally contributes to the ever increasing height of the cells. The acini, once formed, have a fairly constant size.

We also estimated the relative space occupied within a lobule by tubules and acini, respectively, the so-called T/A ratio, where T covers all intralobular duct segments, except the intercalated ducts. For the purpose of this estimation we used a series of Susa-fixed specimens. Sections stained by means of a modification of Wilder's silver impregnation for reticular fibres were found particularly suitable, giving a striking contrast between ducts and acini. Lobular areas were photographed; from the enlarged prints ducts and acini were cut out, collected separately and weighed. Table 3 gives the T/A ratios for the different ages, thus studied, and also the proportion of the total space occupied by tubules. The results confirm

quantitatively the visual impressions obtained. From 6 weeks on the ducts and/or tubules gain on the acini, and in the fully developed gland they take up half, or even more, of the total lobular space. This is due not only to increase in size of cells and accumulation of granules, but to a considerable degree also to cell proliferation. There is from 6 weeks on a shift in mitotic activity from acinar to duct cells. These duct mitoses were seen both in unchanged cells and in cells already containing secretory granules.

Table 3

Weeks	T/A	T as proportion of total
6	0·27	0·21
7	0·33	0·25
9	0·49	0·33
13	0·53	0·35
16	1·10	0·52

Apart from their morphogenetic role the terminal tubules, and centres respectively, also seem to possess secretory activity, judged by their rich granulation and the fact that material with staining reactions similar to those of the granules is found in related lumina and ducts. This secretory activity is only transitory and has ceased by the seventh week. At that time acini are fully formed ready to take over, and also the secretory changes in the convoluted tubules have commenced. In this connexion it is worth recalling the work of Plagge (1938) who found that removal or duct ligation of all four salivary glands (the two submaxillary and the two major sublingual) results in death of newborn rats within 5 days; but if only one of these glands is left intact, the animal will survive. This survival may not only be connected with the secretory function of the terminal tubules, but also with that of the striated ducts. The latter, as is well known, are assumed to be concerned with water transport; and it should be noted that striations are already differentiated at birth.

The formation of the intercalated ducts, 1st and 2nd order, and the nature of the intercalated duct-striated duct junction have already received sufficient comment. As to the granular tubules, the study of the present series proves beyond doubt that it is the convoluted branching system of the intralobular ducts whose cells—from about the sixth week on—are gradually transformed into secretory granule-producing cells; that this transformation, though somewhat erratic at first, proceeds fundamentally in a proximal-distal direction, except for the very 'tip' (i.e. place of junction with intercalated duct), where the change sets in relatively late. The transformation can eventually become so complete that only the most distal short segments remain unchanged, just prior to where they join the interlobular ducts.

Milstein (1950) studied regeneration in the rat submaxillary gland. Two of his observations are of interest in relation to our study. He describes the newly formed acini as staining deeply with eosin and with Altmann's acid fuchsin, and on his photographs they appear heavily granulated. One wonders whether these were not regenerated terminal tubules rather than acini, as he goes on to say that later the acini were found to be basophil. Secondly, he notes that in a regenerated lobule

the granular tubules were not in evidence before the eighth week after operation, which points to a time lag similar to that found in this study of the normal post-natal development.

Whilst the sex dimorphism of the mouse submaxillary gland has, since the work of Lacassagne (1940*a*), been well established and recognized, there is less clear-cut, or even conflicting, evidence on this point concerning the rat. Hammet (1923) noted that the submaxillary gland of rats was, at 150 days, relatively heavier in males than in females, and that lack of thyroid completely inhibited growth of the gland in females, whilst only markedly retarding it in males. Lacassagne (1940*b*) observed that injection of testosterone into female rats caused increase in diameter of the granular tubules (up to 53 μ), though untreated animals (male and female) did not show significant differences in tubule diameters. On the other hand, Grad & Leblond (1949) reported significantly larger tubule diameters in males than in females, at least at day 39 and 150. This was not confirmed by Arvy, Debray & Gabe (1950). Similarly, Raynaud & Rebeyrotte (1950) did not find a sex difference in rats of amylase activity in submaxillary gland pulp, in contrast to that found in mice (Raynaud & Rebeyrotte, 1949, 1950). Screebny *et al.* also deny the existence of a structural sex difference of the rat submaxillary gland, but observed a functional one: proteolytic activity of the glandular extract was markedly higher in males than in females. Our own material allows of the following contribution to this problem. There were no striking sex differences with regard to pace and extent of the maturation of the gland. In one series we measured the diameters of intralobular striated ducts and granular tubules on specimens from litter mates (male and female) which had had the same technical treatment. Up to and including 7 weeks there was no difference in duct or tubule diameters, but from 8 weeks on the diameters of both these structures were on the average 3–4 μ (range 1·5–7·4 μ) larger in the males than in the females, and in no instance did the figures for the female exceed those for the male. These results are, at least in part, in agreement with those of Grad & Leblond (1949).

The analysis of the mechanism of this sex dimorphism revealed the prime importance of the thyroid gland (Grad & Leblond, 1949; Raynaud, 1950; Gabe, 1950*b*; Arvy & Gabe, 1950*a*). Injections of thyroxine or feeding desiccated thyroid stimulate growth and granulation of the convoluted tubules (Arvy & Gabe, 1950*a*; Shafer & Muhler, 1956); thyroidectomy and thiourea have the reverse effects (Arvy & Gabe, 1950*a*; Arvy *et al.* 1950). Oestrogenic hormones have an action antagonistic to thyroxine (Arvy & Gabe, 1950*b*), whereas testosterone acts synergistically (Grad & Leblond, 1949; Shafer & Muhler, 1956). In addition, the existence and intervention of an hypophyseal factor has been evoked. However, in the work dealing with these aspects of the subject, the long drawn-out development of the gland has not always been properly taken into account. Grad & Leblond (1949) refer to, and even give measurements of, the granular tubules at a time (10 days and 39 days) when they are not yet differentiated. Gabe (1950*b*), Eartly & Leblond (1954) and Clark, Shafer & Muhler (1957) studied the effect of thyroxine after hypophysectomy in some series of animals, whose ages at the end of the experimental periods were such that granular tubules could hardly have been expected to have developed, or only very little so. They concluded that hypophysectomy causes

atrophy of the granular tubules, when it would be more exact to say that it causes failure of tubular differentiation or development, at any rate at some of the stages studied.

SUMMARY

1. The post-natal development (from day 1 to week 24) of the rat submaxillary gland has been studied.

2. A brief account is given first of the architecture of the adult gland and attention drawn to the confusing terminology in general use as regards its epithelial components.

3. At birth, acini are not yet formed. The intralobular ducts, already segmentally determined and to some extent differentiated, end in a branching system of terminal tubules, whose cells are highly granulated.

4. From the first week on, acinar cells are 'budded' off from the terminal tubules and proliferate mitotically, gradually forming crescents around the terminal tubules, which become 'centres'.

5. These 'centres', by reduction in size of their cells and loss of granules, are, from the fifth week on, transformed into intercalated ducts (2nd order), and all crescents become acini.

6. At 6 weeks, the phase of acinar development combined with the regression of the terminal tubules is practically complete, and growth and differentiation of the intralobular striated ducts come into the fore, coupled with a shift in mitotic activity from acini to duct cells.

7. With the elaboration of secretion granules these ducts become transformed (in a proximal-distal direction) into the convoluted granular tubules. This transformation is often still incomplete at 4 months.

8. The hitherto obscure intercalated duct-striated duct junction is analysed and described in some detail.

9. Data are given for duct and tubule diameters and tubular/acinar ratios during the post-natal development of the gland.

10. There was no sex dimorphism of the developing gland up to 7 weeks; thereafter duct and tubule diameters were greater in the male than in the female.

11. The importance, particularly for endocrinological work, of the knowledge of the long drawn-out development of the gland is stressed.

This work was carried out during the tenure by one of us (C.R.L.) of a British Medical Association Ordinary Research Scholarship. We wish to thank Prof. J. S. Baxter and Dr M. B. Craigmyle for their criticisms of the manuscript; Mr L. Jones for his conscientious and unflinching help in the preparation of the sections and in the micro-photography; and Miss M. White for the execution of Text-fig. 1.

ADDENDUM

A correction which we had proposed for our abstract (Light- and electron-microscopic observations on post-natal stages of the rat submaxillary gland. By C. R. Leeson and F. Jacoby; this Journal (1958), **92**, 659) was unfortunately incompletely reproduced. For 'special serous' read each time 'granular' and for 'secretory ducts' read 'striated ducts'.

REFERENCES

ARVY, L., DEBRAY, CH. & GABE, M. (1950). Action de la thiourée sur la glande sous-maxillaire du rat albinos. *C.R. Soc. Biol., Paris*, **144**, 111–113.

ARVY, L. & GABE, M. (1950*a*). Action de la thyroidectomie et des injections de thyroxine sur la glande sous-maxillaire de la souris albinos. *C.R. Acad. Sci., Paris*, **230**, 1611–1613.

ARVY, L. & GABE, M. (1950*b*). Action des injections de thyroxine et de folliculine sur la glande sous-maxillaire de la souris albinos. *C.R. Acad. Sci., Paris*, **230**, 2333–2334.

BIXLER, D., WEBSTER, R. C. & MUHLER, J. C. (1958). The effect of pilocarpine on the submaxillary gland of the rat. *J. dent. Res.* **37**, 649–653.

BOERNER-PATZELT, D. (1955–56). Die Mundspeicheldrüsen des Goldhamsters. *Anat. Anz.* **102**, 317–332.

BURKL, W. (1953). Zur Klassifizierung der Schleimdrüsensekrete in der Histologie. *Z. Zellforsch.* **39**, 74–84.

BURSTONE, M. S. (1956). Esterase of the salivary glands. *J. Histochem. Cytochem.* **4**, 130–139.

CLARK, P. G., SHAFER, W. G. & MUHLER, J. C. (1957). Effect of hormones on structure and proteolytic activity of salivary glands. *J. dent. Res.* **36**, 403–408.

DUTHIE, E. S. (1934). Studies in the secretion of the pancreas and salivary glands. *Proc. Roy. Soc.* B, **114**, 20–47.

EARTLY, H. & LEBLOND, C. P. (1954). Identification of the effects of thyroxine mediated by the hypophysis. *Endocrinology*, **54**, 249–271.

FEKETE, E. (1941). Histology, in *Biology of the Laboratory Mouse*, chapter 3, ed. by Snell, Philadelphia: Blakiston Co.

GABE, M. (1950*a*). Action de l'hypophysectomie et des injections d'extraits hypophysaires sur la glande sous-maxillaire du rat albinos. *Arch. Anat. micr.* **39**, 15–34.

GABE, M. (1950*b*). Action de la thyroxine sur la glande sous-maxillaire du rat hypophysectomisé. *C.R. Acad. Sci., Paris*, **230**, 1317–1318.

GAUTIER, A. & DIOMEDE-FRESA, V. (1953). Étude au microscope électronique de l'ergastoplasme des glandes salivaires du rat. *Mikroskopie*, **8**, 23–30.

GLENNER, G. G. & LILLIE, R. D. (1957). The histochemical demonstration of indole derivatives by the post-coupled *p*-dimethylaminobenzylidene reaction. *J. Histochem. Cytochem.* **5**, 279–296.

GRAD, B. & LEBLOND, C. P. (1949). The necessity of testis and thyroid hormones for the maintenance of the serous tubules of the submaxillary gland in the male rat. *Endocrinology*, **45**, 250–266.

HAMMETT, F. S. (1923). Studies of the thyroid apparatus. XV. The growth of the heart, lungs, liver, kidney, spleen, submaxillary glands and eyeballs in male and female albino rats thyro-parathyroidectomized and parathyroidectomized when 100 days of age. *Amer. J. Anat.* **32**, 75–94.

HILLARP, N. (1949). Some critical remarks on the problem of the double innervation of salivary gland cells. *Acta Anat.* **8**, 190–200.

HONDA, R. (1927). The general functional significance of the mitochondria in the submaxillary gland of the adult albino rat. *Anat. Rec.* **34**, 301–312.

KURTZ, S. M. (1954). Cytologic studies on the salivary glands of the rat in reference to the aging process. *J. Geront.* **9**, 421–428.

LACASSAGNE, A. (1940*a*). Dimorphisme sexuel de la glande sous-maxillaire chez la souris. *C.R. Soc. Biol., Paris*, **133**, 180–181.

LACASSAGNE, A. (1940*b*). Réactions de la glande sous-maxillaire à l'hormone mâle, chez la souris et le rat. *C.R. Soc. Biol., Paris*, **133**, 539–540.

LEBLOND, C. P. (1950). Distribution of PAS reactive carbohydrates in the adult rat. *Amer. J. Anat.* **86**, 1–50.

LEESON, C. R. (1956). Localization of alkaline phosphatase in the submaxillary gland of the rat. *Nature, Lond.*, **178**, 858–859.

LEESON, C. R. & JACOBY, F. (1957). Alkaline phosphatase reaction of the myo-epithelial cells in the rat submaxillary gland. *J. Anat., Lond.*, **91**, 579.

LOEWENTHAL, N. (1908). Drüsenstudien. III. Die Unterkieferdrüse des Igels und der weissen Ratte. *Arch. mikr. Anat.* **71**, 588–666.

MILSTEIN, B. B. (1950). Regeneration in the submaxillary gland of the rat. *Brit. J. exp. Path.* **31**, 664–669.

PEASE, D. C. (1956). Infolded basal plasma membranes found in epithelia noted for their water transport. *J. Biophysic. Biochem. Cytol.* **2**, 203–208.

PLAGGE, J. C. (1938). Vital importance of salivary glands to newborn rats. *Amer. J. Physiol.* **124**, 612–619.

RANVIER, L. A. (1886). Étude anatomique des glandes, connues sous les noms de sous-maxillaires et sublinguales chez les mammifères. *Arch. physiol. normale et pathol.* Sér. III, V, **8**, 223–256.

RAYNAUD, J. (1950). Action de la thyroxine sur la glande sous-maxillaire de la souris castrée. *C.R. Soc. Biol., Paris*, **144**, 245–248.

RAYNAUD, J. & REBEYROTTE, M. P. (1949). Différence de l'activité amylasique de la salive des souris mâles et des souris femelles. *C.R. Acad. Sci., Paris*, **228**, 433–435.

RAYNAUD, J. & REBEYROTTE, P. (1950). Activité amylasique des glandes salivaires des rats et des souris. *C.R. Soc. Biol., Paris*, **144**, 636–638.

RUTENBURG, A. M., RUTENBURG, S. H., MONIS, B., TEAGUE, R. & SELIGMAN, A. M. (1958). Histochemical demonstration of β-D-galactosidase in the rat. *J. Histochem. Cytochem.* **6**, 122–129.

SCHAFFER, J. (1908). Zur Histologie der Unterkieferspeicheldrüsen bei Insektivoren. *Z. wiss. Zool.* **89**, 1–27.

SCREEBNY, L. M., MEYER, J., BACHEM, E. & WEINMANN, J. P. (1955). Postnatal changes in proteolytic activity and in the morphology of the submaxillary gland in male and female albino rats. *Growth*, **19**, 57–74.

SHAFER, W. G. & MUHLER, J. C. (1956). The effect of desiccated thyroid, propylthiouracil, testosterone, and fluorine on the submaxillary glands of the rat. *J. dent. Res.* **35**, 922–929.

STORMONT, D. L. (1932). The salivary glands. In *Special Cytology*, Vol. I, ed. by E. V. Cowdry. New York: Paul B. Hoeber.

TUPA, A. (1926). Recherches sur les processus cytologiques dans la glande sous-maxillaire du rat et de la souris. *Bull. Histol. Tech. micr.* **3**, 293–306.

EXPLANATION OF PLATES

All the figures are of rat submaxillary glands, the majority of which was fixed in alcoholic Bouin. This was found to give a convenient degree of shrinkage separating the glandular units thus helping in their analysis and display.

PLATE 1

Fig. 1. 1 day; fix. alc. Bouin, haematoxylin (Hx.) and chromotrope (Chr.) The lobule consists mainly of branching terminal tubules with granulated cells, six of which are in mitosis. Two intercalated ducts are also present, one in L.S., one in T.S. (↓). × 290.

Fig. 2. Adult (6 months); fix. alc. Bouin, Hx. and Chr. Note contrast to Fig. 1. The pale curved elements are the convoluted granular tubules. There are two intralobular striated ducts near the right edge in the middle and one interlobular duct at the very top. Two intercalated ducts are arrowed. Remaining units are acini. × 180.

Fig. 3. Adult; fix. acetone, Gomori's alkaline phosphatase reaction, incub. time 1 hr., to show branching myo-epithelial (basket) cells. × 890.

Fig. 4. 1 day; fix. alc. Bouin, PAS reaction and Hx., to show the fine granules in the cells of the terminal tubules, which are PAS-positive, and a branching intralobular duct. × 335.

Fig. 5. Newborn; fix. alc. Bouin, Hx. and eosin. Intralobular duct narrowing down to an intercalated duct; also terminal tubules and cell 'buds' (↓). × 350.

Fig. 6. 1 week; fix. alc. Bouin, Hx. and eosin. A system similar to that of Fig. 5. Cell buds arrowed. × 560.

PLATE 2

Fig. 7. 1 week; fix. Susa, PAS reaction and Hx. The reaction is of varying intensity in the cells of the terminal tubules and cell buds. Note mitoses in intercalated duct and terminal tubules. × 535.

Fig. 8. 2 weeks; fix. alc. Bouin, Hx. and Chr. One intralobular striated duct in T.S., one intercalated duct in L.S. joined to a terminal tubule showing mitosis. Note weakly acidophil granulation of the terminal tubules. Many acinar cell buds and crescents, in places isolated. × 560.

Fig. 9. 3 weeks; fix. alc. Bouin, Hx. and Chr. A system is shown comprising intralobular and intercalated ducts, heavily granulated terminal tubules, which have become 'centres', and acinar crescents. Note the T-shaped junction between the ducts. ×710.

Fig. 10. 4 weeks; fix. alc. Bouin, Hx. and Chr. A system similar to that of Fig. 9 is shown. Note end-to-end junction of the ducts and the very deep staining of the granules of the centres. ×420.

Fig. 11*a*. 5 weeks; fix. alc. Bouin, Hx. and Chr. Note convolutions of intralobular striated ducts. 'Centres' reduced in size and number. ×305.

Fig. 11*b* (Inset). 5 weeks; fix. Susa, Hx. and eosin. T.S. of terminal tubule with crescents. In the very centre a nucleus of a 'centro-terminal tubular' cell. ×515.

Fig. 12. 6 weeks; fix. alc. Bouin, Hx. and Chr. Remnant of a centre in top right corner. An intralobular striated duct (L.S.) shows ill-defined junction with an intercalated duct. First signs (vacuolization) of transformation of some of the cells of the striated duct (↓). ×365.

Plate 3

Fig. 13. 6 weeks; fix. alc. Bouin, Hx. and Chr. Two systems can be made out; the upper one shows the reduction in size of the cells of the former centres, which have now become intercalated ducts, 2nd order (×). Acinar mitosis arrowed. ×340.

Fig. 14. 6 weeks; fix. alc. Bouin, PAS reaction and Hx. Note two very much 'reduced' (narrowed) centres (↓), the flat cells of which still contain PAS-positive granules. To the left, portion of an intralobular striated duct. ×515.

Fig. 15. 8 weeks; fix. alc. Bouin, Hx. and Chr., to show branching convoluted duct with more advanced transformation into 'granular' tubule. The change is clearly confined to the proximal segments. An ill-defined 'junction' in top left corner. ×320.

Fig. 16. 10 weeks; fix. alc. Bouin, Hx. and Chr. Further progress in transformation of the convoluted tubules. ×180.

Fig. 17. 12 weeks; fix. alc. Bouin, Hx. and Chr. Well-preserved basal striations in inter- and intralobular ducts. Fine granules can be seen in the transformed cells of the convoluted tubules. In top right corner a junction (↓). Note here lack of transformation of the cells of the convoluted tubule. ×335.

Fig. 18. 16 weeks; fix. Susa, azan, to show the large amount of secretion granules in the cells of the convoluted granular tubules. Note also the amount of lobular space now occupied by granular tubules. In the lower part of the field, the transition from granular to non-granular intralobular duct can be seen. ×200.

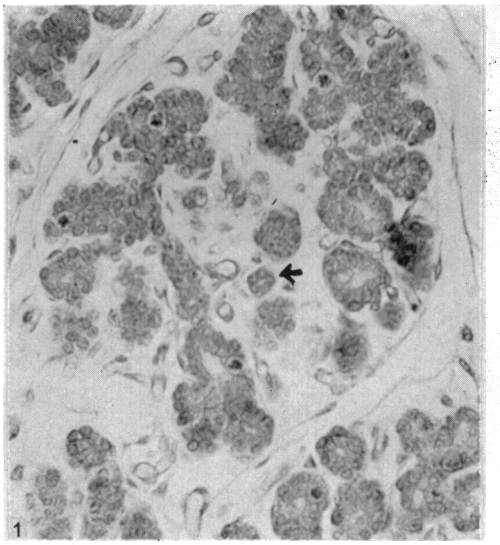
Plate 4

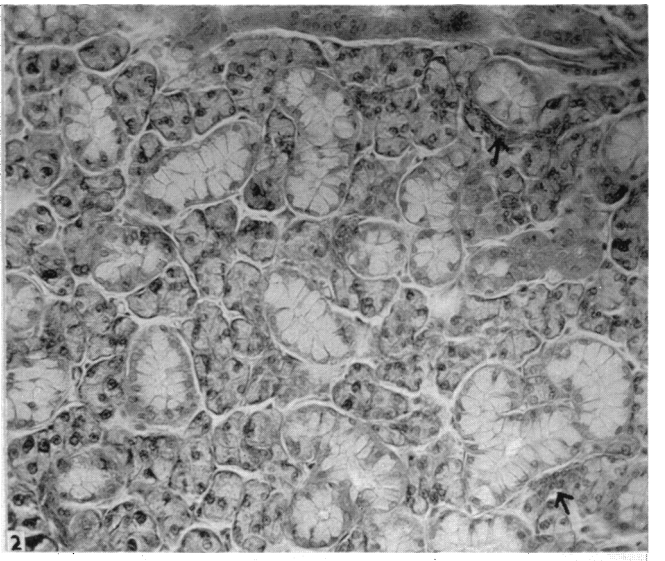
Fig. 19. 4 months; fix. Susa, azan. Most proximal portion of a convoluted granular tubule. Note variation in size of granules and in amount of granules in different cells, particularly the lack of granules in the coiled-in 'tip' of the tubule which just seems to show the 'junction' (↓). ×515.

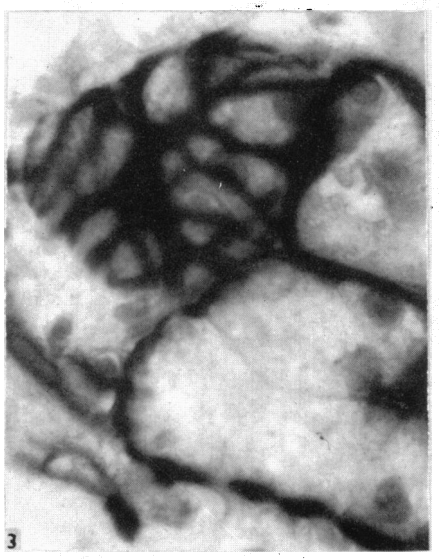
Fig. 20. 7 weeks; fix. Susa, azan, to show two mitoses (↓) in convoluted tubules. All the pale cells of these tubules are in the process of transformation. ×355.

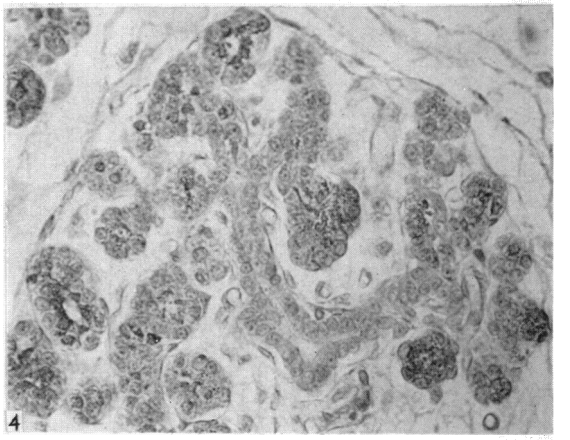
Fig. 21. 14 weeks; fix. alc. Bouin, Hx. and Chr. A branching striated duct is shown. At or near the 'pole' of each branch there is a junction with an intercalated duct. This specimen was not as fully developed as one would have expected from its age. ×490.

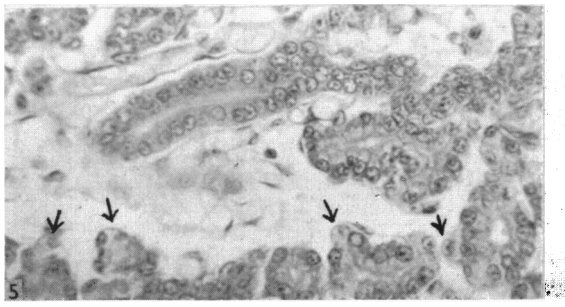
Figs. 22–25. Show various intercalated duct-striated duct junctions (Fig. 22, 8 weeks (×515), fix. alc. Bouin, Hx. and Chr.; Figs. 23, 10 weeks (×355), 24, 4 months (×625), and 25, 6 months (×700) fix. Susa, azan.). All show varying degrees of 'intussusception'; T-shaped junction is indicated in Figs. 22, 23 and possibly 24; the curved course of the intercalated duct around the convoluted granular tubule is well shown in Fig. 25. Note again scarcity of granulation in the cells of the very tip of the convoluted tubule in Figs. 24 and 25.

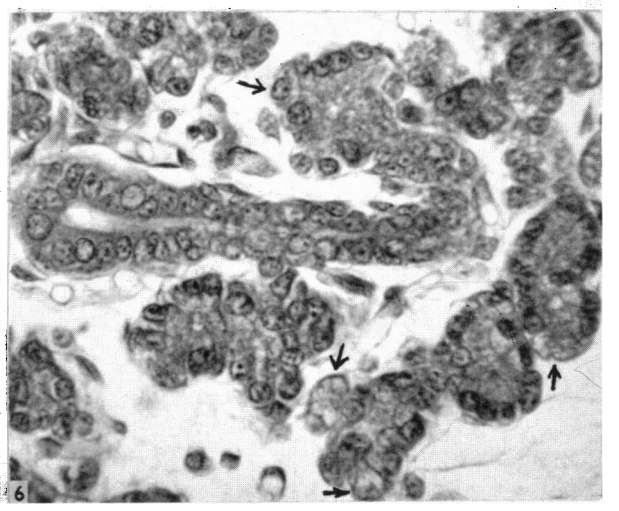






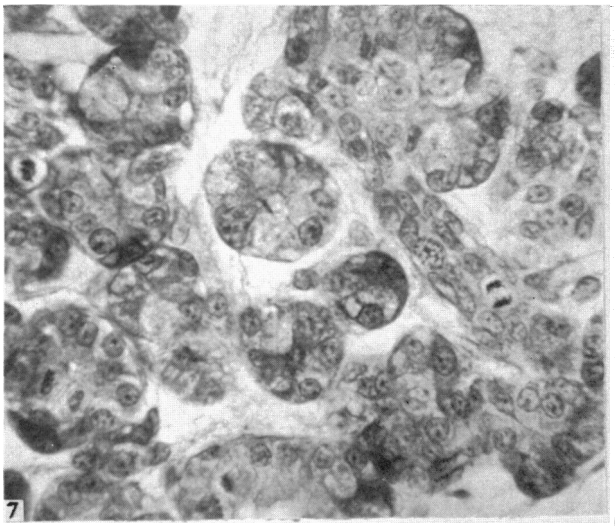


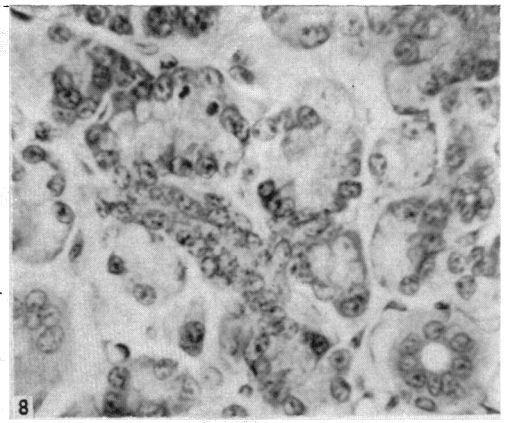


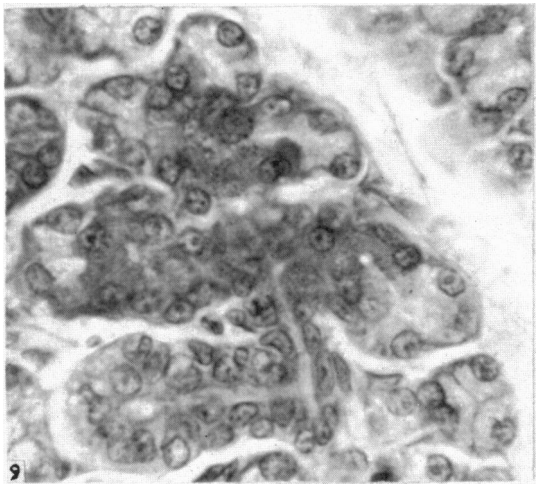


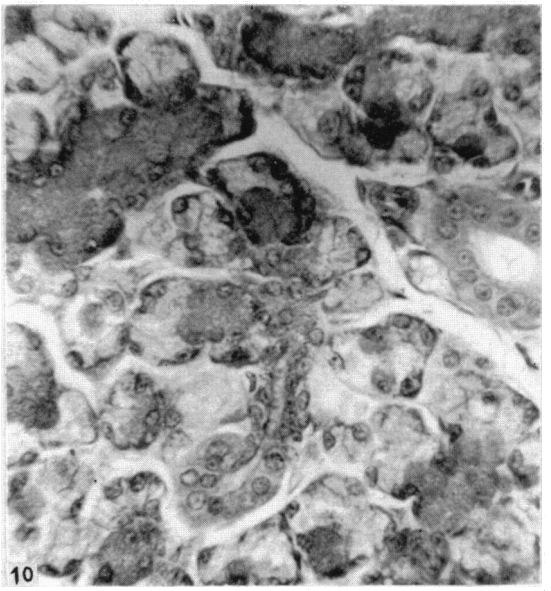
JACOBY AND LEESON—POST-NATAL DEVELOPMENT OF THE RAT SUBMAXILLARY GLAND

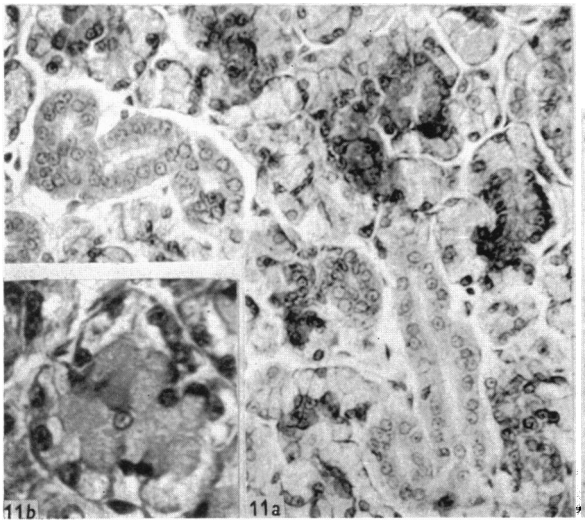
(*Facing p.* 216)

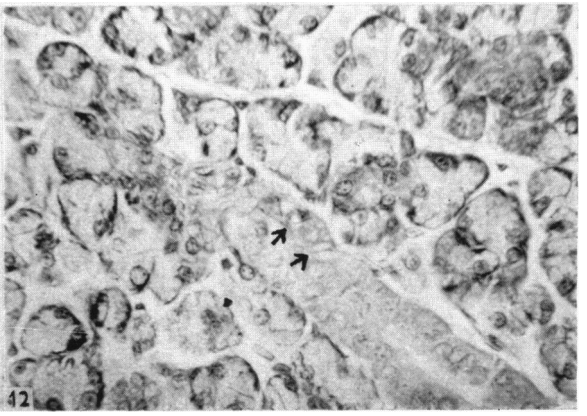

7

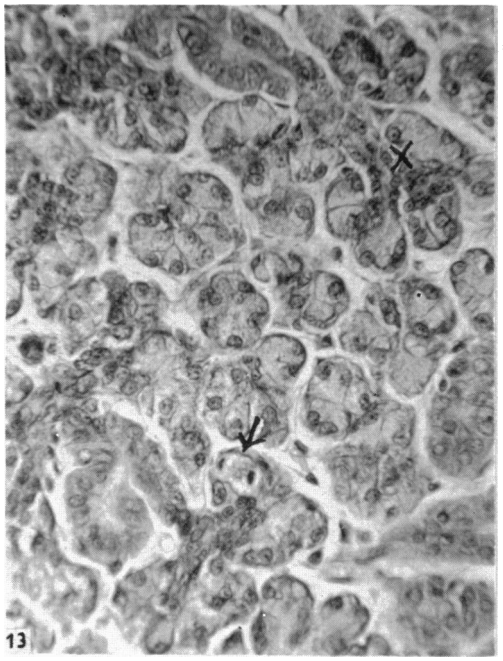

8

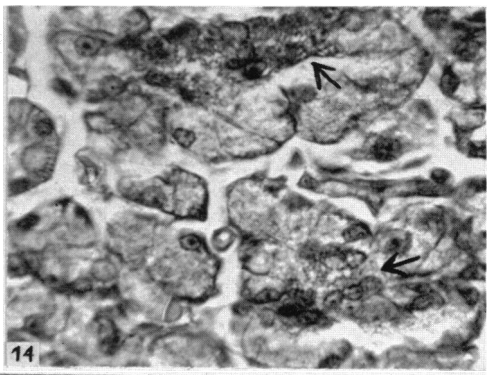

9

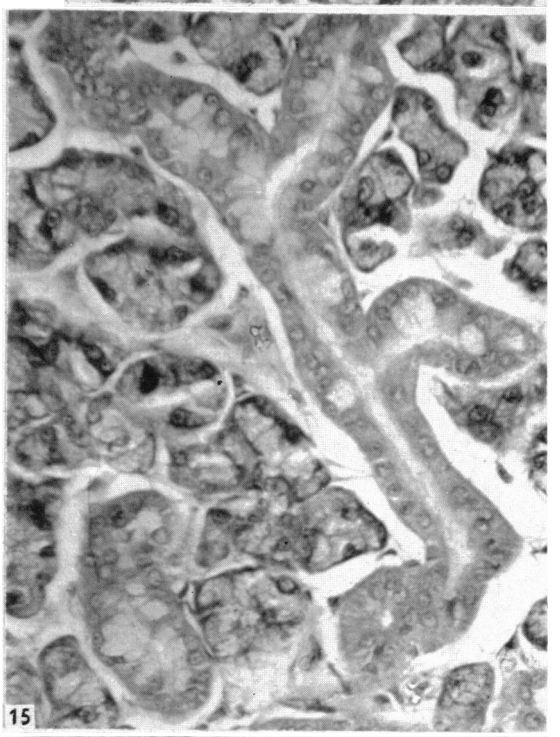

10

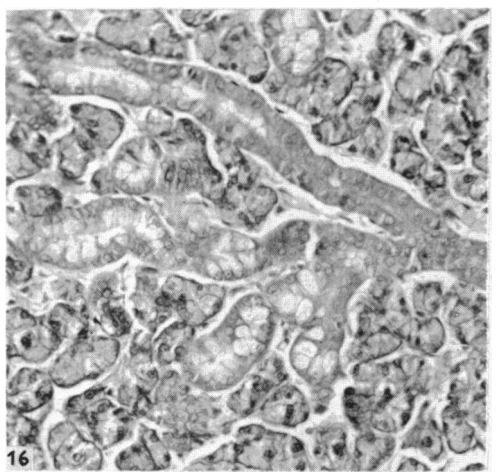

11b
11a

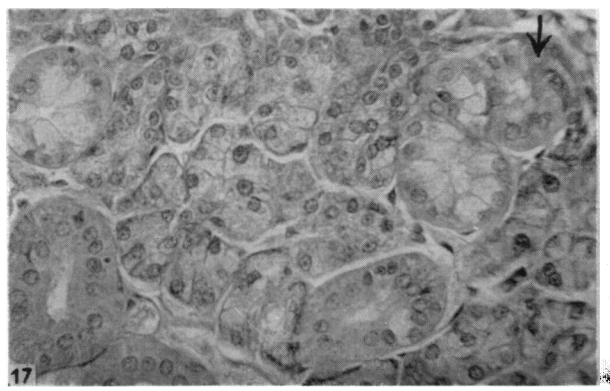

12

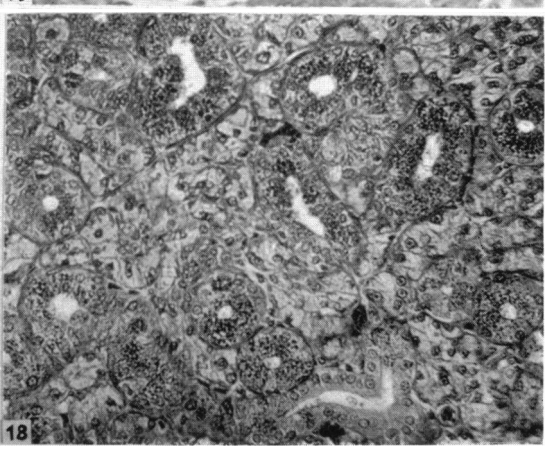

13

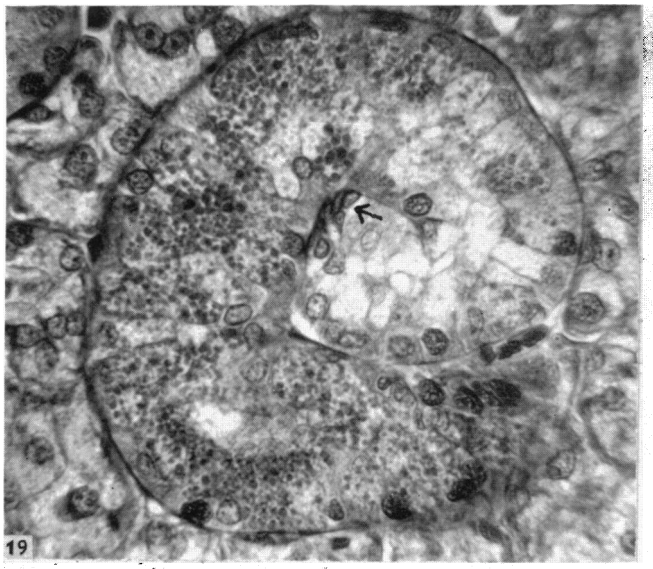

14

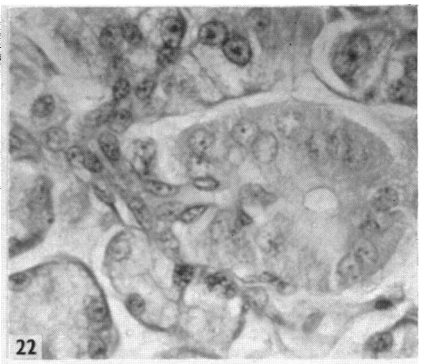

15

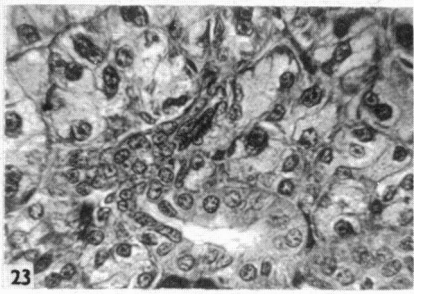

16

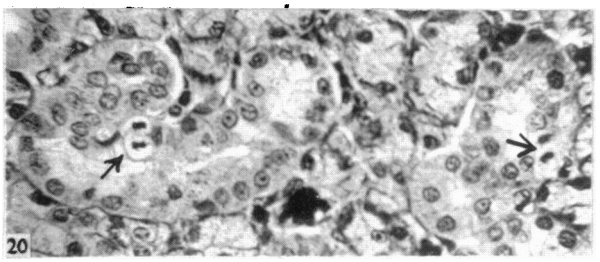

17

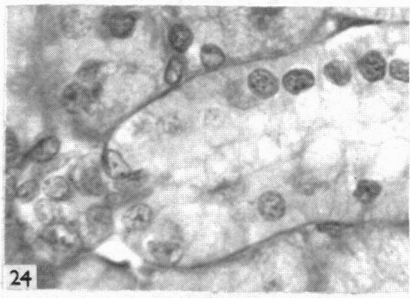

18

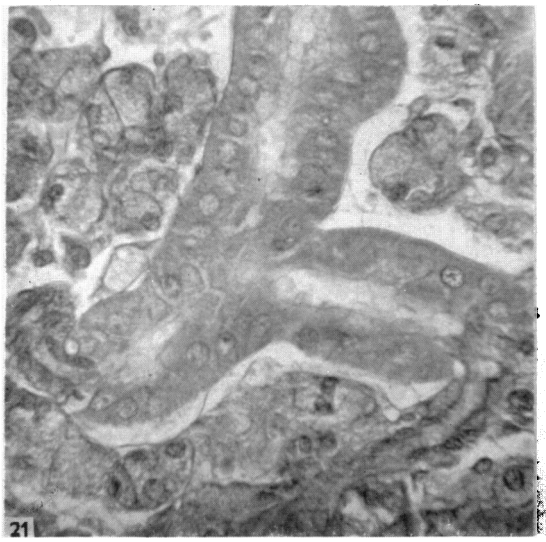


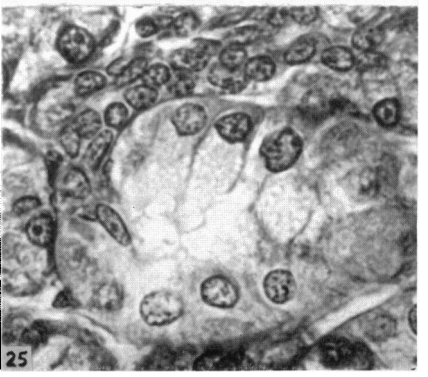












JACOBY AND LEESON—POST-NATAL DEVELOPMENT OF THE RAT SUBMAXILLARY GLAND