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LIGHT AND ELECTRON MICROSCOPIC STUDIES ON CHICKEN INTESTINAL GLOBULE LEUCOCYTES

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Chicken intestinal globule leucocytes (GL) and various intraepithelial migrating cells were light and electron microscopically investigated.

The morphological characteristics of GL were consistent with those of intraepithelial lymphocytes, except for the high cytoplasmic/nucleus ratio, the well-developed Golgi apparatus and the cytoplasmic granules. GL could be light and electron microscopically distinguished from mast cells, plasma cells (Russell body cells), and other migrating cells. Plasma cells and macrophages were located in the epithelium covering the large lymphatic nodules, but not in the ordinary columnar epithelium. However, GL and lymphocytes populated both epithelia. The cellular traffic of GL and lymphocytes across the basement membrane of the mucous epithelium and the penetration of lymphocytes into the intestinal lumen were often found.

The granules of GL, which located in the area neighboring Golgi complexes, were classified into two types; type I consisted only of amorphous matrix (AM), and type II of a marginal zone of fine reticular materials (FRM) in addition to the AM. As the FRM zone became wider, the AM became more irregular in shape. Crystalline or lamellar inclusions were recognized in both AM and FRM. The directions of the crystalline arrays in the AM were all slightly different. Fusion of the granules was rarely found. It was noted that the rough-surfaced endoplasmic reticulum rarely connected with the type II granules. The FRM of the type II granules was released to the extracellular space by emiocytosis. The release of the AM was prevented by the cytoplasmic processes or folds at the outlets of the granules. The secretory products filled the intercellular space between GL and the neighboring epithelial cells.

These results suggested a morphological similarity between GL and natural killer cells, and clarified the formation processes and the fate of the granules of GL.

Key words: chicken intestine, emiocytosis, globule leucocytes, natural killer cells, ultrastructure

INTRODUCTION

Globule leucocytes (GL) were characterized by acidophilic intracytoplasmic granules, pale cytoplasm and lymphocytic nucleus. The predominant appearance in the epithelium of the mucous membrane was observed in various animals.⁵⁰⁾

Though various migrating cells have been proposed as possible sources of GL, the current main theories of the cellular origin have proposed lymphocyte origin^{3,7,18,30,47)} rather than mast cell origin^{23,33,35,48)} in mammals. Morphological investigations in fowls also have fixed their attention on lymphocyte origin, but this problem is still open to discussion. Recently some investigators speculated that mammalian GL may be natural killer (NK) cells.^{5,13)} NK cells, which are a population of lymphocytes with spontaneous cytotoxic activity, have been ultrastructurally investigated with isolated cells from the blood, lung,²⁾ spleen,^{2,38)} liver³⁸⁾ and intestinal epithelium⁴¹⁾ of mammals. The ultrastructural characteristics of mammalian NK cells have been almost completely clarified.^{31,32,39,44)} On the other hand, "pit cells", which are lymphocyte-like cells with cytoplasmic granules, were reported to be located in rat liver.^{25,51)}

Though the ultrastructure of chicken GL has been studied by several investigators,^{18-21,47)} little is clear about the detailed fine structure, the fate and the formation processes of the intracytoplasmic granules.

In this paper, the ultrastructural characteristics of chicken GL are compared with those of mammalian NK cells, and the cellular origin of chicken GL is discussed. The formation processes and the fate of the specific granules of GL are also clarified.

MATERIALS AND METHODS

Animals Fifteen healthy White Leghorn chickens (6-8 weeks of age) of both sexes were used. All of the chickens were reared conventionally and obtained from our colony. They were homozygous at the major histocompatibility locus: blood types B9/B9 and B11/B11.

Light microscopy Duodenum, jejunum, Meckel's diverticulum, ileum and large intestine were fixed with Bouin's fluid, Carnoy's fluid or 2.0% paraformaldehyde-2.0% glutaraldehyde mixture. Paraffin sections 4 μ thick were stained with hematoxylin-eosin (H. E.) stain, Dominici's stain or toluidine blue (pH 7.4) stain. In the eosin staining process, the sections were regressive-stained well enough by 70% ethanol to detect the intensely acidophilic granules in the tissues.

To compare the staining properties of GL with those of other migratory cells which have been considered to be similar to GL, the stainability of the cells with 2.0% paraformaldehyde-2.0% glutaraldehyde fixation and toluidine blue (pH 7.4) stain, were enumerated by the cytophotometric method with the MMSP-TU system (Olympus Optical Co, LTD., Japan).

Electron microscopy Small tissue blocks of descending duodenum and the cervical part of the cecum involving the cecal tonsil, were fixed by immersion at 4°C in 3.0%

glutaraldehyde in 0.1M phosphate buffer at pH 7.4. After postfixation by 1% OsO₄ in 0.1M phosphate buffer at pH 7.4, all tissue blocks were embedded in Quetol-812. Ultra-thin sections were stained with uranyl acetate and lead citrate to be observed by a Hitachi HU-12A electron microscope.

RESULTS

Light microscopical discrimination of globule leucocytes from other migrating cells

Globule leucocytes GL, which were cells 5.0–9.0 μ in diameter, presented a round or oval shape and possessed thick cytoplasmic processes. The nucleus with a diameter of 3.0–5.0 μ , was rich in chromatin, especially just beneath the nuclear envelope. The chromophobic cytoplasm was distinguished from the slight basophilic cytoplasm of neighboring mucous epithelial cells. The cytoplasmic granules were of variable diameter (0.5–3.0 μ), and had a highly refractive nature. They were intensely acidophilic in H. E. stain, faint emerald green or dark blue in toluidine blue stain, and dark red-purple in Dominici's stain. The cytoplasmic/nucleus ratio of GL was higher than that of intraepithelial lymphocytes. Large granule-containing GL were more abundant in the crypt than in the villi (Fig. 1). The maximum number of the visible granules in a cytoplasm was 8.

Intraepithelial lymphocytes Lymphocytes found in the epithelium, were 4.0–5.0 μ in diameter, and presented a round or oval shape. The round or oval nucleus possessed a diameter of 3.0–3.5 μ . The chromatin pattern of the nucleus was similar to that of GL. The cytoplasm was chromophobic and possessed no granules.

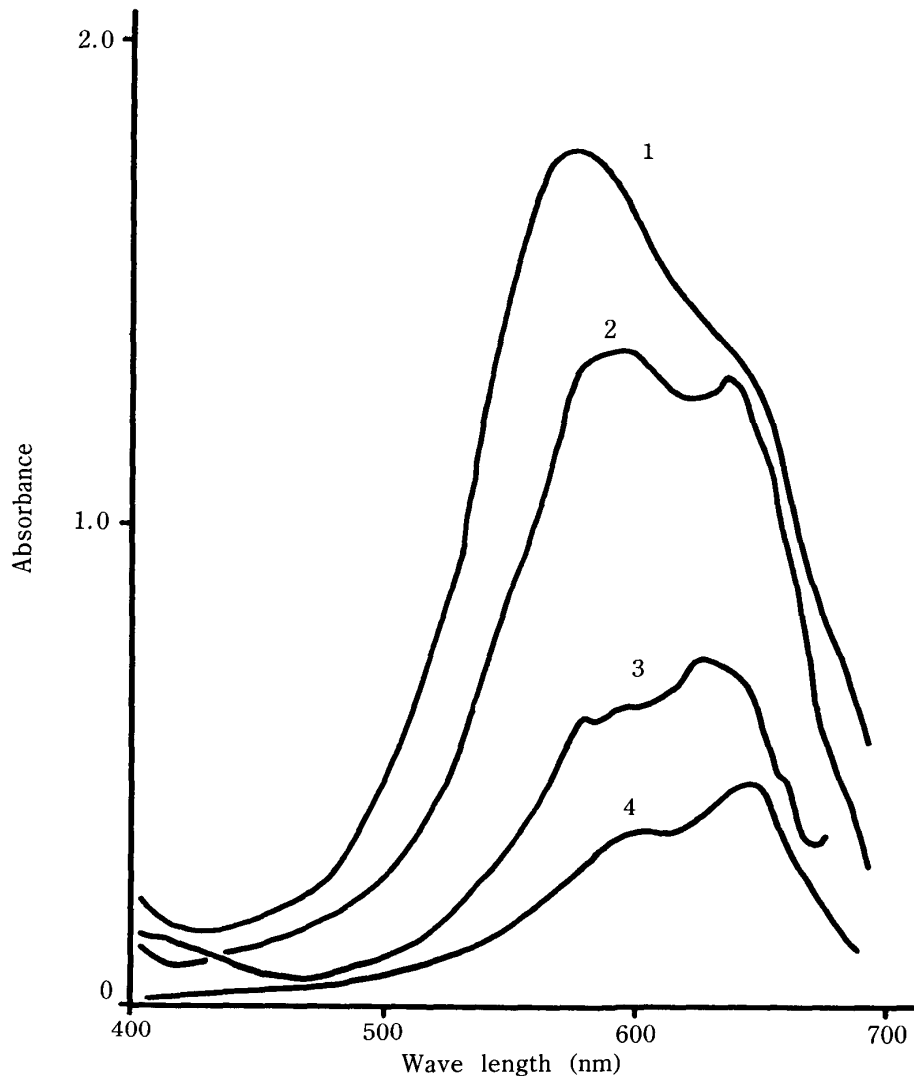
Plasma cells Plasma cells were 4.0–5.0 μ in their longitudinal length and oval or round in shape. The nucleus, with condensed chromatin at the marginal regions, possessed no distinguishable nucleolus. The cytoplasm presented basophilic with H. E. stain, and gray-blue with toluidine blue stain. Russell bodies were slightly acidophilic and slightly red-purple with Dominici's stain.

Mast cells Mast cells, spindle- or irregular-shaped with a longitudinal length of 5.0–18.0 μ , were furnished with numerous cytoplasmic processes. The nucleus, about 5.0 μ in longitudinal length, possessed a small amount of chromatin. The wide cytoplasm was filled with granules with uniform diameters ($\cong 1.3 \mu$), which were unstainable or stained as slightly acidophilic with H. E. stain or as red purple to dark purple with toluidine blue or Dominici's stain.

Eosinophils and heterophils Eosinophils or heterophils were round or irregular in shape, and 6.0–10.0 μ in diameter. Though the nuclei were almost segmented, a few myelocytes with large nuclei and a little chromatin were occasionally recognized. Their cytoplasmic granules were intensely acidophilic, almost unstainable with toluidine blue and presented light red with Dominici's stain. The cytoplasm was filled with spherical granules of uniform size (about 1.0 μ) in eosinophils and large-sized rod-shaped granules in heterophils.

Cytophotometry The absorbance of the nucleus of a smooth muscle cell which was stained (dark blue) non-metachromatically with toluidine blue stain, is shown at 2 in Text figure 1. The spectral curve possessed 2 peaks at 580 nm and 630 nm, at which the absorbance was almost the same. The absorbance of the granules of mast cells with metachromatical staining was higher at 580 nm than at 630 nm. The absorption rate of the granules of GL was lower at 580 nm than at 630 nm. The spectral patterns of erythrocyte with pseudometachromasia were similar to those of GL.

TEXT FIGURE 1 Spectral curves of globule leucocyte and mast cell granules¹⁾



- 1) 2.0%-paraformaldehyde · 2.0-glutaraldehyde fixation and toluidine blue (pH 7.4) stain
- | | |
|--------------------------------|-----------------------------------|
| 1. Granule of a mast cell | 2. Nucleus of a smooth muscle |
| 3. Cytoplasm of an erythrocyte | 4. Granule of a globule leucocyte |

Electron microscopical discrimination of GL from other migrating cells in the intestinal epithelium

GL were also distinguished from other migrating cells in the epithelium at the ultrastructural level.

GL and lymphocytes were sandwiched between the epithelial cells throughout the intestine. The intraepithelial lymphocytes, most of which were small- or medium-sized, were situated at the basal region of the epithelium, but were occasionally found in the apical portion (Fig. 2). Lymphocytes protruding or dropping out with collapsing epithelial cells into the intestinal lumen were extremely rarely found (Fig. 3). Cellular traffic of GL and lymphocytes across the basement membrane of the intestinal epithelium was occasionally observed (Fig. 4).

Plasma cells (including Russel body cells) and macrophages were present within the epithelium the domes of large lymphoid follicles of the cecum, but they were never seen in the ordinary columnar epithelium. In the epithelium covering the lymphoid follicles, none of these cells just protruding into the lumen were seen, but such cells crossing the basement membrane were often found. The Russel bodies, which were surrounded by the membranes of rough-surfaced endoplasmic reticulum (rER), presented a globular shape and were homogeneous with high electron-dense features (Fig. 5), but the matrices were fine and reticular at high magnification.

Mast cells were located at the lamina propria just beneath the epithelium, but never populated the intestinal epithelium. The cells were clearly recognized by several morphological characteristics of the nucleus and by their granules. That is, the large oval nucleus contained a small amount of heterochromatin. The granules mostly presented fine granular or convoluted shapes and were less than 1.0μ in diameter (Fig. 6).

Though no eosinophils and heterophils existed in the ordinary columnar epithelium, a few of them were occasionally observed in the epithelium covering large lymphoid follicles. The density of the matrix of the cytoplasm was higher than that of other migratory cells. The granules of eosinophils were less than 0.7μ in diameter and nearly uniform in size (Fig. 7). Whereas the granules of eosinophils were spherical, those of the heterophils presented a long elipsoidal shape and were possessed of a core with a slightly low electron density.

Macrophages having several lysosomal granules were found in the follicle-associated epithelium, but rarely seen in the ordinary columnar epithelium. The GL crossing the basement membrane were often found at the epithelium covering the large lymphoid follicles (Fig. 8).

Ultrastructure of GL Almost all of GL were spherical and possessed of thick cytoplasmic protrusions prolonged between the epithelial cells. The pattern of the chromatin and the size of the nucleus were similar to the small or medium sized lymphocytes in the same area, but the cytoplasm of GL had a slightly lower electron density. Therefore, the cytoplasm was easily distinguished from that of the neighboring epithelial cells.

The distribution pattern of cytoplasmic organelles was very similar to that of the intraepithelial lymphocytes. The features of GL that differed from the intraepithelial lymphocytes were that the Golgi apparatus was often well developed and a variable number of specific cytoplasmic granules, the maximum number being 12 in one cell. The maximum diameter of the granules existing in a GL of the crypt epithelium was approximately 3.0μ . The granules were distributed in the area proximal to the Golgi apparatus (Fig. 9). In these areas, multivesicular bodies and minute vesicles, which often contained homogeneous substances with high electron-density at the centers, were frequently seen (Fig. 10). As the homogeneous matrices grew, the multivesicular bodies became bigger. Minute vesicles with homogeneous matrices were located throughout the cytoplasm and often situated just beneath the cell surface membrane (Fig. 11).

The cytoplasmic granules of GL were classified into two types due to their fine structures. Type I granules contained only an amorphous matrix (AM) of high electron density, though extremely narrow zone of fine reticular materials of high electron density was often found just beneath the limiting membrane surrounding the granules at a high magnification. Type II granules consisted of the AM and an obvious marginal zone with fine reticular materials (FRM), whose density was generally somewhat lower than the AM, though it was occasionally higher than that of AM (Fig. 12). The AM of type I granules were morphologically the same as those of type II granules. However, the AM of type I granules were almost spherical, while the AM of type II granules were often irregular to some degree. The large granules with irregular-shaped AM appeared to have a wider area of FRM, whereas the granules with the round AM were possessed of very little of FRM. Crystalline inclusions were rarely found in the AM (Fig. 13). The outline of each crystalline inclusion was unclear. The directions of crystalline arrays in the AM were slightly discordant (Fig. 14). At a higher magnification, the crystalline arrays could be resolved into two kinds of parallel layers which consisted of a dense layer approximately 30 A thick and a low-density layer approximately 40 A thick. The FRM of the type II granules also contained lamellar inclusions. This structure presented voluted shapes, comma shapes or irregular-shaped fragments (Fig. 15). The electron density of the lamellar inclusions in the FRM was apparently higher than that in matrices of FRM, and slightly higher than those of the crystalline inclusions in AM. The lamellar inclusions were formed at the margin of the AM of the type II granules (Fig. 16). Two types of inclusions in FRM existed. The major type of inclusions consisted of a dense layer approximately 20 A thick and an electron lucent layer approximately 20 A thick. The other type consisted of a high density band 30–40 A thick, a moderate electron-dense band 20–30 A thick and an electron-lucent band 20–30 A thick (Fig. 17). Occasionally, the granules fused together and grew into larger granules (Fig. 18). The communication of type II granules with rER was extremely rarely found (Fig. 19). The contents of the rER were similar to those of the FRM of type II granules. No vacuoles were seen in either type of granule, but cytoplasmic processes

or folds were found frequently in the granules (Fig. 20). The intragranular cytoplasmic processes in the granules were surrounded by unit membranes and frequently possessed of some cytoplasmic organelles. The intragranular cytoplasmic processes were more recognizable in the type II granules with larger FRM.

Intragranular substances were released to the extracellular space by emiocytosis. The release was exclusively observed in the type II granules which possessed irregular-shaped AM. When the membrane of the type II granules and the cell surface membrane fused, the FRM was released, but the AM was not. At the outlet of the granules, the cytoplasmic process or the fold was situated. At the portion where the granule contents were secreted, the membranes of GL and the neighboring epithelial cells were often difficult to recognize, and the intercellular space was filled with an electron-dense substance which was similar to the FRM (Fig. 21).

DISCUSSION

GL were characterized as cells with acidophilic granules in the lucent cytoplasm by early investigators.⁵⁰⁾ Thereafter, the staining properties of the granules of GL in preparations with basic dye stain caused many discussions with regard to the relation between the cellular origin of GL and mast cells. TONER (1965)⁴⁷⁾ described that the granules of chicken intestinal GL were metachromatic with toluidine blue stain. This metachromatic property with toluidine blue stain was supported by some investigators.^{34,35)} On the other hand, TAKEUCHI et al. (1969),⁴²⁾ CANTIN & VEILLEUX (1972),⁹⁾ TOKASHIKI et al. (1981)⁴⁵⁾ and HUNTLEY et al. (1982)²²⁾ reported that the granules of GL in various mammals possessed slight metachromatic or non-metachromatic properties with toluidine blue stain. The cytophotometric characteristics of GL in this light microscopical observation suggested the non-metachromatic or pseudometachromatic nature of the granules of chicken intestinal GL. Furthermore, the light and electron microscopical appearances of GL were different from those of mast cells. No indication of the cellular transition from mast cells to GL was found. These facts suggested that GL were an independent cell population of mast cells.

In some reports intraepithelial GL were identified using a definition of the metachromatic properties of the granules, neglecting to consider the intensely acidophilic properties of the granules.^{11,16)} On the other hand, the mast cells migrated into the nasal epithelium from subepithelial connective tissue in birth pollen allergy.¹²⁾ The migration of GL into the mucous epithelium was also histochemically and electron microscopically demonstrated in the gall bladder of cattle and sheep.⁴⁶⁾ These facts suggested that strict discrimination between GL and mast cells should accompany the investigation of intraepithelial GL.

Lymphocyte origin for GL is currently main theory in fowls.^{3,7,18,19,47)} Furthermore, NK cells, a population of lymphocytes, have been morphologically speculated to be the origin of the GL in mammals.^{5,13)} The present morphological char-

acteristics of GL, that is, the chromatin pattern of the nucleus and the cytoplasmic organelles except for specific granules, were very similar to those of small- or medium-sized lymphocytes, which confirmed the lymphocyte origin of chicken GL. However, the cytoplasmic/nuclear ratio of GL was large and they contained cytoplasmic granules, in contrast to ordinary small- or medium-sized lymphocytes. These characteristics were very similar to those of NK cells, which were identified as large granular lymphocytes (LGL) and were characterized by a high cytoplasmic/nuclear ratio, numerous protrusions and cytoplasmic processes, granulation of varying intensity in the cytoplasm, and an eccentric and reniform nucleus.^{31,32,39,44)} A morphological characteristic of the intestinal LGL was that they possessed larger granules than those in blood LGL²⁾; this is also consistent with our finding that intestinal GL were furnished with large granules (3.0 μ in maximum diameter). Consequently, the comparison of the morphological characteristics of GL and LGL suggests a homologue between GL and LGL.

In 1970, WISSE⁵¹⁾ found a new unidentified cell during the course of ultrastructural investigations on rat hepatic sinusoids, and these cells were termed "pit cells." SCHEUERMANN (1982)⁴⁰⁾ interpreted them as morphological variants of lymphocytes. The correspondence of the pit cells to NK cells has also become a matter of discussion.²⁵⁾ The pit cells co-expressed many cytological characteristics which GL also possessed. Namely, they possess lymphocyte-like nucleus, pale cytoplasm, well-developed pseudopodia, some multivesicular bodies, scattered formations of rER, few smooth-surfaced endoplasmic reticulum, a well-developed Golgi apparatus, and several characteristic cytoplasmic granules, which exist in homogeneous electron-dense materials and in a narrow/wide halo between the dense materials and the limiting membrane.^{25,26,52)} These characteristics suggest that pit cells are no more than GL. No investigators on the pit cells appear to have noticed the existence of GL with historic interest, nor have investigators of the NK cells.

Because of the morphological variability of the specific granules, the granules have been classified into various types. MURRAY et al. (1968)³⁵⁾ ultrastructurally classified the granules into 4 types: 1) Granules with homogeneous matrices of moderate electron density; 2) Granules in which small areas or rims less electron dense and with more granular matrix were separated from the membrane; 3) Granules in which the matrix was partially lost, leaving paracrystalline structures with electron density similar to the original matrix; and 4) Granules in which the perigranular membranes were lost, leaving paracrystalline structures free within the cytoplasm. These types of the granules were also recognized in GL of rat tracheal epithelium by PEARSAL et al. (1984).³⁷⁾ Our type I granules are similar to 1) granules, and type II granules are likely to be involved 2) and 3) granules. Though there may be some differences in morphological features which are caused by the fixation procedures and/or the animal species, no type-4) granules like those of MURRAY et al. (1968)³⁵⁾ were found in the present study. BAERT and FREDERIX (1985)⁴⁾ also classified the granules into 5 types

in human globule leucocytes. However, most of these types essentially belong to our type I and II granules, though no granules with very electron dense granules in an electron-dense homogeneous matrix were found in chicken globule leucocytes.

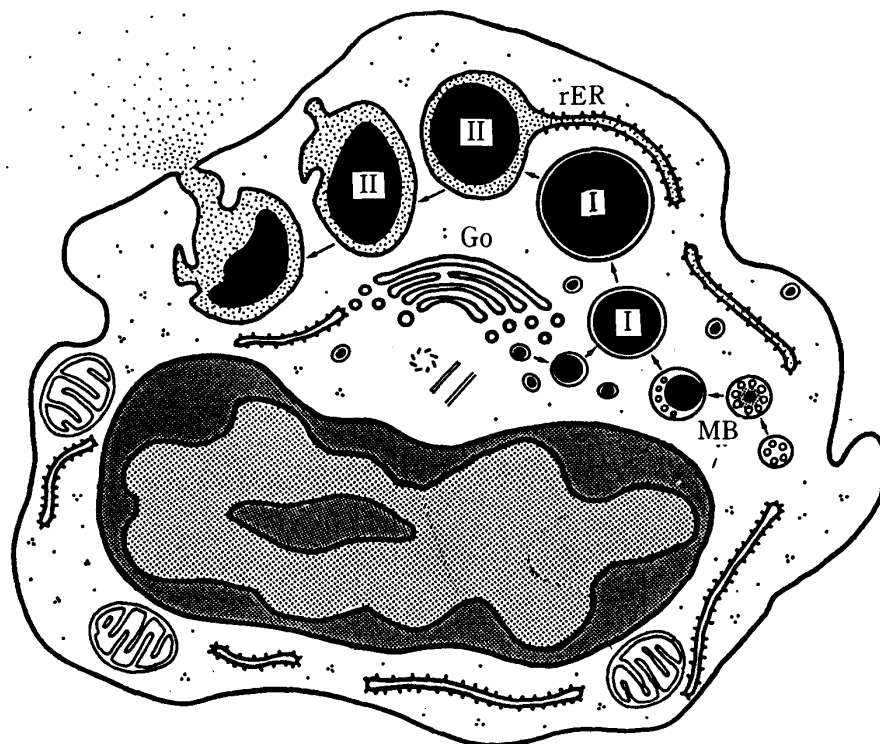
In fowls, HOLMAN (1972)²⁰⁾ divided the specific granules, according to their ultrastructure in the developmental stadia of secondary lysosome, into: 1) Multivesicular bodies which can be proved mainly near the Golgi apparatus and which occasionally contained electron-dense materials. 2) Dense bodies which were almost 1 μ in diameter and filled with medium coarse-grain materials or electron-dense materials. 3) Autophagic vacuoles which sometimes exceeded 1 μ in diameter, were filled with electron-dense materials and possessed great rests of membranes. 4) Vacuolated bodies which were the largest formations of the granules of GL, exceeded 1 μ in diameter and contained some vacuoles. 5) Myelinated bodies which were irregular in shape, did not exceed 1 μ , and contained myelinated formations. Type I and II granules in the present study were considered to correspond to HOLMAN's dense bodies and autophagic vacuoles, respectively. These two types of granules, which were encountered with a high frequency, are considered to be the main features of specific granules of chicken GL. Though cross-views of cytoplasmic folds that were similar to the vacuoles, were occasionally found in type II granules, the vacuoles were themselves never found in the present study. The vacuoles of the vacuolated bodies might also be artifacts which were caused by the effects of the different fixatives.

HOLMAN (1972)²⁰⁾ considered that the lamellar inclusions were great rests of membrane, especially mitochondrial membranes, and the thicknesses of the layers were very similar to those of the unit membrane. The present morphological features of lamellar inclusions in the FRM of the type II granules may be similar to the rest of membranes in autophagic vacuoles.²⁰⁾ However, if the lamellar inclusions in the FRM are homologous with HOLMAN's rest of the membrane, then those lamellar inclusions that consisted of three repeated layers, i. e., the dense and thick layer, the moderately dense layer and the electron lucent layer were similar to those of the myelinated sheath, a specialization of the cell membranes of Schwann cells. However, the fact that the lamellar inclusions were found at the margin of the AM of type II granules suggests that the lamellar inclusions are produced in the granules.

The formation of the granules has not been examined up to now. KENT (1966)²⁸⁾ and HOLMAN (1970a)¹⁸⁾ postulated that the Golgi complex could have a function in the formation of the granules. The present observation that the granules situated around the Golgi complex, confirmed that postulation. The early stage of the formation of the granules appeared to start at the multivesicular bodies or the minute vesicles neighboring the Golgi apparatus. The multivesicular bodies possessing cores like those in AM, were enlarged as the core grew. No minute vesicles which also contained tiny AM, appeared to grow. As the minute vesicles with AM existed beneath the cell surface membrane, the extracellular release of the minute vesicles

may partially occur. These early granules probably fuse with each other to form the type I granules. This postulation is indirectly proved by the fact that the axes of the crystalline structures were slightly different in identical AM, such as that in the formation of juxtaglomerular cell granules.⁶⁾ As the discharge of the granular contents approaches, the rER connect with membranes of the type I granules as CHANDRA et al. (1965)¹⁰⁾ recognized in juxtaglomerular cell granules of rats. Type I granules gradually come to possess an FRM and transform to type II granules. As the FRM becomes larger, the AM of the type II granules is deformed to an irregular shape. This phenomenon is interpreted as the dissolution of the AM. When a part of the membrane surrounding type II granules is locally pulled toward the cell surface membrane and they fuse, the FRM is discharged. No direct discharge of the AM of the type II granules may occur, because the cytoplasmic process or fold prevents the direct secretion of AM. Thus, the serial and hypothetical processes of the formation and the discharge of the specific granules of GL were ultrastructurally demonstrated in the present study as summarized in Text Figure 2.

TEXT FIGURE 2 Formation and discharge processes of granules of a chicken globule leucocyte



I: type I granules, II: type II granules
 rER: rough-surfaced endoplasmic reticulum
 Go: Golgi apparatus, MB: multivesicular body

The fate of the intraepithelial GL of the intestine has not been demonstrated in ultrastructural studies. The absence of intercellular junctions between GL and the neighboring epithelial cells confirms their migratory nature. The speculation that GL may infiltrate into the epithelium from the lamina propria^{4,24,28)} is confirmed by the fact that GL crossing the basement membrane were observed in this study. Though, in the tracheal epithelium of rats, the migration of GL through the epithelium into the tracheal lumen occurred,⁴⁹⁾ we found no ultrastructural evidence of the extrusion of GL in the intestinal lumen. However, the penetration of the intact lymphocytes into the lumen, the traffic of GL across the basement membrane of the epithelium and the non-degeneration of GL in the epithelium suggest two possible fates for the epithelial GL: the first is a return across the basement membrane to the lamina propria and the second is penetration into the lumen by the same route as the intraepithelial lymphocytes.

Mammalian NK cells possess two types of cytoplasmic granules: the first presents an electron-dense, membrane-bound structure, and the second is larger, generally more pleiomorphic, and contain heterogeneous floccular electron-dense materials, including membrane fragments.³⁶⁾ The Golgi apparatus may be the site of the packing of the secretory materials into the granules, which are transported to other places where the concentration of their contents and final maturation occurs.¹⁵⁾ The type II granules, but not type I granules, release their contents toward the extracellular space as a result of stimulus with Sr^{2+} .³⁶⁾ During degranulation, the core of the granules remained in the vacuoles for some time after the matrix disappeared, indicating that dissolution was a slow process.¹⁴⁾ These phenomena of NK cells fit our hypothetical processes of the formation and the discharge of the granules in GL very well.

If chicken GL are homologous with NK cells, it is considered that the intraepithelial GL possess a cytotoxic function^{17,29)}, phagocytotic activity against some bacteria^{1,27)}, the ability to function as antigen-presenting cells⁸⁾ and/or a role in the regulation of B-cell activity⁴³⁾. The significance of the appearance of GL in the intestinal epithelium should be further examined.

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REFERENCES

1. ABO, T., SUGAWARA, S., AMENOMORI, A., ITOH, H., RIKIISHI, H., MORO, I. & KUMAGAI, K. (1986): Selective phagocytosis of gram-positive bacteria and interleukin 1-like factor production by a subpopulation of large granular lymphocytes. *J. Immunol.*, **136**, 3189–3197
2. ALBERTI, S., COLOTTA, F., SPREAFICO, F., DELJA, D., PASQUALETTO, E. & LUINI, W. (1985): Large granular lymphocytes from murine blood and intestinal epithelium: Comparison of surface antigens, natural killer activity, and morphology. *Clin. Immunol. Immunopathol.*, **36**, 227–238
3. BÄCK, O. (1972): Studies on the lymphocytes in the intestinal epithelium of the chicken. *Acta Pathol. Microbiol. Scand.*, A, **80**, 84–90
4. BAERT, J. & FREDERIX, M. (1985): Globule leucocytes in the respiratory epithelium of human upper airways: An ultrastructural study. *Anat. Rec.*, **212**, 143–152
5. BAERT, J. & FREDERIX, M. (1986): Immunohistochemical study on the presence of immunoglobulins in globule leucocytes in the tracheal epithelium of rats. *Acta Anat.*, **125**, 233–237
6. BARAJAS, L. (1966): The development and ultrastructure of the juxtaglomerular cell granules. *J. Ultrastruct. Res.*, **15**, 400–413
7. BJERREGAARD, P. (1975): Lymphoid cells in chicken intestinal epithelium. *Cell Tissue Res.*, **161**, 485–495
8. BROOKS, C. F. & MOORE, M. (1986): Presentation of a soluble bacterial antigen and cell surface alloantigens by large granular lymphocytes (LGL) in comparison with monocytes. *Immunology*, **58**, 343–350
9. CANTIN, M. & VEILLEUX, R. (1972): Globule leukocytes and mast cells of the urinary tract in magnesium-deficient rats. *Lab. Invest.*, **27**, 495–507.
10. CHANDRA, S., HUBBARD, J. C., SHELTON, F. R., BERNARDIS, L. L., PHIL, D. & KAMURA, S. (1965): Genesis of juxtaglomerular cell granules. A physiologic, light and electron microscopic study concerning experimental renal hypertension. *Ibid.*, **14**, 1834–1847
11. CORNISH, J., VANDERWEE, M. A., ORMROD, D. J. & MILLER, T. E. (1986): Mucosal mast cells as a component of the inflammatory response to lower-urinary tract infection. *Int. Arch. Allergy Appl. Immunol.*, **81**, 337–342
12. ENERBÄCK, L., PIPKORN, U. L. F. & OLOFSSON, A. (1986): Intraepithelial migration of mucosal mast cells in hay fever: Ultrastructural observation. *Ibid.*, **81**, 289–297
13. FREDERIX, M. & BAERT, J. (1986): Naphthol-AS-D chloroacetate esterase activity in globule leucocytes in the tracheal epithelium of rats. *Acta Anat.*, **125**, 93–95
14. FREY, T., PETTY, H. R. & MCCONNELL, H. M. (1982): Electron microscopic study of natural killer cell-tumor cell conjugates. *Cell Biol.*, **79**, 5317–5321
15. GROSSI, C. E., CADONI, A., ZIECA, A., LEPRINI, A. & FERGUSON, M. (1982): Large granular lymphocytes in human peripheral blood: Ultrastructural and cytochemical characterization of granules. *Blood*, **59**, 277–283
16. GURSINGHE, C. J. & BIRTLES, M. J. (1985): Light microscopic observations of an intraepithelial granular cell type in the bovine parotid gland. *Acta Anat.*, **124**,

122-126

17. HERBERMAN, R. B., NUNN, M. E. & LAVRIN, D. H. (1975): Natural cytotoxic reactivity of mouse lymphoid cells against syngenic tumors I. Distribution of reactivity and specificity. *Int. J. Cancer*, **16**, 216-228
18. HOLMAN, J. (1970a): Ultrastructure of the intestinal globule leucocytes in the chick. *Acta Vet. Brno*, **39**, 385-390
19. HOLMAN, J. (1970b): Ultrastructure of hepatic globule leucocytes. *Folia Morphol.*, **18**, 347-357
20. HOLMAN, J. (1972): Ultrastructure of specific granules of the intestinal globule leucocytes of the chicken. *Acta Vet. Brno*, **41**, 235-239
21. HOLMAN, J. (1973): Development of globule leucocyte specific granules from lysosomes. *Folia Morphol.*, **21**, 281-283
22. HUNTLEY, J. F., WALLACE, G. R. & MILLER, H. R. P. (1982): Quantitative recovery of isolated mucosal mast cells and globule leucocytes from parasitised sheep. *Res. Vet. Sci.*, **33**, 58-63
23. HUNTLEY, J. F., MCGORUM, B., NEWLANDS, G. F. S. & MILLER, H. R. P. (1984): Granulated intraepithelial lymphocytes: Their relationship to mucosal mast cells and globule leucocytes in the rat. *Immunology*, **53**, 525-535
24. JEFFERY, P. K. & REID, L. (1975): New observations of rat airway epithelium: A quantitative and electron microscopic study. *J. Anat.*, **120**, 295-320
25. KANEDA, K., DAN, C. & WAKE, K. (1983): Pit cells as natural killer cells. *Biomed. Res.*, **4**, 567-576
26. KANEDA, K. & WAKE, K. (1983): Distribution and morphological characteristics of the pit cells in the liver of the rat. *Cell Tissue Res.*, **233**, 485-505
27. KANG, Y.-H., CARL, M., WATSON, L. P. & YAFFE, L. (1987): Immunoultrastructural studies of human NK cells: II. Effector-target cell binding and phagocytosis. *Anat. Res.*, **217**, 290-304
28. KENT, J. F. (1966): Distribution and fine structure of globule leucocytes in respiratory and digestive tracts of the laboratory rats. *Ibid.*, **156**, 439-454
29. KIESSLING, R., KLEIN, E. & PROSS, H. (1975): "Natural" killer cells in the mouse I. Cytotoxic cells with specificity and distribution according to genotype. *Eur. J. Immunol.*, **5**, 112-121
30. KITAGAWA, H., OGATA, K., SUGIMURA, M., HASHIMOTO, Y. & KUDO, N. (1979): Thymus origin of globule leucocytes in chicken. *Jpn. J. Vet. Res.*, **27**: 16-18.
31. LOUGHRAN, T. P. JR., DEEG, H. J. & STORB, R. (1985): Morphological and phenotypic analysis of canine natural killer cells: Evidence for T-cell lineage. *Cell. Immunol.*, **95**, 207-217
32. LUINI, W., BORASCHI, D., ALBERTI, S., ALEOTTI, A. & TAGLIABUE, A. (1981): Morphological characterization of a cell population responsible for natural killer activity. *Immunology.*, **43**, 663-668
33. MAHMOUD, G. S. & PIRIE, H. M. (1982): Ovine bronchopulmonary globule leucocytes I. Morphological and cytochemical studies. *Anat. Histol. Embryol.*, **11**, 205-212
34. MILLER, H. R. P. & WALSHAW, R. (1972): Immune reactions in mucous membrane IV. Histochemistry of intestinal mast cells during helminth expulsion in the rat.

- Am. J. Pathol.*, **69**, 195–206
35. MURRAY, M., MILLER, H. R. P. & JARRETT, W. F. H. (1968): The globule leucocyte and its derivation from the subepithelial mast cell. *Lab. Invest.*, **19**, 222–234
 36. NEIGHBOUR, P. A., HUBERMAN, H. S. & KRESS, Y. (1982): Human large granular lymphocytes and natural killing: Ultrastructural studies of strontium-induced degranulation. *Eur. J. Immunol.*, **12**, 588–595
 37. PEARSALL, A. D., ECHT, R., ROSS, L. M., ROTH, R. A. JR. & DINERSTEIN, R. J. (1984): Morphologic and cytochemical characteristics of amine-containing globule leucocytes in rat tracheal epithelium. *Am. J. Anat.* **170**: 83–99.
 38. REYNOLDS, C. W., DENN III, A. C., BARLOZZARI, T., WILTROUT, R. H., REICHARDT, D. A. & HERBERMAN, R. B. (1984): Natural killer activity in the rat IV. Distribution of large granular lymphocytes (LGL) following intravenous and intraperitoneal transfer. *Cell. Immunol.*, **86**, 371–380
 39. REYNOLDS, C. W., TIMONEN, T. & HERBERMAN, R. B. (1981): Natural killer (NK) cell activity in the rat I. Isolation and characterization of the effector cells. *J. Immunol.*, **127**, 282–287
 40. SCHEUERMANN, D. W. (1982): Proliferation and transformation of lymphocytes in the lung capillaries of the rat: Ultrastructure, acid phosphatase and peroxidase cytochemistry. *Acta Anat.*, **113**, 264–280
 41. TAGLIABUE, A., LUINI, W., SOLDATESCHI, D. & BORASCHI, D. (1981): Natural killer activity of gut mucosal lymphoid cells in mice. *Eur. J. Immunol.*, **11**: 919–922.
 42. TAKEUCHI, A., JERVIS, H. R. & SPRINZ, H. (1969): The globule leucocyte in the intestinal mucosa of the cat: A histochemical, light and electron microscopic study. *Anat. Rec.*, **164**, 79–100
 43. TARGAN, S., BRIEVA, J., NEWMAN, W. & STEVENS, R. (1985): In the NK lytic involved in the mechanism of NK suppression of antibody-producing cells. *J. Immunol.*, **134**, 666–669
 44. TIMONEN, T., RANKI, A., SAKESLA, E. & HAYRY, P. (1979): Human natural cell-mediated cytotoxicity against fibroblast III. Morphological and functional characterization of the effector cells. *Cell. Immunol.*, **48**, 121–132.
 45. TOKASHIKI, S., KAWASHIMA, Y., KUDO, N., HASHIMOTO, Y. & SUGIMURA, M. (1981): Presence of globule leucocytes in the uterus of pregnant goats and their fine structure. *Jpn. J. Vet. Sci.*, **43**, 762–732 (in Japanese with English summary)
 46. TOLEDO, O. M. S., MORALES, C. R., PEREYRA, L. A., JORDAO, T. & MONTES, G. S. (1981): Migrating mast cells in the gallbladder epithelium of cattle and sheep. *Histochemistry*, **72**, 433–442
 47. TONER, P. G. (1965): The fine structure of the globule leucocyte in the fowl intestine. *Acta Anat.*, **61**, 321–330
 48. TONER, P. G. & FURGUSON, A. (1971): Intraepithelial cells in the human intestinal mucosa. *J. Ultrastruct. Res.*, **34**, 329–344
 49. VANDENBERGHE, M. P. & BAERT, J. (1981): Globule leucocytes in the tracheal epithelium of rats. *Acta Anat.*, **111**, 268–276
 50. WEILL, P. (1919): Über die leukocytären Elemente der Darmschleimhaut der Säuge-

- tiere. *Arch. Mikrosk. Anat.*, **93**, 1-81
51. WISSE, E. (1970) : An electron microscopic study of the fenestrated endothelial lining of rat liver sinusoids. *J. Ultrastruct. Res.*, **31**, 125-150
52. WISSE, E., VANT NOORDENDE, J. M., VAN DER MEULEN, J. & DAEMS, W. TH. (1976) : The pit cell : Description of a new type of cell occurring in rat liver sinusoids and peripheral blood. *Cell Tissue Res.*, **173**, 423-435

EXPLANATION OF FIGURES

PLATE I

- Fig. 1 Intraepithelial globule leucocytes (arrows) in the cecal villi (a) and crypt (b). Globule leucocytes present a round or oval shape and the nuclei are rich in chromatin. The chromophobic cytoplasm, containing several acidophilic granules with various sizes, is distinguished from the slight basophilic cytoplasm of neighboring mucous epithelial cells. Globule leucocytes in the crypt epithelium possess larger granules than those in the villous epithelium. H. E. stain x 900
- Fig. 2 Electron micrograph of a globule leucocyte (GL) at the apical portion of the intestinal epithelium. x 12,800

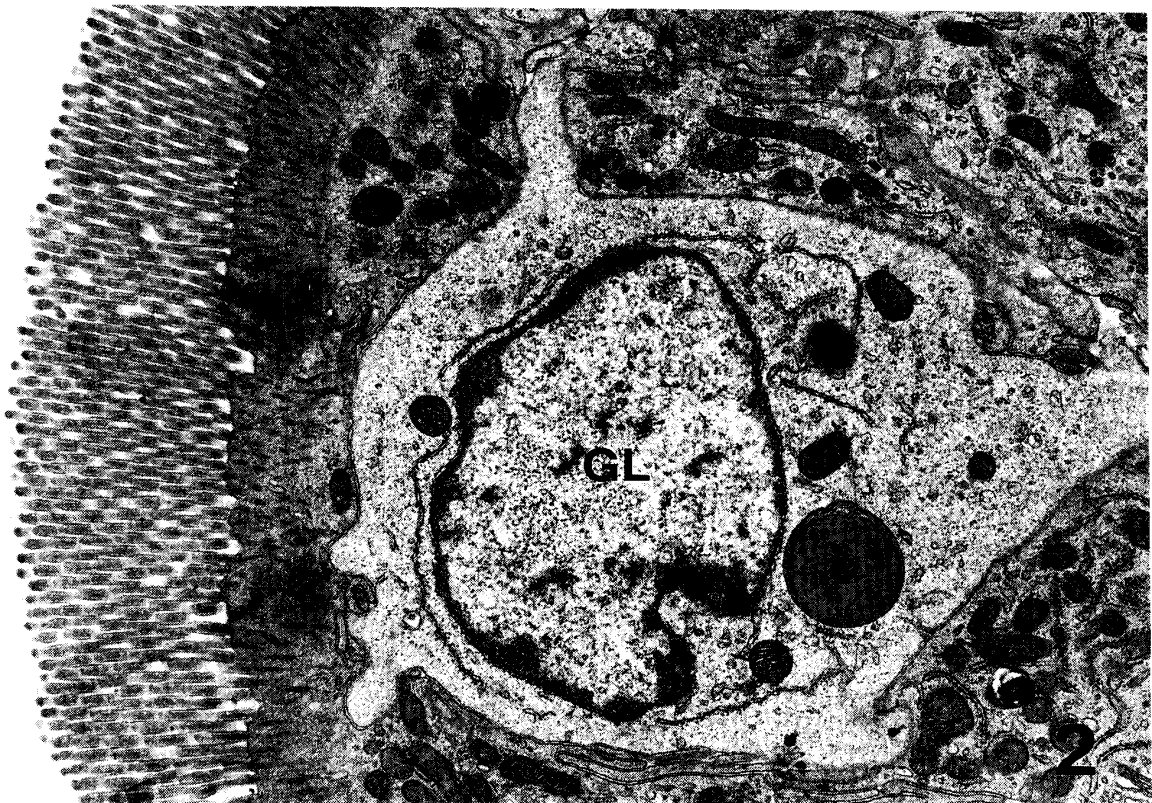
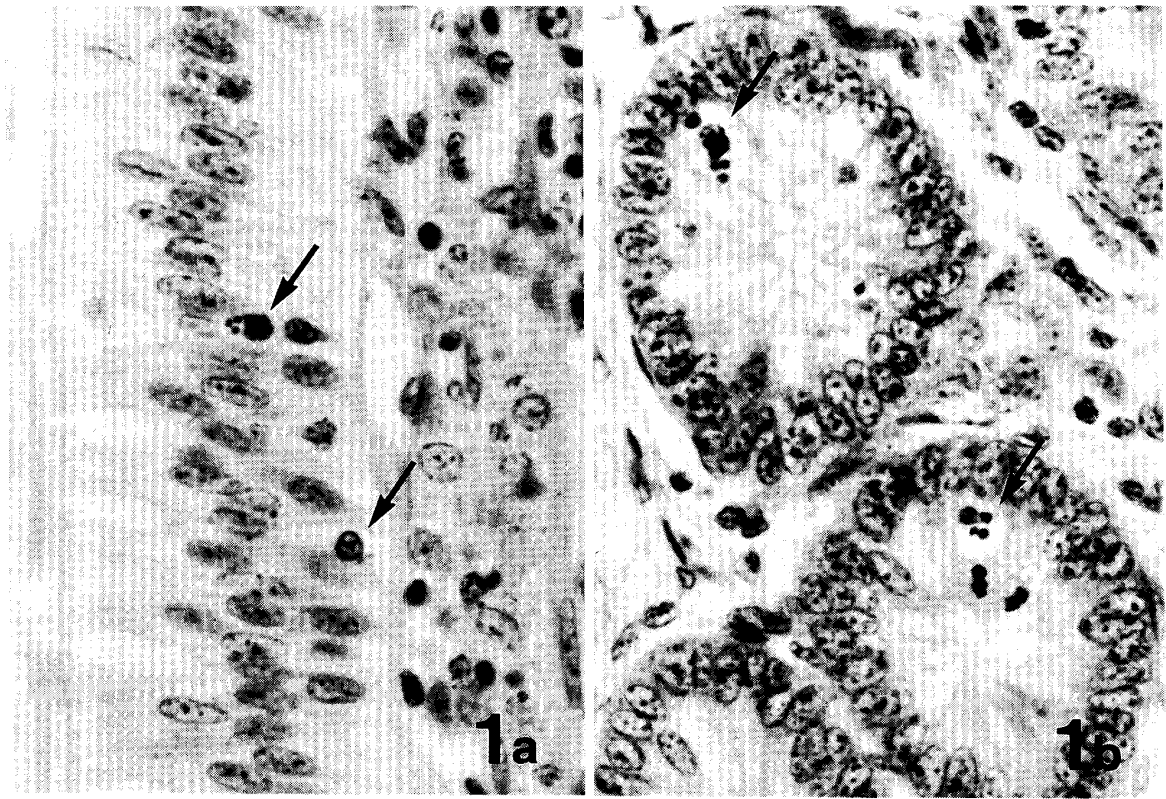
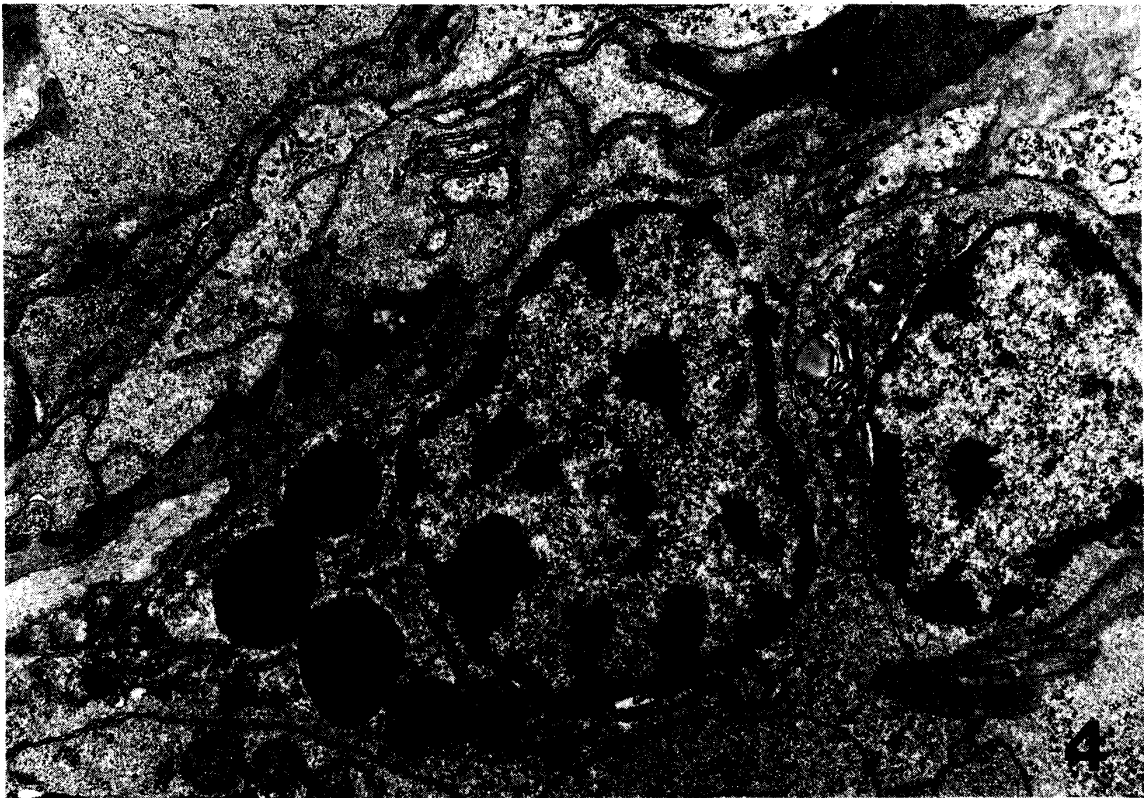


PLATE II

- Fig. 3 The lymphocyte (L) protruding with collapsing epithelial cells into the intestinal lumen. x 15,800
- Fig. 4 A globule leucocyte crossing the basement membrane of the intestinal epithelium (arrows). x 12,600



3



4

PLATE III

Fig. 5 A Russel body cell beneath the cecal epithelium. The Russel bodies are surrounded by the membrane of a rough-surfaced endoplasmic reticulum and the matrices are of the fine reticular type. x 12,600

Fig. 6 A mast cell beneath the cecal epithelium. The matrices of cytoplasmic granules present fine granular or convoluted shapes. The diameter is less than 1.0μ . x 15,600

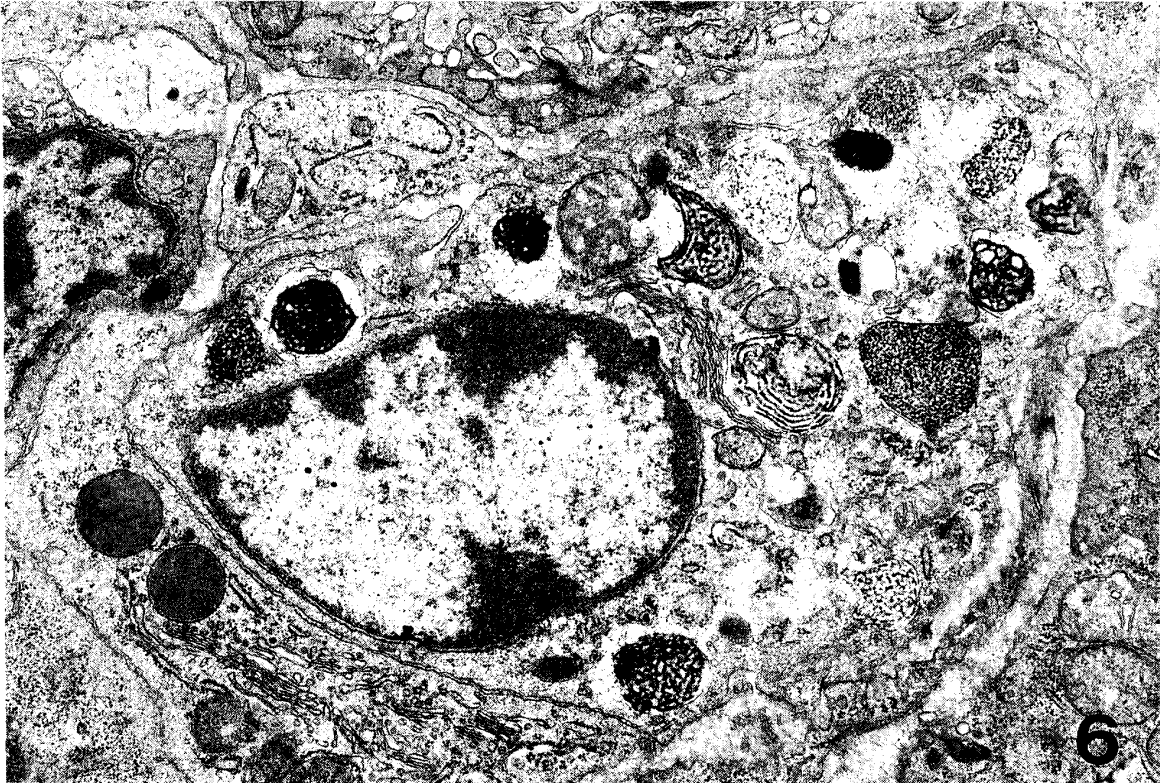
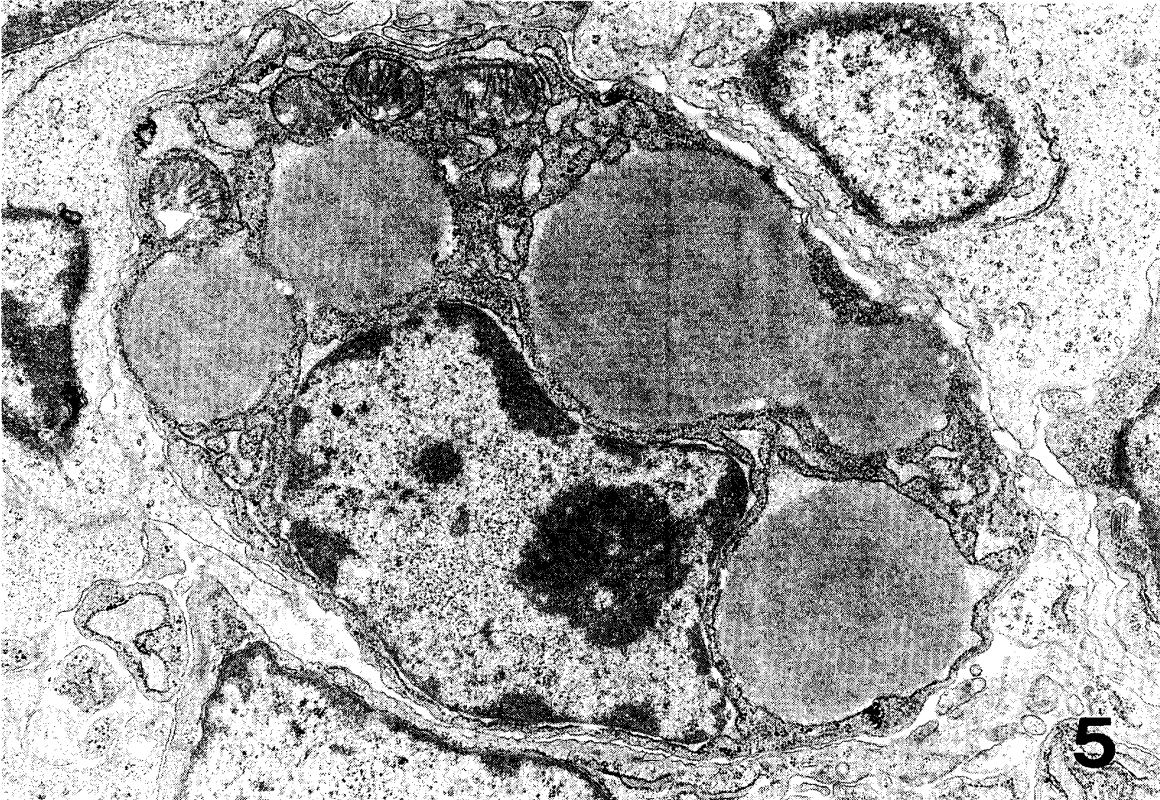


PLATE IV

- Fig. 7 An eosinophil in the lamina propria of the ceca. The dense granules with homogeneous matrices are almost of uniform size. x 12,600
- Fig. 8 A macrophage (between large arrows) and a lymphocyte (small arrows) crossing the basement membrane of the follicle-associated epithelium. x 12,600

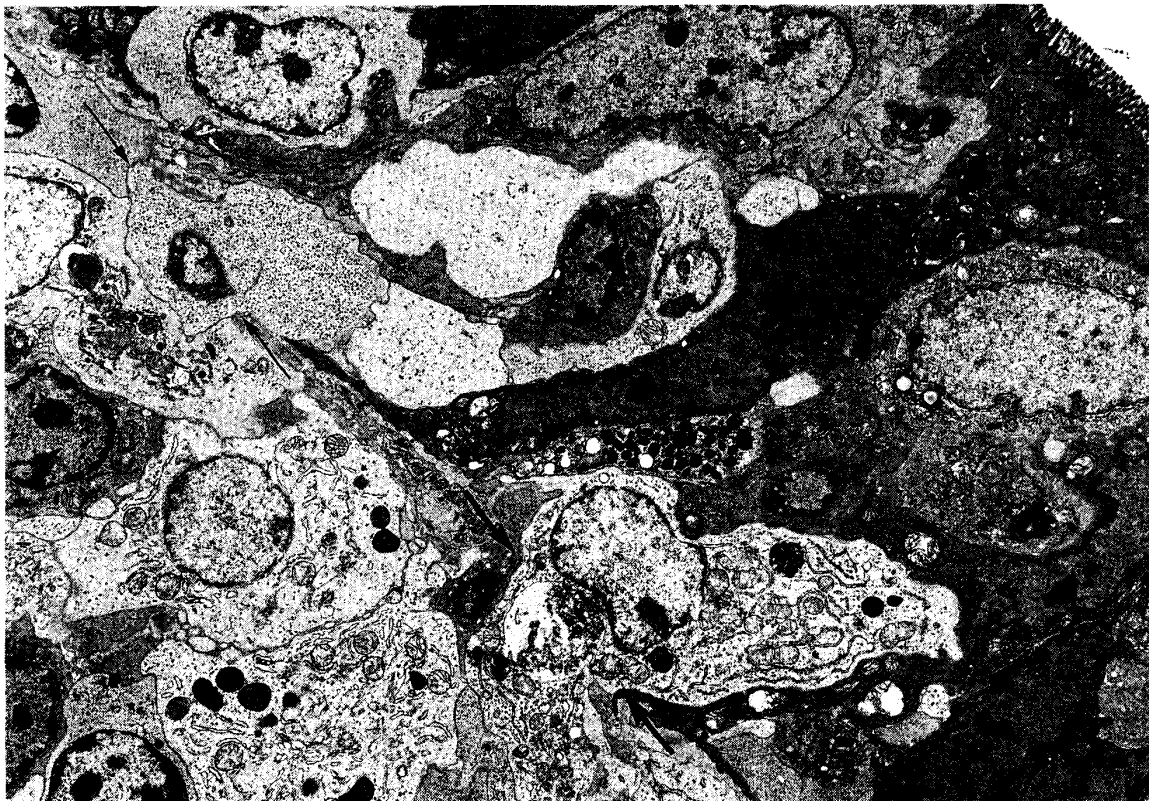
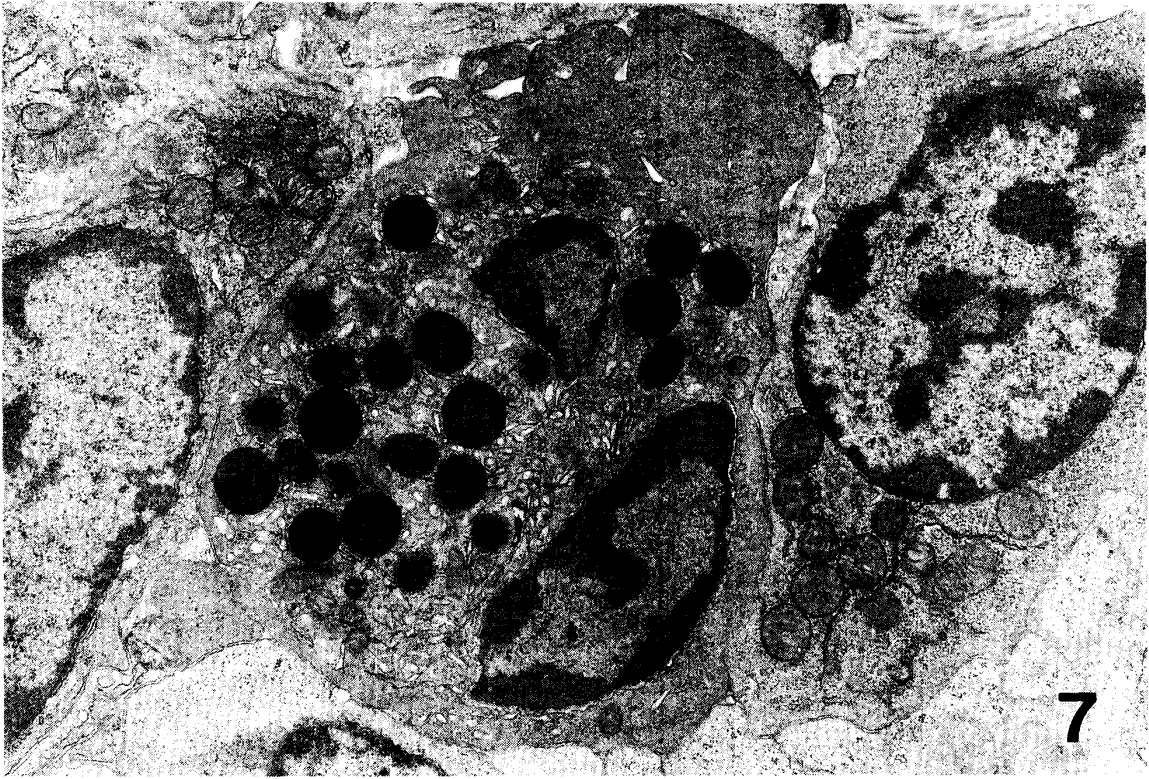


PLATE V

- Fig. 9 The granules are distributed in the area neighboring the Golgi apparatus (Go). x 15,800
- Fig. 10 The multivesicular bodies (arrows) and the minute vesicles (arrow heads) contain dense matrices. x 23,700

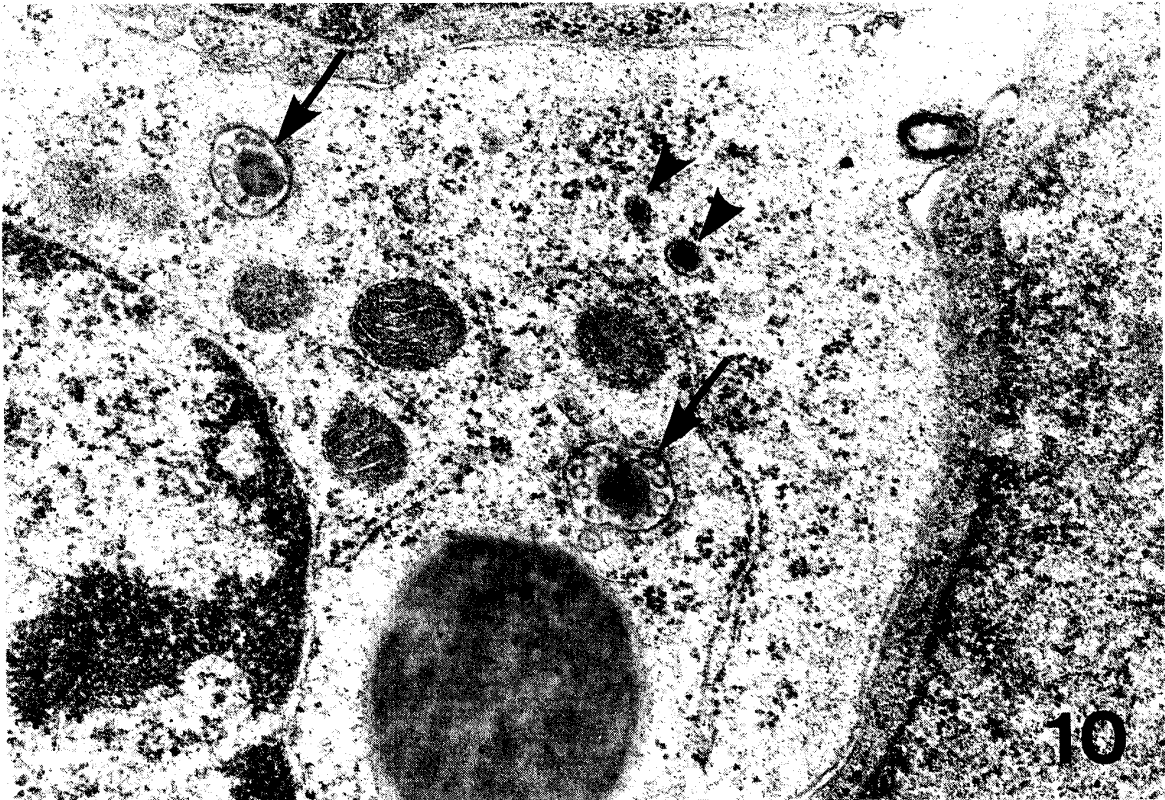
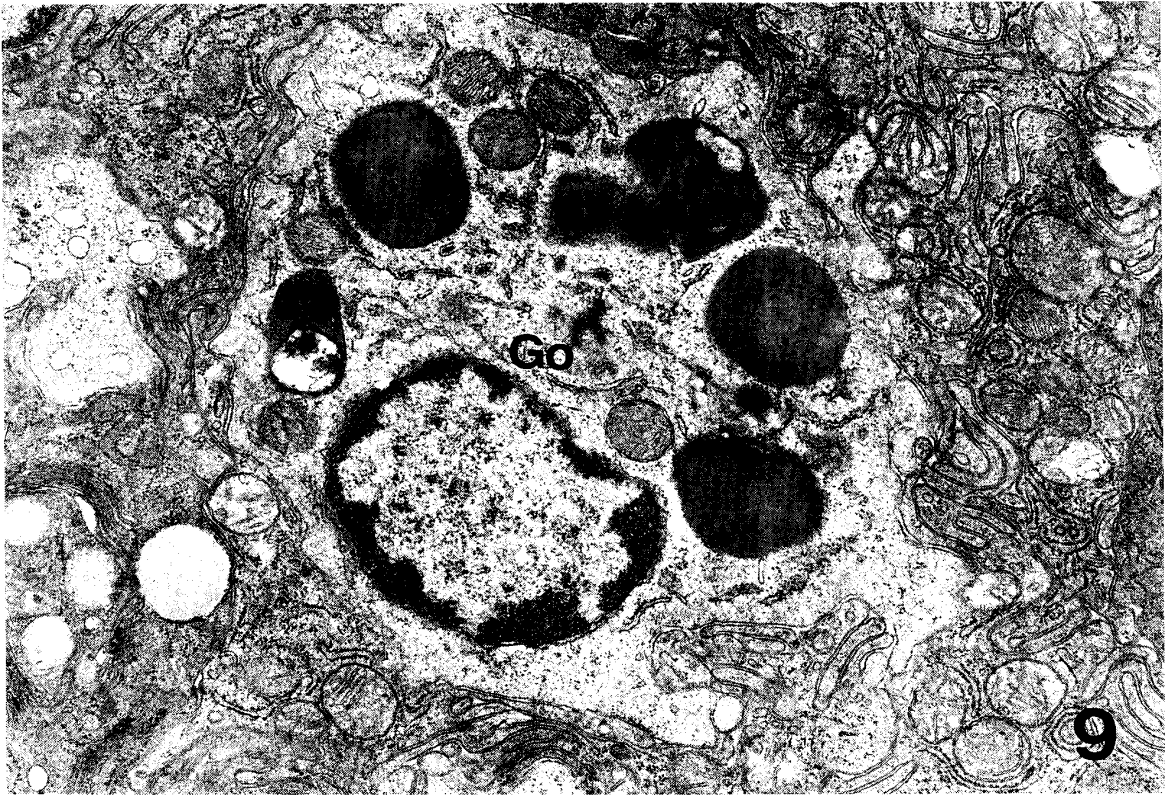


PLATE VI

Fig. 11 Minute vesicles with dense matrices are found throughout the cytoplasm. A granule is located beneath the cell surface membrane (arrow). x 12,600

Fig. 12 Type I granules (I) and a type II granule (II) of a globule leucocytes in the cecal epithelium. The type II granule consists of both an amorphous matrix and a marginal zone with fine reticular materials (arrow). x 23,700

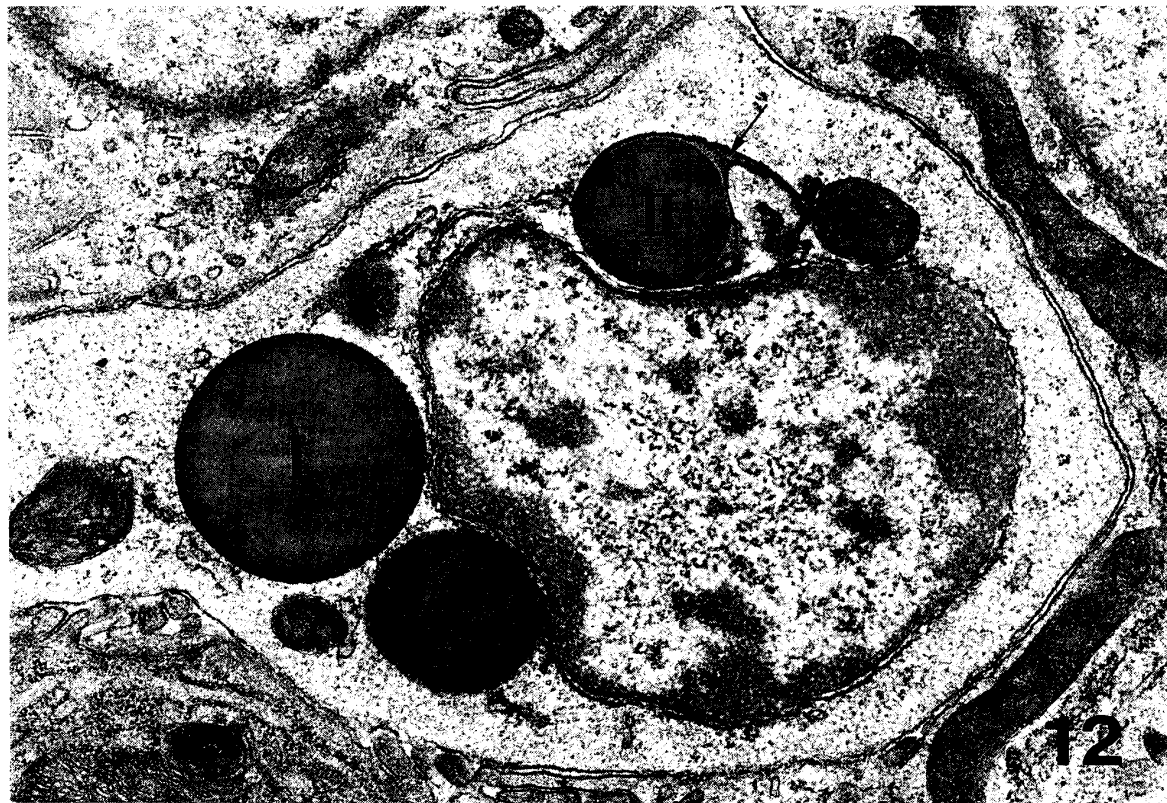
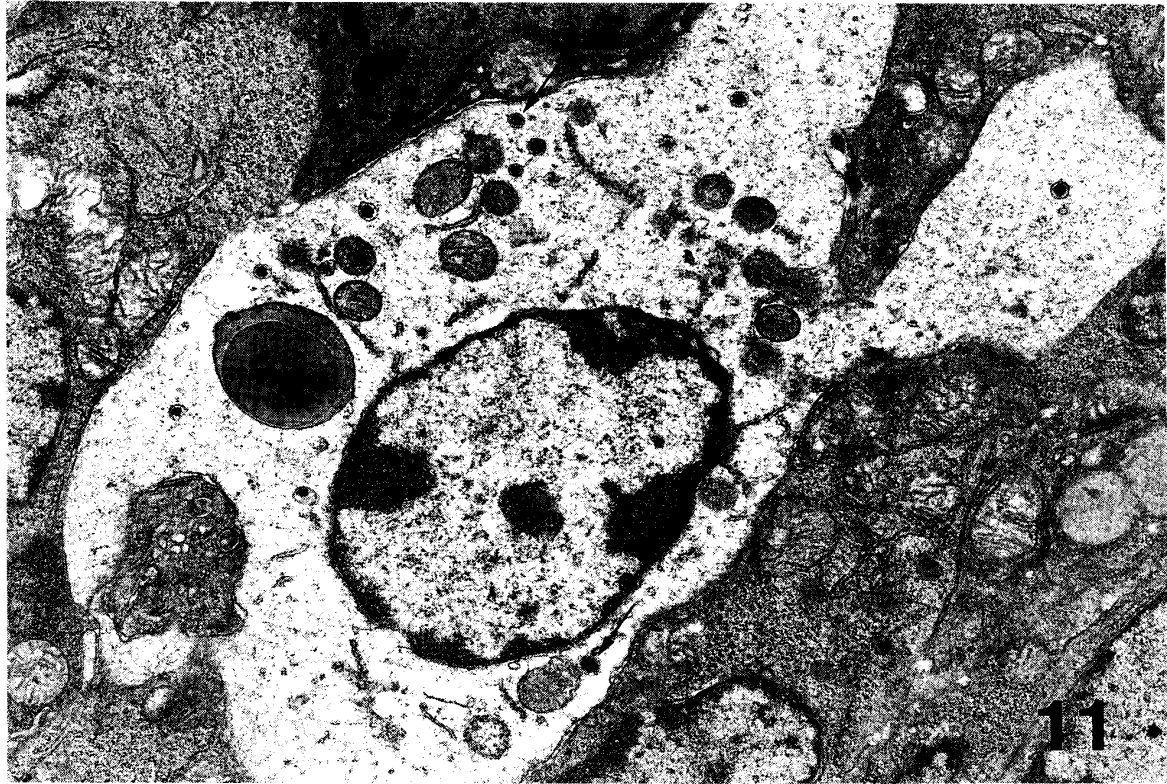


PLATE VII

- Fig. 13 Type II granules of an intraepithelial globule leucocyte of the cecum. The type II granule possesses a wide marginal zone with fine reticular materials (FRM). The oval and amorphous matrix (AM) contain crystalline inclusions (arrow) x 63,200
- Fig. 14 High magnification of type II granules of an intraepithelial globule leucocyte of the cecum. The direction (arrows) of each crystalline array is slightly different. x. 90,000

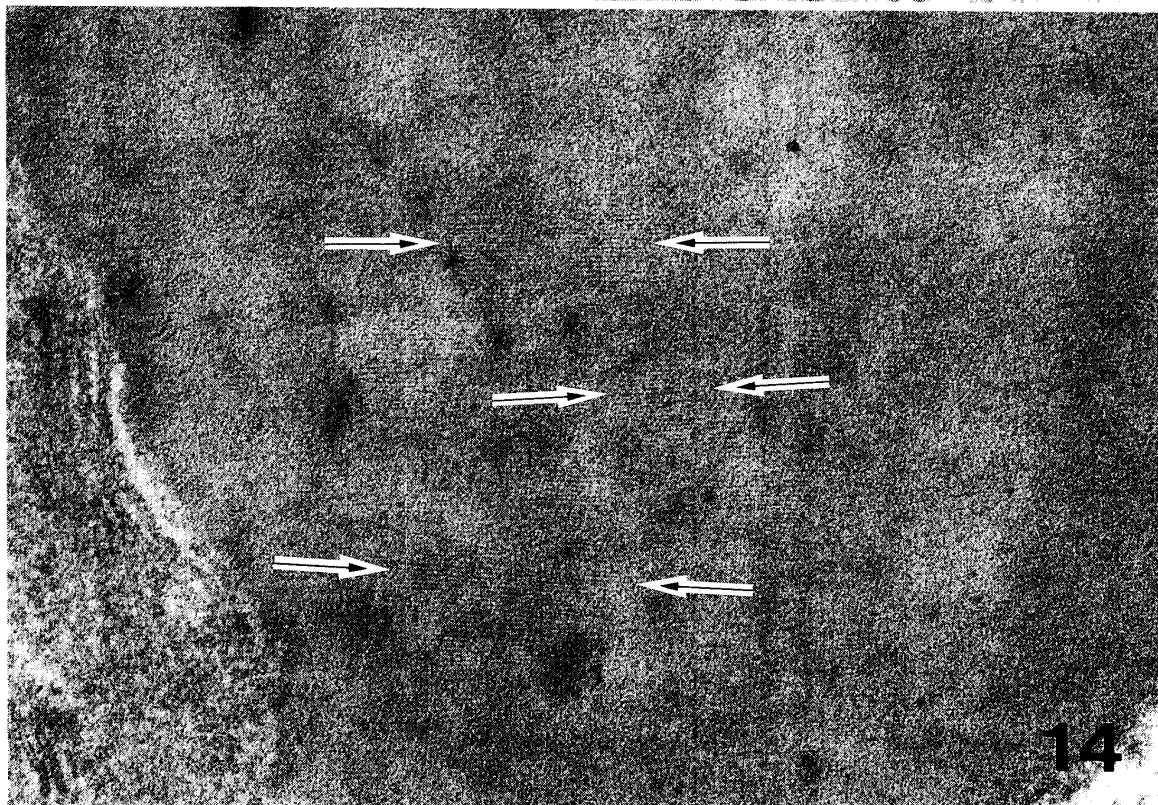
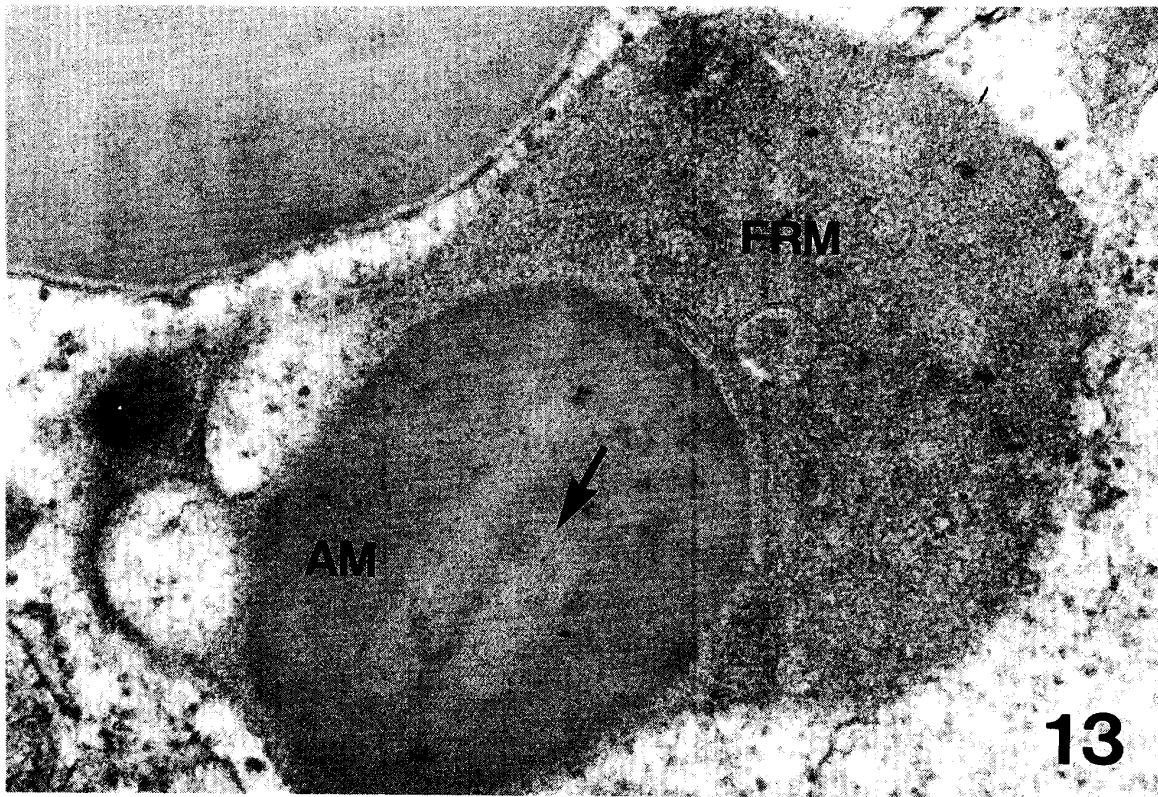


PLATE VIII

- Fig. 15 Type II granules of the intraepithelial globule leucocytes of the cecum. The marginal zone with fine reticular materials contains lamellar inclusions, which present voluted shapes (a) and comma-shaped fragments. a : x 55,300, b : 63,200
- Fig. 16 A high magnification of the type II granules. Single layers of the lamellar inclusion (arrow) are formed at the margin of the amorphous matrix (AM), and the thickness increases successively. a : x 63,200, b : x 110,600

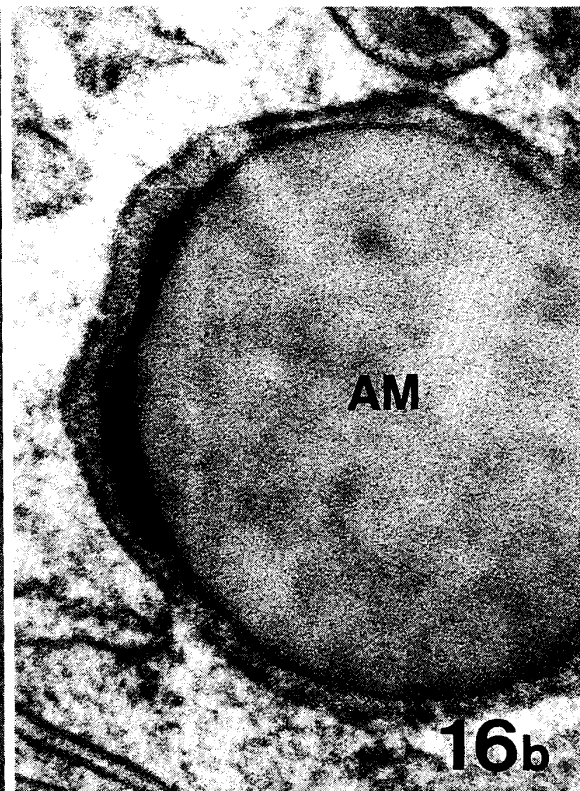
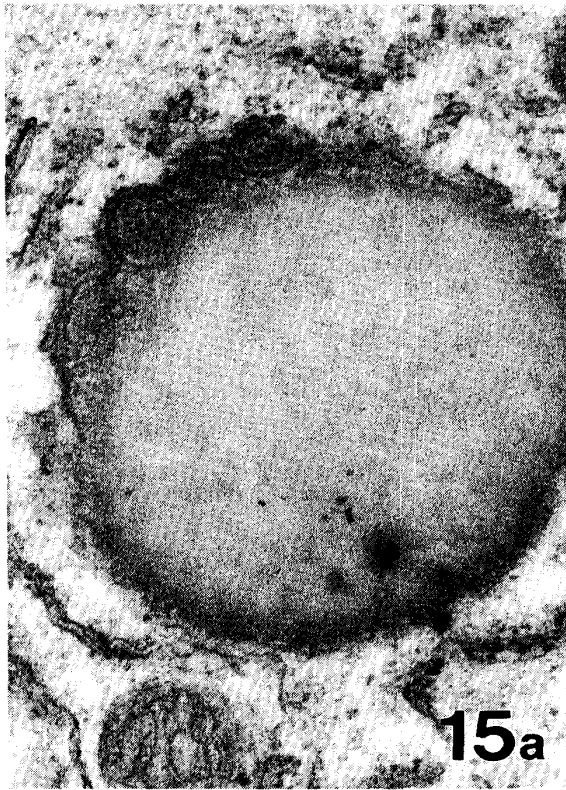


PLATE IX

- Fig. 17 Some lamellar inclusions (arrow) consisted of a high dense band of 30–40A, an electron-lucent band of 20–30A and a moderate electron-dense band of 20–30A. x 110,600
- Fig. 18 Fusion of the granules (arrow) is found in the cytoplasm of the intraepithelial globule leucocyte. x 23,700
- Fig. 19 Communication of the type II granules with the rough-surfaced endoplasmic reticulum. a, b): x 47,400
- Fig. 20 Transverse view of the cytoplasmic processes in the type II granule. x 12,600

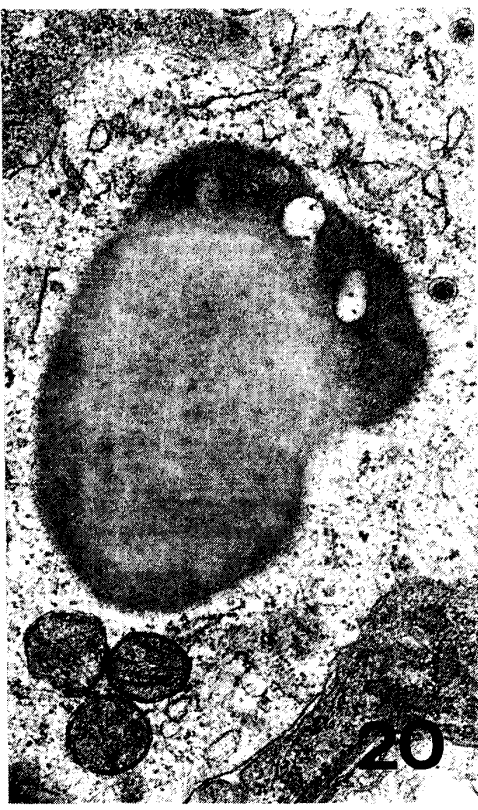
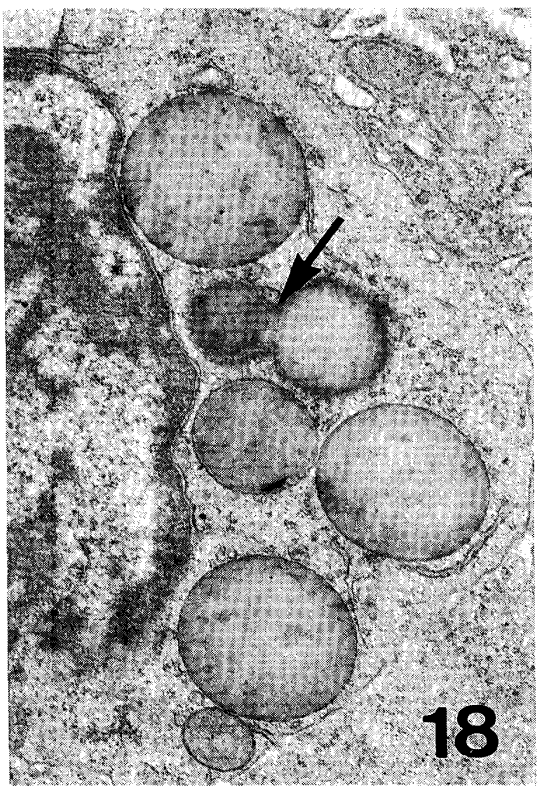
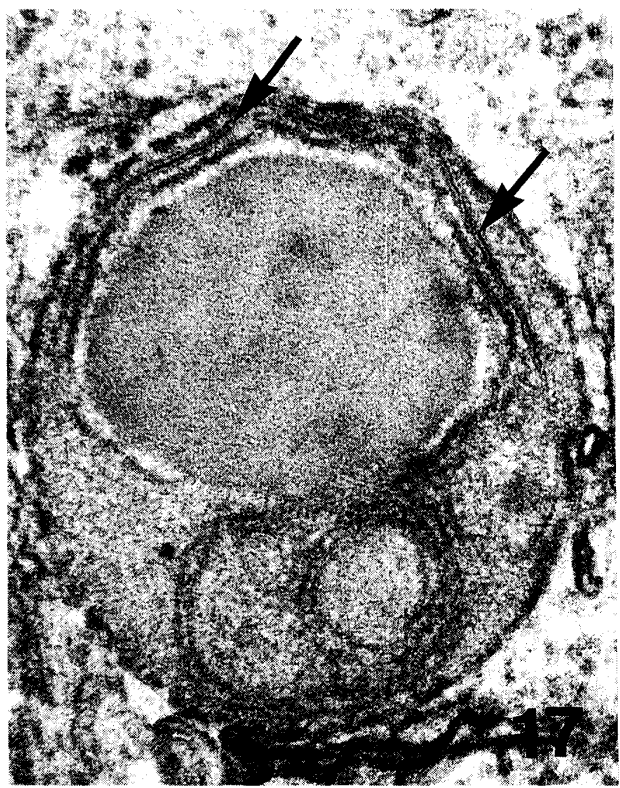


PLATE X

Fig. 21 The release of intragranular substances to extracellular spaces. The cytoplasmic process or fold (arrow) is situated at the outlet of the granule. The intercellular spaces are filled with electron-dense substances (arrow heads). a : x 15,800, b : x 23,700

