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Ultrastructural Studies of Typhoid Cells

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Abstract

To investigate the nature of typhoid cells, three cases of clinically, serologically and histopathologically proven typhoid lesions of the small intestine and regional lymph nodes were studied light and electron microscopically. Light microscopically, typhoid cells were swollen mononuclear cells characterized by abundant amount of eosinophilic cytoplasm and frequent phagocytoses of red blood cells, bacterial clumps and other tissue debris. These cells were pyronin negative. Electron microscopically, these cells showed marked and diffuse dilatation of RER cisternae and disappearance of ordinary cytoplasmic organelles, but frequent phagocytosed materials. The meaning and reason of RER cisternal dilatation and reduction of cytoplasmic organelles were discussed, and are regarded as degenerative process due to bacterial endotoxin. Although there was not enough cytoplasmic organelles to pinpoint the origin of typhoid cells, active phagocytosis and evidences against being either plasmacytic or lymphocytic nature favored retuculoendothelial nature of the typhoid cells.

Introduction

Mallory (1898) made first detail description of typhoid lesion and pointed out that infiltration of plump, round to oval mononuclear cells with abundant cytoplasm is the most characteristic feature of the lesion. These cells are so conspicious that presence of them are almost pathognomonic of typhoid fever (Happs 1971), thus to be named as so-called typhoid cells (Reimann 1937). The nature and origin of these cells are thought to be reticulum cells, reticuloendothelial cells, immigrant macrophages (Robbinson 1974) or immature plasmacytic cells (Goodpasture 1937). Their principal function in typhoid lesion is apparently phagocytosis of bacteria, red blood cells, and other tissue debris (Robbinson 1974).

The causative agent, salmonella typhosa, apparently stimulate these cells to proliferate, multiply within them, and finally destroy them(Mallory, 1898, Hirsh 1956). Salmonella include many species, and they are divided into three group. The first group including, S. typhosa is pathogenic only to human, the second group including S. typhimurium is primarily pathogenic to animals but occasionally produce clinical di sease, and the third group is pathogenic only for animals. Therefore experimental studies were mostly carried out with species pathogenic to animals namely S. typhimurium.

Several electron microscopic studies concerning pathogenesis and nature of typhoid cells were carried out in animal experiment (Yamamoto and Nakano 1960, Yamamoto 1966) but no human cases were studied by electron microscopy. Thus, the present study is to investigate ultrastructural characteristics of human typhoid cells.

Materials and Methods

Three cases of typhoid fever proved clinically, serologically, and histopathologically are studied. In all cases, segmental resections of small intestines including regional mesenteric lymph nodes were performed for the control of intestinal bleeding. The tissue was fixed in 10% neutral formalin and paraffin embedded. Sections from paraffin

blocks were stained with hematoxylin-eosin and methyl-green pyronin methods to study light microscopic features and pyronin reaction of typhoid cells by light microscopic examinations. Small pieces of formalin fixed tissue, about 1 mm^3 size were taken from both intestinal and lymph node lesions. They were double fixed, first with 4% glutaraldehyde in phosphate buffer (pH 7.4) for four hours followed by 1% osmium tetraoxide in phosphate buffer (pH 7.4) for two hours. Dehydration was carried out through graded alcohol and embedded in Epon 812. Several thick sections, 1 μ from each block were stained with basic fuchsin-methylen blue for tissue orientation and selection of the proper field as well as the study of light microscopic features of typhoid cells. The ultrathin sections were made with glass knife and were stained with uranyl acetate and lead citrate. Ultrastructural observations were made with Hitachi HU 11-E Electron microscope.

Results

Light microscopic findings:

All cases showed acute and subacute non-specific ulcerative inflammation of small intestines except numerous numbers of large mononuclear cell infiltration. These cells showed central and slightly peripherally located vesicular nucleus with distinct nucleoli and contained abundant amount of eosinophilic cytoplasm. These cells frequently phagocytosed red blood cells, nuclear fragments and other tissue debris. Beside these large swollen mononuclear cells, large numbers of lymphocytes, some plasma cells and small numbers of polymorphonuclear leukocytic infiltration were also observed. In the regional lymph nodes, cellular reaction was mostly aggregation of large swollen eosinophilic mononuclear cells forming ill-defined granulomatous features. There were also active phagocytosis by theses cells. Some of the cells lost nuclei and were appearently undergoing necrosis. Methyl-green pyronin staining showed negative reaction of these cells of both intestinal and lymph node lesions.

The large mononuclear cells on thick sections from epon block stained with basic fuchsin and methylene blue showed irregular cytoplasmic outline, abundant amount of fuchsinophilic cytoplasm with various degree of fine reticular vacuolation. Phagocytized materials; red blood cells, nuclear debris, amorphous materials, and even bacterial clumps, were more evident and clearly visible than in paraffin embedded hematoxylin-eosin stained sections (Fig. 1, 2, 3, 4).

Electron microscopic findings:

Electron microscopic studies were concentrated on large mononuclear cells. The most conspicuous ultrastructural features of abnormal cells were marked and diffuse vacuolization of cytoplasm due to dilation of rough endopalsmic reticulum cisternae filled with amorphous materials, and frequent phagocytosed materials. Other cytoplasmic organells such as mitochondria, lysosomes and free ribosomes were markedly reduced. The nuclei were mostly round with various degree of degenerative changes, as evidenced by homogenization and loss of chromatin substance, decreased ribosome, various sized vacuole, lysis and fragmentation. Nucleolus was prominent when the nucleus was relatively preserved, but became indistinct as the nucleus underwent degeneration. The numerous cytoplasmic vacuoles were distended rough endoplasmic reticulum cisternae as evidence by ribosomal attachment on outer surface. The vacuoles were filled with amorphous granular substance. Phagocytosed material and myeline figures, red blood cells, and cytoplasmic fragments of other cells. These phagocytosed bacteria were surrounded by clear zone and boundered by membrane. The bacteria maintained relatively intact morphology while the red blood cells were dehemoglobinized. Secondary lysosomes were not observed. Occasional lymphocytes scattered among these cells remained relatively unchanged.

Discussions

Light microscopically, typhoid cells were characterized by swollen mononuclear cells with abundant eosinophilic cytoplasm frequently containing red blood cells, tissue debris and bacterial

clumps. Because of pronounced phagocytic capacity and unlobulated round or oval shape of nucleus, these cells are regarded to belong to reticuloendothelial origin, be it macrophages, monocytes, reticular cells or sinusoidal lining cells (Reiman 1937, Robbinson 1974). Because of a large amount of eosinophilic cytoplasm and frequently eccentric nuclei, possibility of being plasmacytic series was considered by others (Goodpasture 1637). Being a mononuclear cells, a possibility of altered lymphoid cell could also be considered.

The most conspicuous light microscopic features on hematoxylin and eosin stained sections as well as basic fuchsin-methyle blue stained section from epon block in our study were a large amount of eosinophilic cytoplasm with various phagocytosed materials. By methyl-green pyronin staining, these cells gave negative reaction. These cells were actively phagocytic and pyronin negative which are not characteristics of plasmacytic series. Lymphocytic cells were present among and around these abnormal cells, but they were relatively intact and no phagocytic activity is observed. Thus the abnormal cells probably belong to one of the reticuloendothelial cells, although nuclei were round or oval instead of being bean-shaped.

Electron-microscopically, the most striking feature was a marked vacuolization throughout the cytoplasm, with frequent phagocytosed materials, and decrease of ordinary cytoplasmic organelles. The vacuoles were dilatated rough endoplasmic reticulum cisternae as evidenced by ribosomal attachment on the outer surface. The meaning of a marked RER dilatation is not certain, but it is probably an indication of degenerative changes due to the action of endotoxin elaborated by intracellular salmonella organisms, as is the degenerative changes of the nucleus. It is unlikely due to a poor fixation since the other cells in the lesion were well preserved and no such change was observed. Similar marked cytoplasmic vacuolization was also observed by Yamamototo (1966) in an imal experiments with S. enteritidis and was regarded as degenerative process.

Phagocytosed materials were red blood cells, bacteria and other debris. The red blood cells were partially digested and dehemoglobinized, but the cell membrane remained relatively intact. The bacteria was partially degenerated. There was no definite capsular or cell membrane structure, but there were clear space around dense bacterial body. Yamamoto (1966) observed clear triple layered bacterial membrane in degenerating bacteria than in intact one. Other debris were unidentifiable tangles of membranous materials.

Except dilated RER and phagocytosed materials, very little cytoplasmic organelles were present to characterized the nature or origin of cells ultrastructurally. In some of cells a few dense bodies or myelloid figures were found, but no specific granules or secondary lysosomes to suggest macrophages were present. The reason of reduced organelles is not clear. Yamamoto(1966) also noted that displacement or disappearance of cytoplasmic organelles in these cells. Hirsh and Cohn (1960, 1964) and Zucker-Franklin and Hirsch (1964) demonstrated the disappearance of leukocytic granules after the phagocytosis of microorganisms in the process of intracellular digestion. Hirsh (1956) and Weisman (1964) showed that endotoxin lysis the limiting membrane of the lysosome and thereby promote autolysis by releasing digestive enzymes. All of these may bring about the decrease of lysosomal granules and other cytoplasmic organelles.

The presence of relatively intact lymphocytes among aggregates of typhodid cells suggest that lymphocytes takes very little part in human typhoid lesion as was seen in animal experiments (Yamamoto 1966), and there was little evidence to suggest that lymphocytes transform into typhoid cels.

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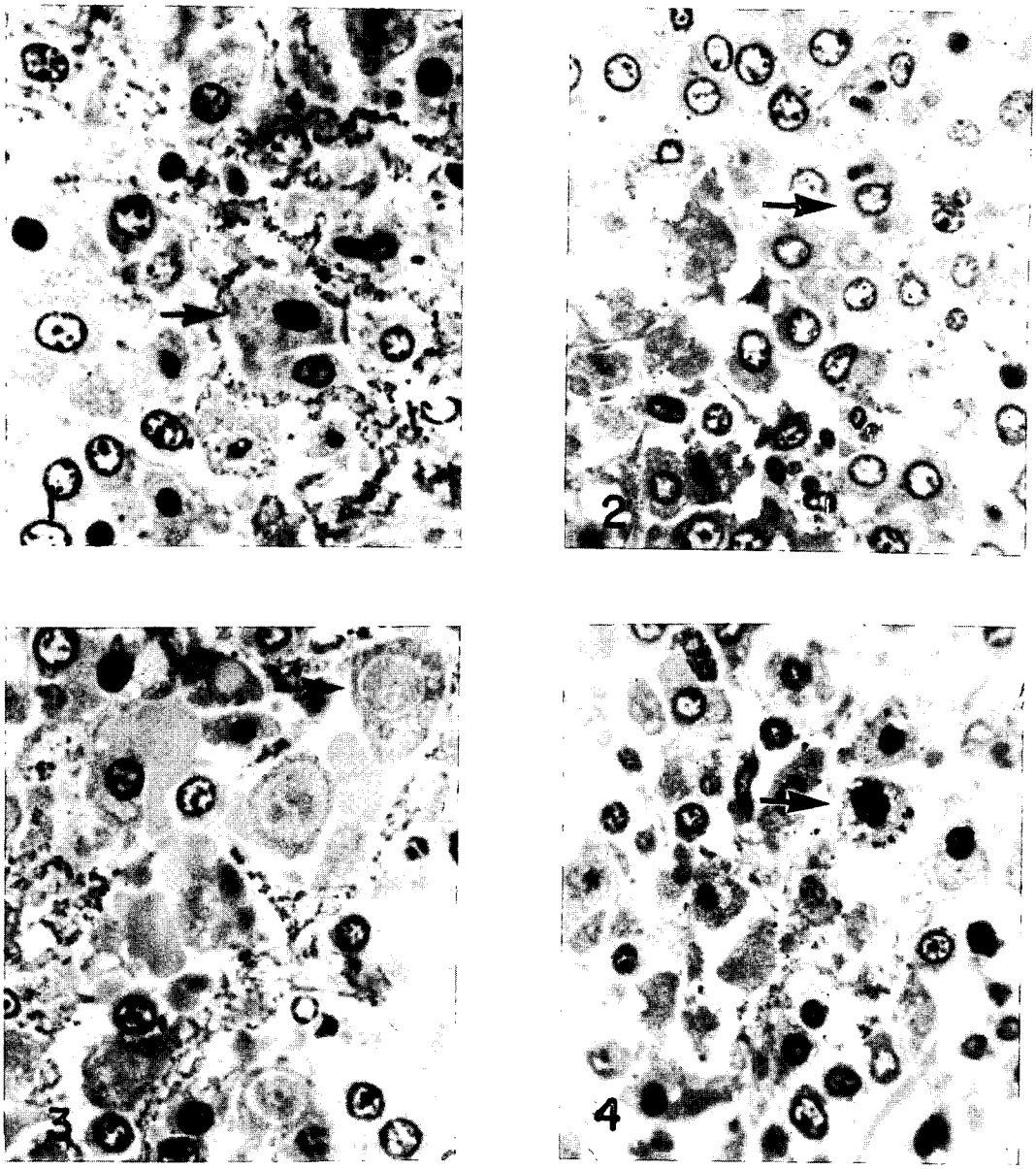

Fig. 1-4: 1 μ thick sections from epon block showing (1)large swollen mononuclear cells, and a phagocytosed red blood cells (↑), (2) a group of swollen mononuclear cells with abundant cytoplasm and phagocytosed debris (↑), (3) several swollen mononuclear cells with finely vacuolated cytoplasm containing large phagocytised debris (↑), (4) perinuclear cytoplamic vacuolization of a mononuclear cell (↑) and scattered small lymphocytes. Basic fuchsin-methylene blue stain, X 1,000.

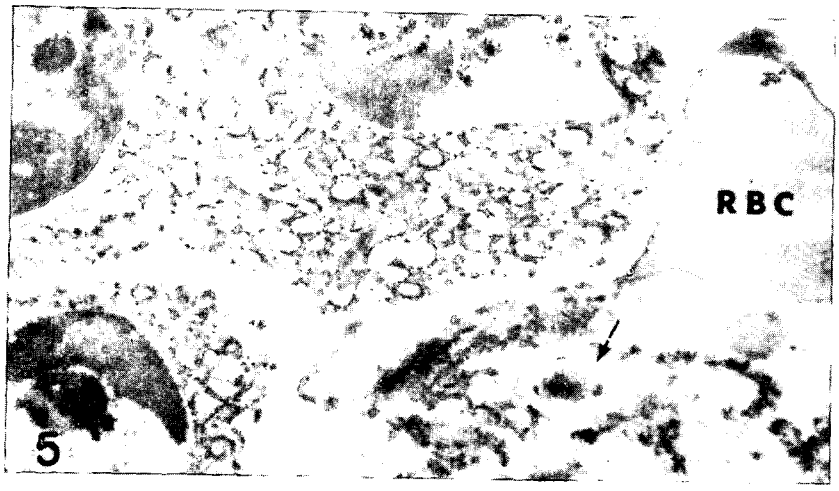

Fig. 5. Typical typhoid cells showing marked vacuolar dilatation of RER cisternae, phagocytosed debris (D), bacteria (↑), and degeneration of nucleus. X 10,700.

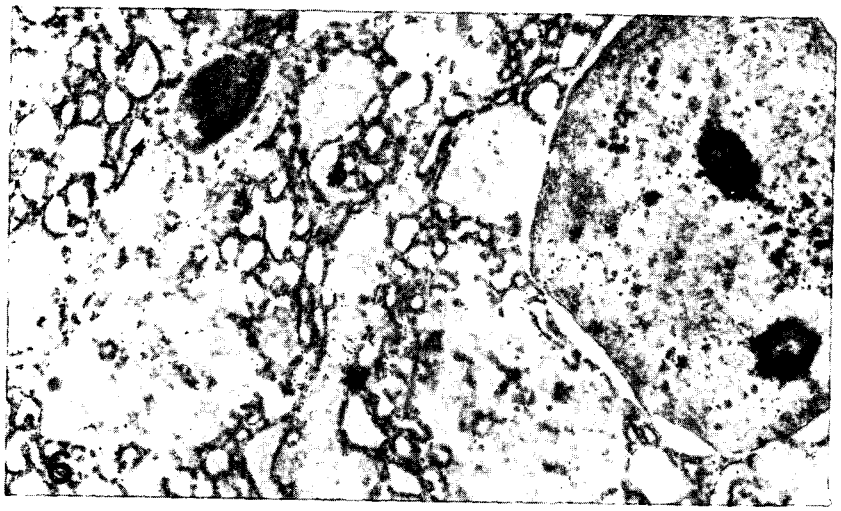

Fig. 6. Higher mangnification showing dilated and interrupted RER, and a bacterial body (↑). X 16,000.

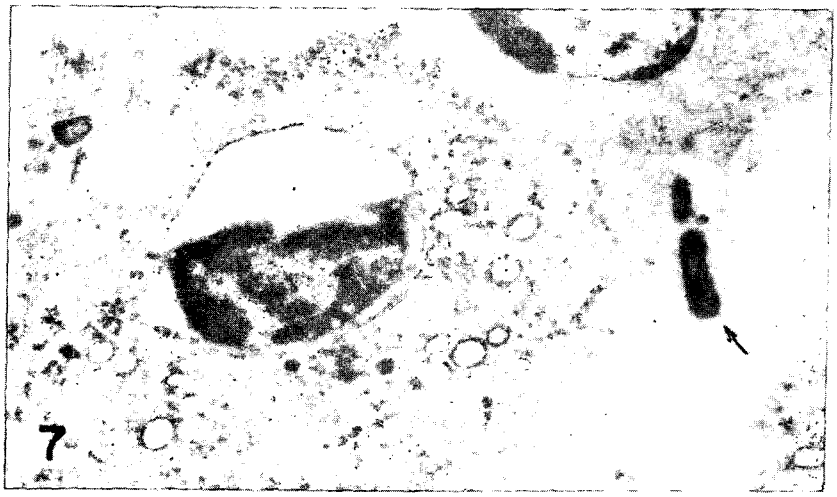

Fig. 7. Early perinuclear vacuolar space, dilatation of RER cisternae, and longitudinal section of bacillus (↑). X 10,700.

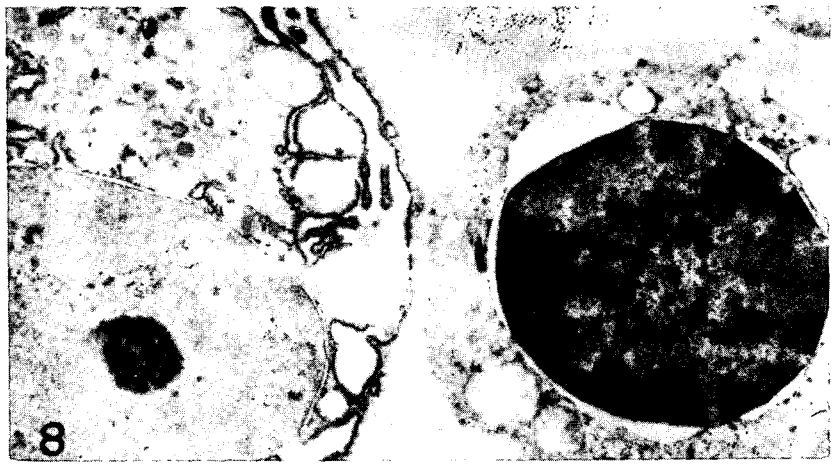

Fig. 8. Marked dilation of RER cisternal with focal cytoplasmic degradation. X 16, 000.

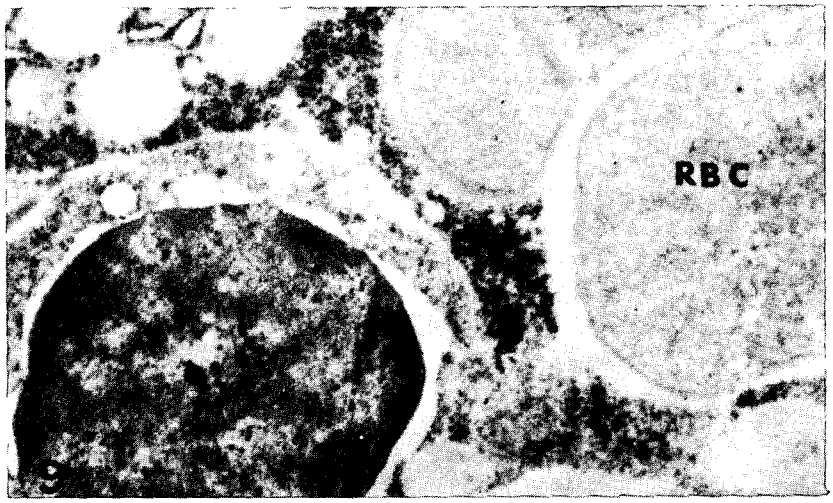

Fig. 9. Two partially dehemaglobinized RBC are seen within cytoplasm. X 22, 500.

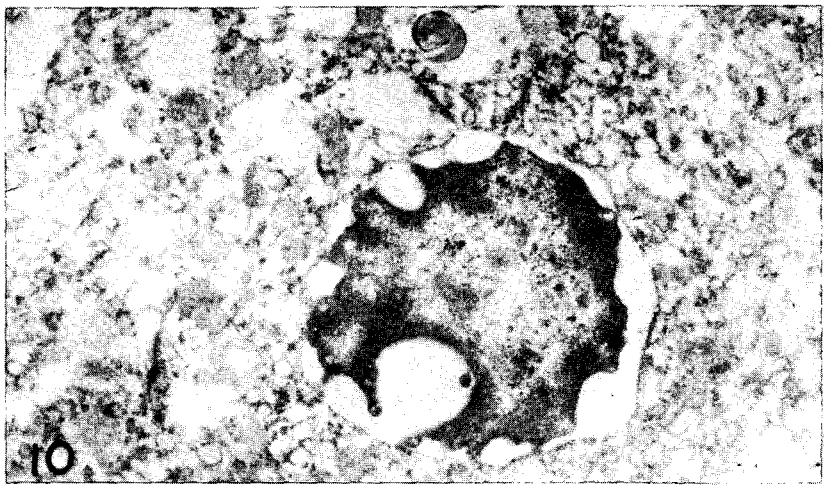

Fig. 10. Marked cytoplasmic degradation vacuolization, and many phagocytosed debris. X 10, 700.