J. Anat. (1970), **107**, 2, pp. 281–299
With 10 *figures*
Printed in Great Britain

Ultrastructure of cells in the human bronchial submucosal glands

BARBARA MEYRICK AND LYNNE REID

Department of Experimental Pathology,
*Institute of Diseases of the Chest, Brompton Hospital, London, S.W.*3

(*Received* 15 *August* 1969)

INTRODUCTION

The ultrastructure of the cells of the bronchial submucosal glands has been little studied in man or animal. In a paper describing the Kultschitzsky cell in the human bronchial glands, Bensch, Gordon & Miller (1965) included a summary of the main features of the two commonly recognized cell types—the serous and the mucous. Sorokin (1965) studied the bronchial glands of the opossum and reported mucous and hydrotic cells, the latter presumably being the counterpart of the serous cell. Recently histochemical methods, combined with light microscopy, have been correlated with organ culture to establish the pattern of uptake of radioactive metabolites by the serous and mucous cells (Lamb, 1969; Lamb & Reid, 1969, 1970), and have underlined the variety of intracellular acid glycoprotein found in these cells.

The purpose of this study is to describe the ultrastructure of the cells included in the human bronchial submucosal glands, particularly the serous cells, which, at this site, were found to have features not previously described for this type of cell. Two further bronchial gland cell types are here reported, the first, a large cell densely packed with mitochondria, the other, a 'clear cell' similar to that seen in the mouse salivary glands (Parks, 1961). Separate light microscopic studies (Meyrick, Sturgess & Reid, 1969) have shown that the cells of the first type line a collecting duct. The others, the 'clear cells', are, it is suggested, small lymphocytes. The following cell types are thus described: (i) serous, (ii) mucous, (iii) collecting duct cells, (iv) clear cells (probably lymphocytes) and (v) myoepithelial cells. Emphasis is on the features characteristic of each cell type rather than on their arrangement and distribution within a gland.

MATERIALS AND METHODS

Suitable specimens of bronchial submucosa were obtained for electron microscopic examination of the main or lobar bronchus from 14 surgical resections (the majority were from lobectomy, the rest from pneumonectomy), and from four biopsy specimens. The resection specimens were from 13 men whose age ranged from 49 to 74 years, and from one woman aged 44. In all the diagnosis was carcinoma. All patients smoked cigarettes save one who smoked cigars, three patients giving 10–15 as their daily cigarette consumption, the others 20 or over. The four biopsy specimens were all from men, whose age ranged from 44 to 73; in two cases a carcinoma of the bronchus was found; in the other two chronic bronchitis was the only diagnosis. The pre-operative medication in all was papaveretum and hyoscine, and for resection the anaesthetic was thiopentone sodium, pancuronium bromide and nitrous oxide.

A strip of bronchus, roughly 2 × 10 mm was excised from the resection specimen immediately on removal from the body and placed into fixative; the region of the tumour was avoided. The biopsy specimens were taken from the bronchoscopy forceps and placed directly into the fixative. Twelve resection specimens were fixed in 3% glutaraldehyde in cacodylate or phosphate buffer and two in 1% osmium tetroxide, prepared with either of the above buffers. For each of the four biopsy specimens primary fixation was carried out in both 3% glutaraldehyde in cacodylate buffer, and in 1% osmium tetroxide in cacodylate buffer. From all specimens 1 mm cubes of tissue were then taken, and dehydrated through graded alcohols. All specimens were embedded in Araldite. Sections were cut with a Huxley or LKB Ultramicrotome, using glass knives. Pale gold sections were then examined after staining with Karnovsky's lead hydroxide (Karnovsky, 1961).

Evaluation of the size of serous cell granules. As serous cell granules vary widely in size without displaying any special pattern of distribution within the cell, it was decided to see whether analysis of their size distribution offered evidence of more than one type of granule. Fourteen micrographs of serous cells were selected at random, each including the basement membrane, nucleus and apex of at least one serous cell. These micrographs were of specimens from six patients. The three cells from each of specimens 1 and 2 came from the same acinus, cells 2*a* and *b* being adjacent. Of the five cells from specimen 3, *a b* and *c* came from one acinus and were immediately adjacent; 3*d* and *e* were from another acinus but in the same section.

The diameter of the granules was measured in millimetres, in a plane parallel to the base of the cell and converted to nm. The basal granules were measured first and then the apical. Only granules larger than 3 mm were measured, but those between 1·5 and 3 mm were counted and included to give the total number of granules for each serous cell. It was felt that measurement of smaller granules measuring less than 1·5 mm was imprecise and these were ignored. Thus, the total number of granules over 100 nm and the size distribution for those larger than 300 nm was estimated for each cell studied.

RESULTS

Serous cell

In cross-section, the serous cell resembles a truncated cone; the cells are arranged around a small central lumen. The nucleus, which is spherical in shape, basal in position and with a prominent nucleolus, is presumed to be small relative to the area of the cell base, because it is rarely included in thin sections prepared for the electron microscope.

Intracellular organelles. The serous cells contain numerous secretory granules, usually concentrated towards the apex of the cell (Fig. 1). Although they are densely packed they do not distend the cell as in mucous cells. Individual granules are usually spherical and, even at the cell apex, completely surrounded by a membrane. The majority of serous granules are electron dense, but a few pale granules occur near the Golgi apparatus. Sometimes a halo effect is seen in the secretory granules (Fig. 2), due to the presence of a less electron-dense region around the central core of the granule, perhaps to a depth of half its radius, and with a granular or fibrillar appearance; the halo may appear only as a crescent. This region of relative pallor is found in granules

of all degrees of electron density, but is not common and usually affects single cells rather than a group, but all granules within a cell. 'Ghost granules' are also seen, similar in size to a serous granule but without a limiting membrane and showing the fibrillar or granular structure throughout. It is suggested later than these represent secretory granules from which the secretion has eluted. All these features are seen

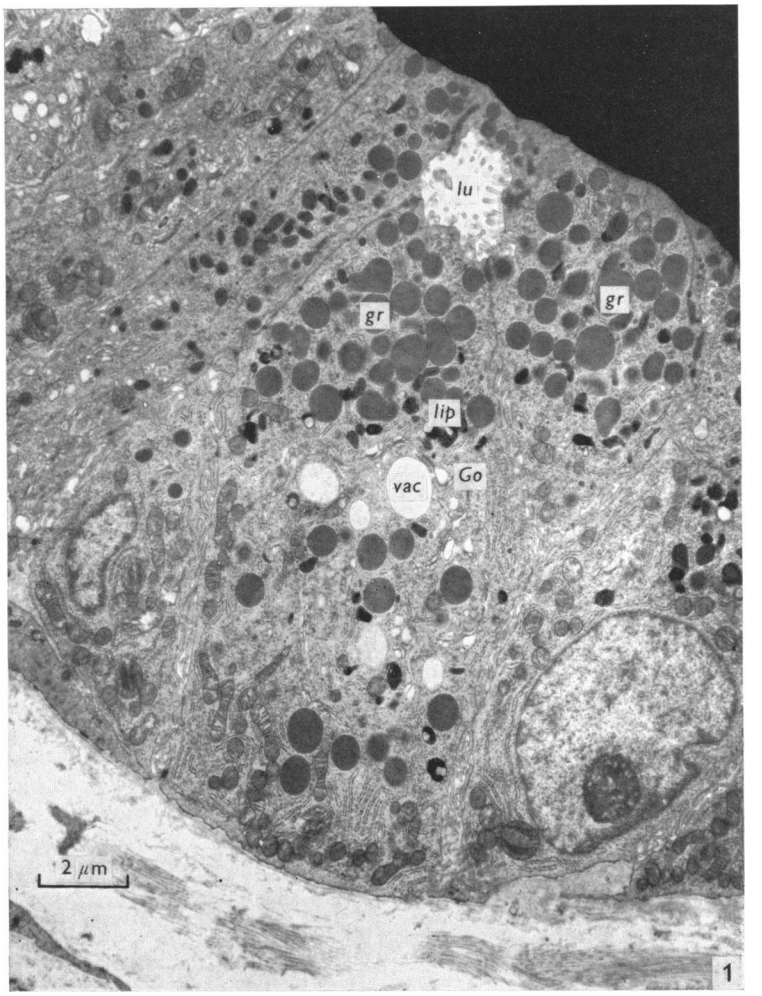
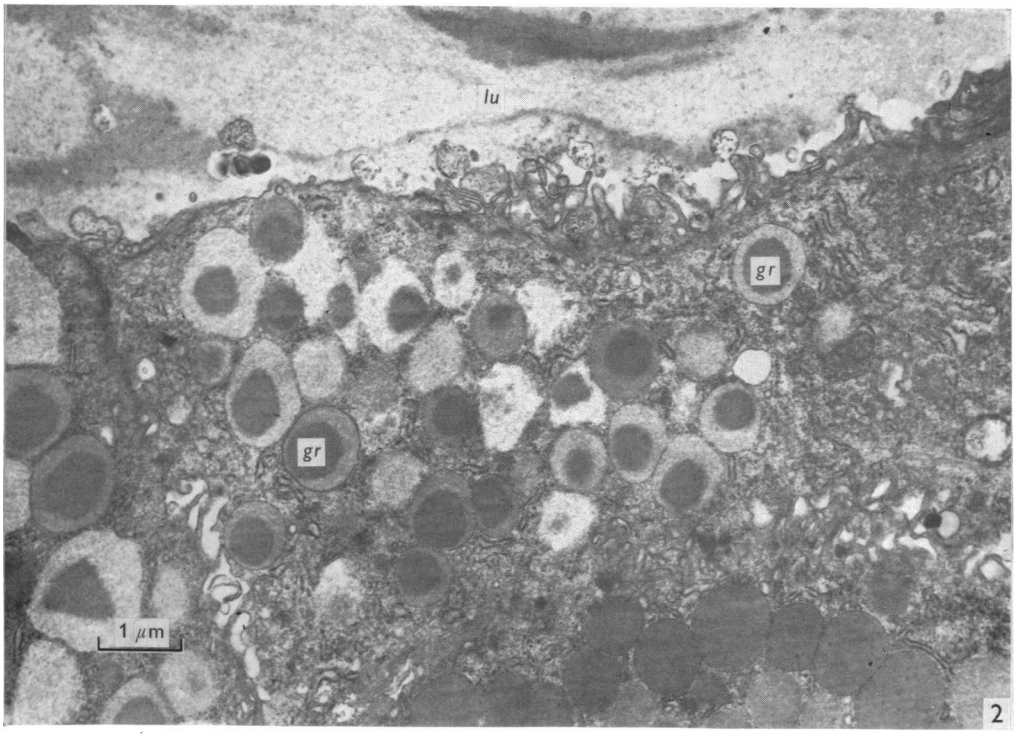
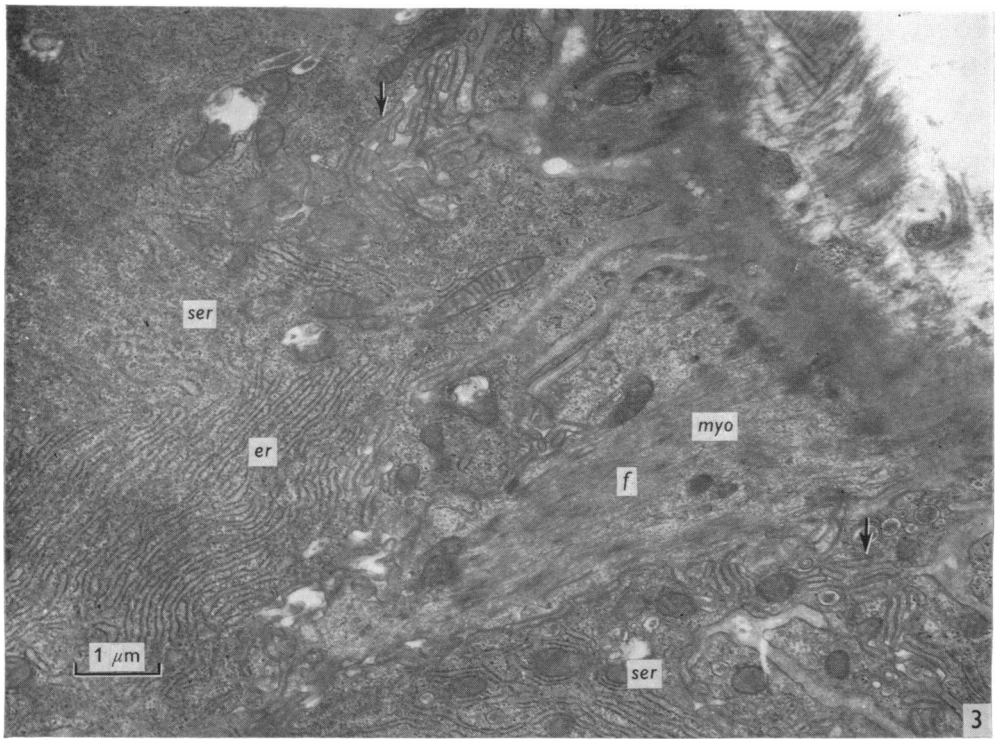


Fig. 1. Section through a serous acinus showing the majority of granules (*gr*) towards the cell apex and their variation in size. Randomly situated lipid granules (*lip*) are seen; large vacuoles (*vac*) occur near the Golgi apparatus (*Go*). The lumen (*lu*) of the acinus is shown. (Fixation Glut. + O_8O_4.)

after glutaraldehyde fixation, but after osmium tetroxide fixation the granules are paler and similar in density, making the limiting membrane more obvious.

Granules are seen close to the unit membrane of the apical cell surface but, while in the mucous cell granules are often seen open and discharging, this has not been


lu
gr
gr
1 μm
2


ser
er
myo
f
1 μm
ser
3

found in the serous cells. Serous granules can occasionally be identified in the lumen, but the surrounding serous cells are usually degenerate.

The majority of serous granules are between 300 and 1000 nm in diameter, but smaller granules of 100 nm are found, and occasionally larger ones, up to 1800 nm.

The mitochondria are long and ovoid; they are mainly concentrated in the base of the cell but a few are found among the granules. Most of the rough endoplasmic reticulum is at the cell base, the cisternae being narrow and grouped in parallel arrays; the density of the intracisternal secretion is the same as that in the paler secretory vacuoles. Free ribosomes are abundant through the cytoplasm.

The Golgi apparatus is well developed and supranuclear, often with dilated lamellae and many associated vesicles. Multivesicular bodies are seen occasionally. Osmiophilic material is found in the apical half of most serous cells (Fig. 1), either in an irregularly shaped body, or as an irregular denser region within an electron-dense secretory granule; occasionally a large pale secretory granule is outlined by a membrane including focal condensations of osmiophilic material (Fig. 1).

Where there are cell projections into the acinar lumen or intercellular canaliculus, numerous vesicles and tubules nearby contain material with an electron density similar to that of the secretory vacuoles.

Cell junctions. The base of the cell is flat where it lies against the basement membrane; but where, as is not infrequent, the cell base is indented by a myoepithelial cell, the contour is plicated (Fig. 3). By contrast the lateral surface is always much folded. Towards the basement membrane the intercellular space is wide, the membrane folds are mainly at right angles to the cell surface and jut into the intercellular space. In the mid-region adjacent cells interdigitate or the intercellular space is narrow. In the apical half of the cell, the intercellular space widens and frond-like cytoplasmic extensions project into it. It is this apical dilatation of the intercellular space that would represent the intercellular canaliculus seen with the light microscope. At the polar extremities of this space tight junctions are found. Some intercellular canaliculi indent the cell membrane to a depth equal to one-third of the cell diameter. These occur in the supranuclear region, often extend nearly to the cell surface and are seen even when the plane of section includes cell base and apex. Secretory granules are found in the cytoplasm surrounding canaliculi and crypts (Fig. 4).

When serous cells surround a large lumen, as is thought to occur at the junction of serous with mucous tubules, not all the above features are present. Inter- and intracellular canaliculi are not usually found. The serous cells have a slightly concave apical surface and cytoplasmic extensions into the duct lumen occur only in the region close to the terminal bars.

Fig. 2. Serous granules (*gr*) exhibiting a 'halo' effect. A dense central core is surrounded by a paler area and then a limiting membrane. Granules of uniform density are seen to the bottom right and the lumen (*lu*) is seen at the top. (Fixation Glut.+O_8O_4.)

Fig. 3. Part of a myoepithelial cell (*myo*) projecting between serous cells (*ser*). Many filaments (*f*) are seen running parallel to the lateral plasma membrane. Numerous interdigitations (arrow) of plasma membranes and arrays of rough endoplasmic reticulin (*er*) are seen in the serous cells. (Fixation Glut.+O_8O_4.)

Granule size. The serous cells show much less uniformity in the size and electron density of their granules, both within and between cells, than do mucous cells. The size distribution of granules within serous cells was analysed to see if this suggested either several 'populations' of cells or of granules within a cell. The granules varied in size from below 100 to 1740 nm. In the subsequent analysis any below 100 nm

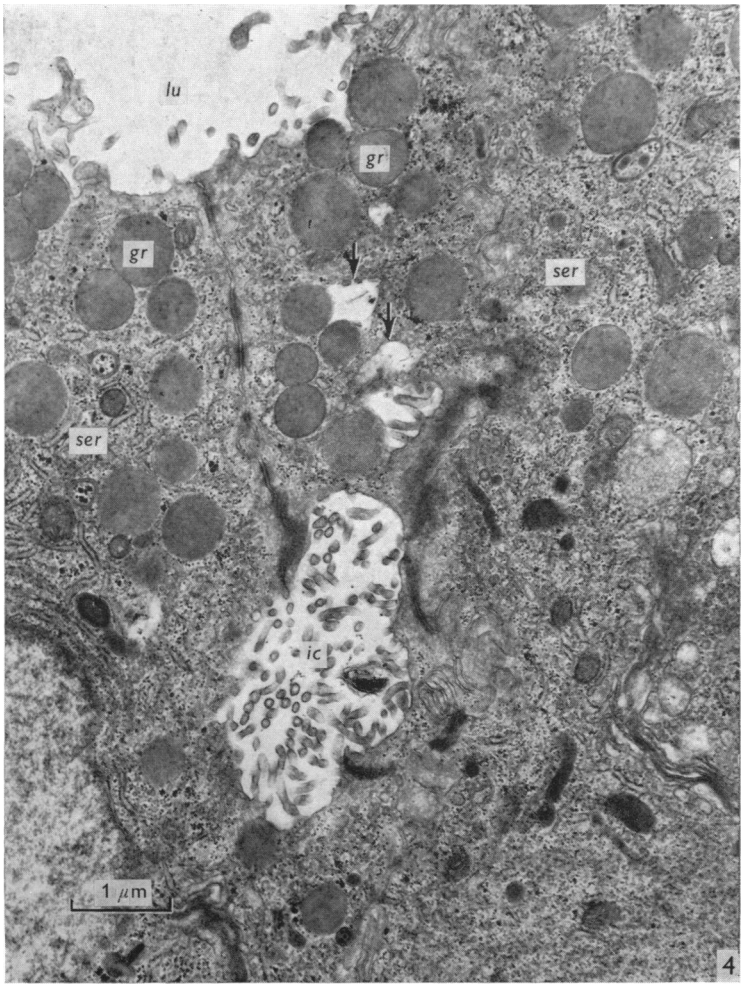


Fig. 4. Section through serous cells (*ser*) showing granules (*gr*) near the lumen (*lu*). An intercellular canaliculus (*ic*) is seen and part of an intracellular canaliculus (arrow). (Fixation Glut. + O_8O_4.)

were ignored. The Table summarizes the size distribution of the granules found within 14 cells.

The total number of granules varied from cell to cell, even within the same section: in the three cells of specimen 3 the numbers lay between 41 and 132. Although in some cells, e.g. those of specimen 2, no granules below 300 nm were seen, in others these

were predominant, up to 76% of the total number falling in this group, as in cell 3*e*. It will be seen in the Table that when granules are numerous those larger than 1300 nm do not predominate in a cell. A single cell does not show the full size range of granules, but within each cell the granules are grouped over a limited size range (see Table). It is striking that when small granules predominate the larger granules are absent. If the granules below 300 nm are not counted the distribution of granules is often Gaussian.

Table 1. *Table of distribution of serous cell granules by size (nm)*

Case and cell no.	Total granules	100–300	to 420	to 540	to 660	to 780	to 900	to 1020	to 1140	to 1260	to 1380	to 1500	to 1620	to 1740
1*a*	98	40	19	11	15	9	2	1	1	1	—	—	—	—
b	56	54	11	17	16	5	7	—	—	—	—	—	—	—
c	79	51	14	18	9	3	5	—	—	—	—	—	—	—
2*a*	67	—	1	5	3	5	15	18	13	13	11	11	1	2
b	31	—	—	10	8	7	25	23	8	13	5	2	—	—
c	54	—	22	22	24	20	8	4	—	—	—	—	—	—
3*a*	41	24	5	8	10	1	10	6	10	4	12	10	—	—
b	70	14	11	23	24	11	11	2	—	2	1	—	—	—
c	119	70	13	7	5	3	2	1	—	—	—	—	—	—
d	118	50	26	19	5	—	1	—	—	—	—	—		—
e	132	76	17	8	—	—	—	—	—	—	—	—	—	
4	153	54	10	13	9	11	2	1	1	—	—	—	—	—
5	79	21	9	14	14	22	12	5	2	1	—	—	—	—
6	37	6	—	5	6	12	14	23	21	9	4	—	—	—

The smaller granules may be very numerous, suggesting that they represent a separate population. An alternative view is that the various sizes of granule represent different stages in a continuous cycle of secretion. Fig. 5 illustrates the distribution of granules within four cells which, it is submitted, represent different stages in a secretory cycle, secretion starting in small granules which increase in size until ultimately they discharge: after this small granules again reform.

Fig. 5(i) represents in a histogram the granules found in cell 4. The total number of granules is high (153), many of them being less than 300 nm in diameter and very few above 800 nm. In cell 1*a* (Fig. 5(ii)) fewer of the granules are below 300 nm, and a higher proportion is found between 300 and 800 nm. The small granules can no longer be seen in cell 2*a* (Fig. 5(iii)), but the many granules still within it are distributed mainly in the large size groups. The same wide range of granule size is found in cell 3*a* (Fig. 5(iv)), but the smaller granules are now reappearing, probably representing the start of another cycle of secretion. The total number of granules has steadily decreased from Fig. 5(i) to (iv). All the cells included in the Table could be regarded as representing some part of such a cycle. The larger the number of granules the more likely is the maximum concentration to be below 600 nm. A cell seems never to be emptied completely of its granules.

In specimens 1, 2 and 3, where more than one cell has been counted from each acinus, the size distribution of the granules suggests that within an acinus the cells

are in the same phase of the cycle. This is most clearly illustrated by cells 2*a*, *b*, *e*: the two cells from specimen 2 were adjacent and are most alike of the three cells from this sample. On the other hand, of the cells from one acinus most difference is found between 3*a*, *b* and *c*, and, as these cells were adjacent, it would seem that close similarity between adjacent cells is by no means constant.

Thus secretory granules of the serous cell appear to pass through a cycle of maturation. They may reach 1900 nm in size but usually between 1000 and 1500 nm they are

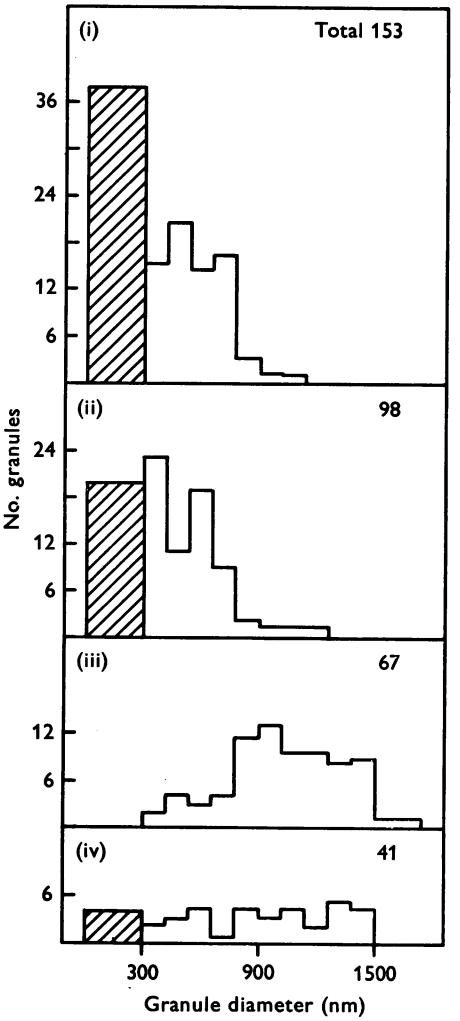


Fig. 5. Pattern of granule distribution (by size) within four of the serous cells included in Table 1. (i) Specimen 4, (ii) specimen 1*a*, (iii) specimen 2*a*, (iv) specimen 3*a*. All granules under 300 nm have been included in one group (shaded). Through the series the small granules reduce progressively, while the larger increase; at the same time discharge occurs with reduction in total number. In (iv) although large granules are still present, the total number is reduced and small ones are reappearing.

secreted. The variation in electron density seen between the granules is found at all sizes and would seem not to change with maturation. This suggests that chemically there are differences in secretion established early in the development of the granule and changing little even as the granule increases in size.

Mucous cell

The mucous cell has a columnar outline, its nucleus is basally situated and irregular in outline, with dense chromatin. The rest of the cell is usually packed with secretory granules of moderate electron density, with slight variation between granules. The size range of the typical secretory granule is between 300 and 1800 nm (Fig. 6), the

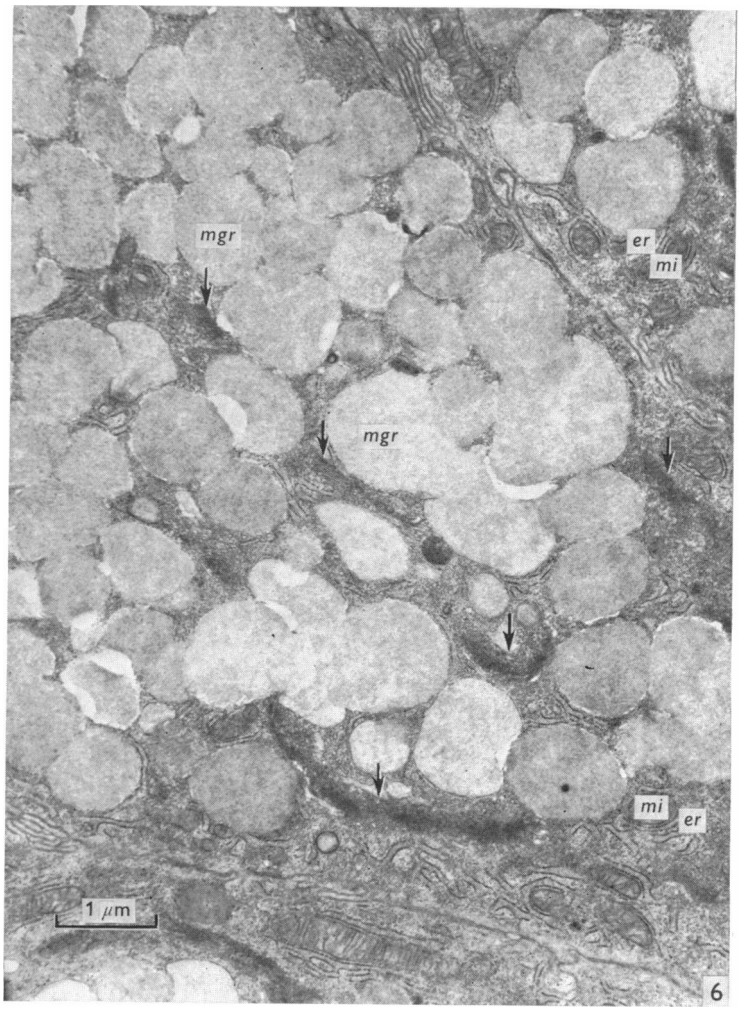


Fig. 6. Part of a bronchial gland mucous cell, showing granules (*mgr*) of different densities and confluence. Groups of Golgi lamellae are seen at arrows and some mitochondria (*mi*) appear to be surrounded by rough endoplasmic reticulum (*er*). (Fixation Glut. + O_8O_4.)

smaller being central and near the Golgi apparatus. The larger granules, near the cell edge, appear to fuse, the limiting membrane no longer being obvious although around the smaller granules a membrane is still evident. The fixative used makes less difference to the appearance of the membrane around the secretory granules than is the case with the serous cells. The Golgi apparatus is easily identified: it lies mainly just above the nucleus and consists of lamellae with many associated vesicles and vacuoles. Groups of lamellae and vesicles, measuring *c.* 30–40 nm in diameter, are also seen more superficially between the larger secretory granules.

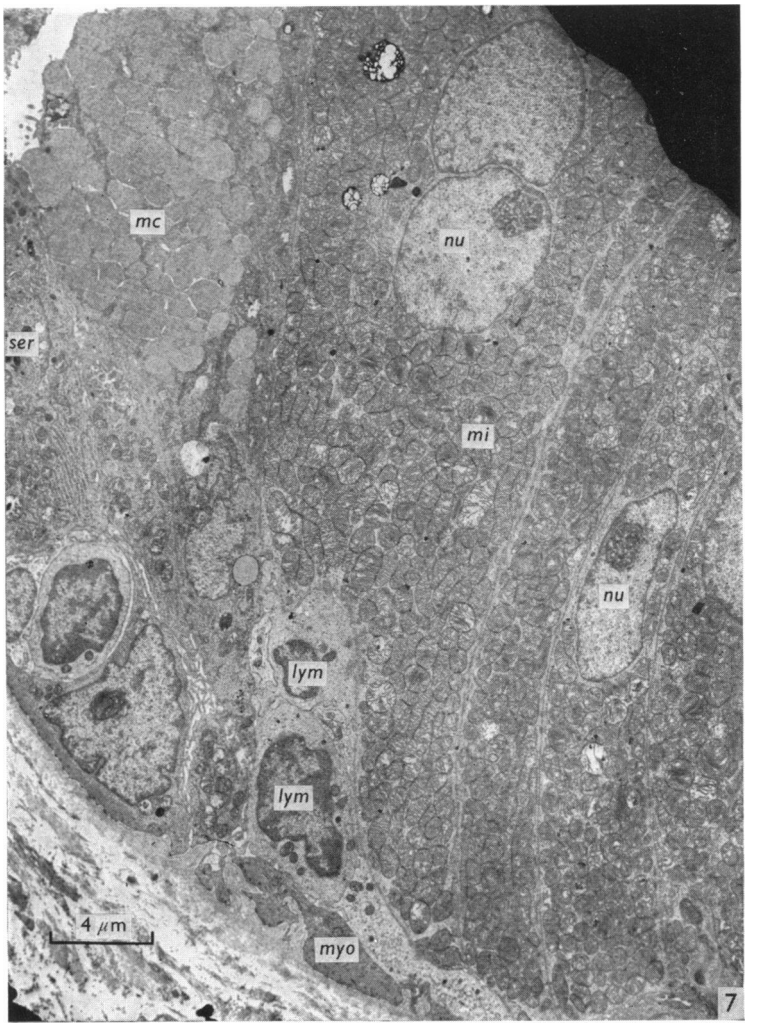


Fig. 7. Section showing part of a collecting duct with two lymphocytes (*lym*) at the base. The collecting duct cells are very tall with centrally placed nuclei (*nu*) and the cytoplasm is densely packed with mitochondria (*mi*). A myoepithelial cell (*myo*) is shown at the base of the collecting duct. Mucous (*mc*) and serous cells (*ser*) and a lymphocyte are seen to the left of the picture. (Fixation Glut.+O_sO_4.)

The other organelles are found between the granules. Mitochondria are frequent and rough endoplasmic reticulum often surrounds a mitochondrion. Free ribosomes and multivesicular bodies are seen. Occasionally lipochondria can be identified (Kurosumi, 1961).

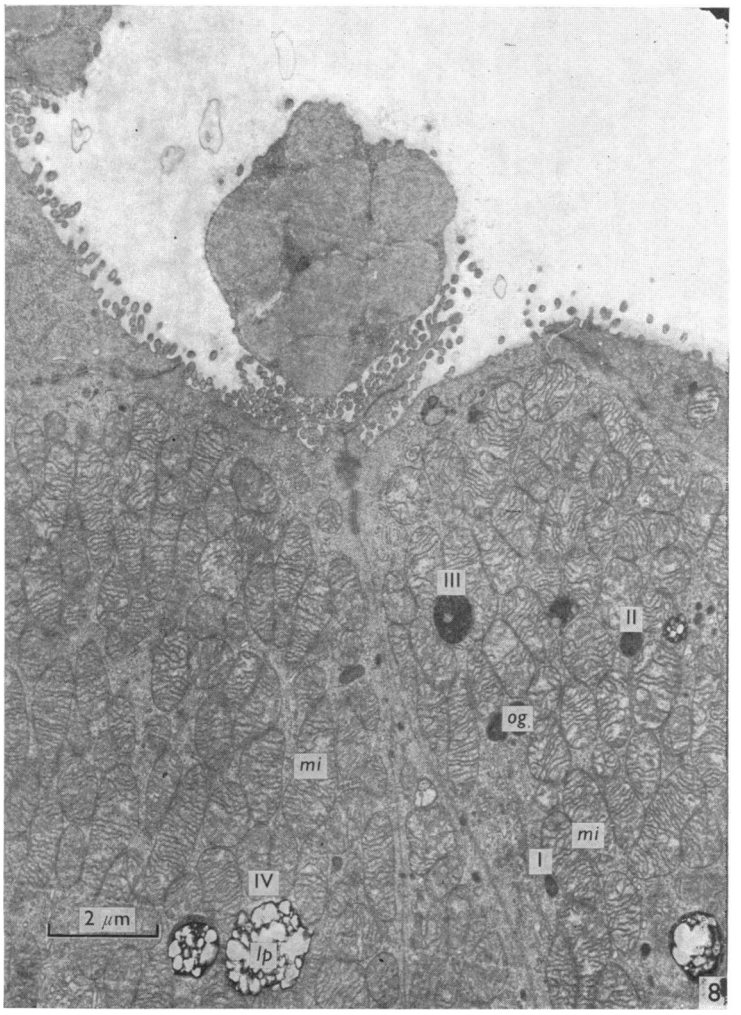


Fig. 8. Collecting duct cells, higher power than Fig. 7, to show numerous mitochondria (*mi*), the majority of which have their long axis in a plane parallel to the lateral membranes. A progression from the osmiophilic granules (*og*) to lipochondria (*lp*) can be seen I–IV. (Fixation Glut. + O_8O_4.)

Cell boundaries. When the mucous cell is distended with secretion its apical (free) edge is smooth and bulges into the lumen; secretory vacuoles may be seen discharging. Occasionally a small cytoplasmic process may protrude into the lumen.

Bleb-like cytoplasmic protrusions from the side of the cell occur toward its otherwise smooth base: these are simple in outline, shallow and with a narrow neck. The cell base and the region near the apex, are smooth except near the latter and terminal

bars and desmosomes are seen. The basement membrane is often separated from the mucous cell by part of a myoepithelial cell.

Bronchial collecting duct cell

Occasionally in sections of the glands a line of tall columnar epithelial cells is seen (Fig. 7). In transverse section the cells appear polygonal. The nucleus is an elongated ovoid, centrally placed and with a prominent nucleolus. These cells are 60–80 μm high and striking because of their densely and uniformly packed mitochondria, mainly orientated to run parallel with the lateral cell membrane. The mitochondria measure up to 3·0 μm long and 1·0 μm wide, with cristae at right angles to the long axis. Intramitochondrial bodies are not found. In the supranuclear region of the cell lies a well-developed Golgi apparatus, containing basket-shaped groups of lamellae, numerous vesicles measuring 40–60 nm in diameter with only an occasional vacuole. Osmiophilic granules, measuring 150–400 nm in diameter, are seen in this area and, in the majority of cells, lipochondria (Kurosumi, 1961). Transitional forms between the osmiophilic granules and lipochondria are seen (Fig. 8). There is little endoplasmic reticulum but free ribosomes are numerous. Around the whole cell circumference, immediately under the cell membrane, lies a narrow zone free from mitochondria and with a fibrillar appearance. The apex of the cell is flat with a few cytoplasmic extensions and contains vesicles in the mitochondria-free zone. The cell edge is smooth, save at the angles of junction, where a group of cell processes interdigitate. Occasionally desmosomes are seen away from the interdigitations.

These cells are found directly against mucous cells but not against serous cells. Myoepithelial cells are found beneath the duct cells but processes from the myoepithelial cells have not been seen protruding between collecting duct cells. 'Clear cells' are more numerous here than in the acini; they lie between myoepithelial and duct cells.

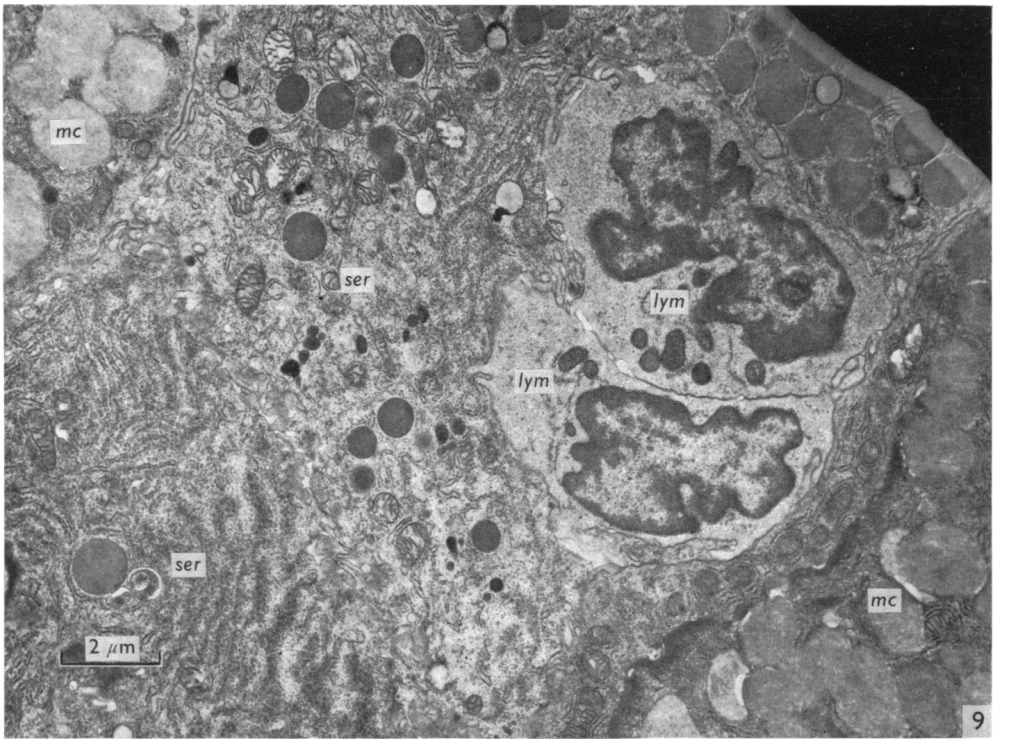
Lymphocyte (clear cell)

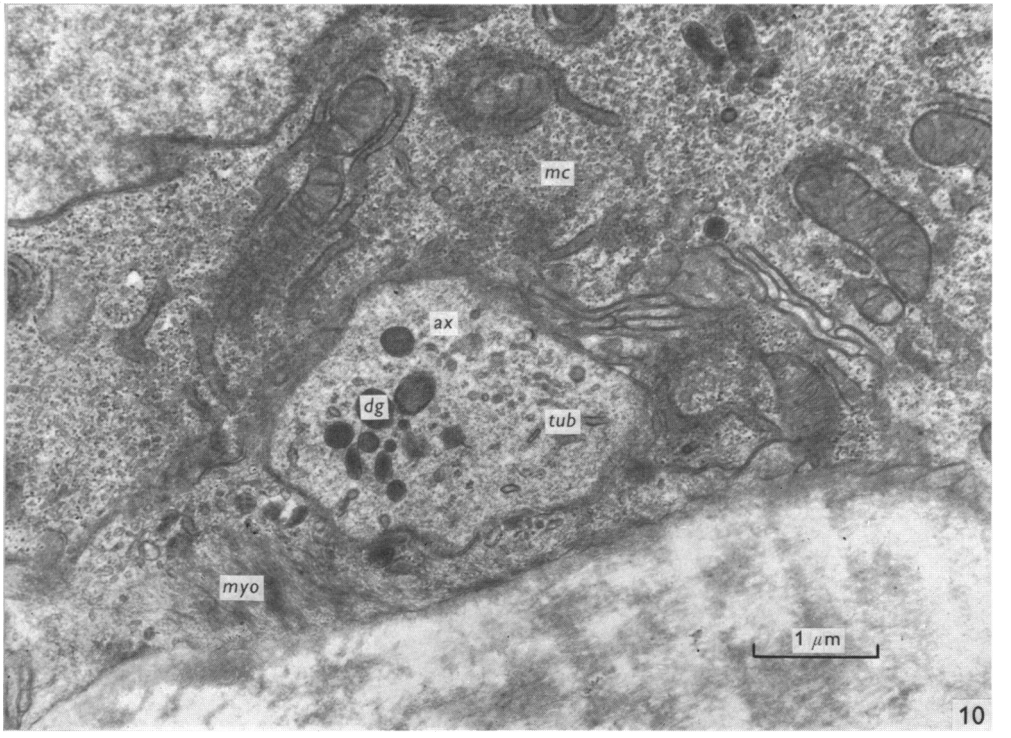
In most sections about half of the acini show at least one 'clear cell' (Fig. 9). These cells are small and have not been seen to reach the lumen, but are found between the cells and the basement membrane associated with the serous, mucous and collecting duct cells. They are identified by their thin layer of cytoplasm, almost free of organelles; in any one section only a few mitochondria and a few cisternae of rough endoplasmic reticulum are seen. In Fig. 9 it can be seen that the cytoplasm includes numerous free ribosomes and polysomes. The nucleus may be indented and often contains a nucleolus. The cell outline is simple with focal regions of interdigitations with surrounding cells.

This cell would seem to be a lymphocyte although it has been described as a special cell type in the mouse salivary gland (Parks, 1961) and as an early myoepithelial cell in the human submaxillary gland (Tandler, 1965).

Fig. 9. Section through an acinus showing mucous (*mc*) and serous (*ser*) cells and two electron-lucent cells, probably lymphocytes (*lym*) within the body of the acinus. (Fixation Glut. + O_8O_4).

Fig. 10. A terminal axon (*ax*) situated between a mucous (*mc*) and a myoepithelial cell (*myo*) showing tubules (*tub*) and dense granules (*dg*). (Fixation Glut. + O_8O_4.)


mc
ser
lym
lym
ser
2 μm
mc
9


mc
ax
dg
tub
myo
1 μm
10

Myoepithelial cells and basement membrane

Myoepithelial cells are found beneath the serous, mucous and collecting duct cells, their processes fitting into basal grooves in the overlying cells (Fig. 4). These cells exhibit numerous fibrils running parallel to the basement membrane. A small amount of rough endoplasmic reticulum, mitochondria and occasional pinocytic vesicles, are also seen. The acinar basement membrane is narrow, and not well defined. The plasma membrane of the adjacent secretory cells is crenated at junctional regions with a myoepithelial cell.

Occasional cells (mast cell and Kultschitsky cell)

Occasionally an acinus includes, internal to the basement membrane, a mast cell: one was also seen near the basement membrane in a collecting duct. The Kultschitsky cell described in the human bronchial gland by Bensch *et al.* (1965) was found only once against the basement membrane and between serous cells.

Innervation and prevalence

Bundles of unmyelinated axons are found between acini. Single terminal axons (Fig. 10) are seen within the acinar basement membrane, between serous cells, collecting duct cells and mucous cells and between any of these and myoepithelial cells. The secretory tubules or acini of the human bronchial submucosal glands consist mainly of three types of cell, the serous and mucous secretory cell and the myoepithelial cell. These were found in all sections studied. The lymphocyte (or 'clear cell') was found in about half of all acini examined. While only three of the specimens, all from lobectomy, included bronchial duct cells, light microscopic studies suggest that they are found in all glands (Meyrick *et al.* 1969).

DISCUSSION

The mucous, serous and myoepithelial cells have all been identified in the human bronchial glands with the light microscope (Fuchs-Wolfring, 1898). The electron microscope has revealed the mitochondria-packed cell and the clear cell and new features of the serous cell. Light microscopic examination of the central region of the gland showed that the mitochondria-packed cells form a duct. The distribution of these cells in the duct is reported elsewhere (Meyrick *et al.* 1969). The clear cell seems to be a lymphocyte: it also can be identified by the light microscope.

Serous cell. The statistical analysis of the size of secretory granules in serous cells did not support the idea that there are distinct granule populations or cell types, but rather that a cycle of secretion occurs within a cell transforming the small granules to large ones. The total number and size range of the granules within a cell support the idea of a cycle of granule development prior to their discharge, leaving a cell with only a few granules before small ones appear and again become numerous.

The way in which the secretory granule is discharged from the cell is still not clear. Caro & Palade (1964) suggested that after fusion of the granule membrane with that of the cell the whole granule is ejected through an interruption of the cell membrane: this type of secretion has been seen occurring from the mucous cell, but as it has not

been seen from the serous cell, it would seem that the secretory product of this cell may elute from the granule, perhaps through the cell cytoplasm, and diffuse across the cell membrane. Secretion may take place from the apical edge into the lumen or laterally to the crypts and the intercellular canaliculi.

The pale fibrillar 'ghost' regions could represent the cytoplasmic framework of the secretory granules. These areas are usually large, which suggests that they occur at the end of the secretory process rather than at its beginning. This cytoplasmic 'skeleton' either collapses quickly or else its interstices are filled by components of the same electron density as the rest of the cell. The halo effect seen in some granules might represent a phase in the discharge of secretion from the granules, part of the secretion already being lost, or the secretion may be of a type to respond by the condensation to the physico-chemical conditions induced by fixation and preparation.

It has recently been shown with the light microscope that the serous cell produces an acid glycoprotein that can be distinguished from that of the mucous cell by its higher content of sulphate relative to sialic acid (Lamb, 1969). The serous cell secretion may be of smaller molecular weight than that of the mucous cell, or it may be that the acid glycoprotein of the serous cell does not become as bulky as the secretion of the mucous cell, perhaps because less water is linked to the molecule.

Using the light microscope Lamb (1969) and Lamb & Reid (1970) have reported a central concentration of AB staining within the granule after sialidase treatment. This may correspond to the more electron-dense central region sometimes seen with the electron microscope.

Electron microscopic studies have been made of serous cells in the salivary and lacrimal glands of the rat (Scott & Pease, 1959), the rat submaxillary gland (Tamarin & Screenby, 1965), human submaxillary gland (Tandler, 1962), murine sublingual gland (Parks, 1961) and in the rabbit pancreas (Meyer & Bencosme, 1965). While intercellular canaliculi are an accepted feature of these cells, as found with both light and electron microscopy, the intracellular crypt-like extensions seen in the human bronchial serous cell have not been described previously in serous cells. They do not achieve the size reported in stomach parietal cells (Winborn & Bockman, 1968).

The role of the intercellular canaliculi is not clear; they may offer a discharge site, for water perhaps, or a storage site, although in either case they increase the area of the cell surface. As the granules of all sizes are found distributed throughout the cell cytoplasm, large as well as small ones are found near lateral and apical surfaces, suggesting that secretion occurs into the intercellular canaliculi as well as from the apex.

The presence of tight junctions between the cell edges is not necessarily a barrier to discharge from the intercellular spaces to the lumen. Loewenstein (1966) has suggested that ion permeability through such regions is at least four times greater than through non-junctional regions.

Mucous cell. There is no difference in electron density in mucous granules as between one cell and another or within a cell which might correspond to the range of types of acid glycoprotein found within some cells by light microscopy (Lamb & Reid, 1969).

Collecting duct cells. The numerous mitochondria in the duct cell are, at first

glance, reminiscent of the striated duct cell in the lacrimal and salivary glands, but the bronchial gland cells lack the extreme folding of the basal plasma membrane which gives the 'striated' appearance. This duct has been identified with the light microscope (Meyrick *et al.* 1969) and found to be a structure about 1 mm long in a normal gland overlying cartilage, and with a volume just over 1 mm^3. All secretory tubules open into this duct, but the distance along which secretion from the various tubules must pass varies widely. The lumen is larger than that in the secretory tubules, the duct lumen being up to 110 μm wide, the average value for the serous tubules only 5 μm and for the mucous tubules 25 μm. It may be that the large duct lumen represents the greater flow along this part; it may act as a site of storage, or the lumen may be wider because this is the site where the mucus is hydrated and swells. A fluid-regulating role has been suggested for the salivary striated duct cell (Junqueira, 1964), and it may be that the bronchial gland duct cell also plays a part in fluid and ionic regulation.

Myoepithelial cell. This cell is found at the base of serous, mucous and collecting duct cells. It would seem likely therefore that its action contributes to the passage of secretion along both types of tubule, mucous and serous, as well as along the collecting duct.

Clear cell. Clear cells have been described in the mouse, but not in the rat, salivary glands (Parks, 1961); in the bronchial glands, these cells have been described here for the first time associated both with the secretory tubules and the collecting duct. In 1965 Tandler reported this cell in the human submaxillary gland and suggested that it was a precursor of the myoepithelial cell. We would suggest that in the bronchial gland, and probably in the submaxillary gland also, this cell is a lymphocyte.

The 'clear cell' is usually scattered singly or in pairs, seeming, with this low prevalence, to be a normal part of the organ structure and not to represent an inflammatory cell response. It is found lying close to the inner aspect of the acinar basement membrane whereas cells of an inflammatory infiltration are usually found mainly between acini. The cell structure seems to be that of an 'intermediate lymphocyte' (de Petris, Karlsbad, Pernis & Turk, 1966; Harris, 1969), that is, it is intermediate between an immunoblast and a small lymphocyte.

The presence of these lymphocytes near the basement membrane in the bronchial gland is similar to that described by Darlington & Rogers (1966) for epithelial lymphocytes in the villi of the mouse small intestine. These authors have shown that these cells comprise about 9 % of the epithelial cells but do not migrate up the villus as the other epithelial cells and, as they lie in the basal third of the epithelium (see also Kelsall, 1946), do not seem to migrate through the epithelium to the surface. These cells have a characteristic pattern of labelling, both with ^{35}S-sulphate and with tritiated thymidine, that is, different from that of blood lymphocytes. No suggestion can at present be offered for the source of these lymphocytes.

The presence of an occasional lymphocyte between duct and secretory cell and basement membrane may be linked with the possible role of the lymphocyte as a growth regulating cell (Carrel, 1922; Loutit, 1962). The increasing evidence that normal organ size must be based on a central as well as a peripheral control, with some feedback between the two, has led to the suggestion that the lymphocyte may

be one of the mediators in the system (Burch, 1968), which would account for its presence.

It is now known that secretory epithelium may produce globulins, different from serum globulins, at least in some respects, and it may be that the epithelial lymphocytes are linked to its production. Secretory IgA has been identified in the intestinal and also the bronchial epithelial secretion.

In a recent study Martinez-Tello, Braun & Blanc (1968) have identified cells of the lymphocytic series by their response to fluorescein-labelled antibody of the IgA, IgG and IgM series. In chronic inflammation of the broncho-pulmonary tree the cells labelled by the IgA and IgG antibody increased in number; those exhibiting IgA activity were found mainly in the bronchial mucosa, and those IgG, in the lymph nodes. The cells labelled with IgM antibody had not increased: in the illustrations these are seen as scattered cells, but it is not possible to be sure whether they lie within the acinar basement membrane. Certainly their study identifies small numbers of cells labelled with IgM antibody that do not appear to be increased in number, even in chronic inflammation, suggesting that their role is different from that of the other globulin producers. It can be suggested, but no more than tentatively, that in the human bronchial submucosal glands epithelial lymphocytes are found, and that this type of lymphocyte may be different from those present in chronic inflammation, and may be related to the production of globulin, perhaps to IgM.

Innervation. Terminal axons have been described in close association with serous, mucous and collecting duct cells. They have not been found near the clear cell.

SUMMARY

The electron microscopic features of the cells comprising the human bronchial submucosal gland are described. *From the serous cell* frond-like projections arise to line the intercellular canaliculi and, from the latter, crypt-like indentations are seen. Analysis of the size and distribution of the secretory granules suggests that differences represent different phases of a secretory cycle, not various populations of cell or granule. Comparison of the features of the *mucous cell* with those of the serous suggests a difference in the way the granules discharge. In the mucous cell the granule can be seen emptying into the lumen whereas no such appearance has been seen in the serous cell. In this the presence of large 'ghost' granules and of the occasional condensation of secretion into the central region of the granule suggests that the secretory product elutes form the granule.

A new cell type, the *collecting duct cell*, is described. It is 70 μm tall, packed with mitochondria with a centrally placed nucleus and lines the central part of the duct system. It presumably influences water and ionic balance of the gland secretion.

The *myoepithelial cell* has been found associated with serous, mucous and collecting duct cells. A cell, here considered as a *lymphocyte* and resembling the 'clear cell' described in the salivary gland, has been found with the secretory and duct cells, in roughly half of the acini seen in section. The lymphocyte is scattered singly or in pairs within the basement membrane but only toward the base of the epithelial cells, thus resembling certain lymphocytes described in the gut. This type of lymphocyte may be concerned with the epithelial secretion of globulin.

Terminal axons have been found within the acinar basement membrane between serous, mucous and myoepithelial cells and toward the base of collecting duct cells. While an occasional mast cell has been seen between the epithelial cells, only one Kultschitzky cell with nucleus has been found.

We are grateful to the following surgeons and their theatre staff for the resection specimens studied here, Messrs O. S. Tubbs, M. Paneth and S. C. Lennox, of the Brompton Hosptial, and for the biopsy specimens to Mr T. A. H. English of the London Chest Hospital: to Dr Bernard Benjamin, Director of Research and Intelligence, Greater London Council, for the statistical help: and to Mr M. S. C. Birbeck for helpful discussion.

The work reported here was included in a presentation to the National Tuberculosis and Respiratory Disease Association at their annual meeting in Cleveland, Ohio, held 24/27 May 1970. This was read by Dr. J. Kleinerman in the absence of Professor Lynne Reid.

REFERENCES

BENSCH, K. G., GORDON, G. B. & MILLER, L. F. (1965). Studies on the bronchial counterpart of the Kultschitsky (Argentaffin) cell and innervation of bronchial glands. *J. Ultrastruct. Res.* **12**, 668–686.

BURCH, P. R. J. (1968). An inquiry concerning growth disease and ageing. Edinburgh: Oliver and Boyd Ltd.

CARO, L. G. & PALADE, G. E. (1964). Protein synthesis, storage and discharge in the pancreatic exocrine cell: an autoradiographic study. *J. Cell Biol.* **20**, 473–495.

CARREL, A. (1922). Growth-promoting function of leucocytes. *J. exp. Med.* **36**, 385–391.

DARLINGTON, D. & ROGERS, A. W. (1966). Epithelial lymphocytes in the small intestine in the mouse. *J. Anat.* **100**, 813–830.

FUCHS-WOLFRING, S. (1898). Über den feineren Bau der Drüsen des Kehlkopfes und der Luftröher. *Arch. mikrosk. Anat. EntwMech.* **52**, 735–761.

HARRIS, M. (1969). The cellular infiltrate in Hashimoto's disease and focal lymphocytic thyroiditis. *J. clin. Path.* **22**, 326–333.

JUNQUEIRA, L. C. U. (1967). Control of cell secretion. In *Secretory Mechanisms of Salivary Glands*, pp. 286–302. (Ed. Schneyer and Schneyer) New York; Academic Press.

KARNOVSKY, M. J. (1961). Simple methods for 'staining with lead' at high pH in electron microscopy. *J. biophys. biochem. Cytol.* **11**, 729–732.

KELSALL, M. A. (1946). Lymphocytes in the intestinal epithelium and Peyer's patches of normal and tumor bearing hamsters. *Anat. Rec.* **96**, 391–409.

KUROSUMI, K. (1961). Electron microscopic analysis of secretion mechanism. *Int. Rev. Cytol.* **11**, 28–31.

LAMB, D. (1969). Intracellular development and secretion of mucus in the normal and morbid bronchial tree. Ph.D. Thesis, University of London.

LAMB, D. & REID, L. (1969). Histochemical types of acidic glycoprotein produced by mucous cells of the tracheo-bronchial glands in man. *J. Path.* **98**, 213–229.

LAMB, D. & REID, L. (1970). Histochemical and autoradiographic investigation of the serous cells of the human bronchial glands. *J. Path.* **100**, 127–138.

LOUTIT, J. F. (1962). Immunological and trophic functions of lymphocytes. *Lancet* ii, 1106–1108.

LOEWENSTEIN, W. R. (1966). Permeability of membrane junctions. *Ann. N.Y. Acad. Sci.* **137**, 441–472.

MARTINEZ-TELLO, J. F., BRAUN, D. G. & BLANC, W. A. (1968). Immunoglobulin production in bronchial mucosa and bronchial lymph nodes, particularly in cystic fibrosis of the pancreas. *J. Immun.* **101**, 989–1003.

MEYER, J. & BENCOSME, S. A. (1965). The fine structure of normal rabbit pancreatic islet cells. *Revue can. Biol.* **24**, 179–205.

MEYRICK, B., STURGESS, J. M. & REID, L. (1969). A reconstruction of the duct system and secretory tubules of the human bronchial submucosal gland. *Thorax* **24**, 729–736.

PARKS, H. F. (1961). On the fine structure of the parotid gland of mouse and rat. *Am. J. Anat.* **108**, 303–329.

PETRIS, S. DE, KARLSBAD, G., PERNIS, B. & TURK. J. L. (1966). Ultrastructure of cells present in lymph nodes during the development of contact sensitivity. *Int. archs Allergy appl. Immun.* **29**, 112–130.

SCOTT, B. L. & PEASE, D. C. (1959). Electron microscopy of the salivary and lacrimal glands of the rat. *Am. J. Anat.* **104**, 115–161.

SOROKIN, S. (1965). On the cytology and cytochemistry of the opposum's bronchial glands. *Am. J. Anat.* **117**, 311–337.

TAMARIN. A. & SCREENBY, L. M. (1965). The rat submaxillary salivary gland. *J. Morph.* **117**, 295–352.

TANDLER, B. (1962). Ultrastructure of human submaxillary glands. I. Architecture and histological relationships of the secretory cell. *Am. J. Anat.* **111**, 287–307.

TANDLER, B. (1965). Ultrastructure of the human submaxillary glands. III. Myoepithelium. *Z. Zellforsch. mikrosk. Anat.* **68**, 852–863.

WINBORN, W. B. & BOCKMAN, D. E. (1968). Origin of lysosomes in parietal cells. *Lab. Invest.* **19**, 256–264.