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Ultrastructural Study of Coelomic Free Cells of Newts *Synops pyrrhogaster*

Takeshi SETO *

Abstract : Characteristic free cells with Russell bodies native to the coelomic cavity of newts were examined by both transmission and scanning electron microscopies. Surface morphology of a Russell cell exhibited close resemblance to the appearance of a monocyte. Bipolar processes of the cell were actually thin, flattened cytoplasmic extensions, which were quite polymorphous membrane architecture. Cross-sectioned ultrastructure demonstrated that the greatly distended vacuole occupied most of the cell volume and cytoplasm seemed to be thinly expanded by the vacuole. Numerous filaments were included in the conspicuous vacuoles, which morphology was similar to the collagenous fiber. Cross-sectiion of the filament viewed a tubular structure with consistent diameter of 40 to 50 nm. Density of intravacuolar fibriles varied possibly by the maturity of Russell cells. The present observation demonstrated that Russell bodies of newt free cells have a quite dissimilar character to those of mammalian plasma cells.

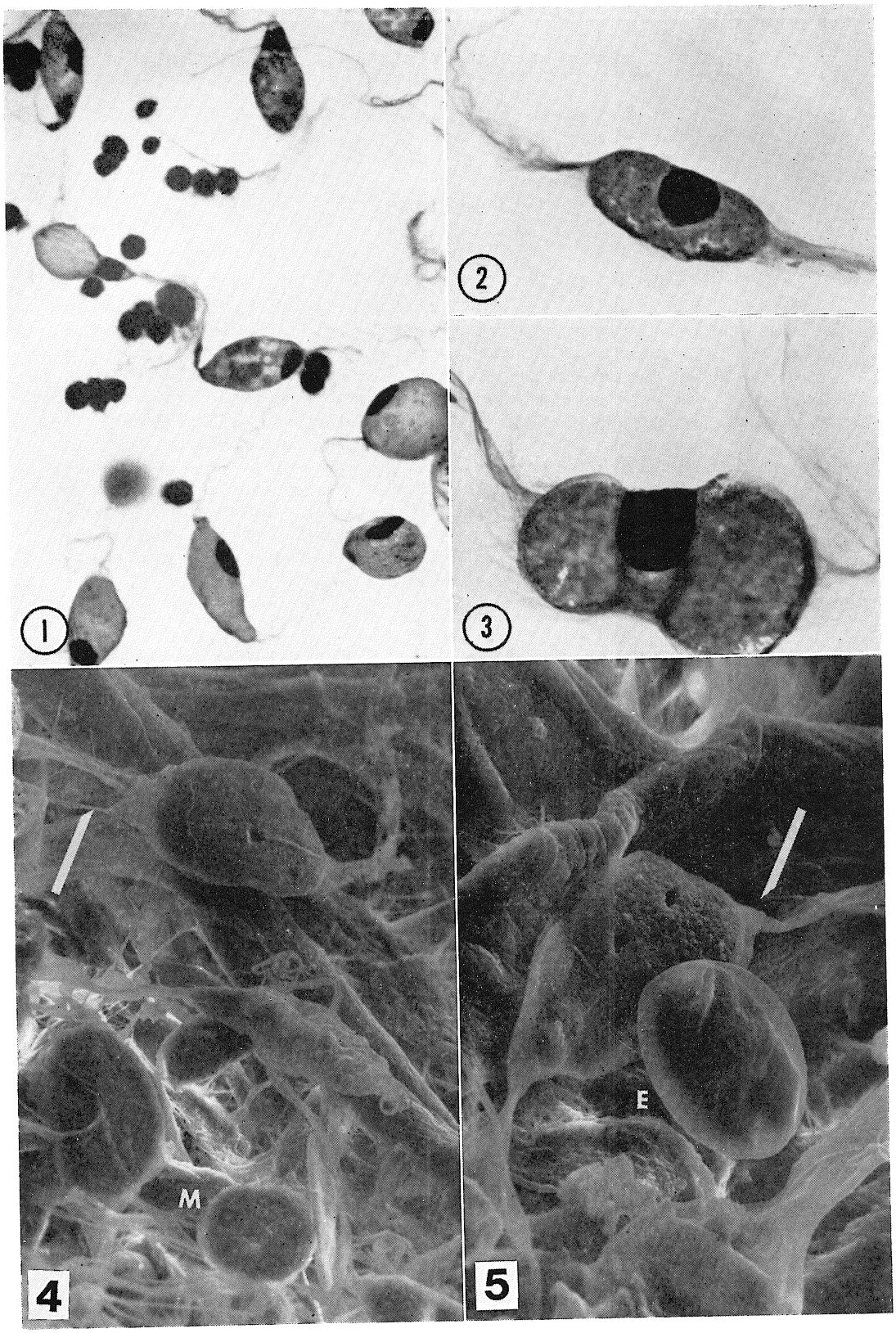
Introduction

In previous studies (Seto 1970a, 1970b, 1971) on certain cytological features of free cells native to the newt coelomic fluid, the author has mainly dealt with the morphological feature and function of the characteristic cells with Russell bodies, abbreviated as a Russell cell. The cell is easily distinguished from other type of free cells on account of having bipolar cytoplasmic processes and greatly distended vacuoles with opaque inclusions.

From a limited examination in previous work (Seto 1971), I have speculated that Russell cells could be originated from monocytes or lymphocytes which were members of coelomic cell population by cellular transformation. It is well known fact that monocytes undergo a characteristic sequential transformation *in vivo* and *in vitro* into macrophages, epithelial cells, and multinucleated giant cells (Sutton and Weiss 1966). However, cytology of the transformation into Russell cells has not been adequately characterized, neither detail structure of the specific free cell has been studied before. It is inevitably needed to study the ultrastructures of the cell to explain the origin and the function of Russell cells in a body cavity and to find similarity or dissimilarity to other free cells.

Present observation was made use of transmission and scanning electron microscopes. Much of my attension has been devoted to two phases ; (1) the surface structure of cytoplasmic processes in order to clear out the aspect of continuity of cytoplasm to the bipolar processes and (2) the internal structure of vacuoles with the ultrathin-sectioned preparation for detection of an uniformal structure of inclusions.

* Department of Biology, Faculty of Education, Shimane University, Matsue, Japan 690.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Materials and Methods

Adult male and female newts, *Cynops* (*Triturus*) *pyrrhogaster*, were collected in the vicinity of Matsue and bred in the laboratory for several months. A routine cytological examination by light microscopy was the same as previously reported (Seto 1970a). Free cells aspirated from the body cavity with a glass capillary were put to use for the smear preparation and for the following processes.

For electron microscopy, the free cells were fixed for 2 hours at 0-4°C in a 2% glutaraldehyde solution in a centrifuge tube. Post-fixation was made by replacing the glutaraldehyde solution with a cold osmium tetroxide solution. Both fixatives were made up in 0.1 M phosphate buffer at pH 7.4. Fixed cells were centrifuged after washing once in distilled water, and the cell pellet was embedded in few drops of 2% agar dissolved in water. Coaggulated agar block was trimmed into small pieces of about one mm^3. Dehydration was carried out in increasing concentration of cold ethanol. The materials were thereafter embedded in Epon 812. Samples were sectioned with glass knives on Porter-Blum ultramicrotome, stained with uranyl acetate and observed with a Hitachi HU-11 electron microscope operated at 75 kV.

For SEM, a drop of coelomic fluid containing free cells directly placed on a slip of filter paper. Then the paper was transferred into a 2% glutaraldehyde solution. Processes of fixation and dehydration with graded ethanol series were conventional. The fine structure of cell surface was preserved against drying artifact by taking the specimens through the critical point drying method using Tanaka's apparatus (Tanaka 1972). The free cells carried with filter paper were then coated with chromium and gold-palladium in a vaccum rotary evaporator. The preparations were viewed with a Hitachi HSM-2 scanning electron microscope operated at 25 kV.

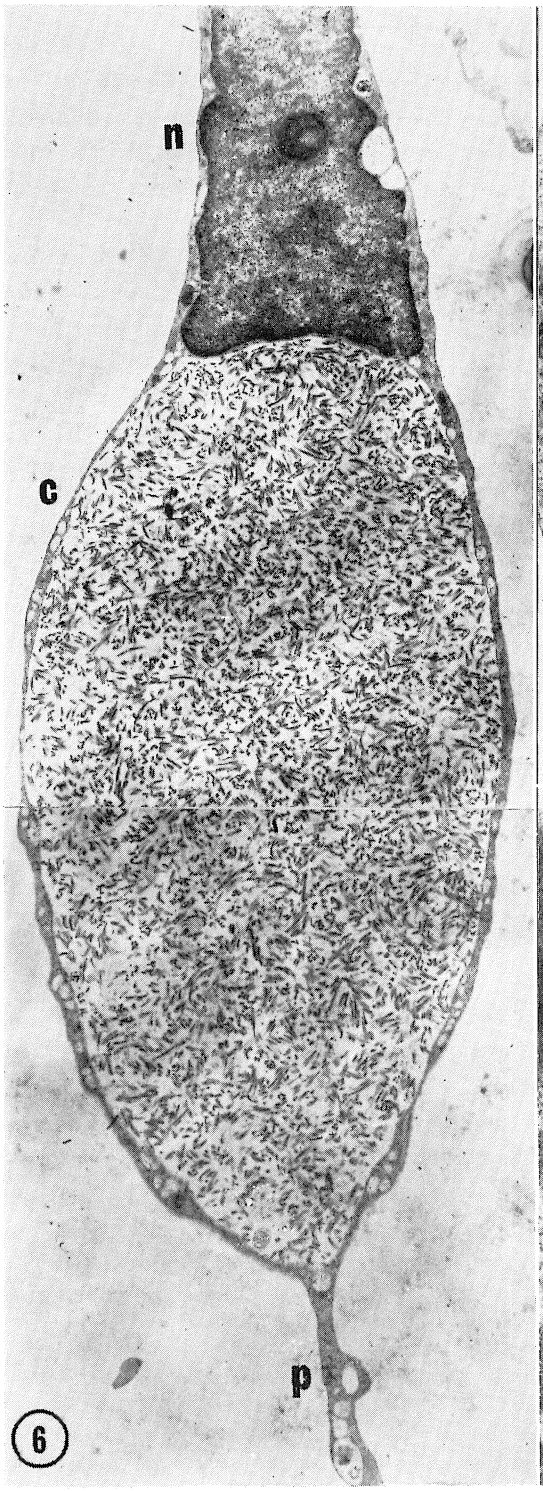
Observations and Discussion

SEM of Russell Cells

Population of coelomic free cells were shown by scanning electron microscopy (Figs. 4 & 5). Among free cells, Russell cells were easily defined by their bipolar cytoplasmic processes. A lymphocyte (Fig. 4) and an erythrocyte (Fig. 5) were simply identifiable ; an oval shape having more smooth surface was a erythrocyte. Significant similarity of surface morphology was found between leukocytes and Russell cells. Vacuolar zone was not recognized as a clear structure of Russell cells in the surface morphology.

Figures 1-3. Light micrographs of free cells in the peritoneal fluid of newts, *Cynops* (*Triturus*) *pyrrhogaster*. Smear preparations, methanol-fixed, and May-Grünwald Giemsa stain. Fig. 1. A variety of cells with Russell bodies (abbreviated as Russell cells) are distinguished among a group of free cells consisting of monocytes, small lymphocytes, and granulocytes. ×300. Figs. 2 & 3. Nuclei of Russell cells locate on the periphery of the cytoplasm or in between vacuoles. 2, ×550. 3, ×800.

Figures 4 & 5. Surface morphology of peritoneal free cells observed by scanning electron microscopy. Bipolar cytoplasmic processes (*arrow*) of Russell cells are quite polymorphous, exhibiting membraneous architecture. An erythrocyte (E) showing oval shape is easily defined. Surface morphology of a monocyte (M) closely resemble in appearance of Russell cells. 4, ×1600. 5, ×3700.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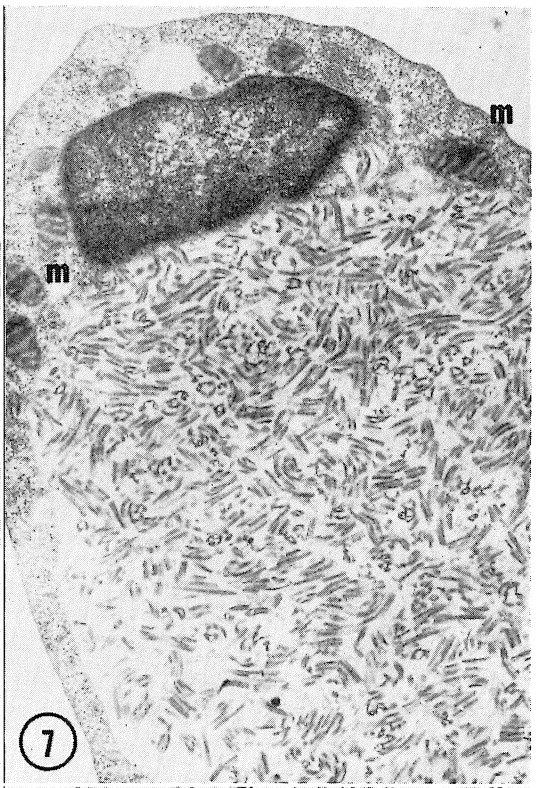
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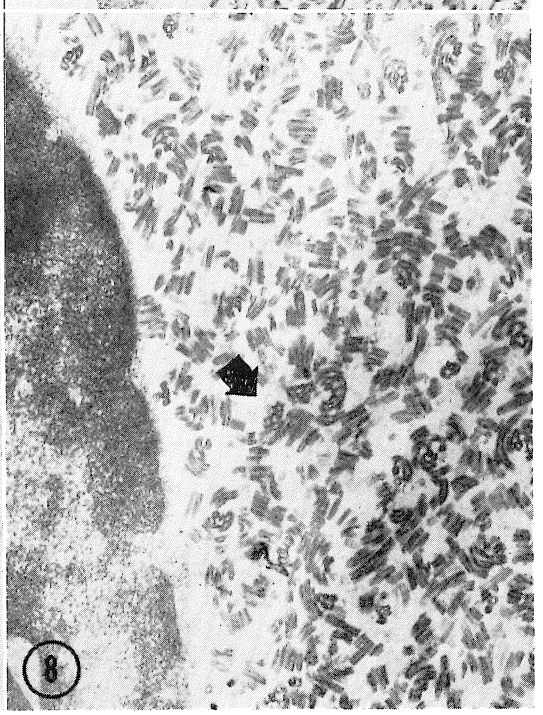
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Although the surface view obtained by SEM did not differ greatly with the smear preparation of light microscopy (Figs. 1-3), the appearance of Russell cell by SEM indicated that bipolar cytoplasmic processes have a continuity of cell surface, suggesting that these processes were differentiated from the part of cytoplasm and cell membrane. It is a matter of interest that the characteristic processes represented not like a typical flagellate structure but thin and flattened membraneous cytoplasmic extension, having no specific axial structure on them.

Ultrastructure of Sectioned Russell Cells

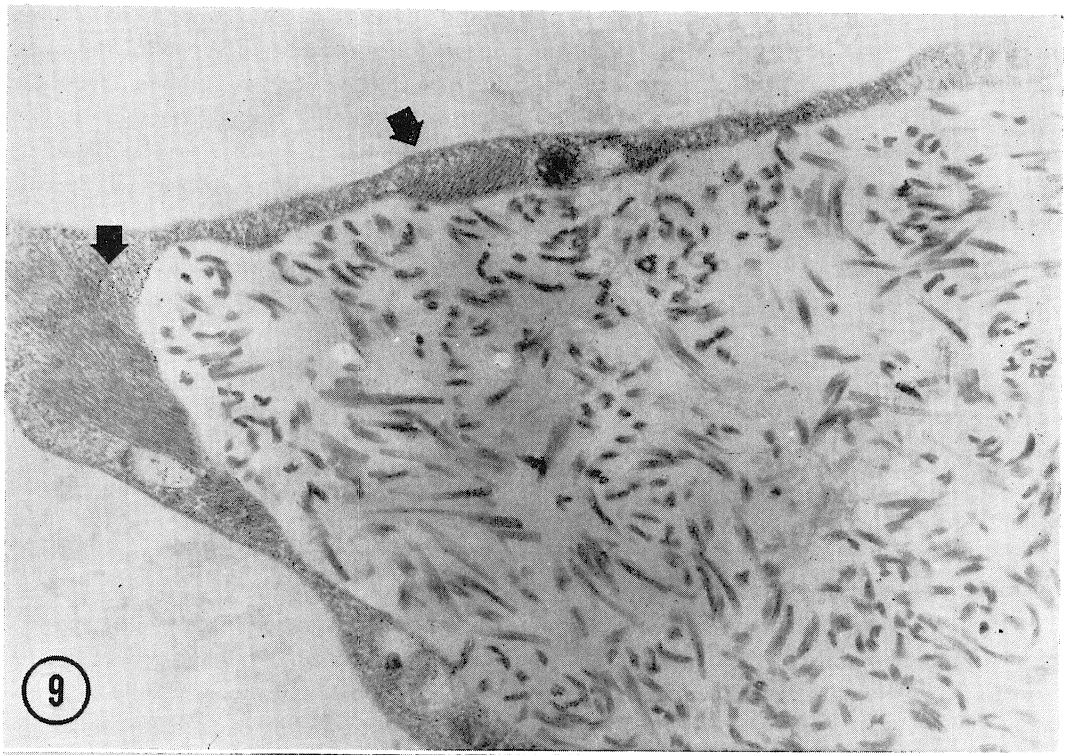
Ultrathin-sectioned view of Russell cells, as revealed with transmission electron microscope, indicated the specific structure which was easily recognized from other free cells. Under light microscope, the vacuole of Russell cells was greatly distended with a mass of dense material. Ultrastructure of the vacuole was demonstrated as membrane-bounded space in the cytoplasm, which occupied most of the cell volume and showing baloon shape. The nuclei located peripheral area of cells. A nuclear pattern consisted of two densities which defined the chromatin and interchromatin material. Nucleoli were not evident. The nuclear envelope did not appear as smooth edge of membrane but irregular outline (Figs. 6 & 8). Nuclear pores were absent.

Cytoplasm presented thin layer feature around the vacuole. Although a few mitochondria were clearly observable, their internal structure was unremarkable. Other cell organelle were not seen in the cytoplasm. Therefore Russell cells contained no organelle required for protein synthesis (ribosomes, nucleoli, nuclear pores) and those required for bounding the product in membrane (Golgi elements, ER). These cells were cytologically considered as the final form of cellular transformation. Number of tiny bubble-like spaces were appeared in the cytoplasm and the number increased in the mature type or degenerating cells (Fig. 10).

The characteristic vacuole contained consistently dense filamentous materials which have uniformal thickness of approximately 40 to 50 nm. The filaments distributed independently in the vacuole and no specific feature of bundles was seen. Bloom and Fawcett (1975) stated on the content of Russell bodies which included in plasma cells of mammals ; the bodies consisted of incomplete immunoglobulin molecules. And according to their speculation, Russell bodies are indicative of an aberrant synthesis or faulty intracellular transport of antibody. Although cytochemical nature of vacuoles of newt Russell cells was studied by Ohuye (1936) to a limited extent, neither description on intravacuolar filaments nor on the nature and incidence of such fibriles in free cells of newts or other vertebrates was available. Present observation revealed the newt Russell cells did not contain any organelle for protein synthesis. It should be noted the existence of essential dissimilarity between both type of Russell bodies.

Mizutani and Nakahara (1961) have attempted a tentative arrangement of Russell cells

Figures 6-8. Electron micrographs of ultrathin-sections of Russell cells. Fig. 6. A longitudinal sectioned view of a Russell cell revealing nucleus (n), cytoplasm (c) and bipolar processes (p). Greatly distended vacuole occupies most of the cell volume. ×6150. Fig. 7. A portion of a Russell cell showing numerous filaments included in the vacuole and typical mitochondria (m) in the cytoplasm. Fig. 8. A portion of a Russell cell exhibiting more details of the fibriles having tubular structure (*arrow*) in cross-sectioned fibriles. ×20000.



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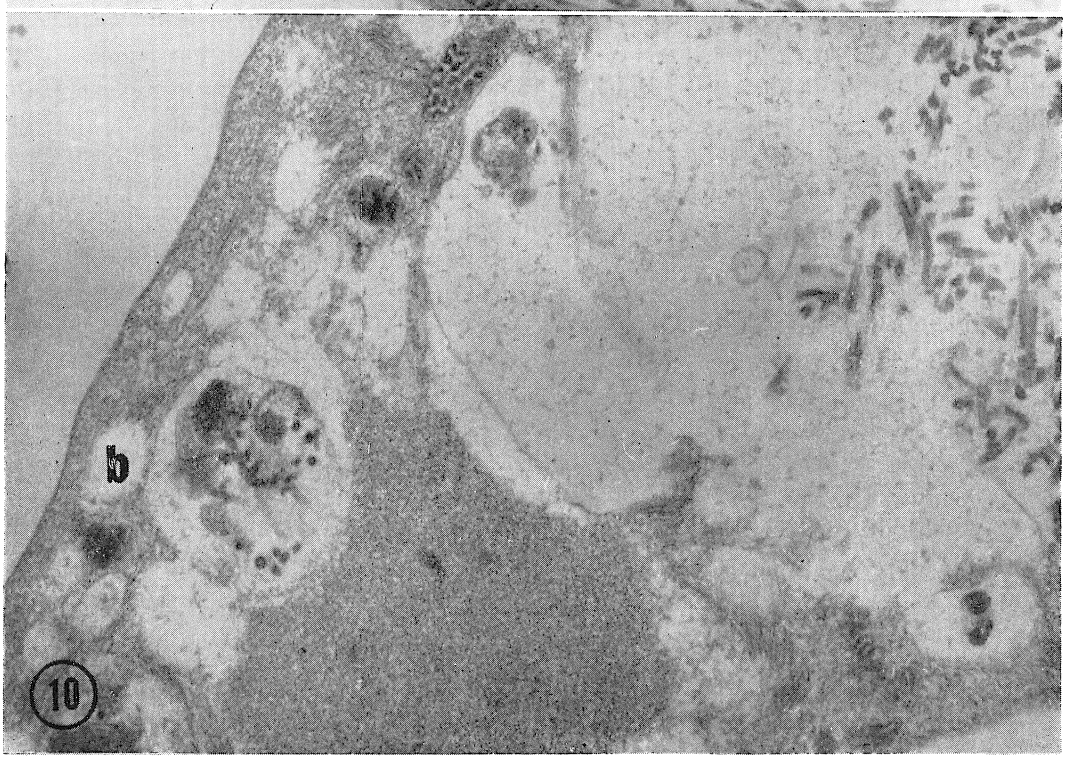

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from immature (or young) type cells to mature (or old) type cells based on a degenerative change of the cytoplasmic inclusions. Concentration of the fibriles under EM observation seemed to be higher in the young type of cells and became lower according as the maturity. The thickness of fibriles did not varied between the young types and the old types, but more fragmented feature was seen in old type cells. In degenerating cells filaments progressively decreased and membraneous structure around the vacuole became obscure (Figs. 9 & 10). Cross-sectioned view of the higher magnification demonstrated the fibriles looked like tubular structure with regular diameter. Such filamentous structure is quite similar to collagen fiber in connective tissues of vertebrates. However, no examination to inquire the reality was performed in this study.

From the above observation, Russell bodies of mammaliam plasma cells and of newt coelomic free cells were recognized to be not identical in nature and fine structure.

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Figures 9 & 10. Electron micrographs of ultrathin-sections of Russell cells. Fig. 9. An old type of the cell indicates less concentration of fibriles in the vacuole than the younger types. Lamellar structure of fine filaments, similar to tonofibrils of human epidermal cells, are seen in the cytoplasm (*arrows*). ×25000. Fig. 10. A portion of possibly degenerating type showing indented outline of the vacuole and small bubble-like spaces (b) in the cytoplasm. Intravacuolar fibriles become sparse and scattered. ×25000.