

## FINE STRUCTURE OF CHICKEN THYMIC EPITHELIAL VESICLES

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### SUMMARY

This paper describes the fine structure of epithelial vesicles as observed in the thymus of the chicken. Three types of vesicles are found. The largest is made up of a stratified columnar epithelium containing lymphoid cells. The lumen contains a fairly light material and cell debris. The lumen-bordering cells are columnar and their microvilli are sometimes developed to such an extent as to appear as a brush border. The wall of the vesicles contains lymphocytes and granular cells with dense granules 100-500 nm in diameter. Smaller vesicles are found which contain no lymphoid cells. They are made of a simple or stratified epithelium containing typical mucous cells, and columnar cells similar to the bordering cells of the large vesicles. The third type is an intracytoplasmic vesicle in epithelial reticular cells, which differs from usual vacuoles by the presence of microvilli and its often unusually large size. The morphology of the first 2 types of vesicles indicates a secretory capability, the function of which is unknown. Data from the literature suggest that the third type produces the factor(s) conferring cellular immunological competence.

### INTRODUCTION

The thymus has attracted much attention in the recent literature, due in large part to its role in the immunological response. This role appears to be mediated by secretions (Trainin, 1974), which in turn appear to be produced by the epithelial moiety of the thymus. The relative importance of the various epithelial components - epithelial reticulum, Hassall's bodies and epithelial vesicles - is still a matter of conjecture.

The ultrastructure of thymic epithelial vesicles has been investigated only in mammals: rat (Lundin & Schelin, 1965; van Haelst, 1967*a, b*; Senelar *et al.* 1973); mouse (Hoshino, 1962; Weiss, 1963; Kohnen & Weiss, 1964; Cowan & Sorenson, 1964; Dung, 1973; Cordier, 1974*a, b*); guinea-pig (Kohnen & Weiss, 1964; Izard, 1965, 1966; Mandel, 1968); hamster (Ito & Hoshino, 1966); monkey (Chapman, 1971); and man (Sebuwufu, 1968). Birds differ from mammals in their lymphoid assets by the presence of the bursa of Fabricius, which, like the thymus, has an epithelial origin. Thus it is of interest to determine whether the presence of the bursa is accompanied by a difference in their thymic epithelial components.

Thymic epithelial vesicles have been observed in the chicken with the light microscope (Hammar, 1905; Salkind, 1915; Hoffmann-Fezer, 1973), but their ultrastructure has not been described in the few electron-microscopic studies of the

chicken's or other birds' thymus glands (Koka, 1960; Ackerman & Knouff, 1964; Clawson, Cooper & Good, 1967; Håkanson, Larsson & Sundler, 1974).

#### MATERIAL AND METHODS

Male and female Leghorn chickens, aged 31–109 days and fed a mixture of powdered Purina Laboratory chow (65%) and Purina Rabbit checkers (35%), were perfused via the left heart ventricle with a mixture of buffered glutaraldehyde (6.25%) and acrolein (2%); the tissues were cut, postfixed in 1% osmium tetroxide, block-stained in 2% uranyl acetate and embedded in Epon. Details of the techniques have appeared elsewhere (Calvert & Isler, 1970; Isler, 1973). Sections were cut with a modified (Isler, 1974) MT-1 Porter-Blum ultramicrotome, stained with lead monoxide (Karnovsky, 1961) and examined in a JEM-7 electron microscope. For light microscopy, thicker Epon sections were stained with a 1% solution of toluidine blue in 1% borax.

#### RESULTS

In chickens the thymic tissue consists of a succession of discrete lobes along almost the entire length of the neck. There may be 4–8 lobes on each side (Panigrahi, Waxler & Mallman, 1971). They may extend quite far caudally. Occasionally we found thymic tissue in close vicinity to or in contact with the thyroid gland, the parathyroid glands, the carotid body and the ultimo-branchial body.

There is great variation in the morphological appearance of chicken thymic vesicles. We tentatively categorize here 3 types of vesicles. Type-1 vesicle is usually a large structure made up of a stratified columnar epithelium containing lymphoid cells (Fig. 1). The cells bordering the lumen are columnar and possess microvilli, sometimes in such abundance as to form a brush border. The microvilli are rich in microfilaments and are densely stained. The apical layer immediately beneath the microvilli is also very densely stained, due mainly to the abundance of microfilaments, most of which are continuous with those of the microvilli. This layer also contains glycogen granules and free ribosomes. Beneath lies another layer conspicuous by its abundance of mitochondria. These mitochondria are long and sinuous, with a dense matrix and randomly oriented cristae. Among them are scattered flat ergastoplasmic sacs. Free ribosomes and glycogen granules fill much of the remaining cytoplasm. The same features are found in the columnar cell of type-2 vesicles and are illustrated in Fig. 5. The Golgi apparatus is usually not prominent. The nucleus has a smooth oval outline, a light granular appearance with a moderate amount of chromatin attached to the membrane, and a number of small nucleoli. The lateral membranes of these cells follow a highly convoluted path and show the usual desmosomes and junctional complexes. Occasionally we found at some distance from the lumen epithelial cells with slender mitochondria (Fig. 1) and others with a deep cleft in their nuclei and a few droplets in their cytoplasm. The nature of these droplets is not certain, but we are inclined to consider them as mucous rather than lipoid, in view of observations made in type-2 vesicles to be described below. No typical mucous cells were found in type-1 vesicles.

The lower half of the epithelium has a much less homogeneous cellular population (Fig. 2). It also contains epithelial cells, recognizable by the appearance of the

nuclei, by filaments, desmosomes and relatively abundant cytoplasm. These, however, differ from the bordering cells by the smooth outline of their plasma membrane. They become flatter as they approach the base. They form the outer limit of the epithelium by extending fine cytoplasmic processes which interpose between the non-epithelial cells of the epithelium and the extra-epithelial tissue. These processes, which are rich in filaments, are lined with a basement membrane. Intermixed with the epithelial cells are lymphoid cells, mostly lymphocytes, characterized by their small size, scanty cytoplasm, lack of filaments and patchy chromatin configuration. These cells are closely apposed to the epithelial cells, with intercellular spaces of about 10 nm, but the 2 cell types are not linked by desmosomes. Occasionally, granular cells are also found (Fig. 3). Their granules are oval, very dense and vary in size from 100 to 500 nm. These cells are rich in mitochondria and smooth endoplasmic reticulum. Their nuclear content is coarser than that of epithelial bordering cells.

The lumen of type-1 vesicles contains mostly a light homogeneous material with some unidentified darker material of various shapes, probably cellular debris. Occasionally, areas with bordering cells deprived of microvilli are found. In these cases, the adjacent part of the lumen contains much more non-homogeneous material, suggesting a recent desquamation of bordering cells.

Immediately under the base of the epithelium are small vessels; usually no connective tissue fibres are present except a small amount of elastic fibres.

Type-2 vesicles are made of a simple or stratified epithelium containing no lymphoid cells but only mucous and non-mucous epithelial cells (Fig. 4). The 2 cell-types have access to the lumen and are attached to each other by desmosomes (Fig. 5). The non-mucous cells in contact with the lumen (Fig. 5) are similar to the bordering cells of type-1 vesicles, with the microvilli, the 2 apical layers (filamentous and mitochondria-rich) and the convoluted lateral membranes. They differ by the occasional presence of one or more intracytoplasmic vacuoles, some of them similar to type-3 vesicles which will be described presently. The microvilli and apical membrane are studded with granular and filamentous material. Coated pinocytotic vesicles and multivesicular bodies are present.

The mucous cells (Fig. 4) have shorter microvilli and an irregularly shaped nucleus, often flat and indented, with larger masses of chromatin attached to the membrane. Their large and abundant mucous droplets contain a finely filamentous material with various density levels. The mucus is secreted into the lumen and may be responsible for the material attached to the microvilli of non-mucous cells. The Golgi apparatus is well developed (Figs. 4, 5). Occasional vacuoles with lytic material are found (Fig. 4). Some of these contain an array of lamellae, a fact suggesting that they may originate from the concentric lamellar bodies observed occasionally (Fig. 4). Filaments are present in the cytoplasm (Fig. 5).

The base of type-2 vesicles is lined by a discontinuous basement membrane. A few vessels, elastic fibres, and other scanty connective tissue elements may be found underneath.

Type-3 vesicles are intracytoplasmic vacuoles occurring in epithelial reticular thymic cells (Fig. 6), and less frequently in cells of epithelial vesicles. They vary

widely in size but differ from ordinary vacuoles by their often unusually large size and the presence of microvilli. The lumen contains filamentous and membranous material. These vesicles may be formed by coalescence of smaller, empty-looking vesicles, as suggested by Fig. 7.

#### DISCUSSION

The type of vesicle most commonly found by electron microscopy in mammals is the type-3 intracytoplasmic vesicle (see references in the Introduction). Their structure is essentially the same as that found in the chicken, except that in some species cilia are found among the microvilli (man, mouse, hamster). These vesicles contain PAS-positive material which, as indirect evidence suggests, may contain a lymphopoietic factor (Trainin, 1974). The fluorescent antibody technique shows the presence of thymosin-containing granules in epithelial reticular cells (Mandi & Glant, 1973), but their relationship with the vesicles is not clear.

Pluricellular epithelial vesicles have also been found in ultrastructural investigations of several mammals and described under various names (cysts, pseudocysts, clefts, crevices, tubules, canals, vacuoles, vesicles), but the fine structure of vesicles as large as those reported here has been described only in rats treated with oestrogens (Senelar *et al.* 1973) or in the genetically abnormal 'nude' mice (Cordier, 1974*a*). None has been found to contain lymphoid cells like those of type-1 vesicles. Also, the types of epithelial cells encountered are not identical, although they vary between mammal species too. The glycogen-rich epithelial cells with interdigitating membrane, desmosomes and filaments is present in most species and is similar to the reticular epithelial cell present throughout the thymic tissue. Ciliated cells have been found in man (Sebuwufu, 1968), guinea-pigs (Kohnen & Weiss, 1964) and mice (Cordier, 1974*a*); we have not found these cells in our material, although they have been observed in chickens with the light microscope (Hammar, 1905; Salkind, 1915). A few mucous cells have been observed in mammals (Izard, 1965, and possibly van Haelst, 1967*b*), but certainly not in such abundance as in type-2 vesicles. 'Nude' mice are an exception and show abundant mucous cells, even in the intervesicular tissue (Cordier, 1974*a*). Perhaps the most interesting cells are the granular cells, as they may be assumed to have a secretory function. In the chicken vesicles we found only one type of granular cell with relatively small granules. These cells were few. In the same species 2 types of granular cells with granules measuring 200–300 nm were described in the thymic cell cords by Håkanson *et al.* (1974), one of them containing 5-hydroxytryptamine. Cells with larger granules were observed in thymic vesicles of oestrogen-treated rats (Senelar *et al.* 1973) or in the 'lethargic' mutant mice (Dung, 1973). The most striking situation occurred in 'nude' mice (Cordier, 1974*a*), where 4 types of granular cells were observed in the wall of the vesicles.

In mammals, some of the smaller vesicles were associated with Hassall's bodies, particularly in the guinea-pig (Kohnen & Weiss, 1964; Izard, 1965; Mandel, 1968) in which these bodies are numerous and well developed, and to some lesser extent in the mouse (Kohnen & Weiss, 1964). In Hassall's bodies, the vesicles usually

appear as a slit-like space between 2 lamellae, with microvilli and occasionally cilia protruding into the lumen. In chickens, typical lamellar Hassall's bodies were not found.

The content of the lumen of the vesicles described in the literature varies from a small amount of scattered material, as in our chickens, to degenerating cells and dense material, sometimes with a crystalline organization (van Haelst, 1967*a*). These cells desquamate from the wall and may be construed as a holocrine secretion.

The function of the pluricellular epithelial vesicles is still hypothetical. Circumstantial evidence is provided by comparison with the situation in the congenitally thymus-deficient 'nude' mouse, characterized by the presence of well developed thymic vesicles, the absence of epithelial reticulum, Hassall's bodies and lymphocytes (Cordier, 1974*a*) and by a lack of cellular immunology (Rygaard, 1969; Rygaard & Povlsen, 1969; Pennycuik, 1971; Wortis, 1971; Pritchard & Micklem, 1972). Since the mouse thymus has both endodermal and ectodermal origins (Crisan, 1935), it has been suggested (Cordier, 1974*b*) that the same hypothetical ectodermal defect may cause the dysgenesis observed in both skin (lack of hair) and thymus. An intact epithelial reticulum is apparently necessary for a normal immunological response. Indeed, it has been suggested repeatedly that the epithelial reticular cells with intracytoplasmic type-3 vesicles may be the site of production of thymic factors (Trainin, 1974) required for immunological competence. The pluricellular vesicles alone do not confer this competence, at least not in the 'nude' mouse. Since their granular and mucous cells obviously have a secretory function, the role of the substances contained in the granules and the mucus remains to be elucidated. In the normal animals they still may contribute to their immunological competence, or they may be related to the endocrine system (Trainin, 1974; Niederer, 1974).

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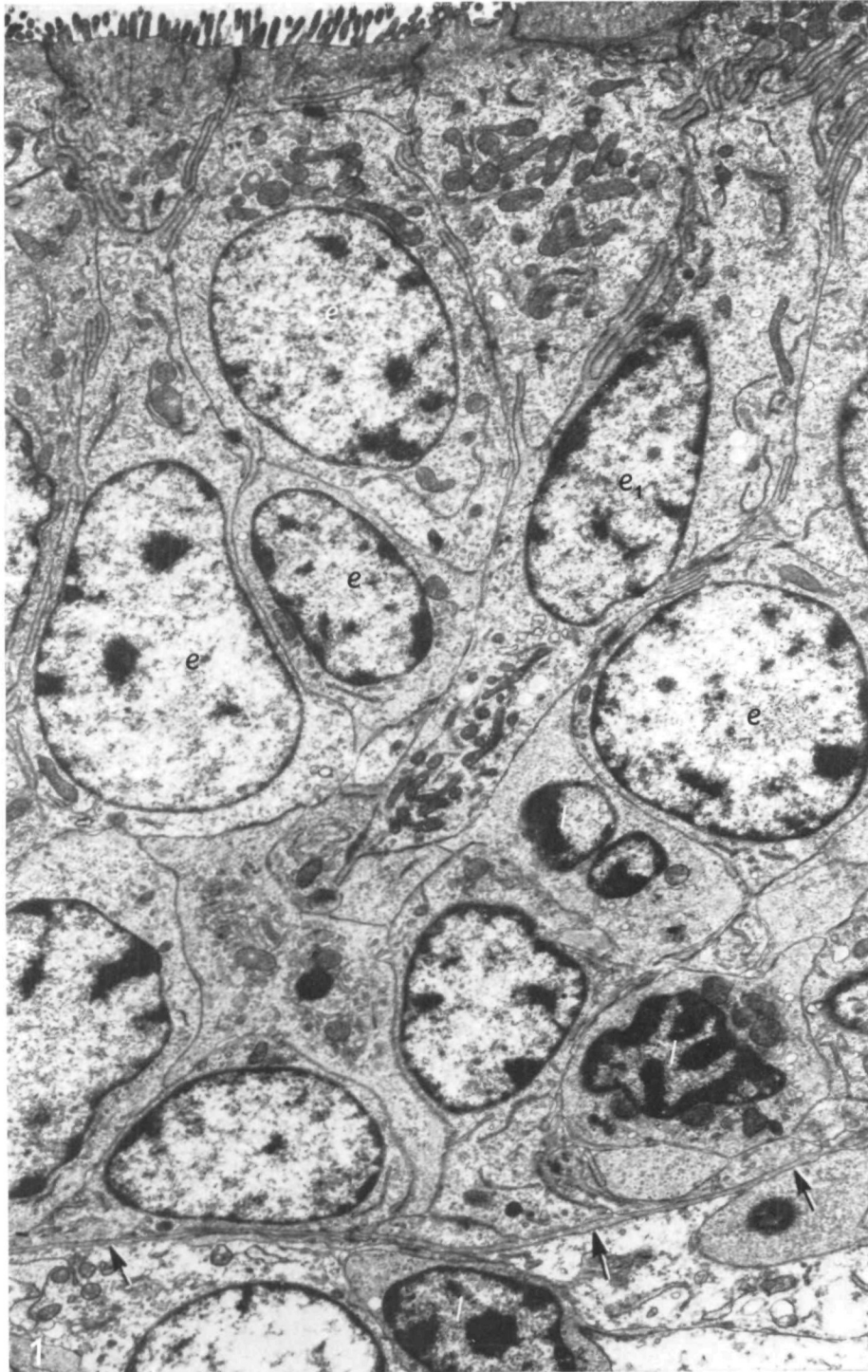


Fig. 1. Type-1 vesicle, the wall of which contains epithelial (*e*) and lymphoid cells, most of the latter being lymphocytes (*l*). The cell *e*<sub>1</sub> contains slender mitochondria and small, light intracytoplasmic vesicles. The cells bordering the lumen (top of micrograph) possess microvilli, a thin dense apical layer and a subjacent mitochondria-rich layer. The lateral membrane follows a convoluted path. Deeper in the wall, the cells have a smooth outline. The base of the wall is lined with processes of epithelial cells covered with a basement membrane (arrows).  $\times 4200$ .

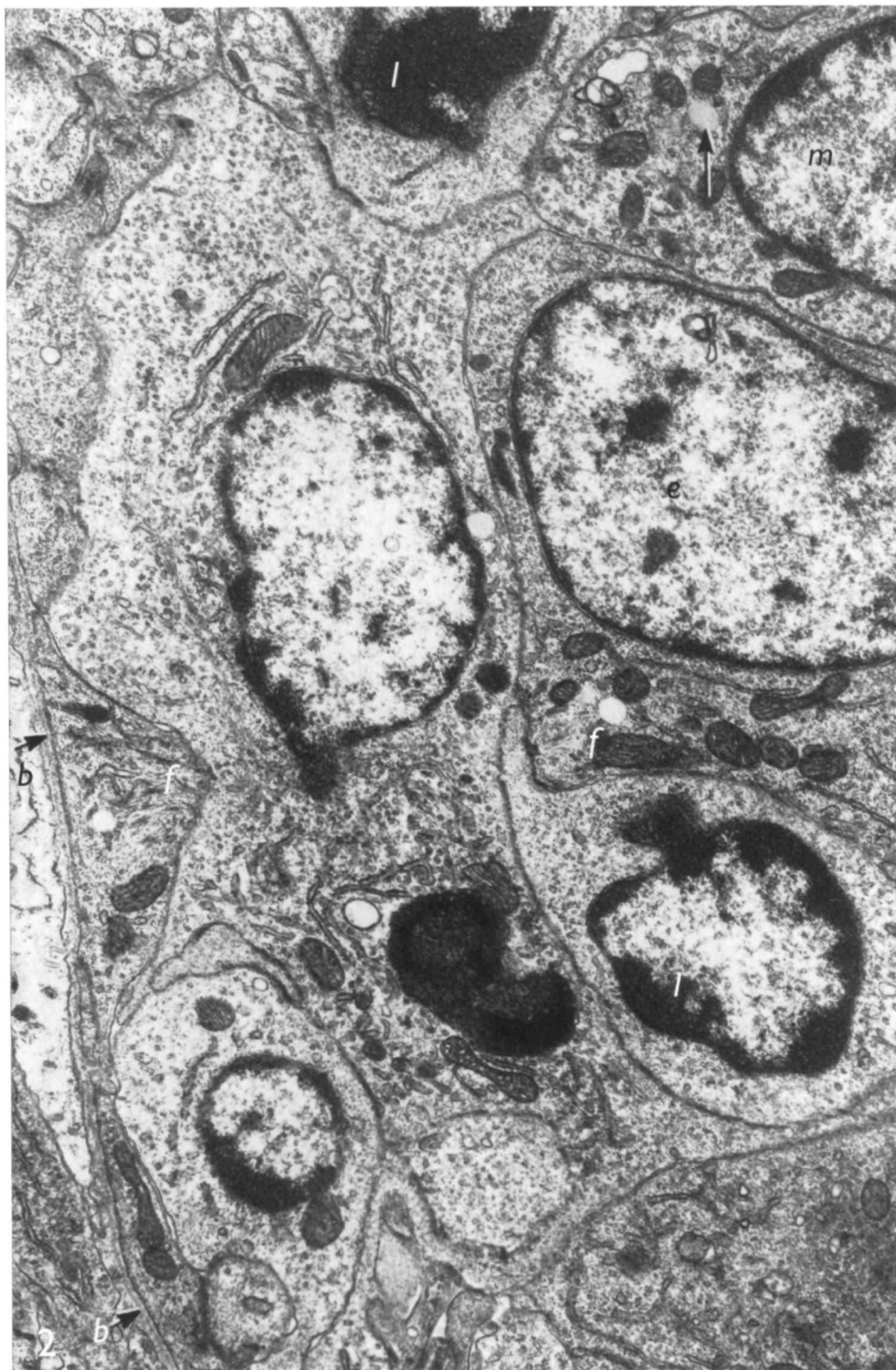


Fig. 2. Base of the wall of type-1 vesicle with the basement membrane (*b*) running vertically at the left. The lymphocytes (*l*) show typical scarcity in the cytoplasm, absence of desmosomes and filaments, and heavy patches of chromatin attached to the nuclear membrane. The epithelial cells (*e*) have only moderate amounts of chromatin on the nuclear membrane and show the same filaments (*f*), mitochondria and ergastoplasmic sacs as observed in lumen-bordering cells. The cell processes in contact with the basement membrane (*b*) are epithelial as indicated by the numerous filaments (*f*). The cell (*m*) at the upper right corner contains one droplet (arrow), probably mucous. The nucleus of this cell possessed a cleft which is not shown in the micrograph.  $\times 10700$ .



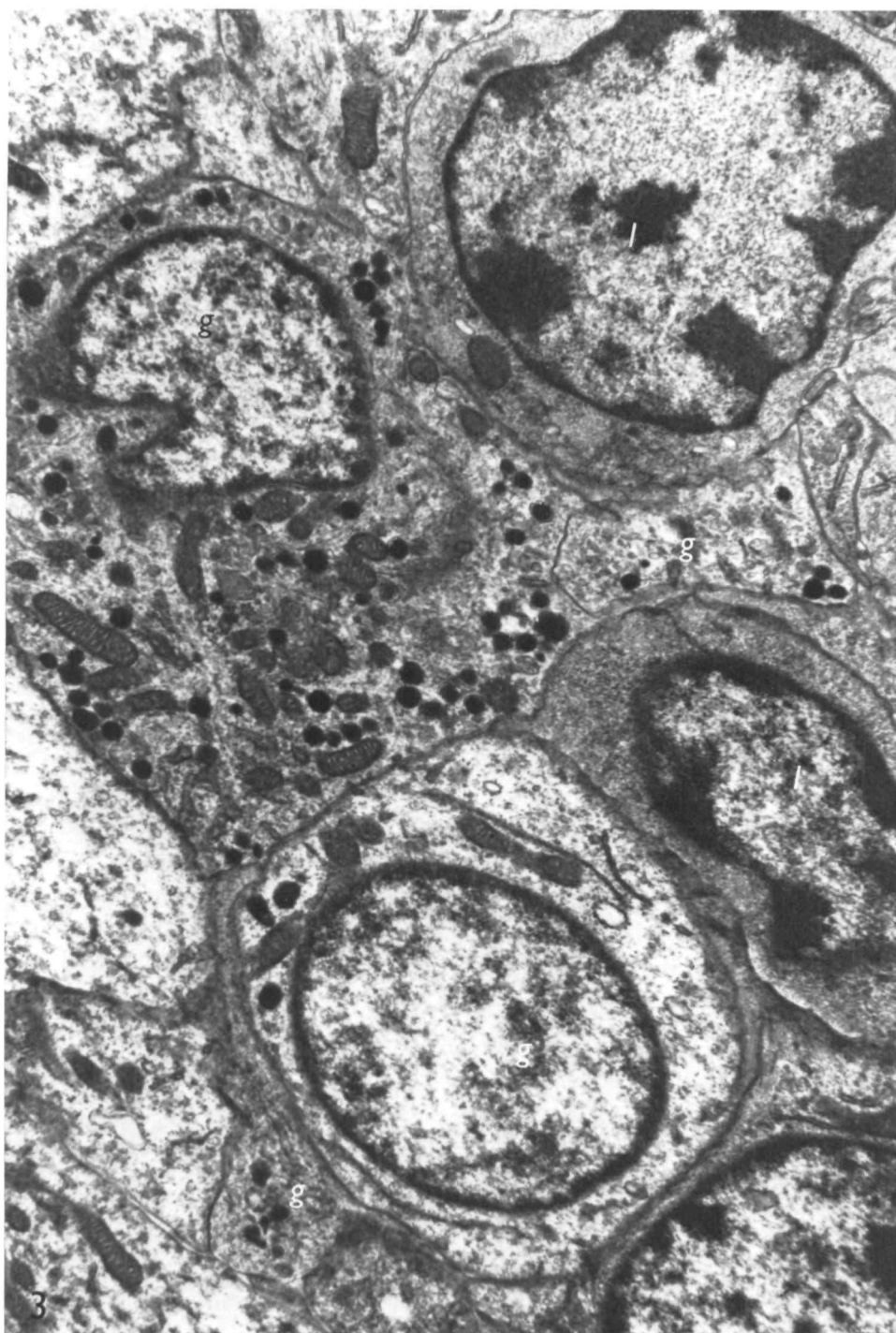


Fig. 3. The wall of type-1 vesicle occasionally contains isolated or groups of granular cells. This electron micrograph shows a group of 4 such cells (*g*), the upper one displaying an indented nucleus. The granules are all very dense but vary widely in size (from 100 to 500 nm). *l*, lymphocyte.  $\times 10700$ .

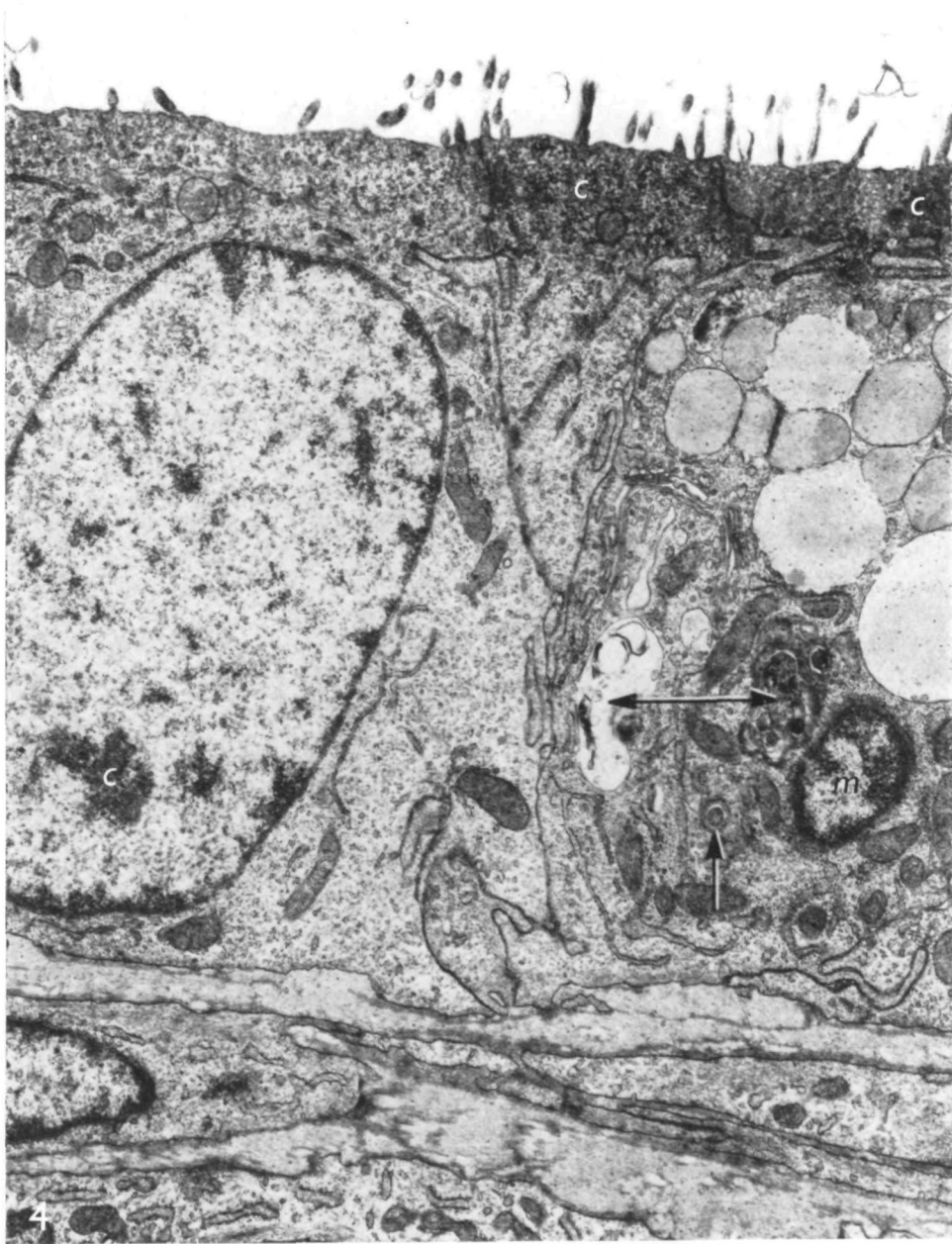


Fig. 4. Type-2 vesicle with a simple epithelium made up of columnar (*c*) and mucous (*m*) cells. Mucous cells have large mucous droplets containing a non-homogeneous material. Two vacuolsomes are present (double-ended arrow). The one on the right has a lamellar appearance, suggesting that it may originate from lamellar bodies (vertical arrow).  $\times 7300$ .

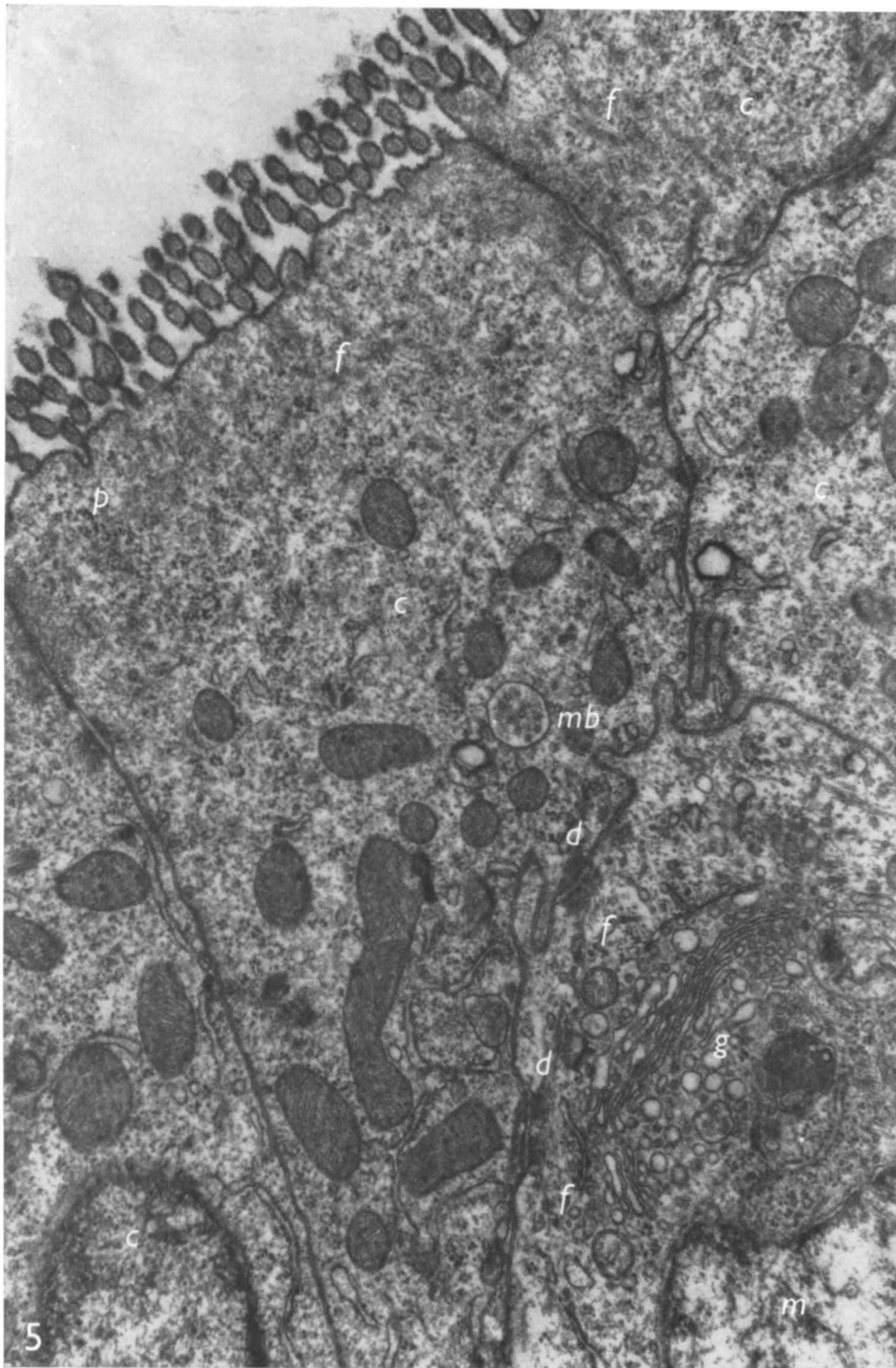


Fig. 5. Type-2 vesicle. The columnar cells (*c*) are similar to the epithelial bordering cells of type-1 vesicle and display the same filamentous apical (*f*) and mitochondria-rich layers. In addition, they possess multivesicular bodies (*mb*), studded microvilli membranes, and pinocytotic vesicles (*p*). The Golgi apparatus is not prominent, in contrast with that (*g*) of the mucous cell (*m*) shown in the lower right corner. Note the desmosomes (*d*) attaching the 2 cell types to each other, and the filaments (*f*) in the mucous cell (*m*).  $\times 18700$ .

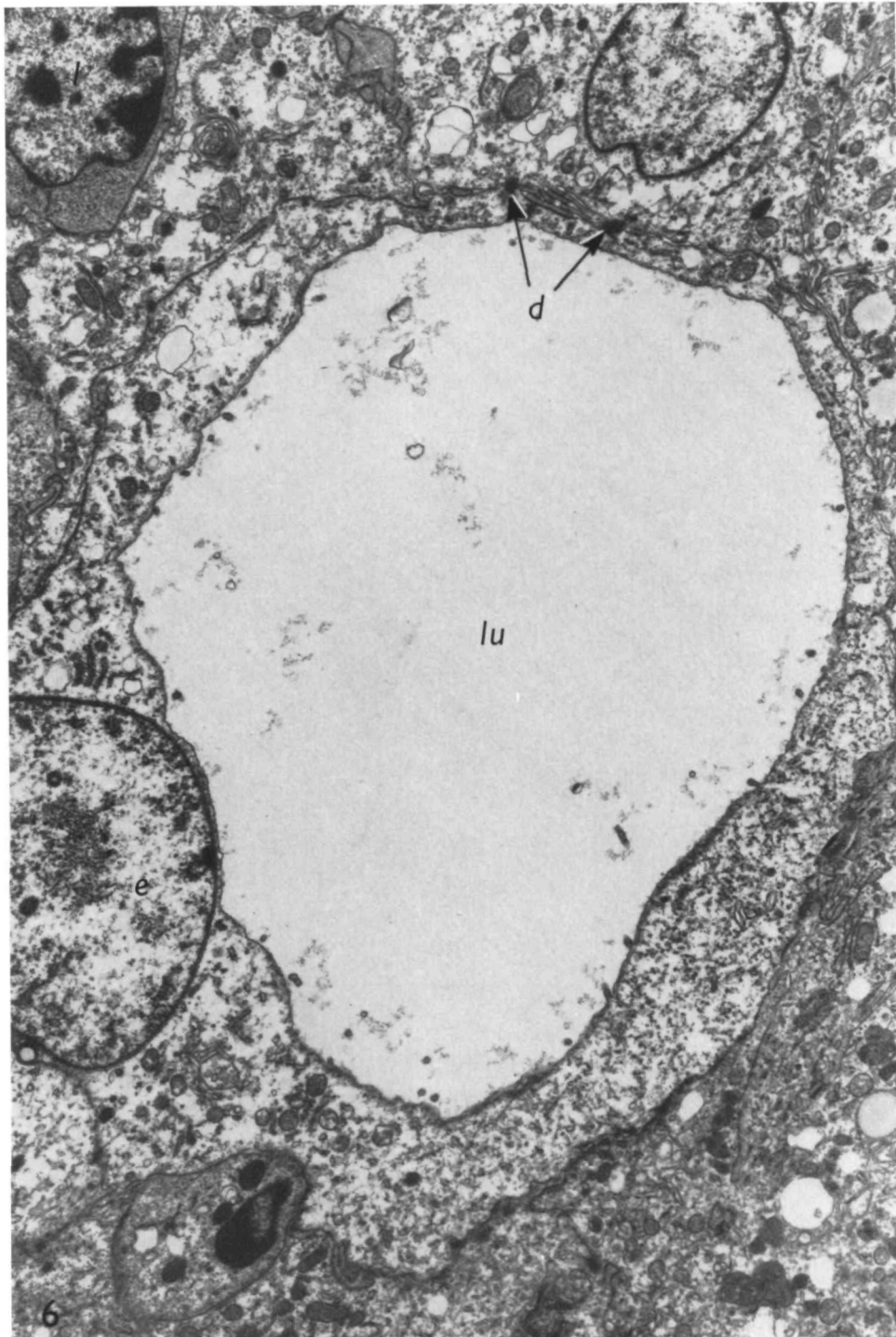


Fig. 6. Intracellular type-3 vesicle in an epithelial thymic cell (*e*). A few microvilli project into the lumen (*lu*), which contains some granular and filamentous material. This cell is attached to neighbouring epithelial granular cells by desmosomes (*d*). *l*, lymphocyte.  $\times 3800$ .



Fig. 7. Epithelial thymic cell (*e*) suggesting how type-3 intracellular vesicles may develop. Here the vesicle (*v*) is small and the microvilli are well developed. The smaller vacuole *v*<sub>1</sub> shows a distinct process of membrane desquamation, which may account for the material found in the lumen of type-3 vesicles. Rows of still much smaller, empty-looking vacuoles are present (arrows). Coalescence of these may result in a larger vesicle such as *v*<sub>1</sub>, still devoid of microvilli. This, in turn, may develop into a typical type-3 vesicle.  $\times 10900$ .