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The effects of castration on the ultrastructure and the iodide-concentrating ability of mouse submaxillary salivary glands

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INTRODUCTION

The submaxillary salivary glands of mice are immature at birth, the convoluted granular tubules (C.G.T.), which are a striking feature of the adult gland, developing at or just before puberty (Brown-Grant & Taylor, 1963). The gland exhibits a sexual dimorphism, the C.G.T. being more prominent in the male but regressing after thyroidectomy or castration. The histological changes occurring in the submaxillary glands following castration have been described by Burgen & Emmelin (1961) and by Shafer & Muhler (1960); ultrastructural changes have been studied by Caramia (1966*a*, *b*).

Radio-iodide is concentrated in both the submaxillary gland and in the saliva in the mouse, in common with several other species, including man (Cohen & Myant, 1959; Brown-Grant, 1961). The site of iodide concentration in the mouse is the C.G.T. (Logothetopoulos & Myant, 1956). This function of the gland also shows sexual dimorphism, the gland–blood ratios for [^{131}I]iodide being higher in males, in which the C.G.T. form a greater proportion of the total gland, than in females (Llach, Tramezzani & Funes, 1960; Brown-Grant & Taylor, 1963). After castration, in spite of the shrinkage and regression of the C.G.T. which results, the ability of the gland to concentrate iodide is significantly *increased*, both *in vivo* (Llach & Tramezzani, 1962) and *in vitro* (Brown-Grant & Taylor, 1963).

In the present work autoradiographic studies of iodide distribution in submaxillary glands of normal mice have been repeated using the isotope ^{125}I. This emits an internal conversion electron of very low energy, permitting higher resolution than was possible in previous studies with ^{131}I (Logothetopoulos & Myant, 1956; Häusler, Bruns & Kutzim, 1965). Improved autoradiographic techniques (Appleton, 1964; Stumpf & Roth, 1964) have also been used to minimize diffusion artefacts. These studies have been extended to the submaxillary glands of castrate mice. In addition, the changes produced in the C.G.T. by castration have been re-examined by electron microscopy. The ultrastructural and autoradiographic data have been correlated in an attempt to explain the anomalous effects of castration on the submaxillary glands of mice.

MATERIALS AND METHODS

The animals were adult male albino mice of the Parkes strain (about 30 g); they received tap water and pelletted diet (Diet 41B, obtained from E. Dixon and Sons, Ltd., Ware) *ad lib*. This diet contains 117 μg/kg iodine. Castration was performed under 'Avertin' anaesthesia and animals were used 3–4 weeks later; gross atrophy of the seminal vesicles at autopsy confirmed the completeness of the operation.

The animals were injected intramuscularly with carrier-free [^{125}I]sodium iodide (Radiochemical Centre, Amersham). The dose varied between 20 and 50 μc per mouse. Animals were killed by bleeding from the aorta, under ether anaesthesia, 2 h after injection. Fragments of submaxillary gland and blood samples were taken for determination of gland/plasma concentration ratios for ^{125}I, using a technique that has been fully described elsewhere (Brown-Grant, 1963).

Autoradiography. Fragments of submaxillary gland were rapidly frozen in isopentane cooled in liquid nitrogen. Sections at 4 or 5 μm were cut from these blocks in a cryostat at -24 °C. Autoradiographs were prepared from these sections by two methods. The first, used in the majority of the experiments, was based on that described by Appleton (1964). Microscope slides were coated with a 3–4 μm layer of Ilford G 5 emulsion by dipping (Rogers, 1967). When dry, these slides were frozen to -24 °C, and the cryostat sections were picked up from the knife by touching them gently with the frozen emulsion layer. The second method was based on that described by Stumpf & Roth (1964, 1969). The cryostat sections were transferred from the knife to a small container and freeze-dried overnight in a cryosorption apparatus (Stumpf & Roth, 1967). The sections were then mounted on emulsion layers at room temperature.

Non-radioactive submaxillary gland was treated in the same way as a control against positive chemography, which was not encountered in these experiments. However, in initial experiments control slides demonstrated very severe negative chemography. This artefact, in which latent images are lost from the emulsion during exposure, due to chemical effects from the tissue section, was controlled by procedures developed as a result of experiments which have been reported elsewhere (Rogers & John, 1969).

It was found that latent image loss by negative chemography could be prevented in our experimental conditions by a combination of four precautionary steps: a developer based on Metol was used (Ilford ID-19); the emulsion layers were thoroughly dried in a desiccator before applying the frozen sections; the sections were kept under conditions of low temperature and humidity, both before and after applying them to the emulsion, to hasten their freeze-drying; and exposure took place either at -40 °C in a deep-freeze, or at the temperature of solid carbon dioxide. In the experiments reported here all four precautions were taken and control slides indicated that negative chemography did not take place. Exposure times varied from 24 h to 33 days. Conditions of exposure and development followed closely the optimum sequence determined by Rogers & John (1969). After development, the sections were stained with Harris's haematoxylin.

Electron microscopy. Tissue fragments were rapidly dissected from submaxillary

glands of four castrate and four control mice, and fixed in 2·5 % glutaraldehyde in 0·085 M cacodylate buffer (pH 7·4). After post-fixation in 1 % osmium tetroxide, the fragments were embedded in Araldite and sectioned on an Ultratome (L.K.B. Instruments, Ltd). Pale gold sections were stained with uranyl acetate and lead citrate and examined in a Phillips electron microscope, model 200.

In addition, 1 μm Araldite sections were cut from the same material and stained in 1 % toluidine blue in 1 % borax for examination with the light microscope.

RESULTS

Iodide concentration

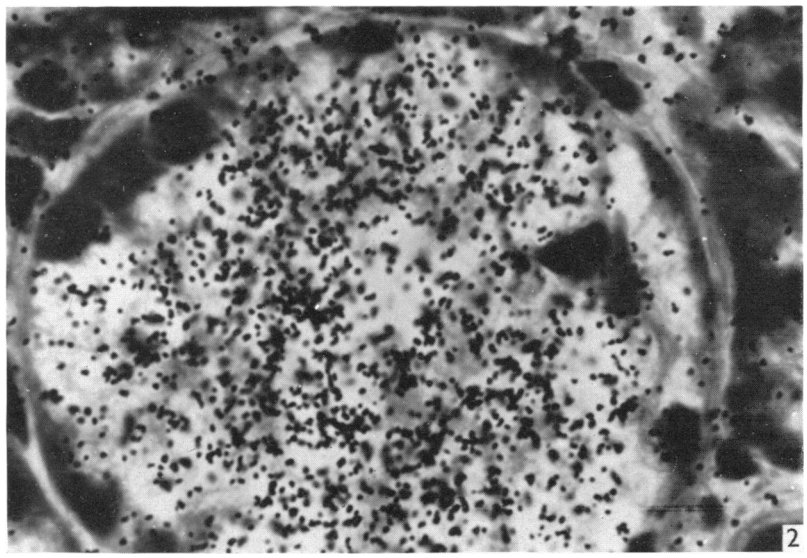
The gland/blood ratios for ^{125}I were determined for fragments of submaxillary gland from four control mice (13·6, 8·2, 5·2 and 2·9) and three castrates (15·3, 8·0 and 7·0).

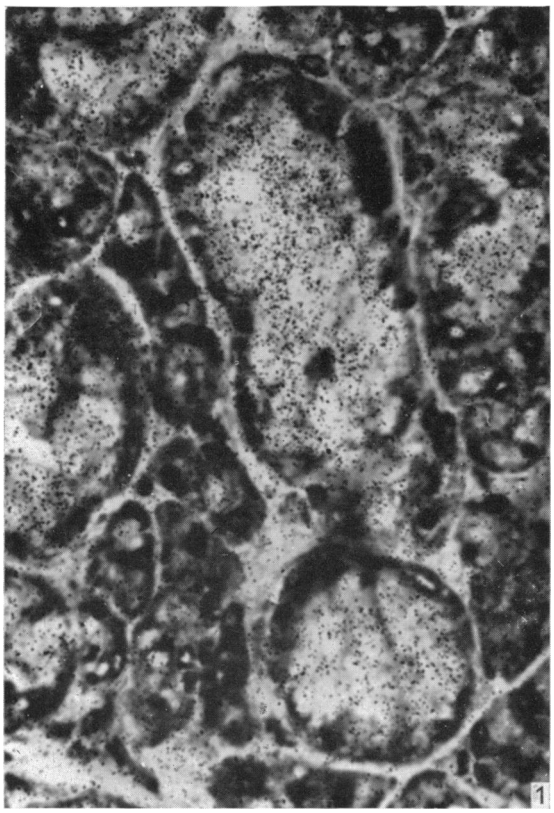
These results confirmed that iodide concentration was occurring, but the values found showed a greater scatter than those reported by Llach & Tramezzani (1962) and by Brown-Grant & Taylor (1963). The previous experiments were carried out with whole glands and the small fragments on which the present counts were based probably introduced variability in the percentage of C.G.T. present in each fragment. The mean value for the castrate mice was higher than for the controls, but owing to the very small numbers and the wide scatter, the difference was not statistically significant. The higher concentration in the glands of castrates was, however, clearly established by Llach & Tramezzani (1962) and by Brown-Grant & Taylor (1963), using larger series.

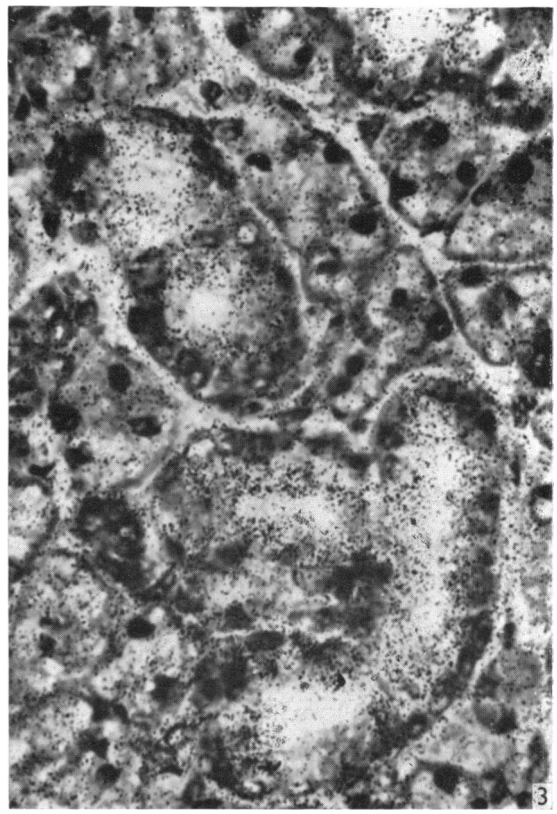
In the experiment in which mouse submaxillary gland was autoradiographed by both the Appleton and the Stumpf & Roth procedures, the distribution of silver grains was the same by the two methods. Resolution was similar, and the qualitative results were comparable. In this experiment, which involved the injection of 40 μC ^{125}I to each mouse, exposure times were 24–48 h. The observed grain densities by the Stumpf & Roth method were uniformly higher than those by the Appleton method. Since negative chemography was excluded in both cases, it is reasonable to attribute this difference in efficiency to the lower self-absorption of the freeze-dried specimens.

In autoradiographs of submaxillary gland from control mice, uniform low-grain densities were seen over all tissues except the cells and the lumen of the C.G.T. and the interlobular ducts (Fig. 1). The highest grain densities lay over the cells of the C.G.T. This labelling was cytoplasmic, often ending abruptly at the basal end of the cell: in many cases the nucleus of the cell could be seen to be unlabelled (Fig. 2). The lumen of the C.G.T. was often relatively small in control mice, and was not always easy to identify. Where it could be clearly recognized, grain densities over the lumen appeared to be less than those over the cell.

In the material from castrate mice, the C.G.T. formed relatively less of the total gland than in control animals: the cells of the C.G.T. however, were as heavily labelled as those of intact animals (Fig. 3): in one case the labelling was much heavier than in the comparable control animal, assessed by visual inspection. With cell shrinkage following castration, the lumen of the C.G.T. was easier to recognize, and was clearly less heavily labelled than the cells themselves (Fig. 3).







The pattern of labelling in the interlobular ducts was the same in control males and in castrates. The smaller interlobular ducts had grain densities over their lumen, which were lower than over the cells of the C.G.T. but clearly higher than over other tissues. This labelling did not end at the apex of the epithelial cells of the ducts with the same sharp resolution that was seen in the C.G.T., but continued over the epithelial cells and fell away to background levels over surrounding connective tissue. In the larger interlobular ducts the grain densities over the duct wall were higher than over the lumen itself. A lymphatic vessel often appeared adjacent to the interlobular duct; the grain densities over this lymphatic were slightly higher than over surrounding connective tissue.

Histological observations

Material prepared for light miscroscopy in paraffin wax showed the cells of the C.G.T. of control mice to be large, with basal nuclei and a rather foamy cytoplasm. The lumen was small and difficult to distinguish. After Araldite embedding and sectioning at 1 μm, clusters of dense secretion granules could be seen at the cell apex (Fig. 4). An occasional cell stained much more darkly than the majority of C.G.T. cells in the Araldite material. No basal striations could be seen in the C.G.T. of control mice.

In castrate mice C.G.T. cells were smaller and the cytoplasm did not have the vacuolated appearance characteristic of the control animals (Fig. 5). The cytoplasm appeared denser and the lumen was clearly defined. The nucleus lay in the centre of the cell, and distinct basal striations could be seen in the majority of C.G.T. cells. Isolated dark cells could be seen with about the same frequency as in control animals.

These findings were fully confirmed by electron microscopy. In the C.G.T. of the control mice the vacuolated appearance under the light microscope could be seen to be due to the abundant rough endoplasmic reticulum (R.E.R.), with many dilated cisternae containing finely granular material (Figs. 6, 8).

The basal cell membrane showed many infoldings, associated with mitochondria; but these usually involved less than half the basal surface of the cell, and did not extend far into the cytoplasm. The membrane foldings and associated mitochondria were not sufficiently regular or extensive to produce basal striations visible with the light microscope (Fig. 4).

In the glands from castrate mice the most striking change was the almost complete absence of the dilated cisternae of R.E.R. (Fig. 7). At higher magnifications the cytoplasm showed occasional isolated lengths of R.E.R. and scattered ribosomes (Fig. 9), but it was clear that the R.E.R. of the intact animals had largely disappeared following

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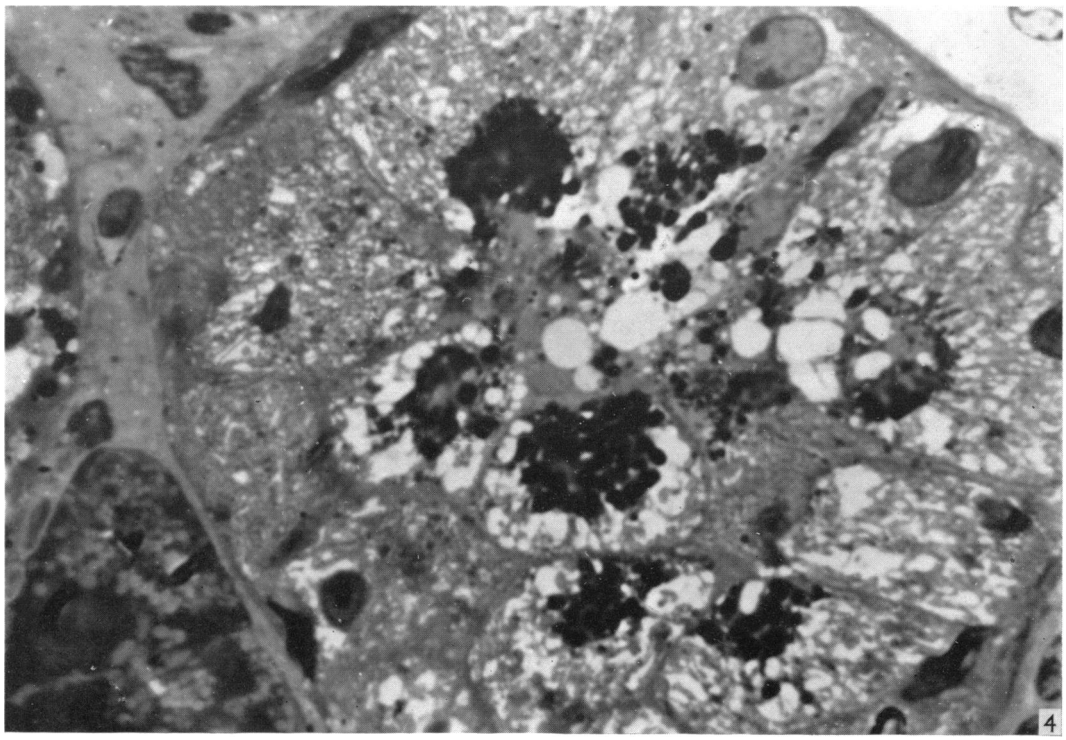
Fig. 1. An autoradiograph of the submaxillary gland of a control male mouse, following an injection of [^{125}I]iodide. Note the high grain densities over the C.G.T. and the uniform, low, grain densities elsewhere. × 280.

Fig. 2. An autoradiograph of the submaxillary gland of a control male mouse, after [^{125}I]iodide, showing the distribution of silver grains over a sectioned C.G.T. Note that the labelling is cytoplasmic, falling off sharply over the nuclei and at the bases of the cells. × 1110.

Fig. 3. An autoradiograph of the submaxillary gland of a castrate male mouse injected with [^{125}I]iodide. The grain density over the cytoplasm is high. The cells are smaller than in the control male, and the lumen is larger. The grain density over the lumen is lower than that over the cytoplasm. × 280.



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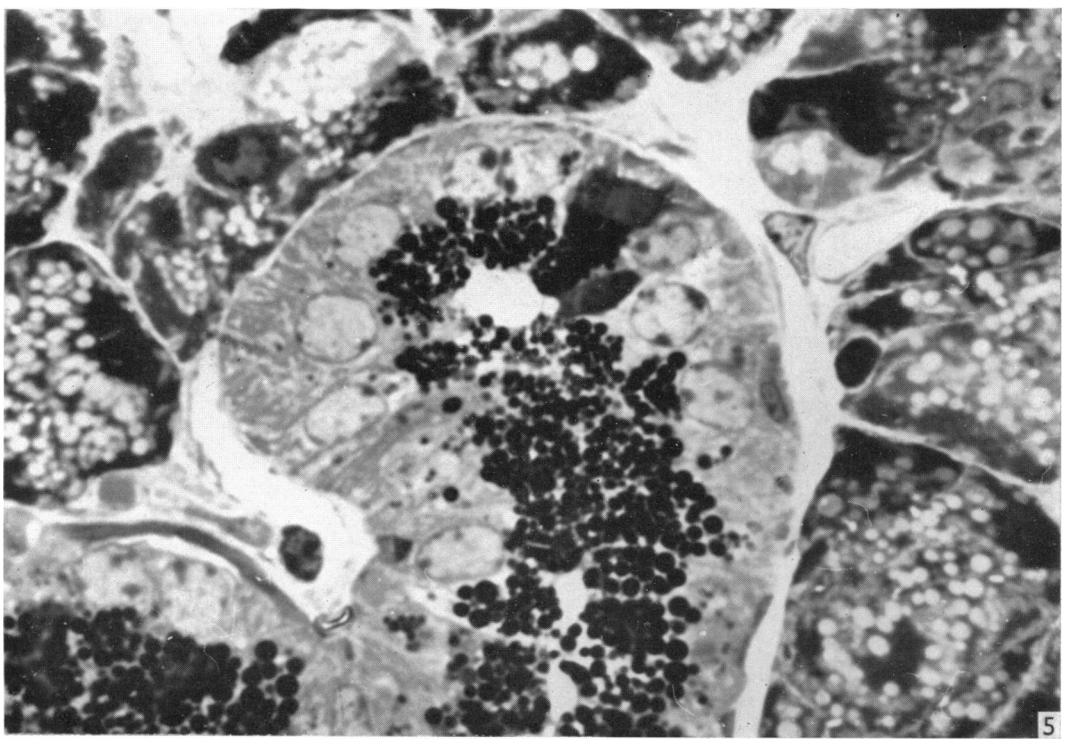

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castration. In view of this marked change it was surprising that the population of secretion granules at the cell apex showed so little change. The infoldings of the basal cell membrane occupied a greater part of the basal surface, and extended further into the cell, than in the control animal, and lay in regular array at right angles to the basement membrane (Fig. 7), with mitochondria between them.

DISCUSSION

The sexual dimorphism of the C.G.T. of mouse submaxillary gland is well documented. Burgen & Emmelin (1961) summarize much of the evidence obtained by the light microscope, including the changes seen in the C.G.T. following castration and thyroidectomy. Caramia (1966*a*, *b*) extended the study of these changes by using the electron microscope. The morphological data presented here confirm and amplify the previous findings. The appearance of the C.G.T. of the control male is indicative of a high rate of synthesis of protein for secretion, with much of the cytoplasm taken up by cisternae lined with R.E.R. (Fig. 6). Following castration the R.E.R. is greatly reduced in amount, producing a change in the appearance of the cells clearly recognizable under the light microscope (Figs. 5, 7). The loss of the R.E.R. from the C.G.T. after castration in the mouse may be related to enzyme levels in the submaxillary gland and the saliva. Junquiera, Fajer, Rabinovitch & Frankenthal (1949) have shown differences in protease content between male and female submaxillary glands in the mouse: the high male levels fall to female levels following castration, and are restored by testosterone administration.

The ability of the submaxillary gland to concentrate iodide is also higher in the male than in the female (Llach, Tramezzani & Funes, 1960; Brown-Grant & Taylor, 1963). The present study confirms the finding of Logothetopoulos & Myant (1956) that the cells of C.G.T. are the principal site of transfer of radio-iodide to the saliva, and, in addition, indicates that cytoplasmic concentration of radio-iodide may be considerably higher than that in the lumen.

Up to this point in the argument it is possible to regard protein synthesis and iodide concentration as related, both responding in similar fashion to testosterone stimulation. This link between the two processes would have provided an interesting parallel to the iodide-concentrating mechanism present in the luminal epithelium of the rat uterus (Brown-Grant, 1967; Brown-Grant & Rogers, 1967), which is also influenced by gonadal steroids. This site of concentration is progesterone-dependent, and develops following progesterone-induced protein synthesis in the epithelial cells: blockage of protein synthesis by administration of cycloheximide prevents the development of iodide concentration in response to progesterone (Rogers, John & Brown-Grant, 1970).

Fig. 4. A microphotograph of 1 μm Araldite section of the submaxillary gland of a control male mouse. The cells of the C.G.T. have basal nuclei and vacuolated cytoplasm. Secretion granules can be seen at the cell apex, bordering the small lumen. No basal striations can be seen in these cells. $\times$ 1500.

Fig. 5. A microphotograph of an 1 μm Araldite section of the submaxillary gland of a castrate male mouse. The cells of the C.G.T. have centrally placed nuclei, prominent basal striations, and apical secretion granules. The lumen is clearly visible. The vacuolated appearance of the cytoplasm, characteristic of these cells in the control animal, has disappeared. $\times$ 1500.

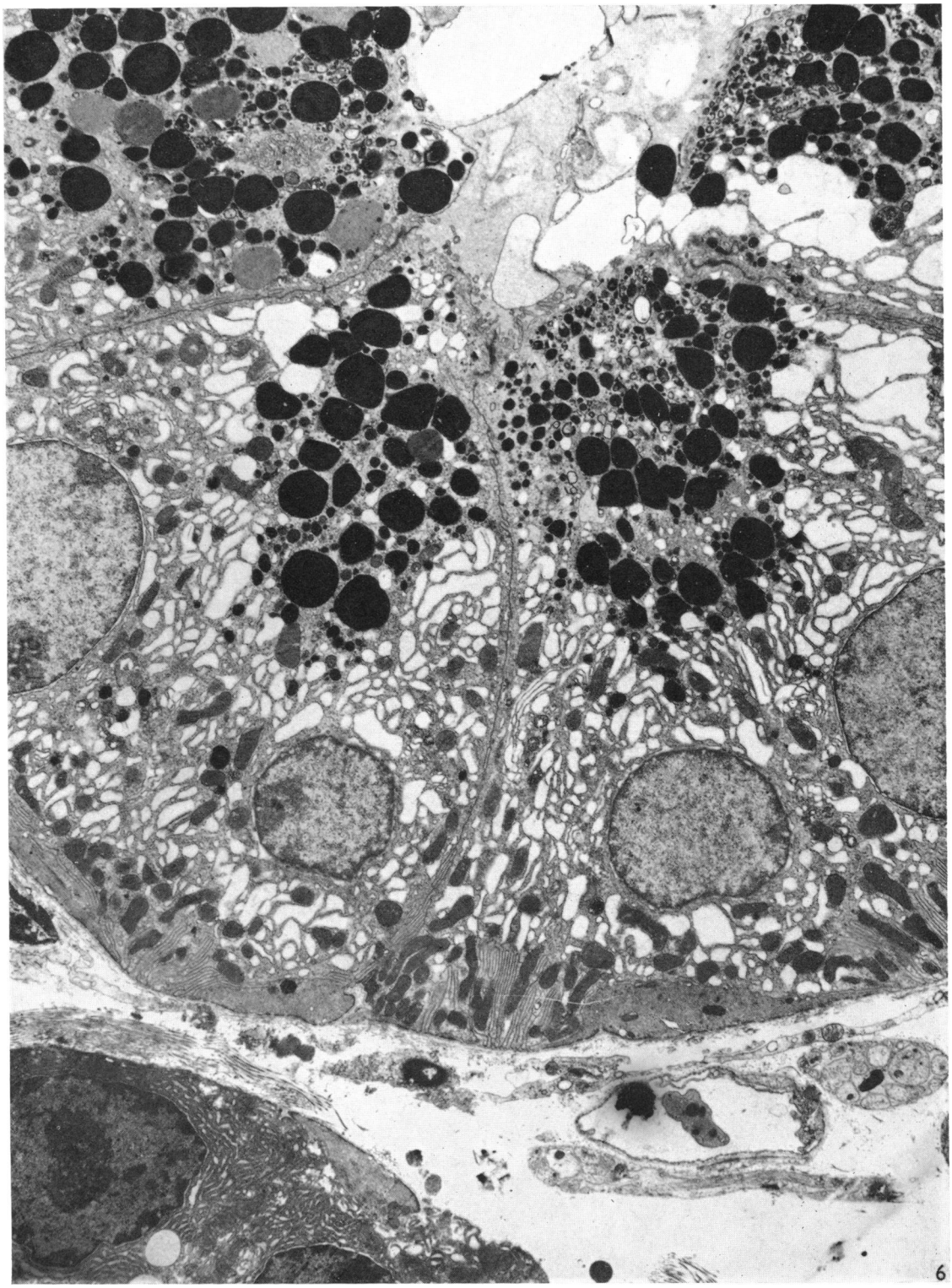


Fig. 6. An electron micrograph of a cross-section through a C.G.T. in a control male mouse. The basal nucleus lies near clusters of mitochondria, which are often associated with infoldings of the basal membrane. Most of the cytoplasm consists of large cisternae of R.E.R. × 3400.

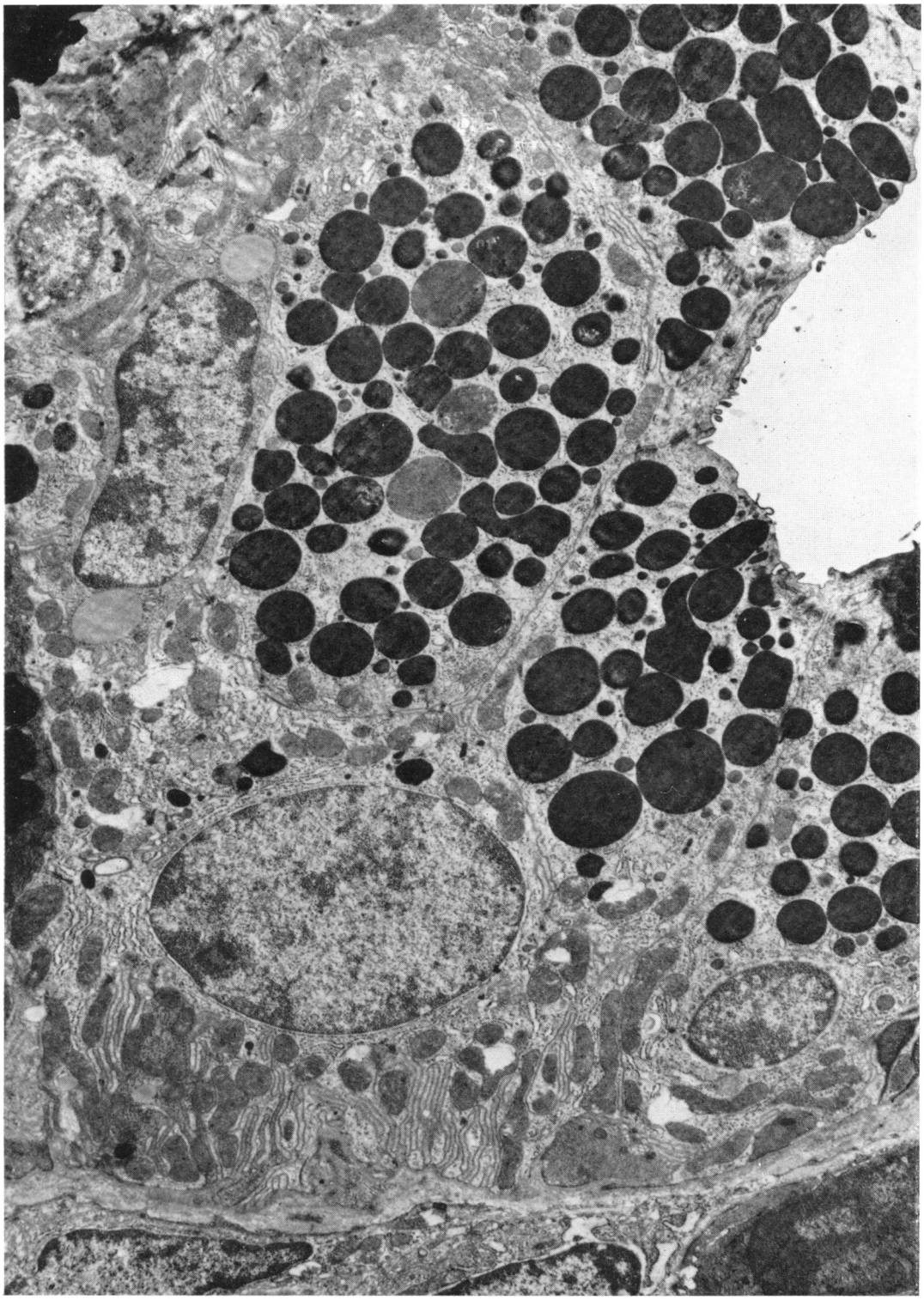
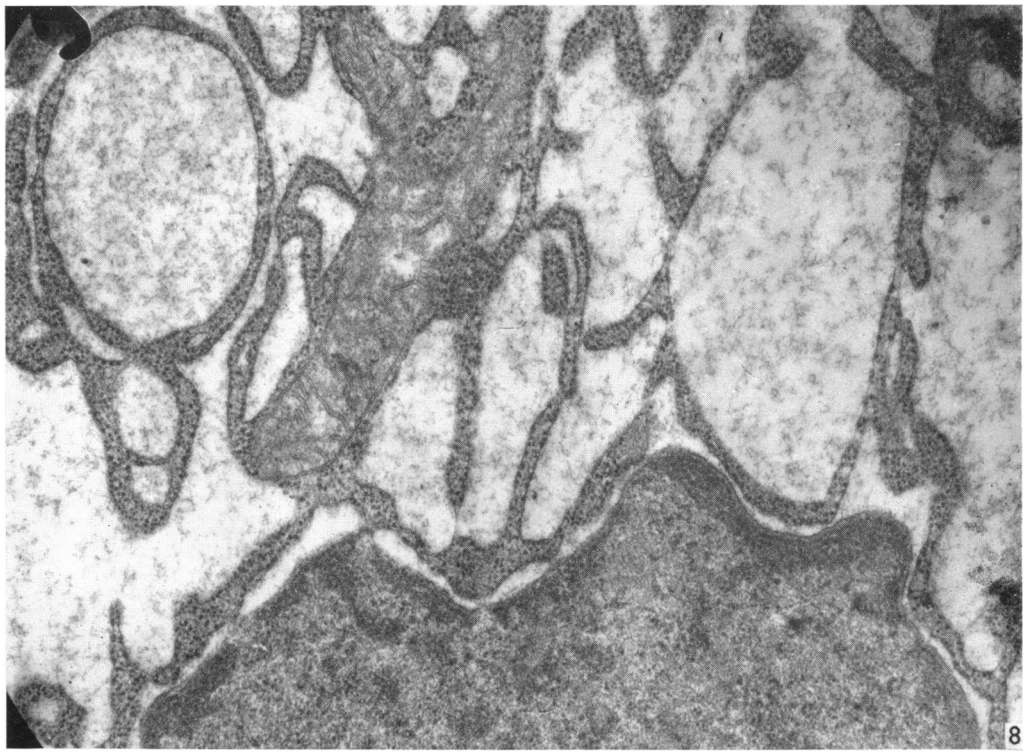
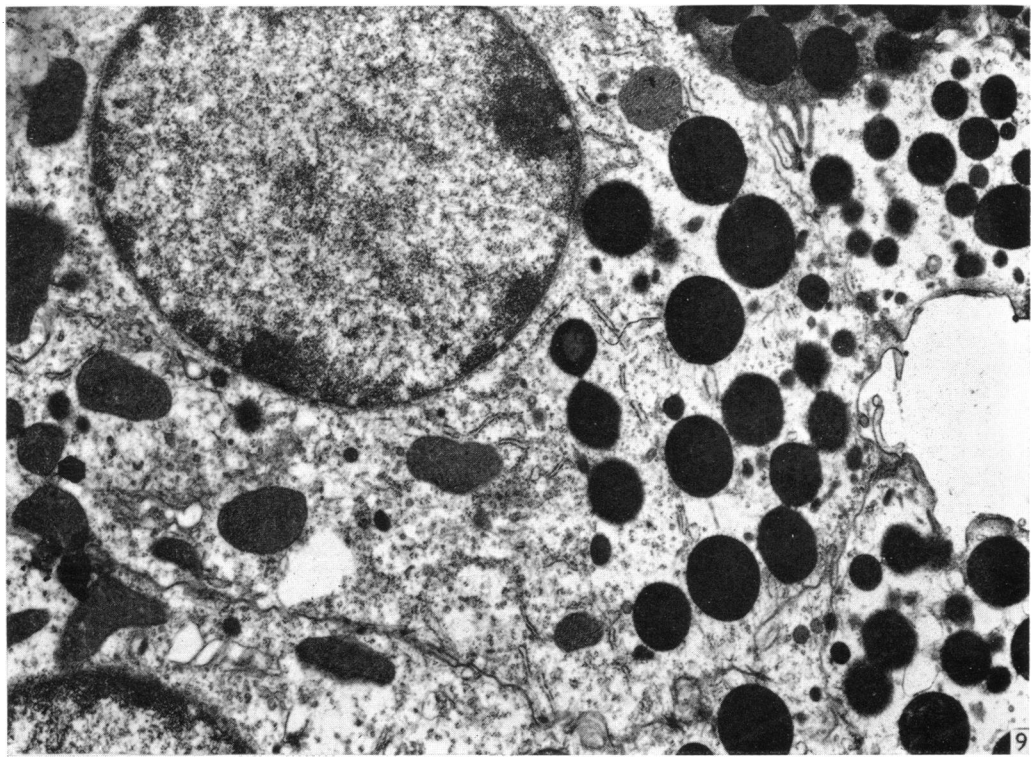


Fig. 7. An electron micrograph of a cross-section through a C.G.T. in a castrate male mouse. Note the central nucleus, the basal masses of mitochondria with infoldings of the basal membrane, and the apical secretion granules. By comparison with the control animal (Fig. 6), there is an almost complete absence of R.E.R. × 2800.





The changes that occur in the mouse submaxillary gland following castration, however, are not consistent with this simple but attractive hypothesis. Llach & Tramezzani (1962) showed that castration results in *higher* concentration of radio-iodide in the gland *in vivo*. They suggested that a reduced saliva flow, with retention of saliva rich in radio-iodide within the gland, might account for this rather puzzling observation, but similar findings *in vitro* (Brown-Grant & Taylor, 1963) cannot be explained in this way. The autoradiographic evidence presented here does not support their suggestion, either. No significant pooling of saliva in the interlobular ducts or C.G.T. was observed in castrate as compared to control males. The cells of the C.G.T., however, were seen to be the principal labelled component of the gland, in castrates as in control mice, even though they were noticeably smaller in castrates (Fig. 3). The observations are consistent with the hypothesis that the cisternae of R.E.R., which are such a priminent feature of the C.G.T. in the control gland, act as an indifferent constituent of the cytoplasm so far as iodide concentration is concerned. Removal of the R.E.R. cisternae by castration appears to have no effect on the mechanism of iodide transport, but effectively increases the amount of radio-iodide per unit weight of gland (Llach & Tramezzani, 1962) by eliminating a diluting factor.

There remains no readily identifiable structure or organelle associated with the phenomenon of iodide concentration. The infoldings of basal membrane with closely associated mitochondria, which are such a prominent feature of C.G.T. cells in the castrate, are known to be present in other sites where ion transport is active. These infoldings are also present in the gland of the control male (Fig. 6), though compressed and distorted by the cisternae of R.E.R. It is tempting to assign to this membrane system the function of active transport of iodide, in the cells of the C.G.T. of the mouse.

SUMMARY

Previous studies have shown that the submaxillary glands of castrated male mice concentrate inorganic iodide to a higher level than those of control males. Glands from both groups of mice have been autoradiographed following an injection of [^{125}I]-iodide, using a technique that permits the localization of diffusible material. In both groups, iodide was concentrated in the cytoplasm of cells of the convoluted granular tubules (C.G.T.). Lower levels of concentration were also seen in the epithelium of the interlobular ducts and in their luminal contents.

Cells of the C.G.T. of normal males contained abundant rough endoplasmic reticulum (R.E.R.) with many dilated cisternae, and apical secretion granules. Infoldings of the basal cell membrane, with many associated mitochondria, were seen. Following castration, cells of the C.G.T. were smaller: the R.E.R. largely disappeared. The infoldings of the basal cell membrane, with their associated mitochondria, formed a conspicuous array visible as basal striations under the light microscope.

Fig. 8. An electron micrograph of the cisternae of R.E.R. in the cytoplasm of C.G.T. cells in the control male mouse, showing the finely granular material filling them. × 11 500.

Fig. 9. An electron micrograph of the apical two-thirds of a cell of the C.G.T. from a castrate male mouse. A few short lengths of R.E.R. are present, and many free ribosomes. The dilated cisternae of R.E.R. characteristic of the control male are absent. × 30000.

These findings are consistent with the hypothesis that active transport of iodide is a function of the cell membrane, which produces a high concentration of this ion in the cytoplasm. Castration eliminates from the cells the cisternae of R.E.R., reducing the volume of the C.G.T. and of the whole gland, without reducing the volume of the cytoplasmic compartment within which the iodide ion is concentrated.

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