J. Anat. (1990), **172**, pp. 115–127
With 4 figures
Printed in Great Britain

Morphometric studies on the development and sexual dimorphism of the submandibular gland of the mouse

N. R. JAYASINGHE, G. H. COPE AND S. JACOB

*Department of Anatomy, Faculty of Medicine, University of Peradeniya, Peradeniya, Sri Lanka and * Department of Biomedical Science, School of Biological Sciences, The University of Sheffield, Sheffield S10 2TN, England*

(*Accepted* 29 *March* 1990)

INTRODUCTION

The mouse submandibular gland possesses two morphologically and biochemically distinct exocrine compartments with different modes of secretory activity. These are the acini and the granular convoluted tubules. The granular convoluted tubule is a specialised type of secretory canal that lies between the intercalated and striated ducts and is well-marked in the submandibular glands of rodents (Young & Van Lennep, 1978). The influence of hormones on the development of the granular convoluted tubule in rodents has attracted much attention since the discovery of sexual dimorphism in the submandibular gland of the mouse (Lacassagne, 1940). After the discovery of kallikrein in high concentrations in the submandibular glands of several species, workers have been able to purify several other biologically active polypeptides from the submandibular gland which are responsible for growth and differentiation. Immunocytochemical findings indicate that these growth factors are localised in the secretory granules of the granular convoluted tubule cells (Goldstein & Burdman, 1965; Schwab, Stockel & Thoenen, 1976). Nerve growth factor (Cohen, 1960), epidermal growth factor (Cohen, 1962), epithelial growth factor (Jones, 1966) and mesodermal growth factor (Barka, 1980) are some of the polypeptides that have been purified from the mouse submandibular gland. The synthesis of these polypeptides has been reported to be androgen-dependent (Lyon, Hendry & Short, 1973; Barthe *et al.* 1974).

In spite of the great interest in the functional aspect of the granular convoluted tubule, the differentiation of the gland and the influence of testosterone on the development of the granular tubule have not been critically analysed by quantitative morphology. The postnatal development and sexual dimorphism (Harvey, 1952; Disher & Elias, 1967; Gresik & MacRae, 1975) and the ultrastructure of the adult mouse submandibular gland (Caramia, 1966; Yohro, 1970; Rogers & Brown-Grant, 1971; Gresik & MacRae, 1975; Chretien, 1977) have only been qualitatively described. Although there have been some quantitative estimates on sexual dimorphism of the adult gland (Smith & Frommer, 1975; Kaiho, Nakamura & Kumegawa, 1975; Murphy *et al.* 1981; Watson *et al.* 1982), postnatal developmental changes have again not been adequately analysed by reliable quantitative techniques.

The present investigation gives a detailed quantitative description of the postnatal development and the sexual dimorphism of the mouse submandibular gland. The changes in the volume proportions of all the structural components of the gland were analysed from birth up to six weeks of age and for the first time the sizes of acinar cells and the granular tubule cells have been measured. The quantitative estimates thus

obtained serve to evaluate precisely the differentiation of various cell types within the gland as well as sexual dimorphism at different stages of development. It is hoped that the results obtained will provide an accurate baseline for further investigations on the response of the gland to hormonal stimulation.

MATERIALS AND METHODS

Swiss Albino mice were maintained in an animal house whose environment was carefully controlled. The animals were exposed to an alternating 12 hours light/dark cycle and the temperature of the laboratory remained constant at 18 °C. Eight male mice and eight female mice were used in each group. Each animal was weighed separately on the day of the experiment. They were killed at birth and at 2, 4 and 6 weeks of age.

A freshly prepared mixture of phosphate buffer and glutaraldehyde stock (25% glutaraldehyde) 9:1 V:V (pH 7·2) was used as the fixative. A perfusion apparatus which allowed the sequential perfusion of two fluids into an animal without an intervening drop in perfusion pressure (Bower, 1981) was used for perfusing the animals. After deeply anaesthetising each animal with ether the chest wall was opened. While the heart was still beating a plastic cannula was inserted into the left ventricle. The right atrium was pierced to allow blood to escape and then the prewash fluid (isotonic saline) was pumped through the cannula for one minute after which the fixative was pumped through for a further three minutes. A pressure of 80–100 mm Hg was maintained while the fluids were perfused through the vascular system.

Both submandibular glands were carefully removed and the glands were further fixed by immersion in the same fixative. One of the pair of glands was diced very finely in ice-cold fixative and left at 4 °C. This tissue was processed for electron microscopy. The other gland, which was intended for light microscopy, was cut into 6–8 pieces and placed in a pot containing the same fixative and left at 4 °C overnight. Each gland was weighed separately after fixation was complete. On the following day the tissues intended for light microscopy were washed 4–5 times in cold buffer. After dehydration the tissue pieces were embedded in JB 4 plastic resin. Tissues were sectioned using a Sorvall JB 4-A Porter–Blum microtome. Semithin sections were cut at 1·5 μm using dry glass knives. Sections were mounted on glass slides and dried at 60 °C on a hot plate. One series of sections was stained with Toluidine Blue for about 30 seconds. After differentiation with 50% ethanol, dried slides (at 60 °C) were mounted in Polymount. A second series of sections from the same block was stained with haematoxylin for one minute. After washing briefly with tap water the slides (dried at room temperature) were mounted in Polymount.

Morphometric analysis

Sampling methods

Five of the best perfused glands were selected for each age group after preliminary observations. One block per animal was chosen at random. Ten sections (1·5 μm thick) were cut and collected from each block at intervals of 100 μm and stained with Toluidine Blue. A total of 10 slides per animal and four fields per section was taken and their components were analysed by point counting. In the second series of sections which were stained with haematoxylin, two slides per animal (per block) were obtained. Four fields per section were analysed for the nuclear content of acinar cells and tubule cells.

For point counting, the slides were examined and analysed directly with a light

microscope fitted with a drawing tube, which was used in reverse of its usual mode. An image of a squared test lattice of 18 mm spacing with 25 line intersections was superimposed onto the field of view seen through the microscope. An unbiased systematic sampling procedure was devised using the same ×40 magnification flat field objective throughout. The magnification of the test lattice was standardised on each occasion by adjusting the length of the drawing tube with reference to a slide engraved with a micrometer scale (Cope, 1982).

Estimation of volume density (V_v)

The analyses were based on a total sample of 1000 test points per animal, 25 per field. Points falling on ten different tissue components of the gland were recorded separately for each slide. The tissue components chosen were granular convoluted tubule nuclei + cytoplasm, granular convoluted tubule granules, granular convoluted tubule lumina, acinar cells, blood vessels, excretory duct, striated duct, intercalated duct, extracellular spaces and other cell types. The mean values obtained from the five glands of the five animals were calculated and the mean of these values was taken as the volume density of a component at each age group. Student's *t* test was applied to compare the results from animals of different developmental groups and between the sexes.

Estimation of mean cell volume ($\bar{V}$ *cell*)

The mean cell volumes of acinar cells and granular convoluted tubule cells were estimated by point count analyses. The mean nuclear profile area was estimated by dividing the total nuclear profile area, determined by point counting, by the number of nuclei present in that area (forbidden line technique, Gundersen, 1977).

$$\text{Mean nuclear profile area } (\bar{A}_{nuc}) = \frac{\text{total nuclear profile area}}{\text{total number of nuclear profiles}}$$

$$\bar{A}_{nuc} = \pi r^2$$

$$\text{Nuclear profile diameter (d)} = 2r$$

$$\text{Mean nuclear diameter } (\bar{D}_{nuc}) = \frac{4d}{\pi} \text{ (Abercrombie, 1946)}$$

$$\text{Corrected nuclear radius } (\bar{R}_{nuc}) = \frac{\bar{D}}{2}$$

$$\text{Mean nuclear volume } (\bar{V}_{nuc}) = \frac{3}{4}\pi(\bar{R}_{nuc})^3$$

(assuming nuclei to be spherical)

$$\text{Mean cell volume } (\bar{V}_{cell}) = \frac{\text{Volume of nucleus}}{V_v \text{ nucleus}} = \frac{\bar{V}_{nuc}}{V_{vnuc}}$$

RESULTS

At birth the submandibular gland consisted of acini and an undifferentiated duct system (Fig. 1*a*). In the duct system, the terminal tubule made up 21 % of the volume of the gland whereas the interlobular and intralobular ducts were of similar

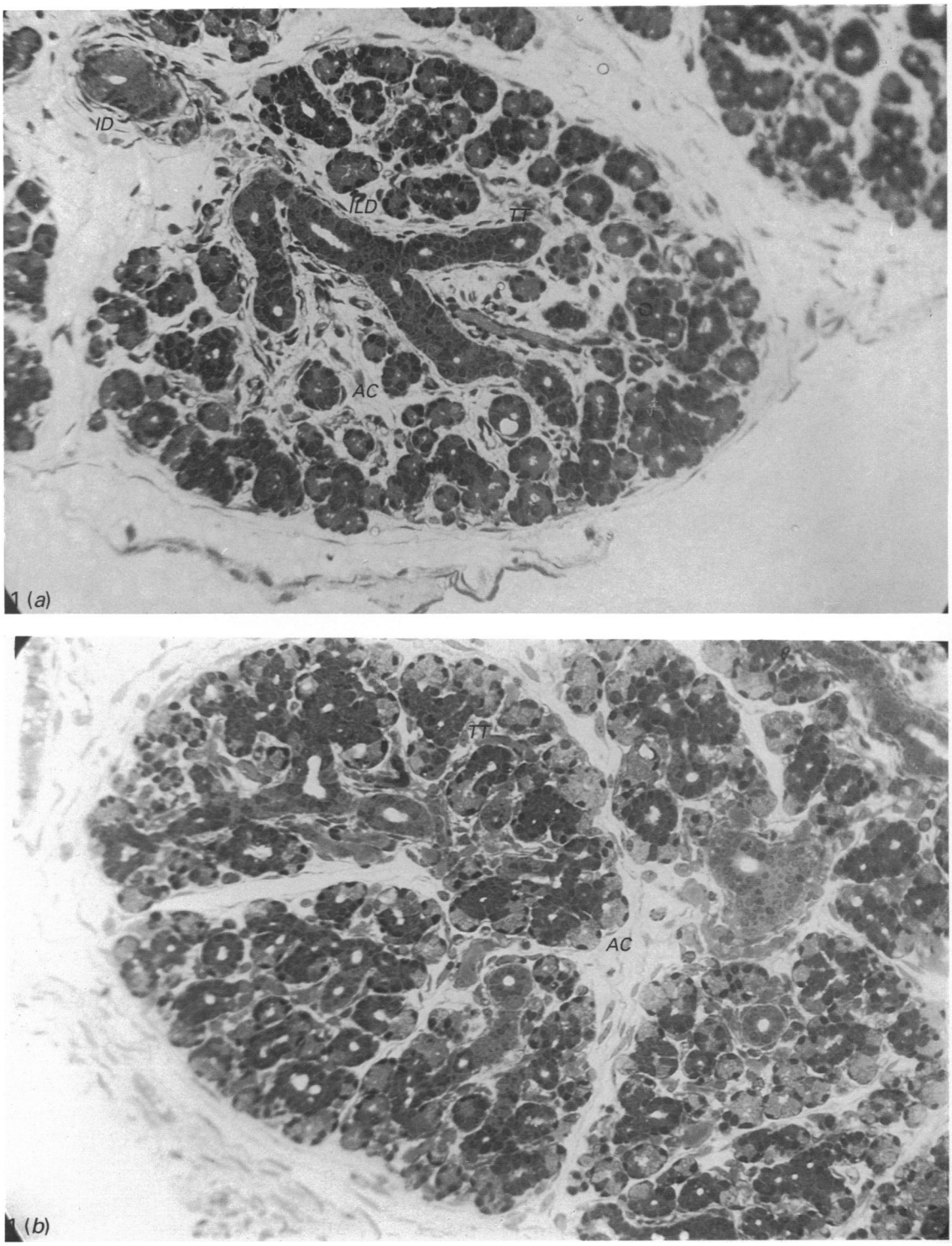

Fig. 1 (*a–b*). Light micrographs of the neonatal male (*a*) and female (*b*) mouse submandibular glands showing the arrangement of the glandular components within a lobe of the gland. The intralobular duct is branching and terminating to form the terminal tubules. Pale clumps of acinar cells can be seen bulging from the periphery of the terminal tubules. Note that the acinar cells are more prominent and the lobules appear more compact in the female gland. *ILD*, intralobular duct; *ID*, interlobular duct; *AC*, acinar cells; *TT*, terminal tubules. ×300.

Table 1. *Mean (± S.D.) percentage volume density (V_v%) (n = 5 animals) of the various tissue components including extracellular spaces in the male and female mouse submandibular gland from birth up to the age of 6 weeks. Comparison of means of a particular parameter between the sexes was tested using Student's t test*

Age and sex	GCT Nuclei + cytoplasm	GCT granules	GCT lumina	Acinar cells	Blood vessels	Inter-lobular duct	Intra-lobular duct	Terminal tubule	Extra-cellular spaces	Other cell types
Male neonatal	—	—	—	23·3 ± 1·7**	12·5 ± 5·6	4·9 ± 0·4	5·2 ± 0·8	21·4 ± 1·7	8·7 ± 0·8	24·1 ± 1·5*
Female	—	—	—	35·6 ± 3·4	12·3 ± 1·0	4·1 ± 0·8	5·4 ± 0·7	21·6 ± 2·9	7·4 ± 0·8	13·3 ± 1·2
	GCT Nuclei + cytoplasm	GCT granules	GCT lumina	Acinar cells	Blood vessels	Excretory duct	Striated duct	Intercalated duct	Extra-cellular spaces	Other cell types
Male 2 weeks	—	—	—	54·9 ± 1·7	3·7 ± 0·8	1·3 ± 0·8	20·7 ± 1·8	14·9 ± 1·3	1·1 ± 0·1	3·4 ± 0·7
Female	—	—	—	53·5 ± 1·3	6·2 ± 0·5	2·9 ± 0·4	17·7 ± 0·8	14·7 ± 0·5	2·5 ± 0·3	2·7 ± 0·4
Male 4 weeks	13·0 ± 3·0*	6·2 ± 2·8*	0·4 ± 0·1	41·7 ± 4·2**	9·5 ± 1·3	1·1 ± 0·3	13·6 ± 1·9	6·6 ± 0·8	3·3 ± 0·2	4·5 ± 0·3
Female	7·1 ± 0·7	1·0 ± 0·2	0·1 ± 0·0	61·9 ± 1·7	3·7 ± 1·0	2·6 ± 0·8	11·3 ± 1·2	9·7 ± 1·3	2·2 ± 0·6	3·6 ± 0·9
Male 6 weeks	21·3 ± 1·4****	24·3 ± 2·1****	1·6 ± 0·1	29·0 ± 2·1****	6·1 ± 0·6	0·9 ± 0·5	4·4 ± 0·5*	5·5 ± 0·9	3·0 ± 0·8	3·8 ± 0·5
Female	9·8 ± 0·8	2·1 ± 0·6	0·3 ± 0·0	56·9 ± 1·7	5·6 ± 1·2	1·9 ± 0·5	8·7 ± 1·2	6·0 ± 0·5	3·7 ± 0·4	4·4 ± 0·6

Note: *$P < 0.02$, **$P < 0.01$, ***$P < 0.002$, ****$P < 0.001$.
Although the excretory, striated and intercalated ducts appear in the same column as the interlobular ducts etc. it is not suggested that they correspond developmentally.
GCT, granular convoluted tubule.

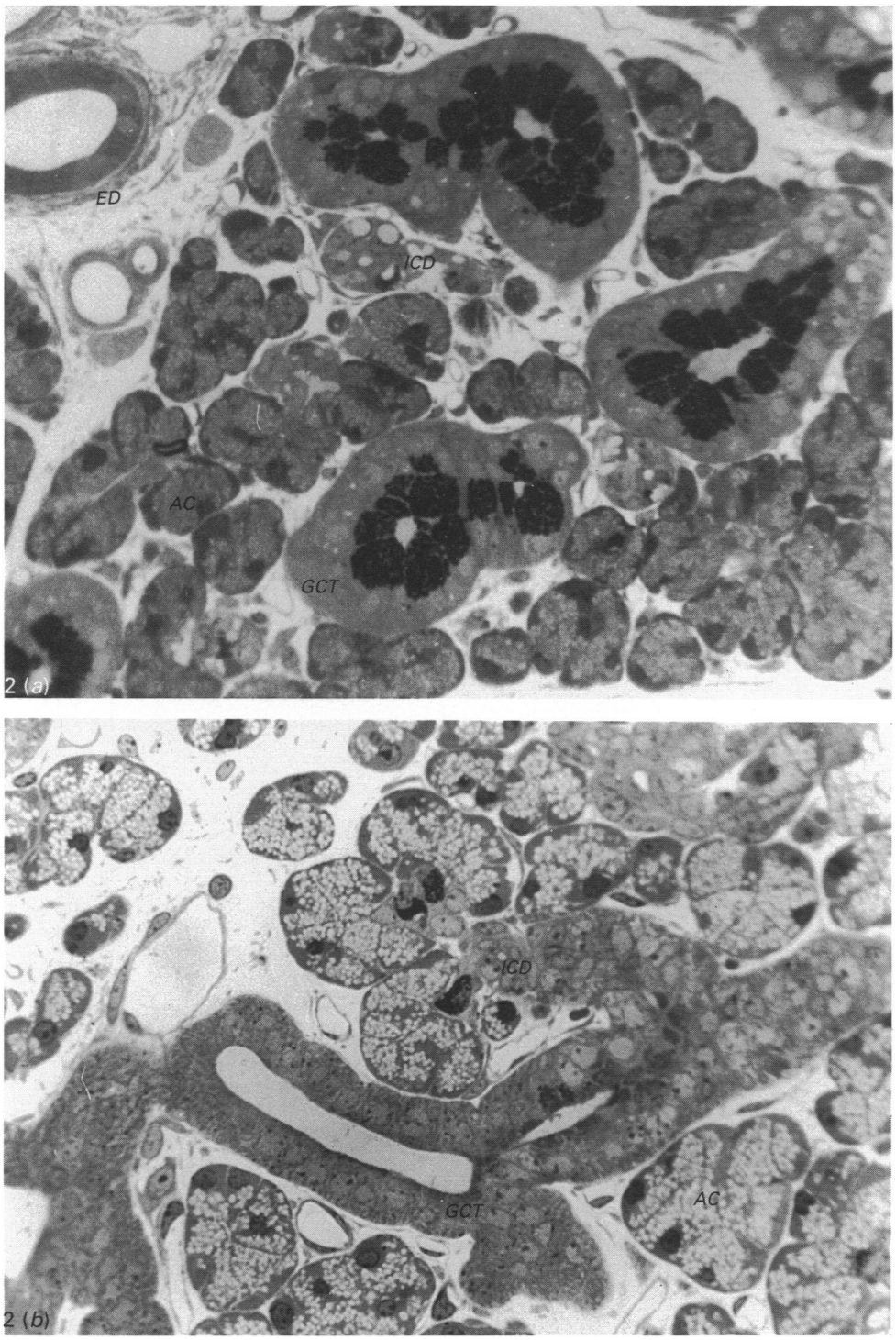

Fig. 2 (*a–b*). Light micrographs of male (*a*) and female (*b*) mouse submandibular glands at 4 weeks of age. The granular convoluted tubule contains numerous darkly stained secretory granules in the male gland. The cells of the granular tubule of the female gland contain fewer secretory granules and for the most part consist of basal striations. *AC*, acinar cells; *ICD*, intercalated duct; *GCT*, granular convoluted tubule, *ED*; excretory duct. × 750.

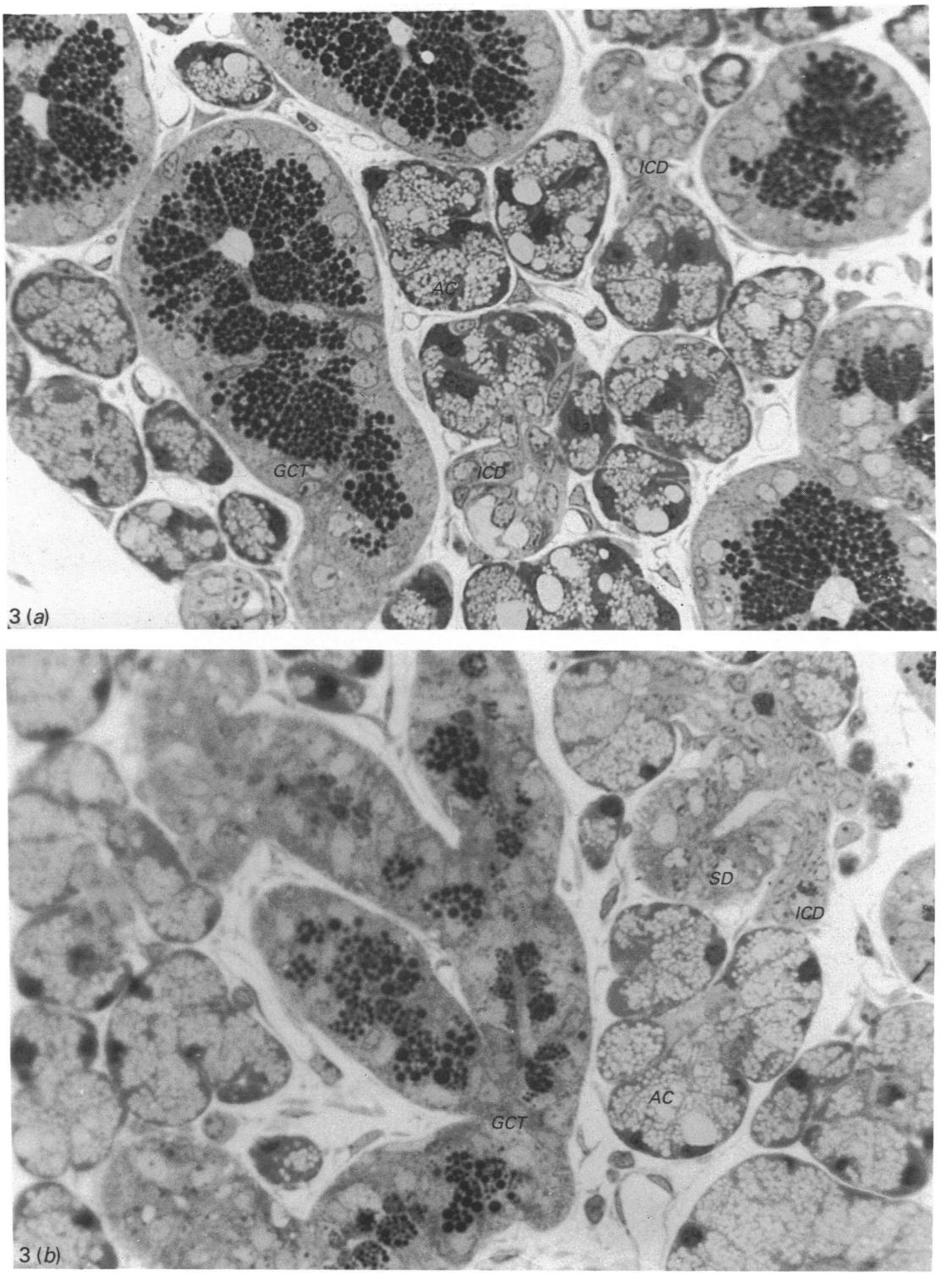

Fig. 3 (*a–b*). Light micrographs of male (*a*) and female (*b*) mouse submandibular glands at 6 weeks of age. The granular convoluted tubule occupies a relatively large proportion of the gland space and the cells are packed with dark secretory granules in the male gland. Note that the granular tubule is less convoluted in the female gland. The acini occupy a relatively larger proportion of the gland space in the female. *AC*, acinar cells; *ICD*, intercalated duct; *SD*, striated duct; *GCT*, granular convoluted tubule. × 750.

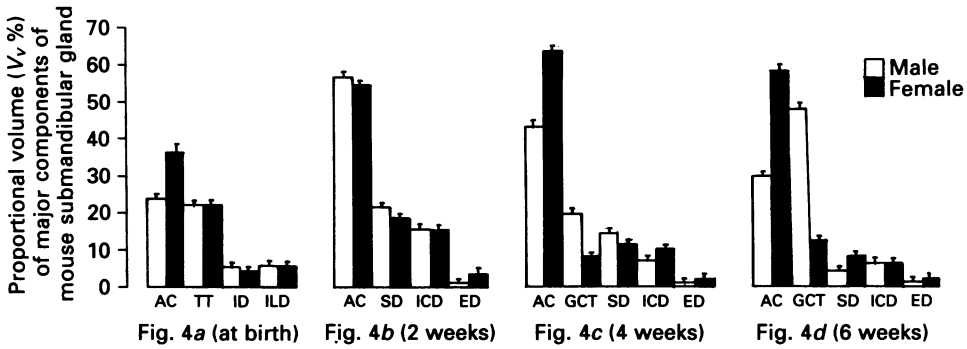

Fig. 4 (*a–d*). A comparison of developmental changes between the sexes in the major components of the submandibular salivary glands of mice from birth up to 6 weeks of age. Values represent the mean (± S.D.) percentage volume densities (V_v%) of the tissue components. The statistical significance of differences between the sexes is given in Table 1. Abbreviations for all figures: *AC*, acinar cells; *TT*, terminal tubule; *ILD*, intralobular duct; *ID*, interlobular duct; *SD*, striated duct; *ICD*, intercalated duct; *ED*, excretory duct; *GCT*, granular convoluted tubule.

Table 2. *The mean nuclear diameter ($\bar{D}_{nuc}$), the numerical areal density (NA_{nuc}) of the nuclei and the cell volumes of the acinar cells of the male and female mouse submandibular gland from birth up to 6 weeks of age. For calculation of cell volume see Materials and Methods*

Sex	Age	$\bar{D}_{nuc}$ Acinar cells	NA_{nuc} Acinar cells	Acinar cell volume (μm^3)
Male	Neonatal	9·5041	0·013714	748
Female		10.5208	0·088679	1308
Male	2 weeks	8·6251	0·007576	1230
Female		10·1521	0·007951	1378
Male	4 weeks	10·3783	0·004181	2679
Female		8·8039	0·004252	2235
Male	6 weeks	9·9536	0·00322	3331
Female		9·3931	0·003612	2807

proportions (5%) of the total gland volume. The acini and the other types of cells (connective tissue cells, undifferentiated cells etc.) were of similar proportions to those of the terminal tubule cells (23%) at this stage in the male gland. The female gland at birth was similar to that of the male in many respects (Fig. 1*b*) except that the volume proportions of acini were 12% higher and the other cell types were 11% lower when compared with those of the male gland (Table 1; Fig. 4*a*).

At 2 weeks of age the duct system had become differentiated into intercalated, striated and excretory ducts. The granular convoluted tubule could not be recognised in the duct system at 2 weeks of age. Many of the constituents of the female gland represented similar volume proportions to those of the male at this age (Table 1). Sexual dimorphism was, therefore, not evident in the gland at 2 weeks of age (Fig. 4*b*).

The granular convoluted tubule had become evident in the gland in both sexes by

Table 3. *The mean nuclear diameter ($\bar{D}_{nuc}$) and the numerical areal density (NA_{nuc}) of the nuclei and the cell volumes of the granular convoluted tubule cells of the male and female mouse submandibular gland at 4 and 6 weeks of age. For calculation of cell volume see Materials and Methods*

Sex	Age	$\bar{D}_{nuc}$ GCT cells	NA_{nuc} GCT cells	GCT cell volume (μm^3)
Male	4 weeks	11·2642	0·005124	2373
Female		9·8517	0·010029	1060
Male	6 weeks	9·6604	0·003488	2995
Female		10·0806	0·005981	1842

Table 4. *Mean ($\pm$S.D.) weights of the male and female mice and the mean ($\pm$S.D.) weights of their submandibular glands from birth up to the age of 6 weeks ($n = 5$ animals)*

Sex	Age	Weight of animal (gm)	Weight of gland (mg)
Male	Neonatal	1·52 $\pm$ 0·1	2·88 $\pm$ 0·1
Female		1·55 $\pm$ 0·1	2·74 $\pm$ 0·5
Male	2 weeks	6·46 $\pm$ 1·2	15·44 $\pm$ 0·3
Female		6·70 $\pm$ 0·5	19·60 $\pm$ 0·3
Male	4 weeks	18·05 $\pm$ 4·0	71·57 $\pm$ 8·8
Female		18·06 $\pm$ 2·3	73·86 $\pm$ 6·2
Male	6 weeks	31·35 $\pm$ 2·5	110·26 $\pm$ 12·5
Female		21·89 $\pm$ 1·8	88·32 $\pm$ 5·1

4 weeks of age (Fig. 2*a*, *b*). However in the male it already occupied a proportionate volume of 19% whereas in the female it was only 8%. The volume proportions of acini were significantly lower in the male than in the female at this age (Table 1; Fig. 4*c*). The proportions of acini, striated ducts and intercalated ducts all occupied a significantly lower proportion of the gland at 4 weeks than at 2 weeks of age in the male gland (Table 1). However, the proportions of acini increased in the female gland between 2 and 4 weeks of age (Table 1).

At 6 weeks of age, in the male gland, the great increase in proportion of granular tubule cells was mainly due to the remarkable increase in the content of the granules within the cells. In the female gland the granular convoluted tubule appeared to be smaller in size and less convoluted and the secretory granules seemed fewer than in the male (Fig. 3*a*, *b*). Quantitatively, the proportion of granular convoluted tubule cells in the female gland was half that of the male at 6 weeks and the granule content was only 10% that of the male. The proportion of acini decreased again in the male between 4 and 6 weeks of age. The proportion of acini, however, remained high in the female and was nearly twice as high as that in the male at 6 weeks. The striated duct also formed a higher proportion in the female than in the male (Table 1; Fig. 4*d*).

The cell volumes of acinar cells and granular convoluted tubule cells of male and female submandibular glands are summarised in Tables 2 and 3 respectively. At birth the cell volume of acinar cells in the female was twice that of the male gland. At 2 weeks the acinar cell volume between the two sexes was almost the same. At 4 weeks and at 6 weeks the acinar cell volume of the female gland had a slightly lower value than that of the male (Table 2).

At 4 weeks of age the cell volume of granular convoluted tubule cells of the female gland was less than half that of the male while at 6 weeks of age the cell volume of these cells was 40% lower in the female gland when compared with that of the male (Table 3).

Table 4 summarises the weights of the male and female animals and the weights of their submandibular glands from birth up to 6 weeks of age. At birth, at 2 weeks and at 4 weeks the weights of the female animals were similar to those of the male. However, the weight increase of the female animals from 4 to 6 weeks of age was 27% lower than the weight increase of the male animals between these two ages. The weight of the female gland at birth, at 2 weeks and at 4 weeks was similar to those of the male. The weight of the female gland at 6 weeks of age was 20% lower than that of the male.

DISCUSSION

This paper describes the changes in the volumes and proportions of the principal cell types of the mouse submandibular gland from birth to 6 weeks of age. This period is of particular interest because during this time sexual dimorphism of the gland is established.

At birth the gland is relatively undifferentiated. The acini can be seen to bud off from the terminal tubules but the duct system is poorly developed (Harvey, 1952). It differentiates at the expense of the terminal tubule which eventually disappears. The duct first differentiates into the intercalated, striated and excretory components. Subsequently, the granular convoluted tubule is formed. This portion has variously been reported to appear by 45–60 days (Harvey, 1952), 28 days (Disher & Elias, 1967) or 20 days (Gresik & MacRae, 1975) in mice. This last estimate agrees with our findings which show that the tubule is not present at two weeks but is apparent at four weeks of age although it is not fully developed at this time. Srinivasan & Chang (1979) analysed the growth of the gland by measuring total DNA levels at various times and by counting nuclear profiles and the number of thymidine-labelled cells in each category. Although this technique does not take into account changes in cell size, the results seem broadly to agree with ours. At one week of age they report that the gland consists of 36% acinar cells, 26% intercalated duct cells, 13% juxta-acinar cells (terminal tubule cells) and 12% striated duct cells. Clearly therefore, by one week, the tubule has begun to differentiate but the granular convoluted tubule is not yet present.

Sexual dimorphism becomes evident within the gland at four weeks of age and the granular convoluted tubule develops rapidly in the male mouse between four and six weeks of age. This rapid development coincides with an increase in plasma testosterone levels following attainment of puberty. However, this development is not evident in the glands of females. In females the greatest change occurs between birth and two weeks of age and continues until the fourth week, but this is not specifically associated with the development of the granulated tubule.

In the adult male mouse submandibular gland, five distinct regions can be seen: the acinus, the intercalated duct, the striated duct, the granular convoluted tubule and the excretory duct (Yohro, 1970; Gresik & MacRae, 1975; Gresik, 1980). A similar

organisation exists in the female gland although the granular convoluted tubule is not as well-developed and many of the cells contain basal striations reminiscent of those seen in striated duct cells. Srinivasan & Chang (1979) report that the adult male gland consists numerically of 47% granular convoluted tubule cells, 28% acinar cells, 12% intercalated duct cells and 1% striated duct cells. Our estimates of its composition at 6 weeks of age, based on cell volumes, show that the proportions are 45% granular convoluted tubule cells, 29% acinar cells and about 5% each for the intercalated ducts cells and the striated duct and excretory duct combined. Broadly therefore our estimates agree, although other reports would suggest that the proportion of the granular convoluted tubule is often much higher in the adult gland, particularly in the male. For example, Smith & Frommer (1975) report that the granular convoluted tubule occupies 65% and 36% of the gland in adult male and female mice respectively, Kaiho *et al.* (1975) 55% and 23% respectively, Murphy *et al.* (1981) 56% and 19% respectively and Watson *et al.* (1982) 60% and 23% respectively. These variations could be due to differences in fixatives and fixation procedure, errors incurred due to section thickness or due to the different methods used in quantifying the structural components of the gland. Our results (not presented here) indicate that although the gland continues to increase in size at least until 10–12 weeks of age there is no significant change either in the size of the cells or of the relative proportions of the various gland components beyond 6 weeks of age. An exception to this occurs during mating and pregnancy when significant changes occur in the granular convoluted tubule of both sexes (Jacob, unpublished analyses).

The granular convoluted tubule is not present in human glands. However, epidermal growth factor has been localised both in the duct cells (Elder, Williams, Lacey & Gregory, 1978) and in the serous cells of the acini (Kasselberg, Orth, Gray & Stahlman, 1985; Poulson *et al.* 1986). In addition to these biologically active polypeptides the submandibular glands of some species have been shown to secrete a group of pheromones.

Androgen-dependent hypertrophy has been observed in the granular duct of the mature pig submandibular gland (Booth, 1977). An odorous steroid is synthesised in the duct of this gland and is subsequently secreted into the saliva as a pheromone. The presence of a pheromone in the saliva of the mature pig facilitates the adoption of the mating stance in oestrus (Booth, 1977; Read, Melrose & Patterson, 1974; Perry, Patterson, MacFie & Stinson, 1980). Although tubular development is stimulated by testosterone in the male mouse (Harvey, 1952; Disher & Elias, 1967; Chretien, 1977) the secretion of the tubular cells has not yet been shown to be involved in promoting sexual attraction nor is there any evidence of the conversion of steroids to pheromones. Quite why, therefore, sexual dimorphism of the gland is so marked in the mouse remains to be elucidated.

SUMMARY

A light microscopic morphometric analysis of the development of the mouse submandibular gland has been carried out from birth up to the age of 6 weeks. At birth the bulk of the gland consists of approximately equal volume proportions of acinar, terminal tubule and non-secretory cells. The granular convoluted tubule is absent at birth. The neonatal female gland resembles that of the male in many respects. With the regression of the terminal tubule at 2 weeks of age the duct system of the gland is seen to differentiate into excretory, striated and intercalated ducts. The volume proportions of the gland constituents of the female are similar to those of the male at 2 weeks. At this age, the acini occupy 55%, the striated duct 20% and the intercalated duct 15%

of the total gland volume. Sexual dimorphism is clearly evident in the gland at 4 weeks of age when the duct system is seen to differentiate to form its granular convoluted tubule component. The granular tubule occupied 19% of the gland volume in the male but only 8% in the female at 4 weeks. The proportions of acini are only 41% in the total gland volume of the male mouse but 62% in the female at 4 weeks. In the male gland the proportions of granular convoluted tubule increase from 13% to 21% between 4 and 6 weeks and the secretory granule content of these cells from 6% to 24%.

At 6 weeks of age the volume proportion of granular convoluted tubule in the male is 45% and that in the female is only 12%. At this age the acini occupy a proportion of 30% in the male gland as opposed to 57% in the female gland. At 6 weeks the volume of granular convoluted tubule cells is 40% lower in the female (1842 μm^3) than in the male gland (2995 μm^3).

This work was done at the Department of Biomedical Science, University of Sheffield. We wish to thank Maureen Tune for photographic assistance. The financial support from the Association of Commonwealth Universities in the United Kingdom is gratefully acknowledged.

REFERENCES

ABERCROMBIE, M. (1946). Estimation of nuclear population from microtomic sections. *Anatomical Record* **94**, 239–247.

BARKA, T. (1980). Biologically active polypeptides in submandibular glands. *Journal of Histochemistry and Cytochemistry* **28**, 836–859.

BARTHE, P. L., BULLOCK, L. P., MOWSZOWICZ, I., BARDIN, C. W. & ORTH, D. N. (1974). Submaxillary gland epidermal growth factor: a sensitive index of biologic androgen activity. *Endocrinology* **95**, 1019.

BOOTH, W. D. (1977). Metabolism of androgens *in vitro* by the submaxillary salivary gland of the mature domestic boar. *Journal of Endocrinology* **75**, 145–154.

BOWER, A. J. (1981). A simple, inexpensive apparatus to allow sequential perfusion of an animal with several different fluids without an intervening drop in perfusion pressure. *Journal of Microscopy* **122**, 103–104.

CARAMIA, F. (1966). Ultrastructure of mouse submaxillary gland. 1. Sexual differences. *Journal of Ultrastructure Research* **16**, 505–523.

CHRETIEN, M. (1977). Action of testosterone on the differentiation and secretory activity of a target organ: the submaxillary gland of the mouse. *International Review of Cytology* **50**, 333–396.

COHEN, S. (1960). Purification of a nerve growth promoting protein from the mouse salivary gland and its neurocytotoxic antiserum. *Proceedings of the National Academy of Sciences of the USA* **46**, 302–304.

COHEN, S. (1962). Isolation of a mouse submaxillary gland protein accelerating incisor eruption and eyelid opening in the new-born animal. *Journal of Biological Chemistry* **237**, 1555–1562.

COPE, G. H. (1982). Stereological techniques and their application to the gastrointestinal tract and its glands. In *Techniques in the Life Sciences* (ed. D. A. Tichen), *Digestive Physiology* **210**, 1–33. Elsevier Scientific Publishers Ireland Ltd.

DISHER, L. & ELIAS, J. J. (1967). The effect of testosterone injections on submaxillary salivary gland structure of castrated mature and immature male mice. *Journal of Oral Therapy* **4**, 128–139.

ELDER, J. B., WILLIAMS, G., LACEY, E. & GREGORY, H. (1978). Cellular localization of human epidermal growth factor. *Nature* **271**, 466–477.

GOLDSTEIN, M. N. & BURDMAN, J. A. (1965). Studies of the nerve growth factor in submandibular glands of female mice treated with testosterone. *Anatomical Record* **151**, 199–208.

GRESIK, E. W. (1980). Postnatal developmental changes in submandibular glands of rats and mice. *Journal of Histochemistry and Cytochemistry* **28**, 860–870.

GRESIK, E. W. & MACRAE, E. K. (1975). The postnatal development of the sexually dimorphic duct system and of amylase activity in the submandibular glands of mice. *Cell and Tissue Research* **157**, 411–422.

GUNDERSEN, H. J. G. (1977). Notes on the estimation of the numerical density of arbitrary profiles: the edge effect. *Journal of Microscopy* **111**, 219–223.

HARVEY, H. (1952). Sexual dimorphism of submaxillary glands in mice in relation to reproductive maturity and sex hormones. *Physiological Zoology* **25**, 205–222.

JONES, R. O. (1966). The *in vitro* effect of epithelial growth factor on rat organ cultures. *Experimental Cell Research* **43**, 645.

KAIHO, M., NAKAMURA, T. & KUMEGAWA, M. (1975). Morphological studies on the synthesis of secretory granules in convoluted tubules of mouse submandibular gland. *Anatomical Record* **183**, 405–420.

KASSELBERG, A. G., ORTH, D. N., GRAY, M. E. & STAHLMAN, M. T. (1985). Immunohistochemical localization of human epidermal growth factor urogastrone in several human tissues. *Journal of Histochemistry and Cytochemistry* **33**, 315–322.

LACASSAGNE, A. (1940). Dimorphism sexual de la glande sousmaxillaire chez la Souris. *Comptes rendus de la Société de biologie* **133**, 180–181.

LYON, M. F., HENDRY, I. & SHORT, R. V. (1973). The submaxillary salivary glands as test organs for response to androgen in mice with testicular feminization. *Journal of Endocrinology* **58**, 357–362.

MURPHY, R. A., WATSON, A. Y., MCCARTHY, M., PAPASTAVROS, M., NAUTRA, M. & FROSSMANN, W. G. (1981). Submandibular glands in mice with muscular dystrophy: studies with nerve growth factor. *Anatomical Record* **200**, 177–194.

PERRY, G. C., PATTERSON, R. L. S., MACFIE, H. J. H. & STINSON, C. G. (1980). Pig courtship behaviour; pheromonal property of androstene steroids in male submaxillary secretion. *Animal Production* **31**, 191–199.

POULSEN, S. S., NEXØ, E., SKOV OLSEN, P., HESS, J. & KIRKEGAARD, P. (1986). Immunohistochemical localization of epidermal growth factor in rat and man. *Histochemistry* **85**, 389–394.

REED, H. C. B., MELROSE, D. R. & PATTERSON, R. L. S. (1974). Androgen steroids as an aid to the detection of oestrus in pig artificial insemination. *British Veterinary Journal* **130**, 61–67.

ROGERS, A. W. & BROWN-GRANT, K. (1971). The effects of castration on the ultrastructure and the iodide-concentrating ability of mouse submaxillary salivary glands. *Journal of Anatomy* **109**, 51–62.

SCHWAB, M. E., STOCKEL, K. & THOENEN, H. (1976). Immunocytochemical localization of nerve growth factor in the submandibular gland of adult mice by light and electron microscopy. *Cell and Tissue Research* **169**, 289–300.

SMITH, R. J. & FROMMER, J. (1975). Quantitative morphology and carbohydrate histochemistry of the mouse submandibular gland following prepubertal castration. *American Journal of Anatomy* **144**, 137–148.

SRINIVASAN, R. & CHANG, W. W. L. (1979). The postnatal development of the submandibular gland of the mouse. *Cell and Tissue Research* **198**, 363–371.

WATSON, A. Y., RADIE, K., MCCARTHY, M., LARSEN, P. R. & MURPHY, R. A. (1982). Thyroxine reverses deficits of nerve growth factor and epidermal growth factor in submandibular glands of mice with muscular dystrophy. *Endocrinology* **110**, 1392–1400.

YOHRO. T. (1970). Development of secretory units of mouse submandibular gland. *Zeitschrift für Zellforschung und mikroskopische Anatomie* **110**, 173–184.

YOUNG, J. A. & VAN LENNEP, E. W. (1978). *The Morphology of Salivary Glands*. London: Academic Press.