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ULTRASTRUCTURAL CHANGES IN THE BACTERIAL VEGETATIVE ENDOCARDITIS*1

BY

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ABSTRACT

Electron microscopic study of the endothelium of the bacterial valvular endocarditis, induced by the single intravenous inoculation of *Streptococcus faecalis* in the chicken, revealed cellular changes ranging from the relatively early changes consisting of marked increase of pinocytotic vesicles, much more population of free ribosomes, cystic dilatation, shrinkage and collapse of endoplasmic reticulum and mitochondria, predominant lysosomes, phagocytosis, edematous cytoplasm, pseudopodia multiplication and microvilli deformation of cytoplasmic processes, alienation of the intercellular junctions, and estrangement of basement membrane from the endothelial cells, to the final high alterations consisting of cytoplasmic lysis, desquamation, and vegetative formation on the endothelial cells of the mitral valve. These various lesions occurred at 2, 4, 6, 12, and 24 hr, and 2, 5, 7, and 10 days after bacterial inoculation. The changes varied in different locations on the valve. Most of them were found frequently on the auricular surface at the line of closure. The bacterial vegetation developed on the valves of 7 of the 8 chickens from 7 to 10 days after inoculation.

The present study has suggested that both bacteria-active and bacteria-passive endothelial cells are present in the valvular endocardium by the selective sensitivity to bacterial injury.

INTRODUCTION

Although rather many studies[1-10] have demonstrated the endocardial changes in experimental endocarditis, the production of bacterial endocarditis has been usually done with repeated injections of bacteria or with a single injection of bacteria after injuring previously the heart valve or vascular vessels, such as aortic vena caval shunts and AV shunts of fistulae[2-9].

It is more reasonable to introduce the bacterial endocarditis in experimental animals without the previously damaged valves or circulatory systems, or with a single injection of bacteria, for electron microscopic observation on the minimal initial changes as well as regressive degeneration

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and desquamation of endothelial cells and subendothelial stromal edema in vegetative endocarditis. In addition, it is very difficult to distinguish the actual initial changes involving the endothelial cells from the secondary degenerative changes and some artifacts produced in duration of the pathological examination.

The present study reports the ultrastructural changes of the initial lesions in bacterial endocarditis produced successfully in the unmodified chickens by a single intravenous injection of the suspension of non-hemolytic *Streptococcus faecalis*.

The varying degrees of cellular damages in bacterial endocarditis ranges from early edematous changes such as reactive increase of pinocytotic vesicles and ribosomes population, swelling of mitochondria, enlargement of endoplasmic reticulum, significant peripheral cytoplasmic processes or thickened microvilli, and predominant lysosomes in the endothelial cells, to necrotized degeneration and desquamation of endothelial cells resulting in bacterial vegetation.

Materials and Methods

A total of 42 female white Plymouth-Rock chickens, weighing approximately 3,000 g, were used in this study. Thirty-six chickens were inoculated intravenously with a single dose of 1.0 ml of non-hemolytic *Streptococcus faecalis* suspension (5×10^7 microorganisms). The bacteria were isolated originally by Jortner[11] from the field cases of bacterial endocarditis in the chicken. The detail of bacterial suspension has been reported in a previous publication[12]. A second group of 4 control chickens was given injection of 1.0 ml bacteria free normal saline. A third group of chickens was not inoculated. One each from three groups was scarificed by concussion at 2, 4, 6, 12, and 24 hr, and at 2, 5, 7, and 10 days after inoculation as shown in Table 1.

The heart was removed promptly, surveyed rapidly under the binocular microscope, and fixed in 4% phosphate-buffered glutaraldehyde for 30 minutes. After the fixation, selected areas of the mitral valve were taken for electron microscopic study and refixed in 4% phosphate-buffered glutaraldehyde for 2 hr at 4°Cels., washed in phosphate buffer, refixed in 1% phosphate buffered osmium tetroxide at pH 7.4 for 1 hr, dehydrated throguh a graded series of ethanol, and acetone, and embedded in Epon 812[13]. Thin sections were cut on a Porter-Blum I microtome, stained with 1% uranyl acetate[14] and lead citrate[15], and observed with Hitachi HU-10 B electron microscope. Thick sections of 1.0–0.5μ were stained with Toluidine Blue for trimming and selection of appropriate areas of valvular tissue

Table 1. Sacrified time after inoculation and classification of valvular lesions in the 36 chickens that developed endocarditis and control chickens

Experimental groups of chickens		Period (days) from inoculation to sacrifice	Number of chickens	Valvular lesions**				
				0	I	II	III	IV
Control groups	No inoculation	0	2 each	2	0	0	0	0
	Saline inoculation	2 (hr)		0	2	0	0	0
		4		1	1	0	0	0
Bacteri inoculation group		2 (hr)	4 each	1	3	0	0	0
		4		1*	2	1	0	0
		6		1*	2	1	0	0
		12		0	2	2	0	0
		24		0	1	2	1	0
		2 (days)		1	1	1	1	0
		5		1	0	1*	2	0
		7		0	0	0	1	3
		10		0	0	0	0	4*

* Died.

** The light microscopic degree of valvular lesions.
0: Non-remarkable, I: Focal edema, II: Diffuse edema, III: Desquamation of endothelial cells and vegetation, IV: Bacterial vegetation.

before thin sectioning for electron microscopy. For the optic microscopy, the remaining portions of the mitral valve were fixed in 10% neutral Formalin and embedded in paraffin. Paraffin sections were stained with Hematoxylin-Eosin, phosphotungstic acid-Hematoxlin, periodic acid-Shiff procedure, Alcian Blue and Toluidine Blue at various levels of pH, and Elastica-van Gieson combination. The valvular vegetation was stained with Gram-Towort stain for bacteria.

The heart blood and the vegetation of heart valve were examined bacteriologically in a careful manner so as to avoid extraneous contamination before the pathological examination.

Results

Optic microscopy:

The chicken heart had no distinct muscular component within the mitral valve, while the tricuspid valve was a muscular fold. The mitral

valve was a thin and membranous fibrous structure and composed of three layers; (a) an auricular and ventricular endothelial layer, (b) zona spongiosa, and (c) zona fibrosa as the central core.

Control chickens:

The second and third groups showed almost non-remarkable changes in the heart valve, chorda tendinae and papillary muscles, except for slightly increased metachromatic change of Toluidine Blue stain in the subendothelial edematous stroma of the heart valve of the sacrificed chickens in the second group.

Inoculated chickens:

The inflammatory reactions in the heart valve appeared to parallel the progressive course from 2 to 24 hr after the inoculation (Figs. 1 and 2). There were focal or diffuse edematous swelling and focal proliferation of overlying endothelial cells, and slight proliferation and degeneration of subendothelial stromal cells. The loose subendothelial ground substance of the zona spongiosa demonstrated a marked metachromasia.

The heart valves from 48 hr to 5 days after the inoculation showed uneven, nodular edematous swelling miniature desquamation of the overlying endothelial cells associated with marked edema in the stroma of zona spongiosa and slightly in the zona fibrosa (Figs. 3 and 4).

The chickens 7 and 10 days after the inoculation had a bacterial vegetation on the valves (Figs. 5 and 6). Not only bacterial endocarditis of the tricuspid valves but also myocarditis and glomerulonephritis were seen in the chickens with bacterial vegetation on the mitral valve.

Electron microscopy:

Control chickens:

The details of ultrastructure of the normal mitral valve of the chicken have previously be shown by Mitomo *et al.*[16] The endothelium of heart valve resembled closely that of vascular vessels, but the shape and size of valvular endothelial cells varied considerably from those of other vascular channels. The valvular endothelial layer was thicker than that of the vascular channels[17]. From the valvular endothelial cells, thin villous-like cytoplasmic projections extended into the cardiac cavity. Their cellular junctions frequently had complicated patterns in the form of interdigitating folds, and considerable overlapping of the adjacent endothelial cells. The basement membrane varied with its location in the valves; at the ventricular surface, it was scanty or obvious but very distinct at the auricular surface. Oval or rounded mitochondria were scattered in the cytoplasm of

the endothelial cells. Golgi apparatus was located at the perinuclear region. Numerous rough-surfaced endoplasmic reticulum and free ribosomes, pinocytotic vesicles, multivesicular bodies, and fine filamentous plexus were a regular feature of the cytoplasm. The zona spongiosa had amorphous ground substance including collagen fibers and bundles, and elastic fibers. Their amount, composition, and configuration varied according to different location within the valve.

Inoculated chickens:

The ultrastructural changes of the endothelial layer showed similar general appearances depending the progressive stages in the groups of inoculated chickens.

No abnormalities were seen in any of the control chickens of the second group injected with a normal saline. The endothelial cells at the line of closure on the auricular surface appeared to be slight increased in the pinocytotic vesicles and multi-vesicular bodies.

As the first observation on the bacteria-inoculated chickens, the chickens sacrificed at 2, 4, and 6 hr after the bacterial inoculation showed focal edematous changes such as various shapes and sizes of predominant pinocytotic vesicles, slight enlargement of endoplasmic reticulum, swelling of mitochondria, irregular distribution of free ribosomes, slightly rough fine filaments in the cytoplasm, and slight dilated Golgi apparatus in the low electron density of edematous cytoplasm, associated with elongated and extended villous-like cytoplasmic processes, pseudopodia multiplication, slightly irregular dilatation of intercellular spaces between the adjacent endothelial cells, and minute edema around the basement membrane beneath the endothelial cells. These lesions were encountered in the occasional endothelial cells of the auricular surface.

The chickens 12 and 24 hr after inoculation had rather more intensive edematous changes similar to early groups of chickens from 2 to 6 hr after inoculation, associated with focal or diffuse edema in the subendothelial stroma beneath the basement membrane. There were occasional sinusoidal dilatation of the spaces between the intercellular junctions at the long and thick overlapping areas of endothelial cells. In short contact of the cells, junction areas of adjacent cells were completely closed by the formation of an invagination or twist of cytoplasmic processes of endothelial cells. A small amount of lysosomal vacuoles were also more remarkable in the phagocytic activity, showing irregular size and shape, with amorphous electron-dense material or fibrillar debris (Fig. 7).

The chickens 2 and 5 days after inoculation showed more advanced changes in the endothelial and subendothelial cells. The changes in the

auricular and ventricular surfaces appeared generally to go together (Figs. 8–12) but the fine ultrastructural lesions of endothelial cells were variable in different portions of the valvular endocardium. In some chickens, the changes on the auricular surface were predominant but in other cases the ventricular surface showed a more intensive change. In some instances edematous alterations appeared to be more localized and limited but the others were diffuse and extensive, along the line of closure or at the free edge of valves. There were marked increase of pinocytotic vesicles, predominant multivesicular bodies, various sizes and shapes of vacuoles and vesicles, regressive degeneration of rough surfaced endoplasmic reticulum and mitochondria, and low electron density of cytoplasm with ribosomal dislocation (Figs. 8 and 9). Particularly in mitochondria, swelling, enlargement accompanied by pleomorphism, increase or decrease in the number of cristae, elongation, disordered arrangement, or loss of cristae, fragmented cristae unattached to the inner membrane, fibrillar inclusion, diffuse increase in the desification of the matrix, and absence of the matrix granules (Figs. 8–10) were demonstrated. There were marked cisternal dilatation, fragmentation, vesiculation, and disappearance of endoplasmic reticulum and Golgi apparatus, and degranulation of rough surfaced endoplasmic reticulum in the edematous endothelial cells and subendothelial stromal cells. In some endothelial cells, the nuclei were irregular in shape, and arregated chromatin with clump formation along the nuclear membrane (Fig. 10).

There was a definite basement membrane clearly separating the overlying endothelial cells from the subendothelial ground substance due to marked edema (Fig. 8). The endothelial basement membrane showed wide separation and meandering, and formed widely dilated spaces containing scanty, fragmented collagen fibers and bundles, predominantly in the occasional areas on the auricular surface of the valve. The basement membrane including small localized spaces beneath the endothelial cells appeared to be rough and fragmentary.

The stratified subendothelial cells had edematous changes similar to the overlying endothelial cells in some ventricular surfaces (Fig. 9).

There were marked increase in electron density of extended and elongated thin endothelial cells, separation of endothelial intercellular junction, destruction of endothelial cells resulting in vegetation formation consisting of platelets, aggregation of fibrin, leucocytes, erythrocytes, etc., in the heart valve from a chicken 5 days after inoculation (Figs. 12 and 16).

Bacterial vegetation was found at the line of closure of the auricular surface of the valve from chickens 7 and 10 days after inoculation (Figs. 13, 14, and 15). There were bacterial colonies scattered in the amorphous

electron dense mass including destroyed cellular debris, bacterial phagocytic leucocytes, erythrocytes, and fibrin aggregation on the denuded endothelial layer of the valves.

Microbiological examination:

Macroscopic vegetation on the mitral valves and cardiac blood were cultured for non-hemolytic *Streptococcus faecalis* at autopsy. Seven of the 8 chickens, 7 and 10 days after inoculation, showed positive cultures in the vegetative tissue and cardiac blood. Vegetative tissues from the chickens 5 days after inoculation gave negative culture, but one of these 4 chickens showed positive culture from the cardiac blood. Cultures were all negative in cardiac blood from control chickens.

Discussion

It is extremely difficult to obtain a fine structure of the endocardial endothelial cells in early inflammatory damages without any artefact. The present study was performed in an attempt to explain the fine structure of endothelial cells, with special attention to early changes in the overlying endothelial cells of the valve surface under stress and early stages of alterations in vegetative endocarditis.

Numerous electron microscopic studies[18-40] have been made on endothelial cells of large arteries, small arteries, venous vessels, or capillaries in acute inflammation by a large number of investigators for the analysis of morphological aspects of exudation and cellular migration. Many investigators have made observations on permeability in the endothelium induced by physiological injuries[18,24,27,29,31] or drugs[21,22,36]. There are very few investigations[1,32] on the early endothelial changes due to microbiological causes. No ultrastructural observations on the endothelial cells of the valve surface are available, except for some description by a few authors[1,12,41-45]. Therefore, many studies have shown the ultrastructural changes in exudation or permeability of the capillary in early inflammation[23,25,26,28,29,30,38]. These findings have indicated that this inflammatory process extends to the perivascular or pericapillary tissue, starting with the migration of leucocytes in the blood stream through the endothelial cells. On the other hand, in the heart valve, the initial inflammatory lesions before leucocytic permeation have been considered to arise from the edematous subendothelial ground substance[1] in the valve corresponding with the perivascular or pericapillary fibrous tissue. It is very difficult to differentiate whether the primary lesion or the endocardium in the vegetative endocarditis is the high reactive activity of endothelial cells to injury or

subendothelial edema. According to the present observations, the endothelial and subendothelial changes seem to go together or to vary in different portions of the valvular endocardium.

The earliest endothelial changes seem to suggest a reversible phase, showing an increase in number of mitochondria, irregular accumulation of ribosomes with high activity, slight dilatation of endoplasmic reticulum, extension of Golgi apparatus, slightly predominant pinocytotic vesicles, and slight multiplication of microvilli and pseudopodia of the membrane within 12 hr after the inoculation. An additional feature of these early changes in the endocardium is the localized minimal appearance of edema between or beneath the endothelial cells and the basement membrane in the subendothelial region of valves. In this stage, the endothelial cells seem to show no significant changes in the inflammatory processes, except for occasional dilatation of mitochondria. Degeneration of mitochondria appears to be one of the characteristic early markers of cell damage[46].

Edematous structural changes are found in occasional endothelial cells as the inflammatory response of this phase (12–24 hr after inoculation). These edematous lesions in the endothelial cells of valves is compatible with the delayed and prolonged vascular reaction in acute inflammation, but endothelial phagocytosis and leucocytic penetration seen in the damaged vascular vessels demonstrated by many workers[28–32,46] could not be found in the valvular endothelial cells.

As inflammatory lesions progress (2–5 days after inoculation), the endothelial changes become more evident and varied. The edematous endothelial alterations, with or without subendothelial edema, can result in two main types; one of them produces progressively more increase in edematous swelling, and vacuolation and multiplication of cytoplasmic processes, and the other, marked atrophic retraction, fragmentation, and opacification of the cytoplasm. The former includes dilatation of the endoplasmic reticulum, enlargement of mitochondria, marked increase in various vacuoles and vesicles, multivesicular bodies and pinocytotic vesicles along the basal cell membrane, invagination of cell membrane, and widening of intercellular gaps in the electron-lucent cytoplasm of the overlying endothelial cells (Figs. 8 and 9). These changes are seen not only in the endothelial cells but also in the subendothelial cells (Figs. 9 and 10). The latter changes remain as marked atrophy or disappearance of intracytoplasmic organelles in the retracted, electron-dense cytoplasm, associated with spatial continuities between cardiac lumina and the basement membrane by widened intercellular junctions of endothelial cells. When the inflammatory changes are more advanced, the damaged endothelial cells of both types seem to disappear by desquamation. After the desquamation

of overlying endothelial cells, the basement membrane is denuded with the subendothelial ground substance immediately in contact with the blood stream in the cardiac lumina, and breaks down secondarily, but it is not clear whether both the basement membrane and endothelial cells are destroyed together, or whether the basement membran is first destroyed and then endothelial cells to be lost later.

The endothelial cellular changes of vascular or capillary permeability in inflammation have been observed often by means of non-toxic carbon particles[22,27,28,30], representing the relationship between the endothelial cells and the basement membrane, and the intercellular gaps.

In the heart valve, various changes between the basement membrane and endothelial cells have been demonstrated by the presence of virus particles found incidentally in the chicken heart valve[12] and by the subendothelial accumulation of injected ferritin[47]. Ts'ao and Glagov[34] have emphasized that basal endothelial attachment was normally associated with fine fibrillar connections between the plasma membrane and underlying basement membrane segments. The exact process or mechanism by which desquamation occurs is not clear from the present findings and from these descriptions about changes between the damaged endothelial cells and the basement membrane.

The affinity of platelets for the exposed collagen fibers at the denuded underlying connective tissue of the valve surface has been described by Nakao[48], Zucker and Borrelli[49], Spae, *et al.*[50], Hovig[51], Hughes and Tonks[52], and by Ashford and Freiman[53]. Non-bacterial thrombotic vegetation of the valves from a chicken 5 days after the inoculation is evident in the present study. In this group, one of 4 chickens gave positive culture of the cardiac blood. Bacterial vegetation was seen in 7 of 8 chickens 7 and 10 days after the inoculation.

A mass of bacteria is present in the vegetation containing fibrin, collagen fibrils, platelets, amorphous fibrinoid material, acid muco olysaccharides, polymor honuclear leucocytes filled with bacteria in vacuoles, and other fragmented cellular debris. The present study revealed that bacterial infection occurred subsequently on the thrombotic vegetation of the valve and that the initial lesion of this bacterial endocarditis was undoubtedly non-bacterial, as Angrist suggested[1].

Although the initial injury induced by *Streptococcus faecalis* was not uniforly manifested among the endothelial cells of the valves, some of these cells showed a high sensitivity to bacteria at the earliest stage, characterized by increased permeability and electron-lucent cytoplasm, and other endothelial cells appeared still inactive at the beginning but later underwent

progressive necrotized degeneration, showing retraction and disappearance of intracytoplasmic organelles, and electron-dense cytoplasm. These variations in endothelial cell-sensitivity to bacterial injury are similar to the immediate and delayed vascular reactions in inflammation shown by Cotran[29,30] and Majno, *et al.*[21,22]. According to them, the immediate type of vascular permeability was localized in medium sized venules and delayed type in small venules and capillaries, with endothelial damage and occasional necrosis. There are some structural differences in valvular endothelial cells, compared with those lining vascular channels, some of which may reflect physical conditions and function, such as mobility, extensibility, and contraction[16,17]. In fact, Mitomo, *et al.*[16] have demonstrated that the basement membrane varies with its location in the valves; it is distinct on the atrial side but often scanty and obscure on the ventricular surface.

The present study has suggested that both bacteria-active and bacteria-passive endothelial cells are present in the valvular endocardium by their selective sensitivity to bacterial injury. The bacteria-active endothelial cells appear to be compatible with the endothelial cells in the delayed and prolonged vascular reactions in inflammation, and the bacteria-passive endothelial cells, the immediate progressive degenerated.

Although many experimental studies in the vegetative endocarditis produced with a number of microorganisms, such as staphylococci and streptococci or bacterial toxins used, have implicated allergic sensitization, local infection mechanisms, or local Schwarzman phenomenon in the valve, the initial edematous lesions of the subendothelial region in the formation of valvular vegetation would seem to occur without evidence of the infection or antigen-antibody mechanism[2,54,55].

The exact pathohistogenesis of the initial lesion in vegetative endocarditis is still not clear; the initial lesion relates to the subendothelial stromal connective tissue and the endothelial changes may be subsequent and secondary. However, the basteria-active endothelial cells appear to play a role of initiating the valvular endocarditis, with or without the initial subendothelial edematous change, and the bacteria-passive endothelial cells, promoting the valvular endocarditis, followed from the initial subendothelial edematous changes.

This study may well help the understanding of the pathogenesis of all endocarditis, suggesting valvular vegetation formation through several different pathologic ways in the endothelial cells.

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Explanation of Figures

Plate 1

Fig. 1. Auricular surface at the closure line of the mitral valve from a chicken 2 hr after inoculation. Slight focal edema in the subendothelial region of the zona spongiosa (Sp). Endothelial layer (E). Hematoxylin-Eosin stain (HE), ×200.

Fig. 2. Auricular surface of the mitral valve from a chicken 24 hr after inoculation. The focal high cellularity characterized by marked hypertrophic endothelial cells and attached erythrocyte accumulation. HE, ×200.

Plate 2

Fig. 3. Auricular surface at the line of closure of mitral valve from a chicken 2 days after inoculation. Focal desquamation of the overlying endothelial cells (E) resulted in shallow ulcer (arrows) with amorphous material accumulation in the basis, and hypertrophic swollen fibroblasts scattered in the edematous subendothelial region (Sp). HE, ×250.

Fig. 4. A similar portion in the heart valve of the same group shown in Fig. 3. An uneven, nodular edematous swelling of the subendothelial region of zona spongiosa (Sp), with minimal desquamation of endothelial cells (E), and slight inflammatory stromal round cells (arrows) infiltration. HE, ×250.

Plate 3

Fig. 5. Bacterial vegetation on the auricular surface at the free edge of the mitral valve from a chicken 7 days after inoculation. HE, ×100.

Fig. 6. Higher magnification of Fig. 5. Bacterial vegetation on the valvular surface with massive bacterial colonies (arrows) and reactive inflammatory cells scattered beneath the vegetation. HE, ×600.

Plate 4

Fig. 7. Ventricular surface at the free edge of the mitral valve from a chicken 24 hr after inoculation. The endothelial cells and subendothelial region show marked edematous changes. Marked decrease and degeneration of the intracytoplasmic organelles; disappearance of free ribosomes, dilatation of endoplasmic reticulum (er), swelling of mitochondria (m), and various large or small sized vacuoles and vesicles are seen in the endothelial cell and subendothelial region. Nucleus (N). Cardiac ventricular cavity (V). ×20,000.

Plate 5

Fig. 8. Auricular surface at the line of closure of the mitral valve from a chicken 48 hr after inoculation. One of expansively lifting endothelial cells by marked subendothelial edema includes marked increase of pinocytotic vesicles (Pv) along the basal cytoplasmic membrane, marked swelling of mitochondria, and thickened microvilli. Occasional collagen fibers and fibrils (arrows) are encountered in the dilated space between the overlying endothelial cell and its basement membrane (Bm). Auricular cavity (A). ×31,000.

Plate 6

Fig. 9. Ventricular surface at the free edge of the mitral valve from a chicken 48 hr after inoculation. The stratified endothelial cells (E1 and E2) and subendothelial cells demonstrate edematous changes similar to Fig. 8. Marked increase of pinocytotic vesicles (arrows), irregular dilatation of granular endoplasmic reticulum (er), amorphous electron-dense material in mitochondria (m), and scattered small vesicles (v). Collagen bundles and fibers are located in the subendothelial intercellular regions. ×35,000.

Plate 7

Fig. 10. Ventricular subendothelia region of the mitral valve from a chicken 5 days after inoculation. More severe changes are characterized by cystic dilatation of endoplasmic reticulum (er), loss of cellular organelles, irregular condensation of nuclear chromation, and lysis of cytoplasm. Rough arrangement of collagen bundles (c) scattered around the vacuolated cell. ×20,000.

Fig. 11. The same specimen as Fig. 10. The thin retracted endothelial cells (E) with low electron density of cytoplasm, and vacuolar and autolytic subendothelial cells are seen in the markedly edematous subendothelial ground tissues including loose arrangement of collagen bundles and fibers (c). Ventricular cardiac cavity (V). ×14,000.

Plate 8

Fig. 12. Auricular surface at the line of closure of mitral valve from a chicken 5 days after inoculation. Leucocytes with different forms of vacuolar systems, in which most vacuoles (Ly) contain various fragmented debris showing lysosomal nature, are attached to the extended endothelial cells with dilated granular endoplasmic reticulum (er), multivesicular body (arrow), and lytic cytoplasm in the subendothelial region. ×26,000.

Plate 9

Fig. 13. Bacterial vegetation on the auricular surface at the line of closure of the mitral valve from a chicken 7 days after inoculation. Mass of bacteria (Ba) located at the lower portion of the micrograph. Bacterial phagocytic leucocytes with peripheral aggregation of nuclear chromatin are surrounded by amorphous electron-dense material including destroyed cellular debris and fibrin. ×9,800.

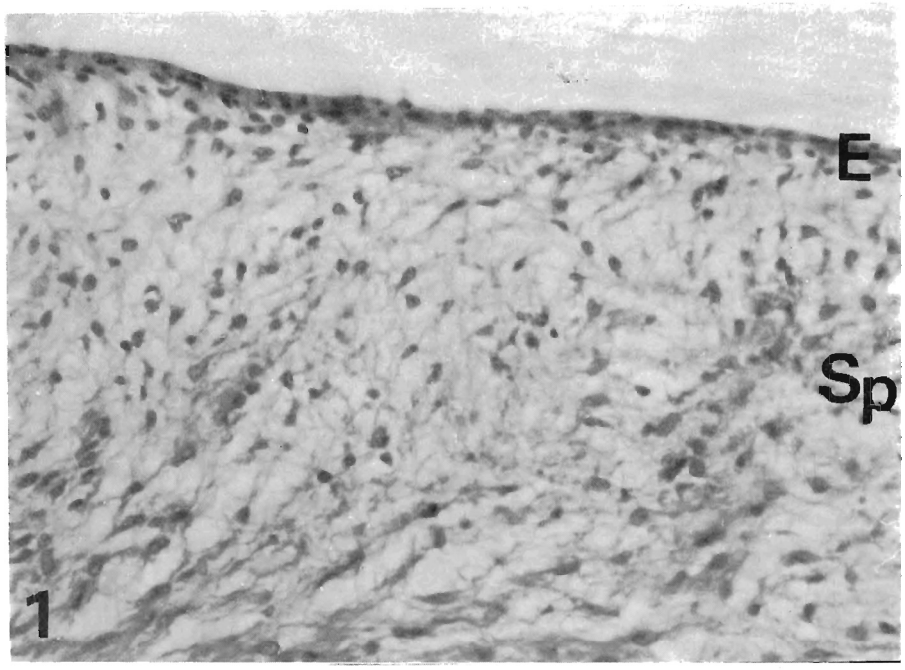
Plate 10

Fig. 14. The same specimen as Fig. 13. Edematous leucocyte accumulation in the bacterial vegetation includes bacteria phagocytic lysosomes (Ly), enlargement of mitochondria (m), dilated endoplasmic reticulum (arrows, er), and marked lysis of electronlucent cytoplasm. ×36,000.

Plate 11

Fig. 15. Higher magnification of the lysosomal region in Fig. 13. Bacteria (white Ba) are present in the amorphous electron-dense material surrounded by a limited double membrane. ×98,000.

Fig. 16. Auricular surface of the free edge of the mitral valve from a chicken 5 days after inoculation. Non-bacterial vegetation includes fragmented platelets (Pl), leucocytes (W), cellular debris (d), fibrin (f), and erythroctes (R). ×12,000.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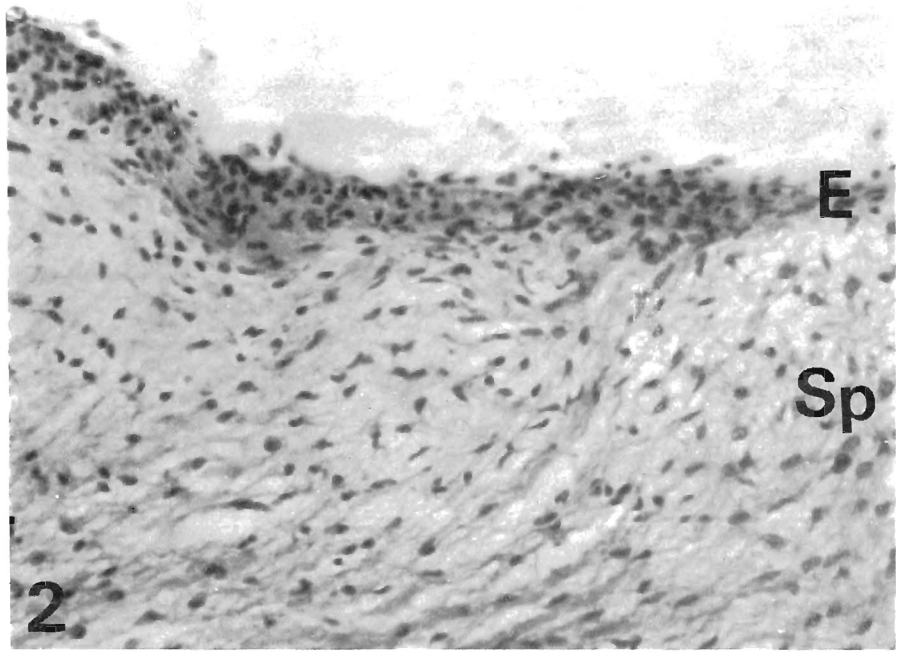
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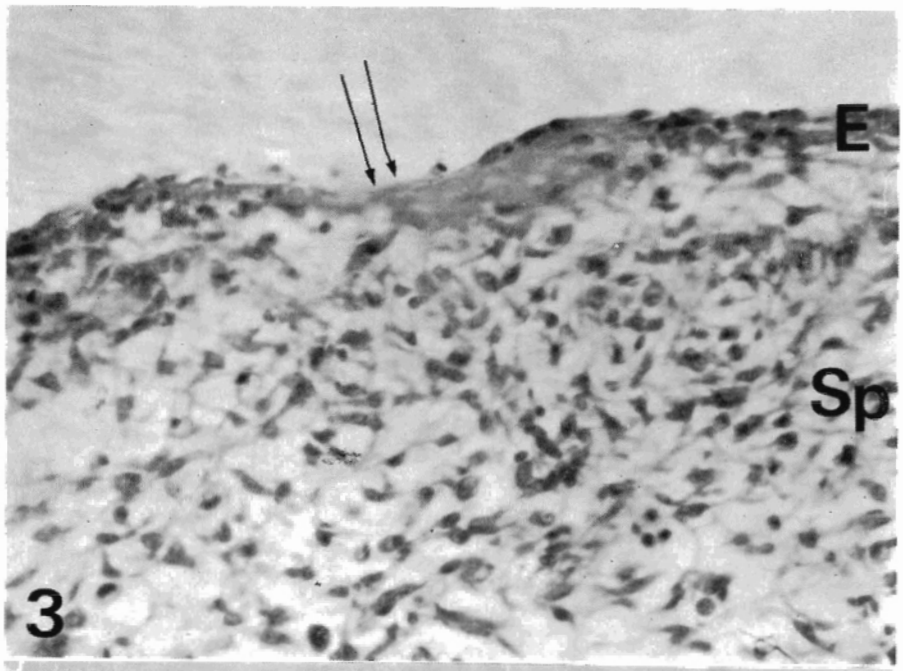
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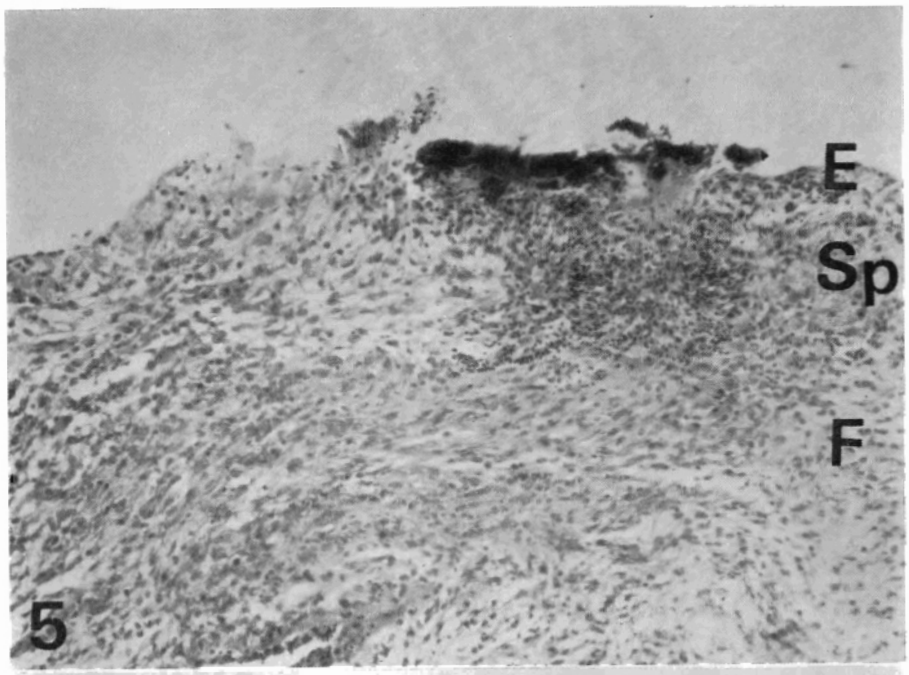
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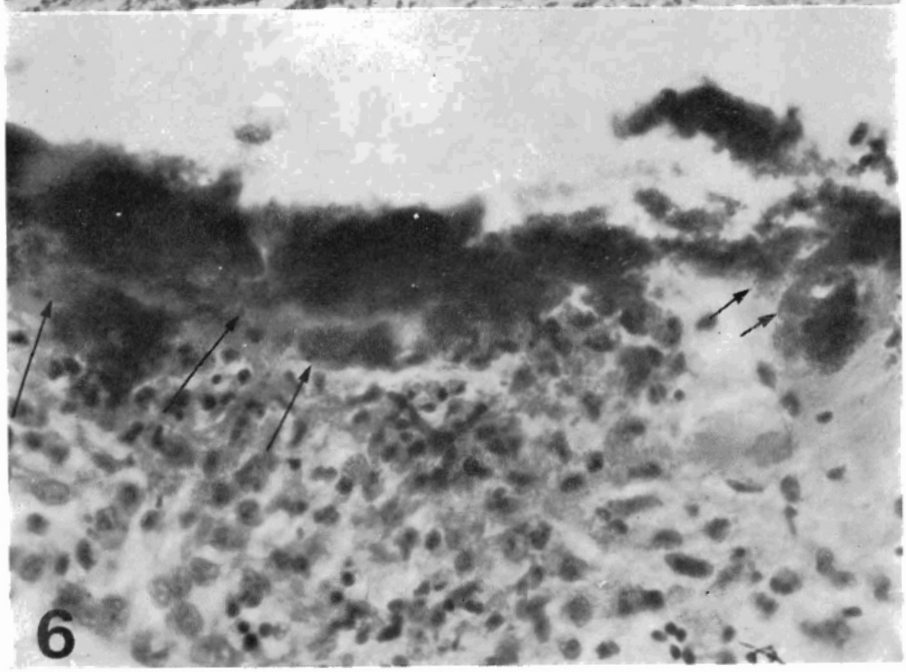
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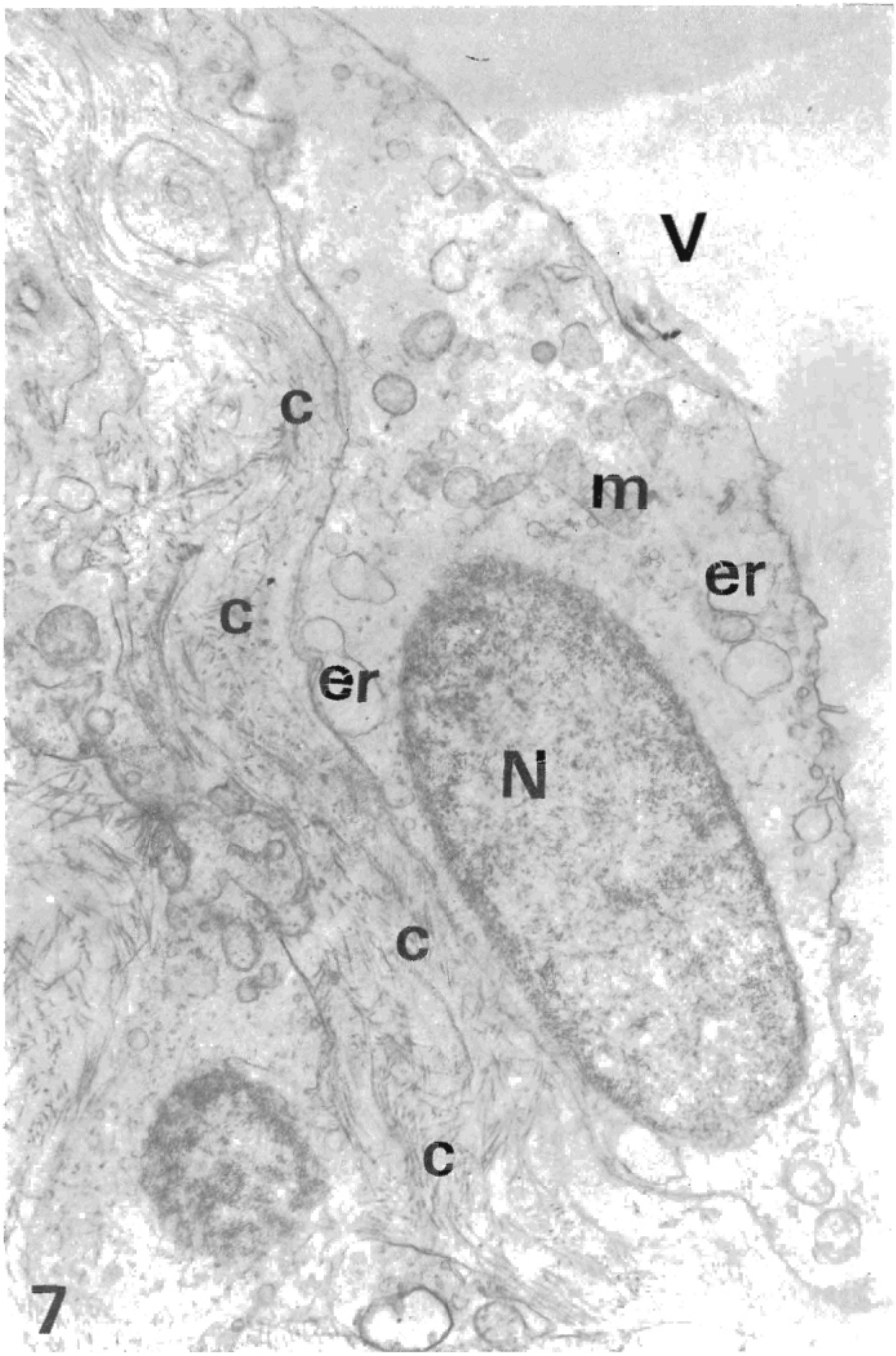
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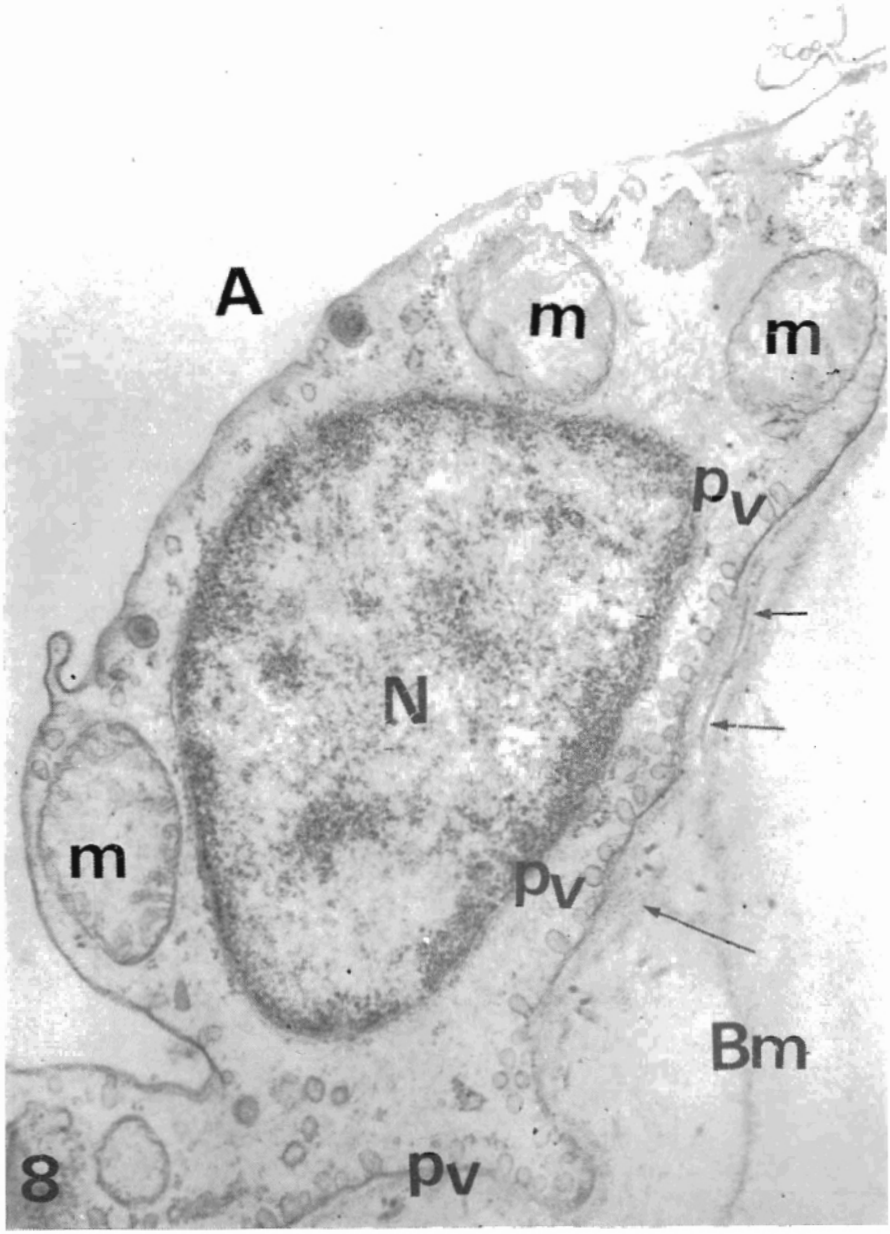
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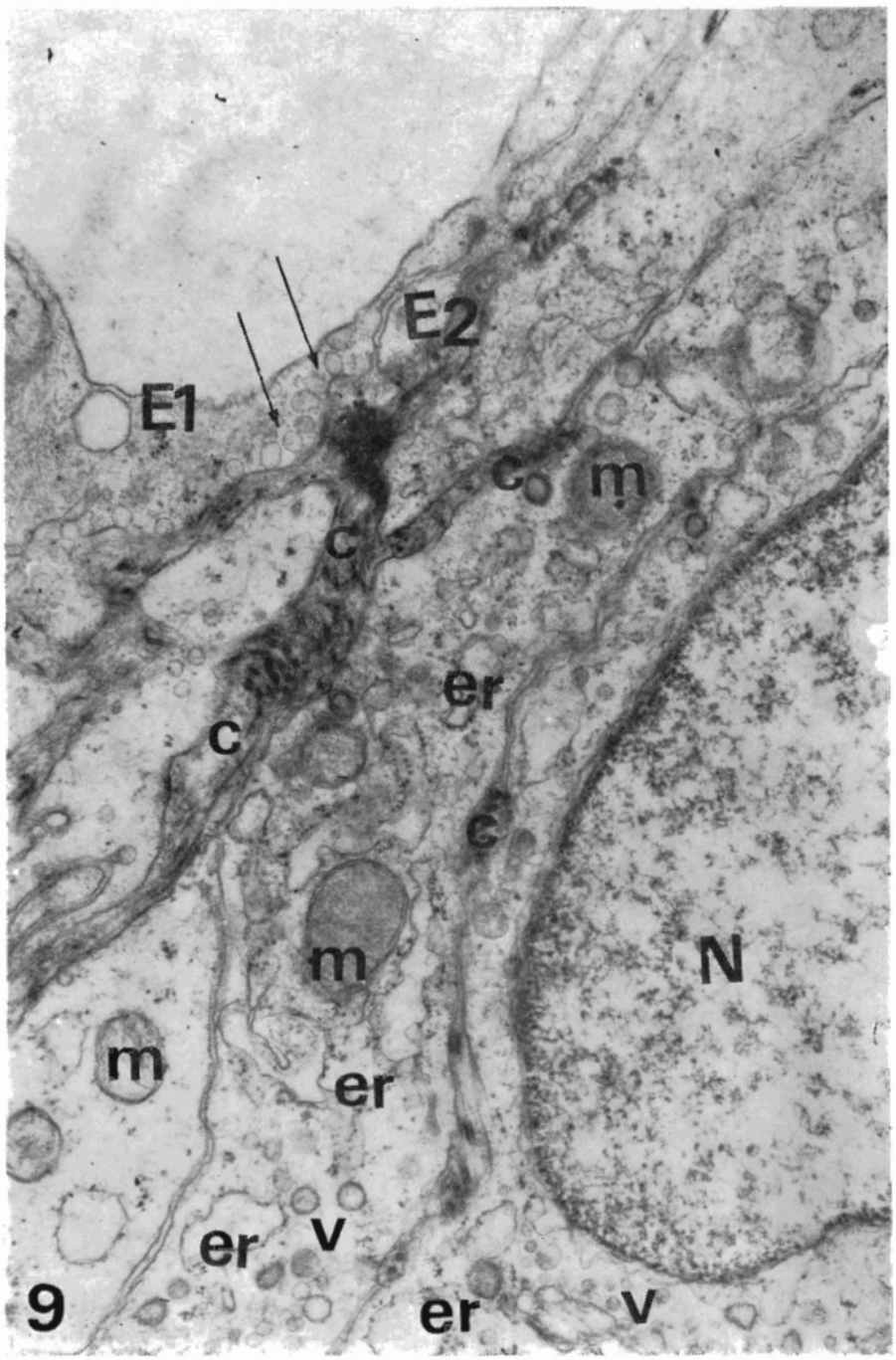

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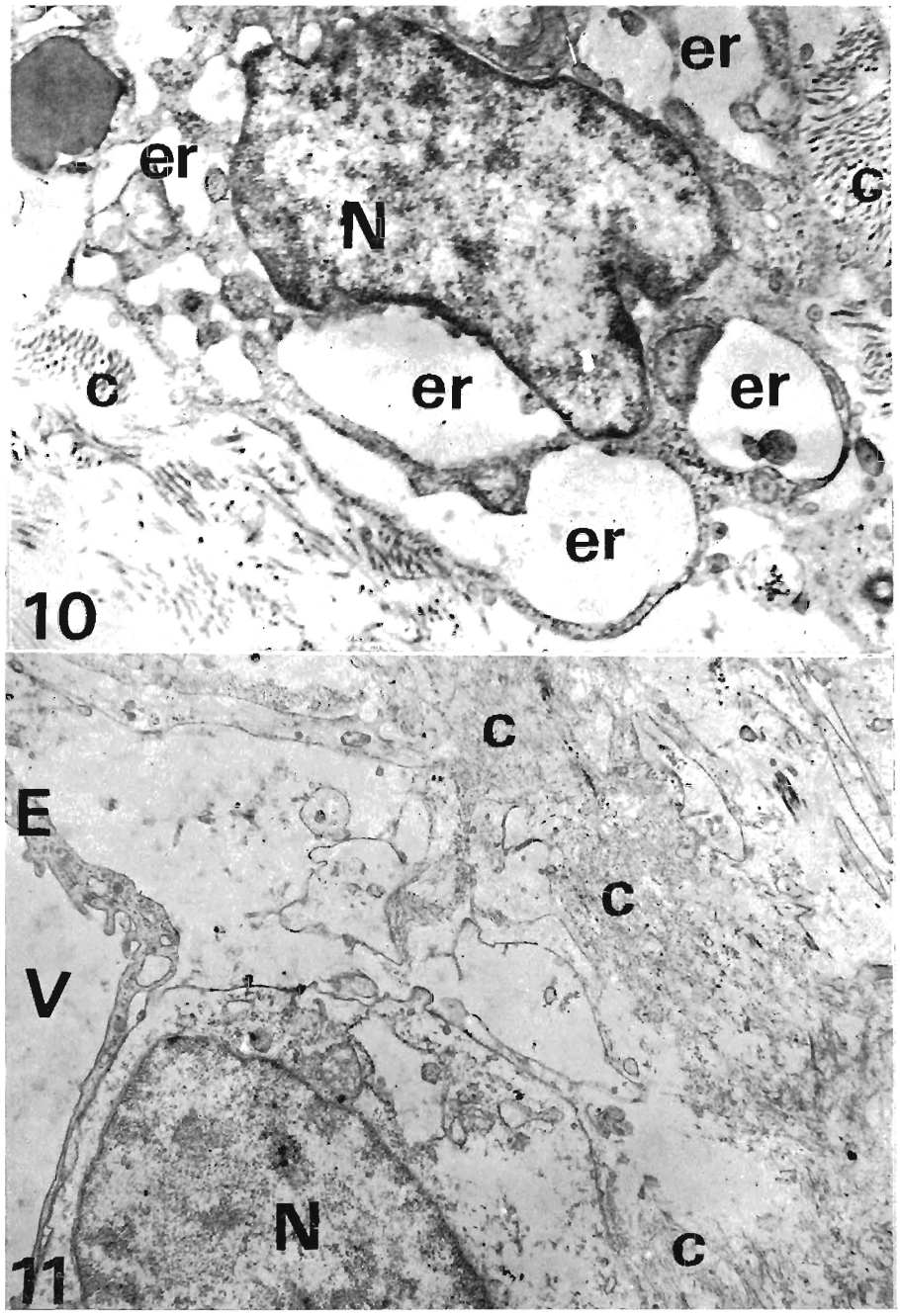
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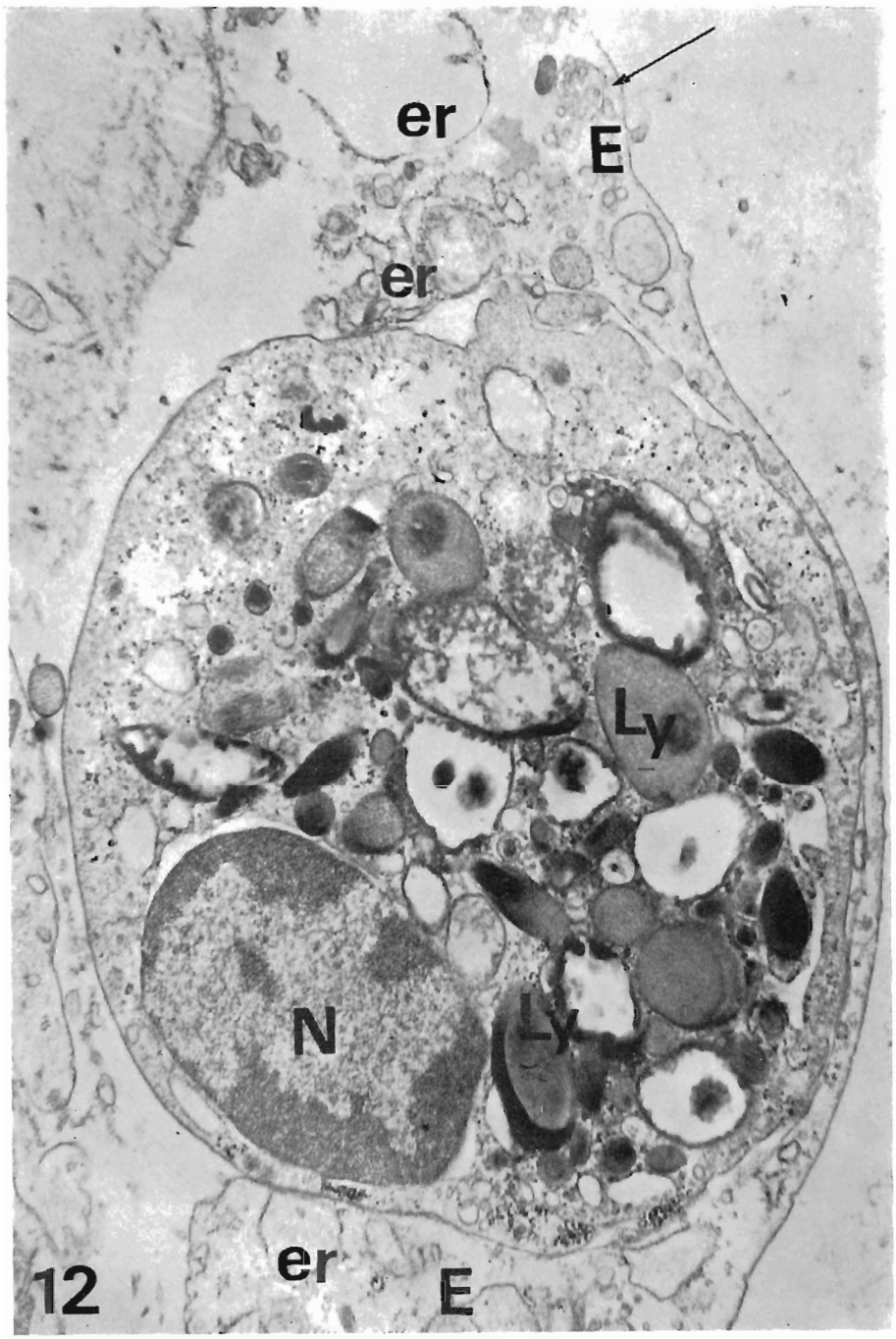
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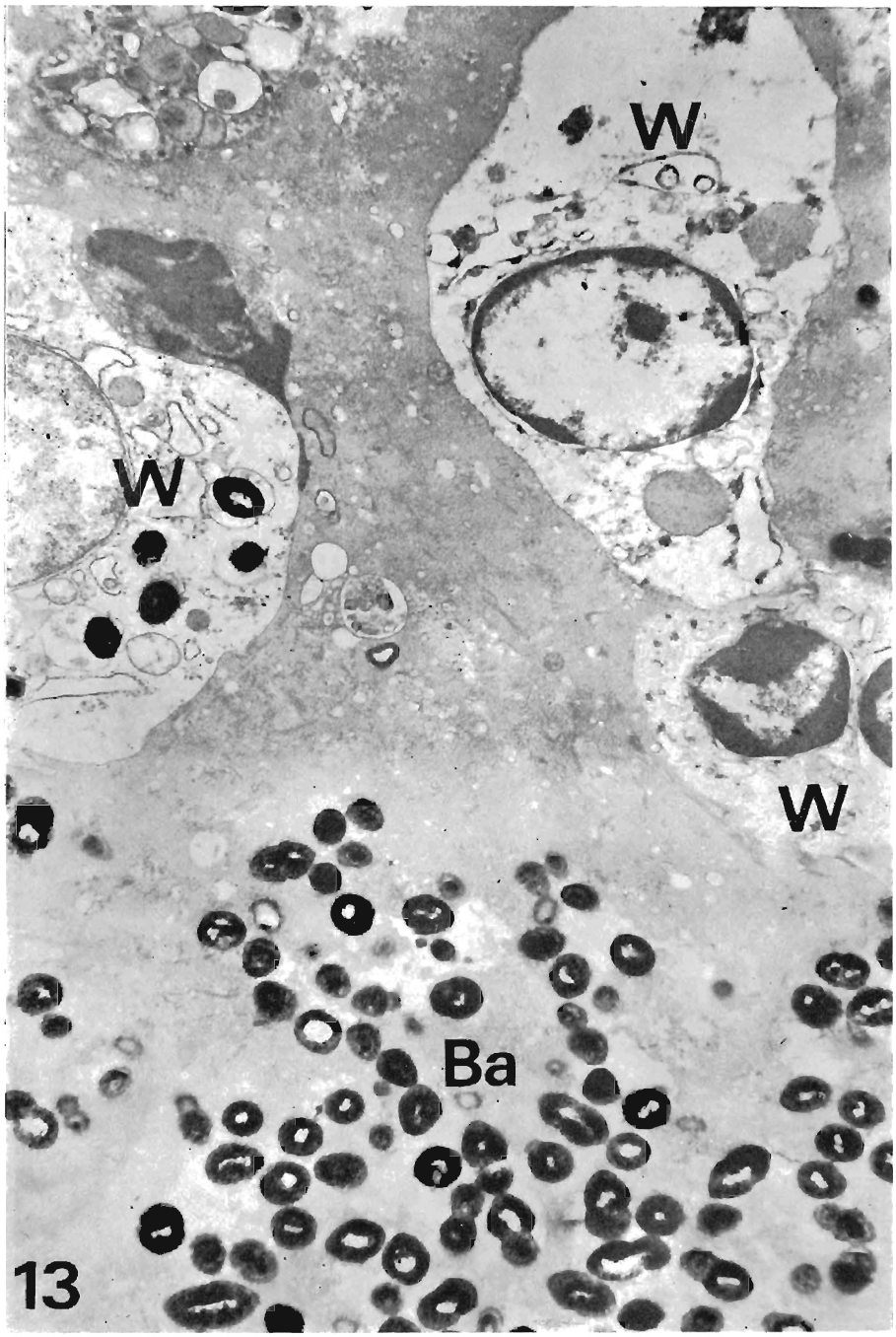
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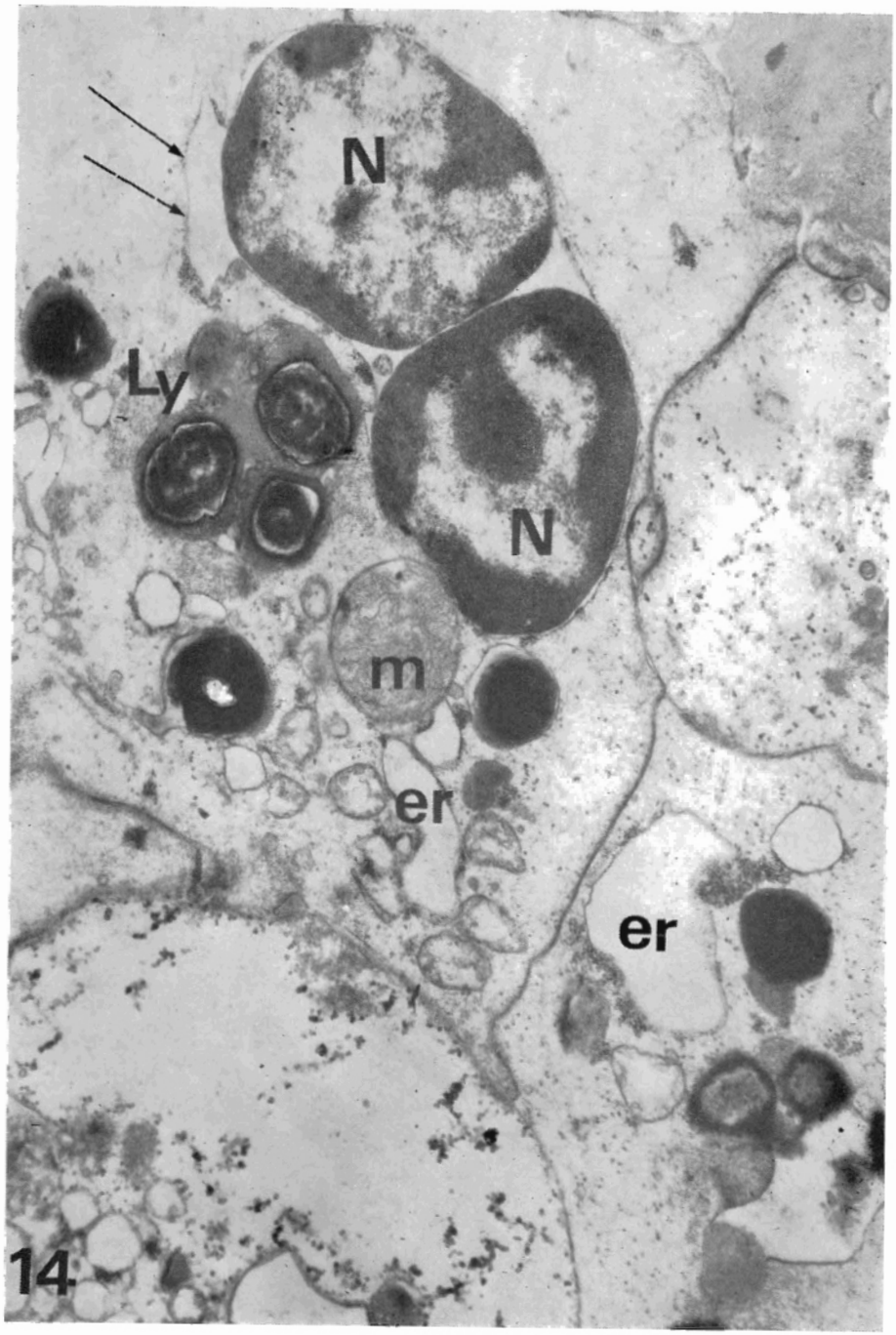
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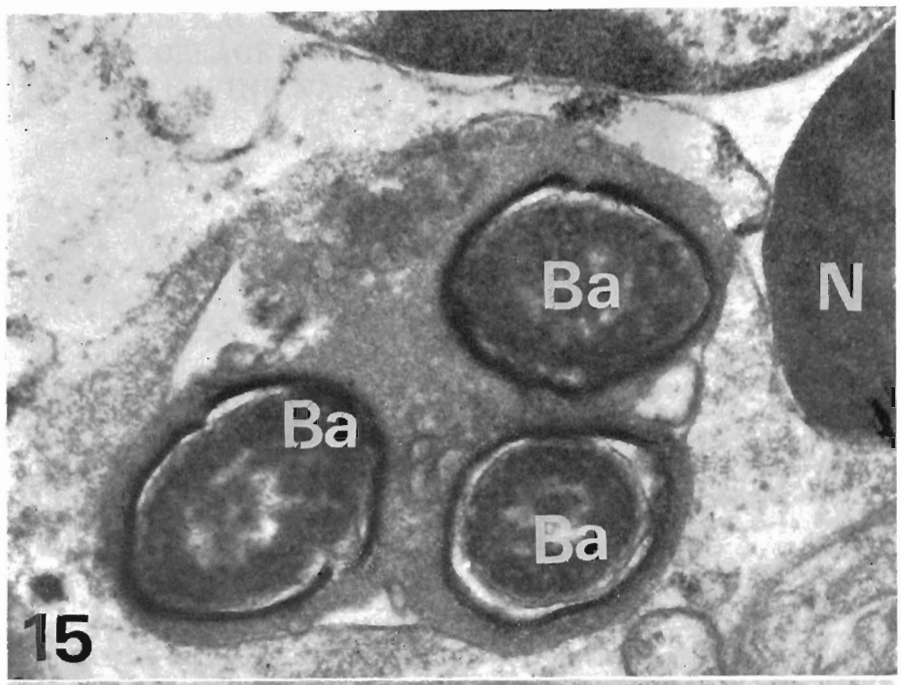
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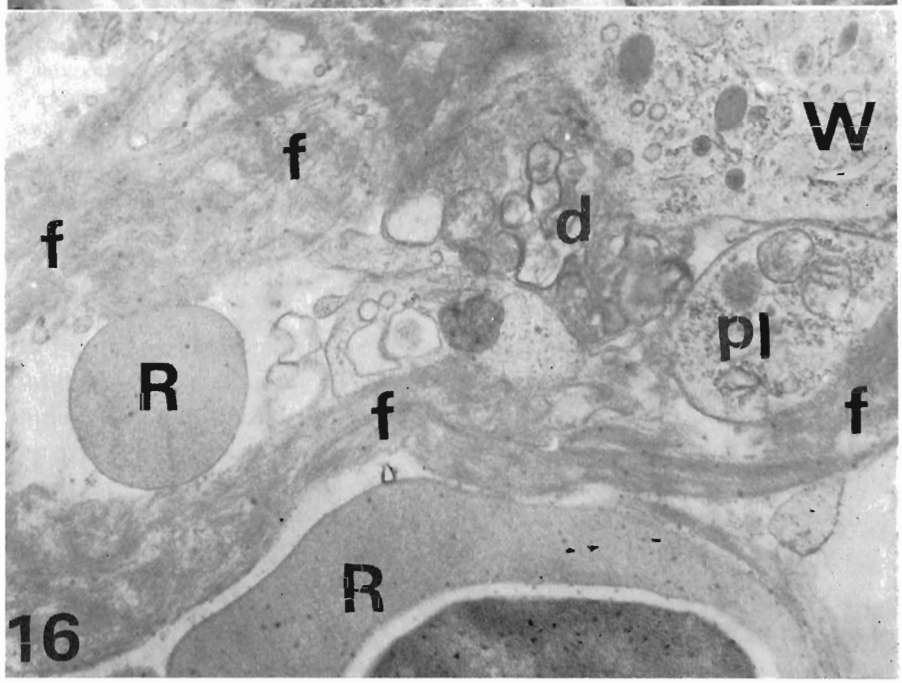
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