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BURSA OF FABRICIUS CONTAINING VIRUS-LIKE PARTICLES AN ELECTRON MICROSCOPIC OBSERVATION*[1]

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A bursa of Fabricius containing numerous virus-like particles was examined. The structures and cells of the bursa seemed to be normal histologically, though the bursa was larger in size for the age of the chicken and under the electron microscope was seen to contain virus-like particles. The particles, about 800 Å in size, were mainly found in the medulla of lymphoid follicles and were composed of a central dense nucleoid, surrounded by an envelope. Macrophages, which appeared to be epithelial in origin, in the medulla showed cytoplasmic inclusions or viroplasts and the budding of the plasma membrane.

Thus, a similarity between the particles and the avian leukosis tumor virus, and the site of virus replication in the bursa have been discussed.

INTRODUCTION

It was strongly suggested that the bursa of Fabricius and the thymus are "central lymphoid organs" in the chicken, essential to the ontogenetic development of adaptive immunity in that species[4,6]. It was also confirmed that the bursa has a close-relationship with visceral lymphomatosis[7,8,9], but there is no evidence regarding the actual site of virus growth in the bursa.

In this paper, a case of bursa of Fabricius which contains numerous virus-like particles is reported with the aim of clarifying virus synthesizing cells in the bursa.

MATERIAL AND METHODS

A bursa of Fabricius of a White Leghorn hen, 17 weeks old, was used as the material for electron microscopic observation. The hen had a considerably large bursa, about 2 cm in diameter, for her age, but there were no other special pathological findings of other organs macroscopically.

The pieces of the bursa were fixed in 1% osmic acid (MILLONIG) and embedded in Epon 812 (LUFT) in the routine way. They were cut on Hitachi UM-3 ultramicrotome

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using glass knives. The sections were stained with uranyl acetate (WATSON) and/or lead citrate (REYNOLDS) and examined under a JEM-4 CHD electron microscope. The thicker sections were stained with toluidine blue for the purpose of ascertaining the area to be sectioned for electron microscopy.

As a control, a bursa of a 13-week-old chicken was electron microscopically examined. The bursa and other organs were histologically observed; they were fixed in Carnoy's fluid, embedded in paraffin, cut 5 μ thick and stained with hematoxylin-eosin, pyronin-methyl green, toluidine blue and PAS reaction.

RESULTS

1 Histological findings

In the bursa of Fabricius were not found any special changes; it had several longitudinal folds in which there were numerous lymphoid follicles. In the follicles, the cortex and medulla were separated by a slightly waved, thin basement membrane, on which a layer of epithelial cells was found. The cortex was packed with numerous cells of the lymphocytic series and the medulla loosely contained lymphocytes within a meshwork of epithelial reticular cells (fig. 1). Mitotic cells were often observed both in the cortex and medulla (fig. 2). Proliferation of subepithelial connective tissue and degeneration of cells were hardly found in the bursa; the bursa appeared to belong to the middle and last stage of growth (YAMADA's type III). In the medulla, a considerable number of epithelial reticular cells and macrophages contained a PAS positive substance in their cytoplasm.

The liver, kidney, lung, spleen and caecum appeared to be normal. Small foci of lymphatic tissue were observed perivascularly in the liver, peribronchially in the lung and subepithelially in the ureter, but did not contain germinal centers. The white pulps of the spleen and subepithelial lymphoid follicles of the caecum contained sharply circumscribed germinal centers, though the centers were not so different from those normally found, in structure, size and number.

2 Electron microscopical findings

In the lymphoid follicles of the bursa, the cortex was mainly packed with small and medium-sized lymphocytes. The small lymphocytes included more numerous polysomes than those of other mammalian lymphatic organs, though other organelles were similar (fig. 3). Reticular cells including cell debris and mitotic cells were also observed in the cortex. A few plasma cells appeared only near the interfollicular connective tissues. The cortex and medulla were separated by a basement membrane, on which epithelial cells were connected with one another by desmosomes (fig. 4). In the medulla, cells of lymphocytic series were loosely scattered in a meshwork of epithelial reticular cells as observed histologically (fig. 5). Large lymphocytes (blast cells), mitotic cells and macrophages of the medulla were more numerous than those of the cortex (figs. 6 & 7). The nuclear bodies were hardly found in nuclei of lymphocytes in the bursa.

Virus-like particles were found in the cortex and more numerously in the medulla. The particles had an envelope and a central dense core just similar to the nucleoid of some

viruses. The particles were about 800 Å in diameter and the central cores measured about 400 Å (fig. 11). They mostly lay scattered in the intercellular space, but were often in contact with the cell surface of macrophages, and some of them were found in vacuoles of the cells (fig. 7). No special relationship between the virus-like particles and the lymphocytes was found in this case (fig. 6). On the cell surface of the macrophages, the particles appeared to be formed by budding from the cell membrane (figs. 7 & 10).

The macrophages sometimes contained bundles of tonofilaments in their cytoplasm. A close-relationship by interdigitation was occasionally observed between the blast cells and the macrophages relating to the particles (figs. 8 & 9).

No virus-like particles were seen in the bursa of the control.

Discussion

A case of bursa of Fabricius which contained numerous virus-like particles was examined. The bursa did not show any special histological changes in comparison with YAMADA's findings, though it was larger in size for the age of the chicken. The electron microscopic structures of cell components of the bursa also appeared to be similar to those reported by ACKERMAN and SAISHOJI & UEGAMA.

Recent reviews indicate evidences that the bursa of Fabricius is the major organ of immunologic competence in the chicken[4,6]. Furthermore, the organ clearly has a close-relationship with visceral lymphomatosis[7,9], so in this report, the present writers focused their attention on numerous virus-like particles found in the medulla of the bursa. The particles, about 800 Å in diameter, were composed of a central dense nucleoid, surrounded by a zone of low electron density which in turn was surrounded by an envelope. The particles were found in cytoplasmic vacuoles including a dense substance (viroplasts) and on the surface of the cell membrane of the macrophages, though most of the particles were extracellularly located. Furthermore, occasional budding of the plasma membrane was observed in the macrophages.

DMOCHOWSKI stated that cells synthesizing the avian leukosis tumor virus show viroplasm or viral matrix formation, cytoplasmic inclusion or viroplasts and the budding of the plasma membrane. Appearance of the virus-like particles and macrophages relating to the particles found in the present observation appeared to indicate an essential similarity with those of the avian leukosis tumor, though no viroplasm was distinguished.

Similar particles were also reported in the medulla of the bursas of the adult and embryonal domestic ducks, but there are no detailed descriptions[10,12].

PETERSON et al.[7,9] confirmed that visceral lymphomatosis is prevented in bursectomized virus-infected chickens. They also found that thymic lymphocytes are well developed prior to the time of hatching, whereas bursal lymphocytes

remain large and possibly relatively immature for the duration of the bursa's existence, and suggested that immature lymphoid cells in the bursa may be more susceptible to a visceral lymphomatosis virus than those in the thymus[8].

SADLER & EDGAR reported that the growth of birds bursectomized at 5 days of age was less depressed by fowl pox vaccination than that of non-bursectomized birds, and suggested the possibility that the bursa may be an important site of fowl pox virus growth in the host.

The present writers' findings seem to support the suggestion of SADLER & EDGAR; a morphological barrier of epithelial cells and a basement membrane seems to make the medulla of the bursa into a special environment like the thymus, even if it may be not complete. The results obtained indicate that the medulla of the bursa, especially the macrophages which appear to be epithelial in origin, seems to be the site of better replication of the virus in young chickens. If so, the bursectomy has to remove an important site of replication of some viruses. Furthermore, it is important that the bursa may be a site of tumor virus formation in latent or subclinical infections in the young chickens.

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EXPLANATION OF PLATES

All scales in the figures are shown at 1 μ.

PLATE I

Fig. 1 Lymphoid follicle of bursa
A basement membrane separates the cortex from the medulla. The cortex is packed with numerous cells of lymphocytic series. The medulla loosely contains lymphocytes within a meshwork of epithelial reticular cells. Epon section; toluidine blue stain $\times$ 340

Fig. 2 High magnification of figure 1
Blast cells are observed in large numbers in the medulla. A layer of epithelial cells (E) is found along the basement membrane (BM). Mitotic cells (arrows) and macrophages are also seen. Epon section; toluidine blue stain $\times$ 850

Fig. 3 Cortex is mainly composed of small and medium-sized lymphocytes. Mitotic cells (MC) and plasma cells (P) are also observed. Extracellular virus-like particles (arrow) are seen among these cells.

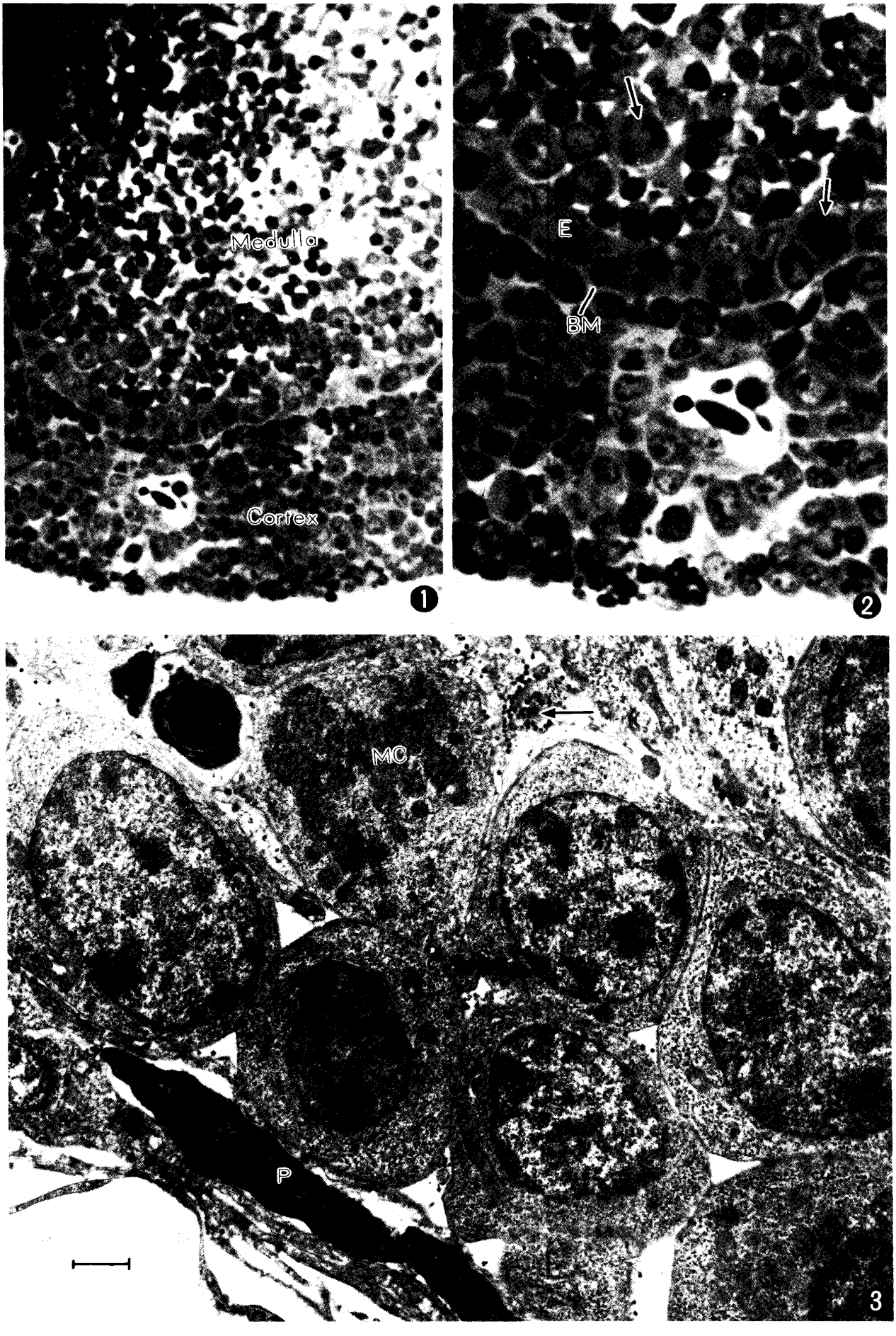
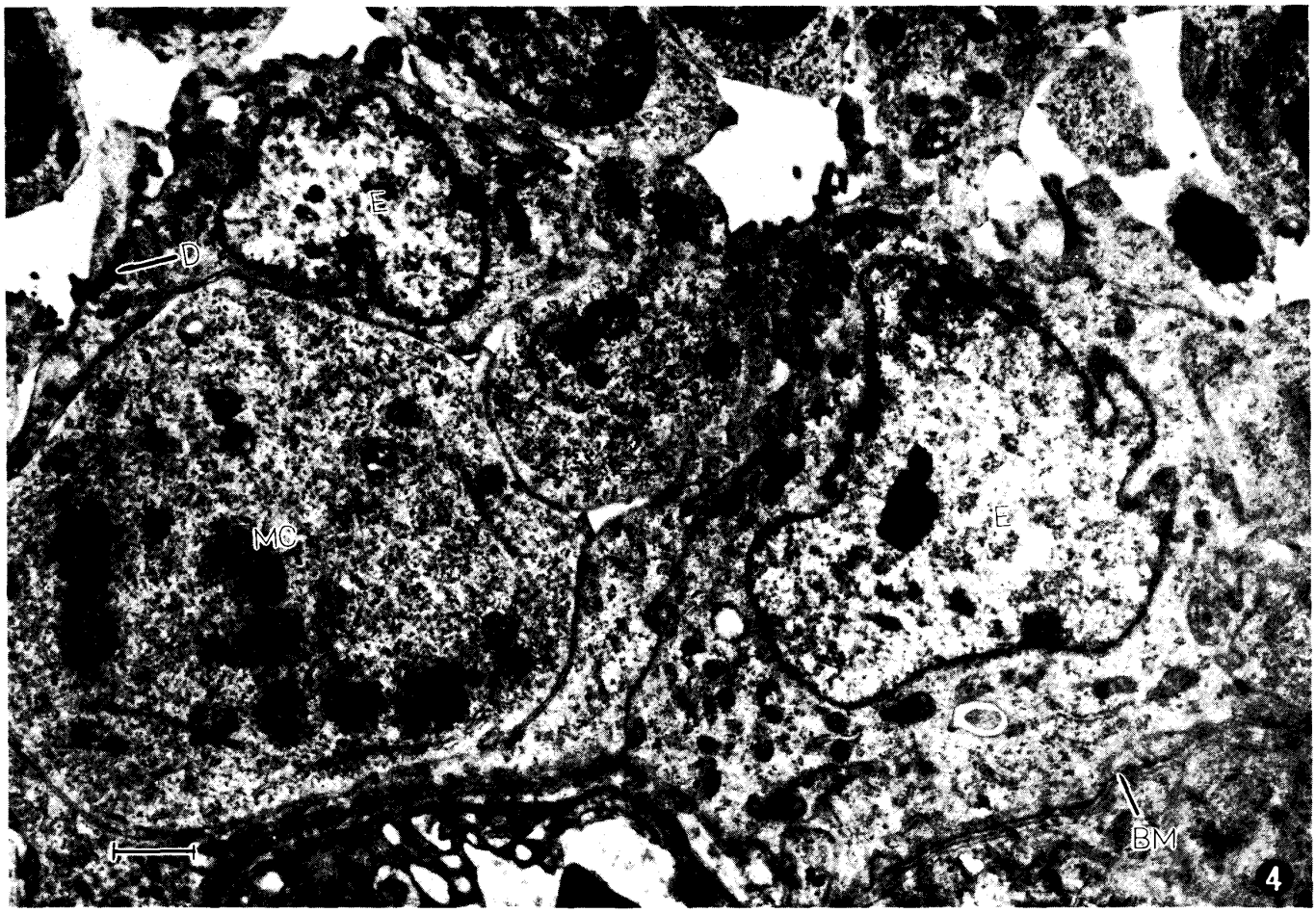

Medulla
Cortex
1
E
BM
2
MC
P
3

PLATE II

Fig. 4 Cortex and medulla are separated by a basement membrane (BM) on which epithelial cells (E) are located to connect to each other by desmosomes (D). A mitotic cell (MC) is found between the epithelial cells.

Fig. 5 In the medulla, lymphocytes are scattered in a meshwork of epithelial reticular cells (E). The epithelial cells have tonofilaments (T) and desmosomes (D). There are numerous virus-like particles extracellularly.


E
D
MC
E
BM
4

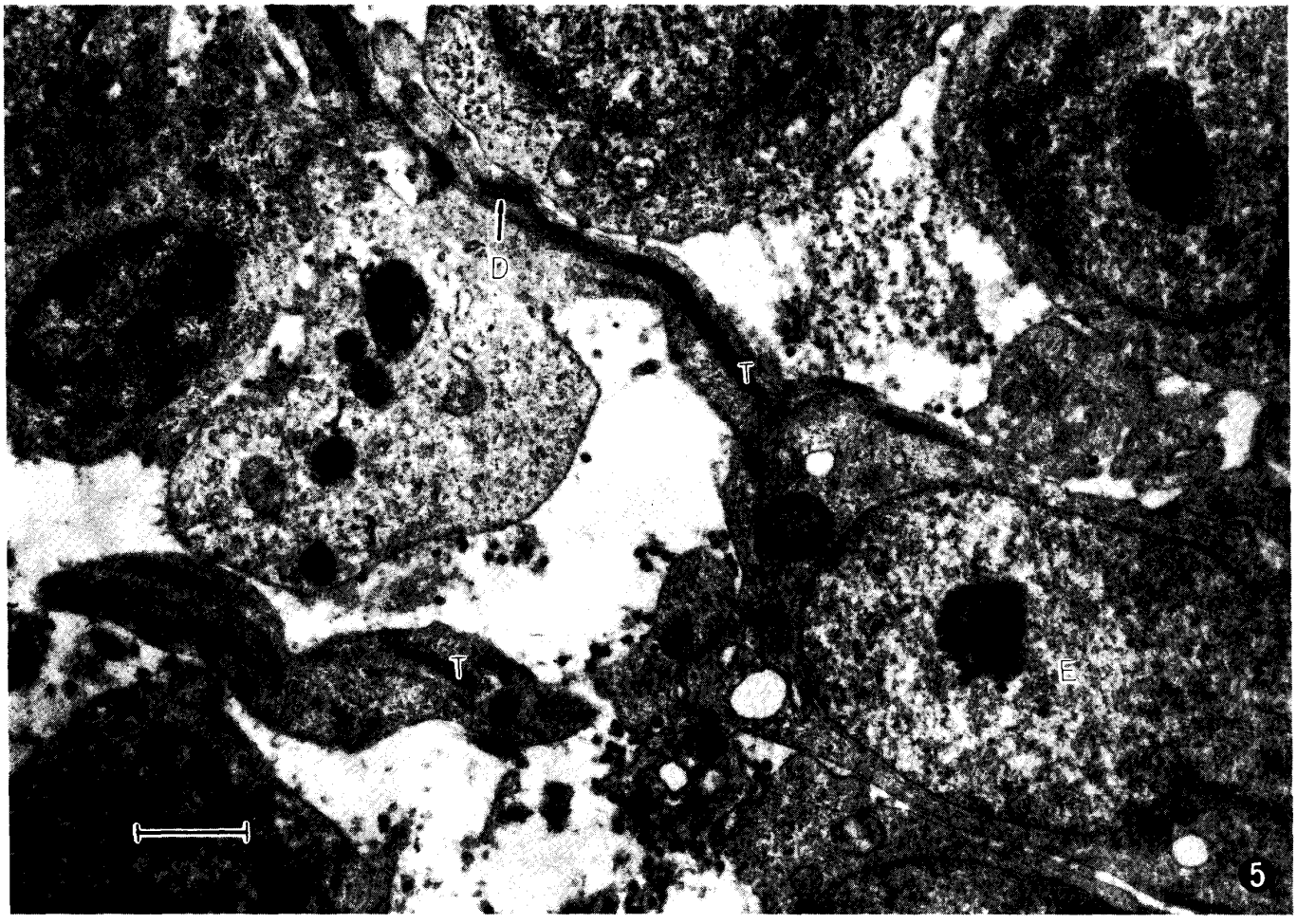
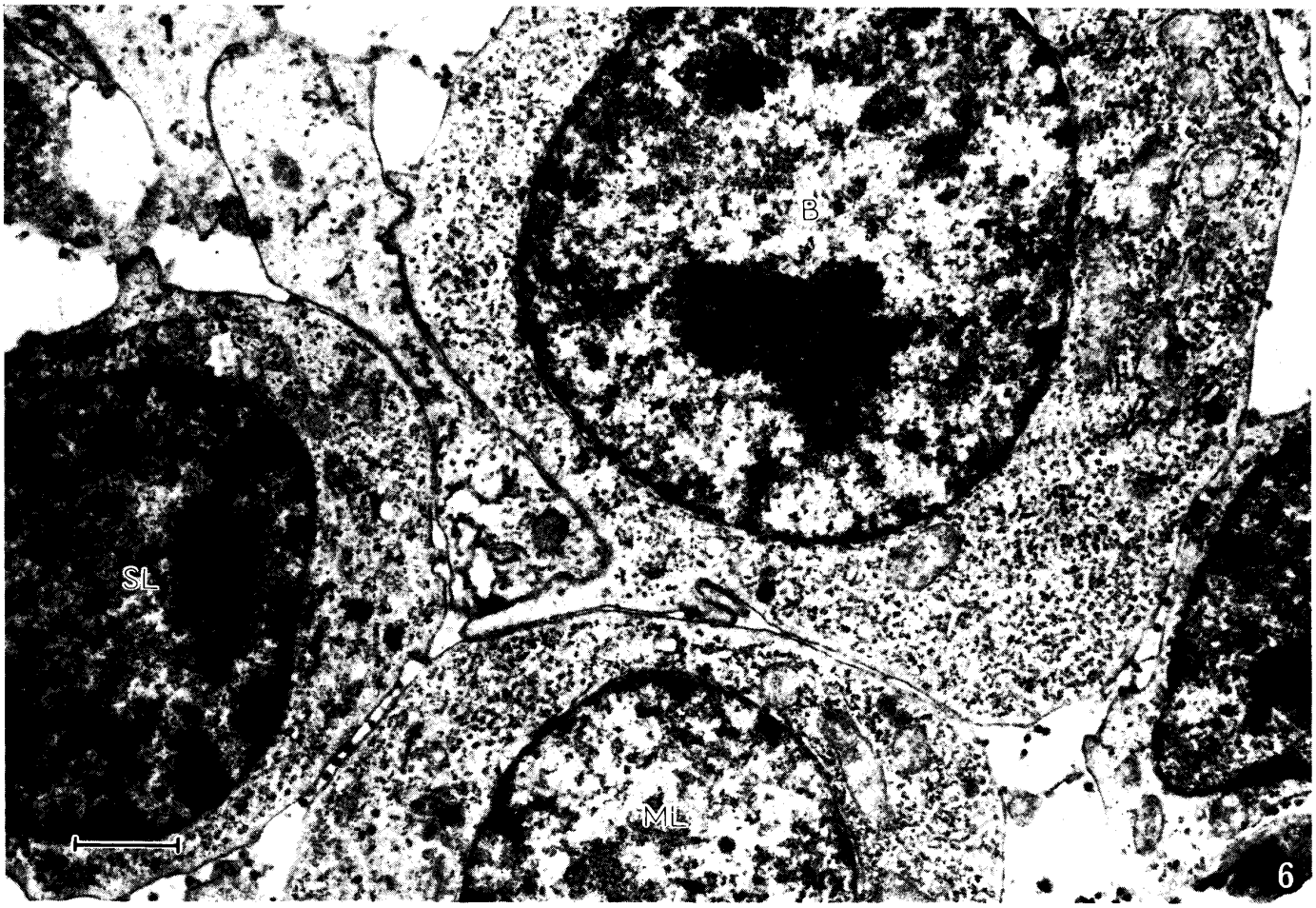

D
T
T
E
5

PLATE III

Fig. 6 Lymphocytes in the medulla
Small (SL),medium (ML) and blast cells (B) are seen. No special relationship is found between virus-like particles and the cells of the lymphocytic series.

Fig. 7 Macrophage (M) in the medulla
The cell contains some dense bodies (DB), probably cell debris. Numerous virus-like particles are in contact with the cell surface of the macrophage. Some of the particles are found in the cytoplasmic vacuole (V) or viroplast and budding formations (arrows) are often observed.


B
SL
ML
6

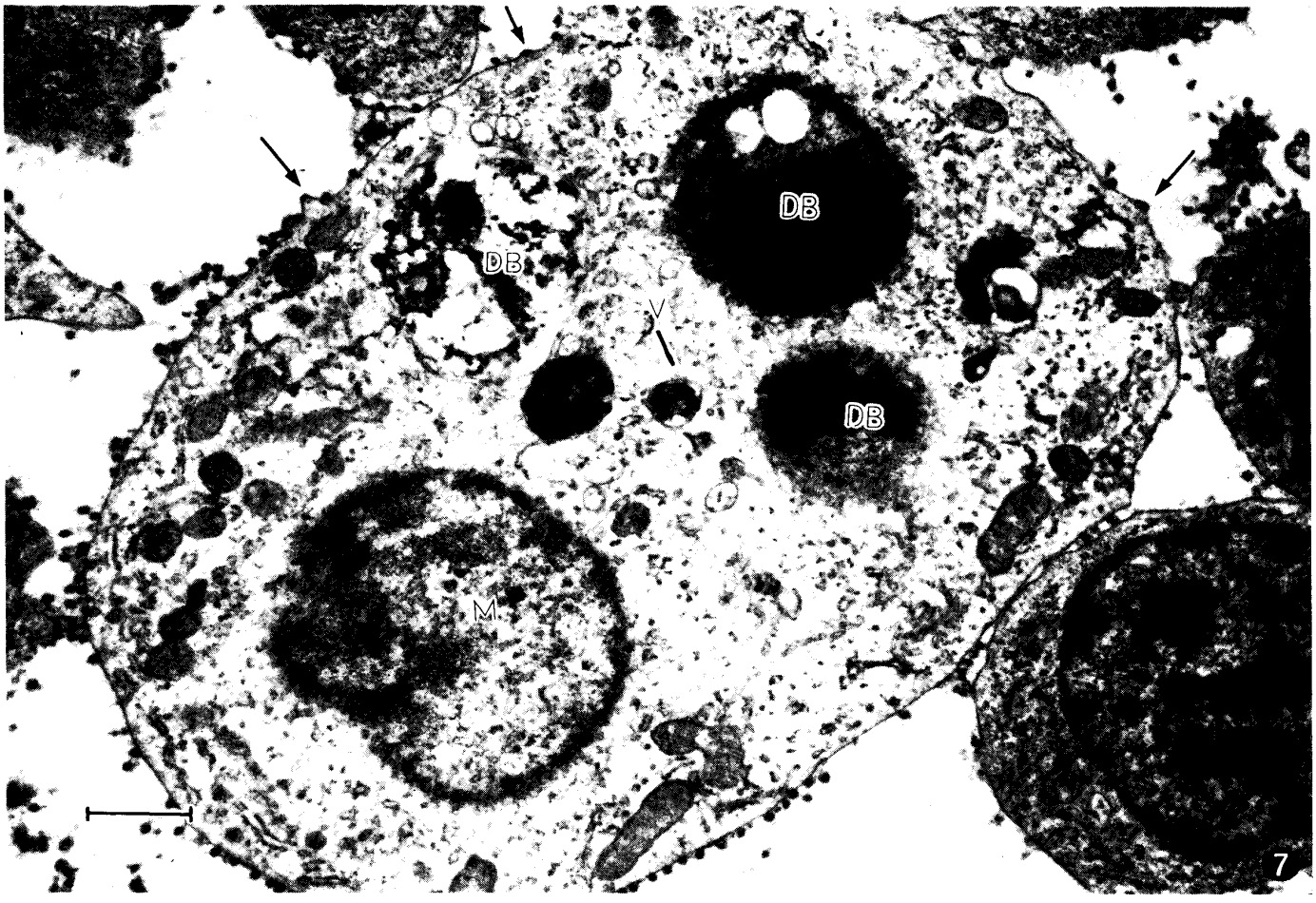
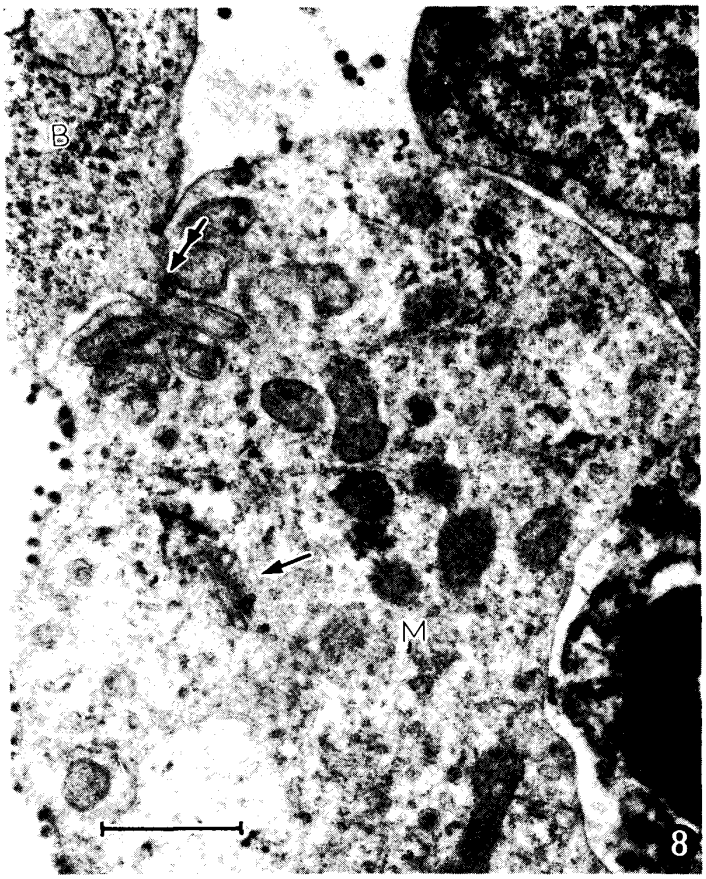
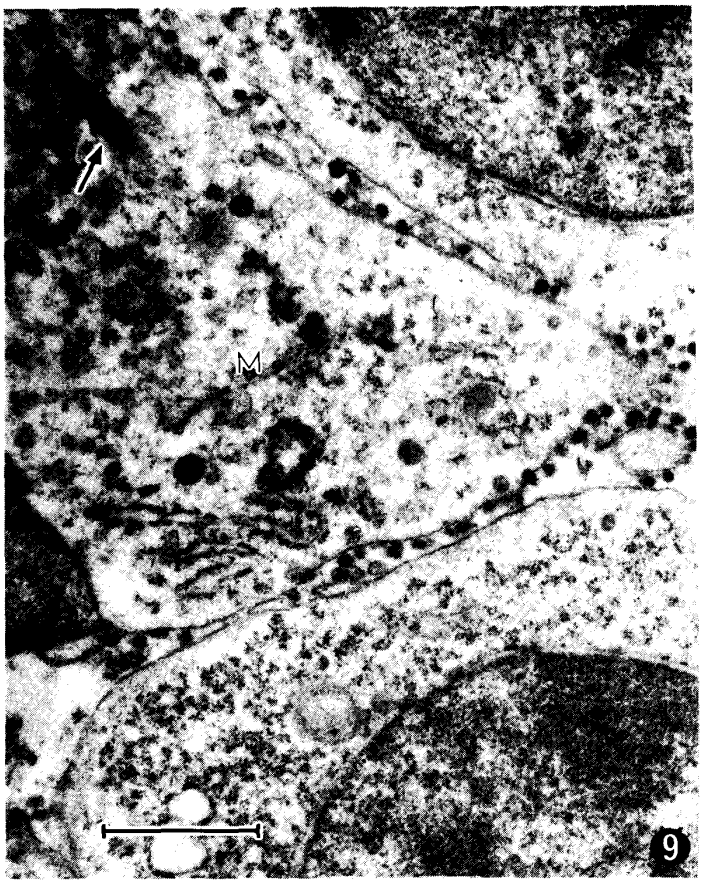

DB
DB
DB
M
7

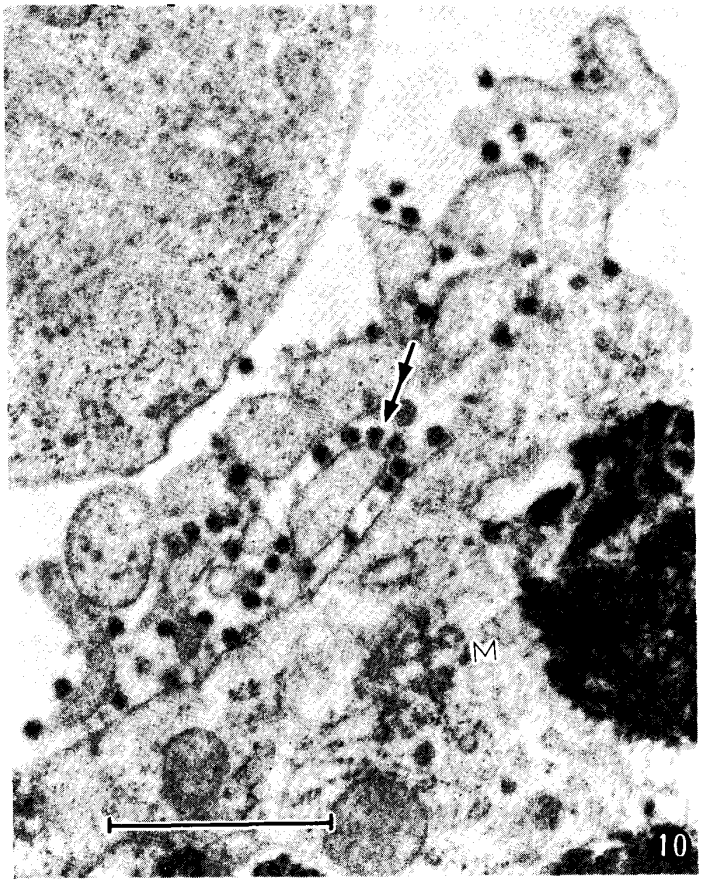
PLATE IV

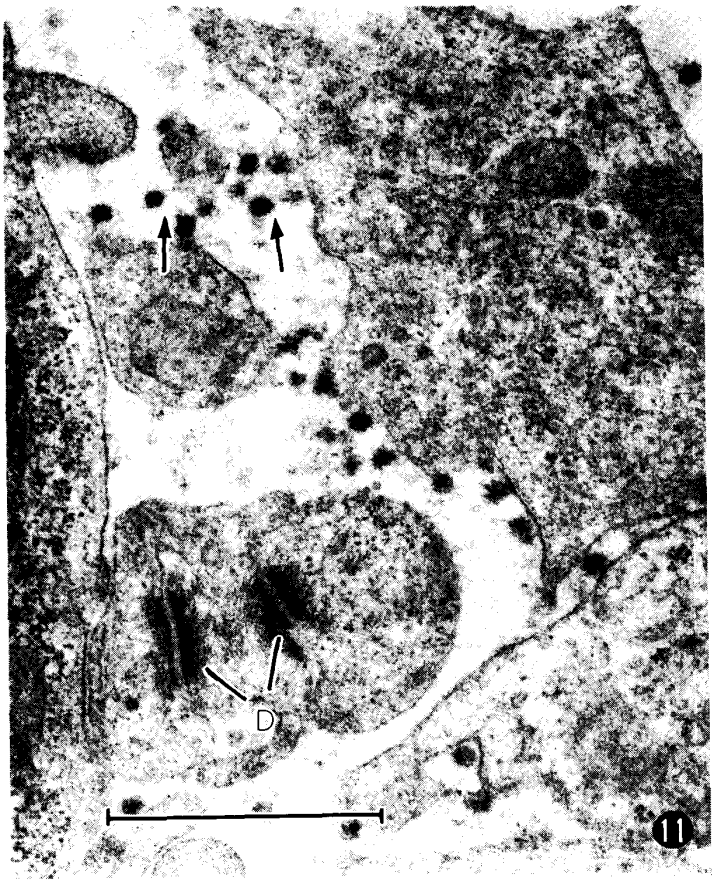
Figs. 8 & 9 Macrophages relating to virus-like particles sometimes contain small bundles of tonofilaments (arrow). Occasional close-connection by interdigitaion (double arrow) between the blast cell (B) and the macrophage (M) is found.

Figs. 10 & 11 Virus-like particles, about 800 Å in size, are composed of a central dense core or nucleoid, surrounded by an envelope (arrows). Budding formation of cell membrane of macrophages is also seen (double arrow).


B
M
8


M
9


M
10


D
11