INFECTION AND IMMUNITY, Feb. 1978, p. 649-658
0019-9567/78/0019-0649$02.00/0

Vol. 19, No. 2
Printed in U.S.A.

Electron Microscopic Study on Phagocytosis of Staphylococci by Mouse Peritoneal Macrophages

KAZUO IWATA,* YAYOI KANDA, AND HIDEYO YAMAGUCHI

Department of Microbiology, Faculty of Medicine, University of Tokyo, Tokyo 113, Japan

Received for publication 6 August 1977

Macrophages from the mouse peritoneal cavity were examined by electron microscopy at various time intervals up to 24 h after intraperitoneal administration of each of three strains of staphylococci different in virulence for mice: high-virulent, low-virulent, and avirulent strains. After engulfment, avirulent bacteria were highly liable to intracellular digestion, resulting in almost complete degradation within 24 h after injection, whereas high-virulent bacteria were more resistant to digestion, some showing figures suggestive of a dividing process; the gross configuration of most of the ingested bacteria was relatively well preserved over the 24-h period. Time-dependent morphological changes of low-virulent bacteria were intermediate. Among the most distinct cytoplasmic responses to the ingested bacteria was the formation of phagocytic vacuoles around them, the type of which was dependent on the staphylococcal strain infected; ingestion of avirulent bacteria led to formation of vacuoles in which the bacteria were surrounded by a halo of amorphous material of moderate density, which may be the lysosomal content. In contrast, larger vacuoles developed after ingestion of high-virulent bacteria and contained only a small quantity of such amorphous materials. Both types of phagocytic vacuoles were seen around the low-virulent bacteria ingested. Some degenerative changes were found in the macrophages ingesting high- or low-virulent bacteria, but were not in those ingesting avirulent bacteria. Thus, resistance to intracellular degradation, as well as cytotoxicity toward phagocytes of staphylococcal strains, can be correlated with their virulence.

Studies have been made on host resistance to staphylococcal infection in which mice have primarily been used. In this widely used model, the outcome of inoculation of staphylococci by the intraperitoneal route has been shown to be governed by two principal host responses to the injected bacteria, phagocytic uptake and intracellular killing, in which peritoneal macrophages play a predominant role, in particular, early in infection. It implies that resistance of a given strain of staphylococcus to phagocytosis may be the determinant of its pathogenicity. Koenig and Melly (16) reported that high virulence of Smith diffuse staphylococcus for mice can be directly related to possession of capsular antigens that retard phagocytosis. Correspondingly, Suganuma et al. (24) have recently demonstrated the existence of capsular structures with a related strain of *Staphylococcus aureus* by means of thin-section electron microscopy. Data are also available to indicate that once phagocytized, *S. aureus* is usually subjected to killing by both macrophages and neutrophiles (1, 14, 16-18, 21, 23), whereas no evidence has been provided that this organism is capable of continuous multiplication within phagocytes.

It has been established that the intracellular mechanisms involved in the response of macrophages to ingested microorganisms depend on the interaction of phagosomes and lysosomes (25, 26). However, the ultrastructural aspect of this interaction during infection by staphylococci has not yet been studied systematically, although some aspects have been reported in human leukocytes in vitro (6, 7) and in rabbit liver reticuloendothelial cells in situ and in vivo (9). There are bacteriological data on phagocytosis of staphylococci by human leukocytes that show that the number of viable bacteria of virulent strains increases within the phagocytes, whereas that of avirulent strains decreases (22).

The purpose of this study is to describe the ultrastructure of phagocytic and postphagocytic events in normal mouse peritoneal macrophages during the early stage of infection with each of three staphylococci strains different in virulence for mice and in several biological characteristics: a high-virulent, coagulase-negative, mannitol-positive nonhemolytic strain, E46; a low-virulent, coagulase- and mannitol-positive hemolytic Smith-type strain, E111; and an avirulent, coagulase- and mannitol-negative nonhemolytic

strain, E241. The relationship of these staphylococcal strains of graded virulence to the peritoneal macrophages that have phagocytized them were examined on ultrathin sections by an electron microscope. Comparison of principal biological characteristics, as well as pathogenicity, among these strains has been described previously (10–13).

MATERIALS AND METHODS

Strains and culture. The three strains of *Staphylococcus* used were freshly isolated from clinical specimens in our laboratory (10). E46 was conventionally designated as the high-virulent strain because it had the highest virulence for mice of all our staphylococcal stock cultures. Its 50% lethal dose value for 4-week-old DD/YF male and female mice weighing 15 ± 1 g upon intraperitoneal injection was 1×10^8 viable cells when determined at 48 h after challenge. Marked sepsis appeared to be the cause of death. E111 was chosen as the low-virulent strain and E241 as the avirulent strain; the 50% lethal dose value of the former was 1.5×10^9 viable cells when determined under the same assay conditions as described above, whereas the latter showed no lethal effect on mice when 1.5×10^9 viable cells were injected. Based on the data on biological characterization, E111 and E241 were identified as typical strains of *S. aureus* and *S. epidermidis*, respectively. Strain E46 was regarded as a variant of *S. aureus*.

Cultures of these strains were all maintained and transferred every 2 months on heart infusion agar slants. Each strain was grown in heart infusion broth at 37°C with vigorous aeration. Eighteen-hour cultures were centrifuged at 8,000 × *g* for 15 min to harvest organisms. They were then resuspended in the same fresh broth and standardized to an approximate concentration of 10^9 organisms per ml of broth.

Animals and experimental infection. Four-week-old male DD/YF mice, weighing between 16 and 20 g, were injected with an intraperitoneal inoculum of 2×10^8 viable staphylococci in a volume of 0.2 ml.

Preparation of specimens for electron microscopy. The peritoneal macrophages were washed out with the aid of Hanks balanced salt solution as described by North and Mackaness (19), at times varying from 30 min to 24 h after injection. They were washed twice with the same solution by centrifugation, and then the pellet was fixed for 2 h in 2% (vol/vol) glutaraldehyde buffered in 0.1 M phosphate (pH 7.2), washed in the same buffer, and postfixed in 1% (wt/vol) osmium tetroxide buffered in 0.1 M *S*-collidine, pH 7.0. The pellet was dehydrated in a graded series of ethanol and embedded in Epon 812. Thin sections were cut on an LKB ultramicrotome, collected onto collodion- and carbon-coated grids, and poststained with 1.5% (wt/vol) uranyl acetate and lead citrate. Electron microscopy was performed with a JEOL JEM-7 electron microscope.

RESULTS

The fine structure of normal mouse peritoneal macrophage presented here was essentially identical with that reported by several other investigators (2, 3, 8, 19). The irregular outline of the macrophage was characterized by numerous invaginations of the cell membrane, which conferred a fenestrated appearance on the peripheral cytoplasm (Fig. 1). The nucleus was deeply indented, and chromatin was clumped around the nuclear periphery as an irregular, dense band. The ample cytoplasm was rich in small vesicles, many of which contained electron-dense materials. Granular endoplasmic reticulum was disposed in several parallel strands, usually around one pole of the nucleus or sometimes sparsely distributed at random through the cytoplasm.

Infection with the low-virulent strain. Samples taken within 1 min after the intraperitoneal injection of low-virulent staphylococci indicated that many of bacteria were encircled by projecting pseudopods, but no organism was seen within the major cytoplasmic body, indicating that entrapment of cocci by macrophages had occurred. Subsequently, bacteria were apparently pulled back toward this cytoplasmic mass for engulfment (Fig. 2). The number of intracellular bacteria increased with increasing time. Thirty minutes after infection, many staphylococci were found in phagocytic vacuoles (Fig. 3). Formation of the phagocytic vacuole was most frequently seen at this stage of infection, although some were found throughout the experimental period.

Phagocytic vacuoles appeared to be lined by a trilaminate membrane that contained one or several bacteria. Two types of phagocytic vacuoles were regularly present in the cytoplasm of macrophages ingesting low-virulent cocci, as was described by Dumont and Robert (4): a "tight type" in which the membrane was directly apposed over the cell wall of the bacterium and a "loose type" in which a wide space was present between the membrane and the cell wall. This peribacterial space was occasionally occupied by abundant amorphous or floccular material of moderate density or by membranous profiles. In addition, giant vacuoles of unilocular or lobulated form sometimes appeared in the central portion of the cytoplasm. They were considered to result from fusion of several loose-type phagocytic vacuoles and often contained more than one bacterium. The electron-translucent peribacterial space became larger as bacteria grew and was filled by a variable quantity of low-density material. Where bacteria grew or multiplied vigorously, the space appeared to be completely electron-lucent, forming a clear zone. Most of the bacteria present in these vacuoles appeared morphologically unaltered, and frequent occurrence of septation figures in such

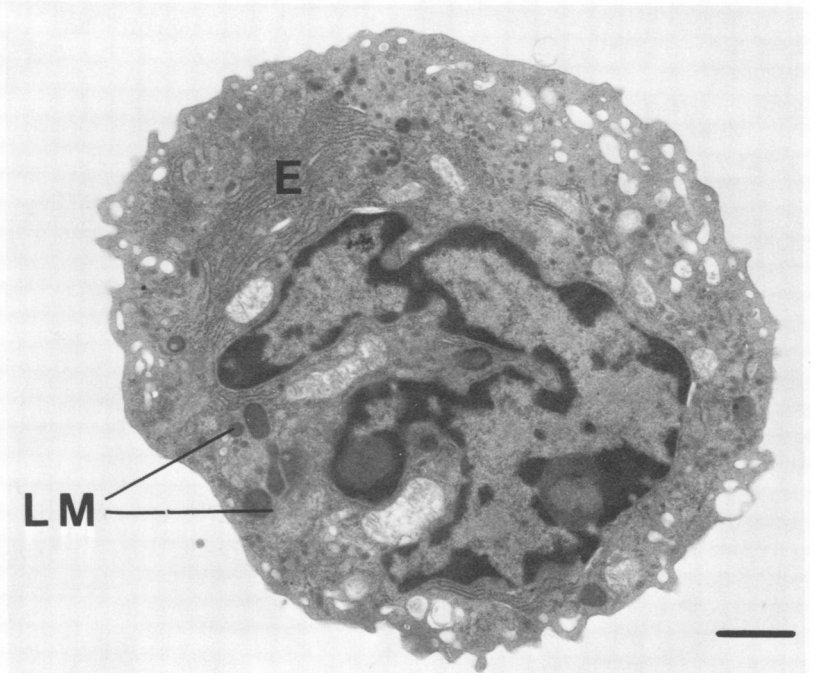


FIG. 1. *Peritoneal macrophage of normal mouse obtained by peritoneal lavage. There is a great amount of granular (rough) endoplasmic reticulum (E). Frequent electron-dense vesicles (LM) occur throughout the cytoplasmic area. Numerous mitochondria are relatively small.*

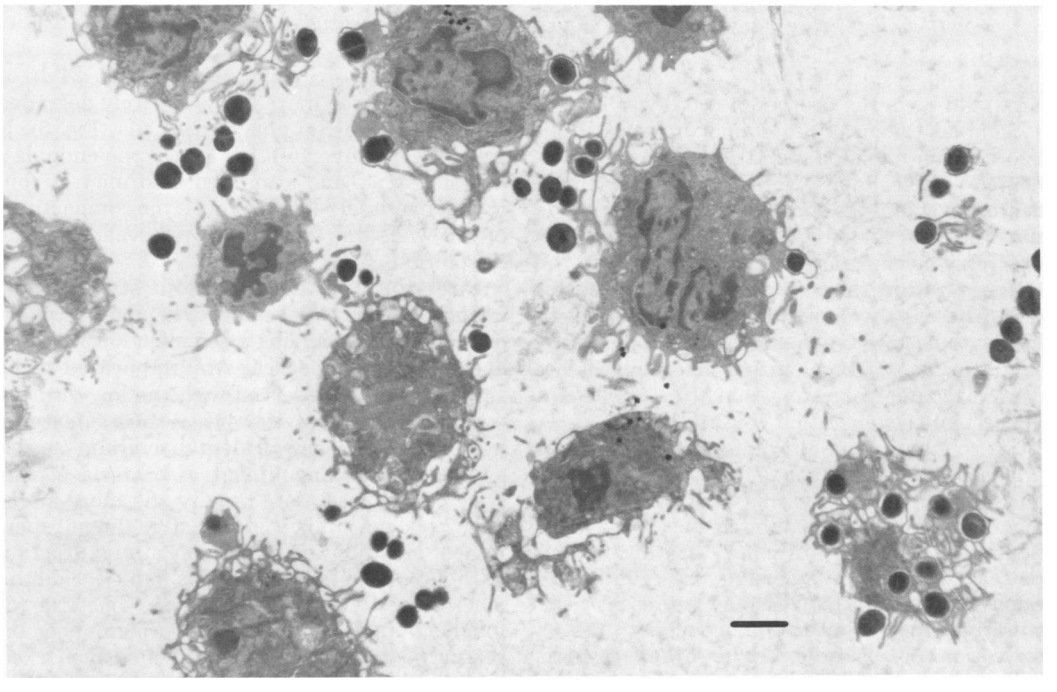


FIG. 2. *Macrophage fixed 1 min after injection of low-virulent cocci. Some bacteria are already encircled by cytoplasmic processes. Several other bacteria are completely ingested and occupy small phagocytic vacuoles.*

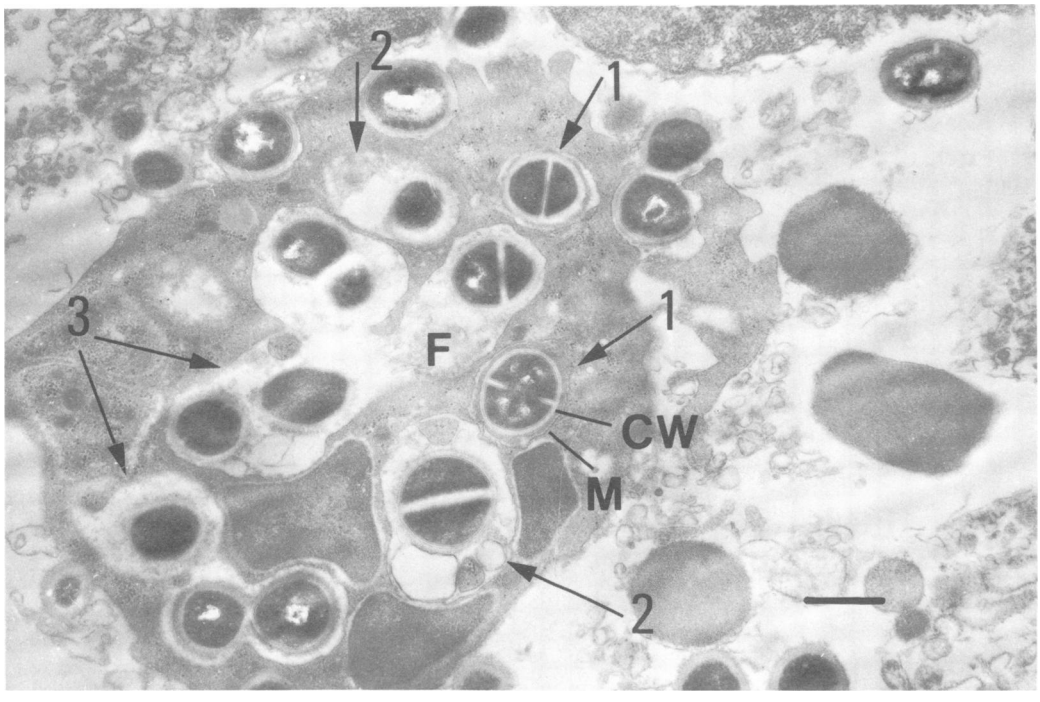


FIG. 3. *Macrophage fixed 30 min after injection of low-virulent staphylococci. The cytoplasm contains many phagocytic vacuoles (phagosomes) in an area devoid of organelles. Some phagocytic vacuoles (arrow 1) are of a tight type, with no appreciable space between the cell wall (CW) of the bacteria and the membrane of the vacuoles (M). Other phagocytic vacuoles are of a loose type, appearing to be unilocular (arrow 2) or multilocular (arrow 3). Floccular material (F) of low density is observed inside the vacuole, but there is no indication that other dense material has been added to the phagocytic vacuole (cf. Fig. 6).*

bacteria suggested that some of ingested bacteria would be in the process of cell division.

Samples taken at subsequent intervals from 1 to 4 h after injection indicated increasing numbers of intracellular bacteria with conversely decreasing numbers of bacteria lying free outside the cell. Size and frequency of giant vacuoles progressively increased; a good portion of the bacteria within the vacuoles still appeared intact, whereas the configuration of all species of intracytoplasmic organelles but with free ribosomes in many phagocytizing macrophages became obscure, suggesting that they underwent appreciable degeneration (Fig. 4).

Six to eight hours after infection, all types of phagocytic vacuoles started to decrease in size and number, and the cytoplasm of phagocytizing macrophages usually exhibited the normal configuration of all constituents, together with a pattern of increased density and granularity. Such cytological changes may represent a restorative response of the phagocytes to the intoxication of bacterial origin. Many bacteria within the vacuoles showed an irregular contour and a decrease in the density of the cytoplasmic constituents, although some others appeared to be viable cells. Samples taken after 24 h of infection showed more of the severely degraded bacteria within the phagocytic cells (Fig. 5). It was reflected by extensive loss of the endoplasmic substance and a marked deformity in contour of bacterial cells. However, the compactness of cell walls was fairly well preserved, avoiding dissolution.

Infection with the avirulent strain. Some cytological aspects of phagocytic and postphagocytic events occurring after infection with the avirulent staphylococcal strain appeared to be similar to those observed with the low-virulent strain. Nevertheless, there were several features peculiar to the low-virulent bacterium as described below. Engulfment of bacteria of this strain took place more rapidly and more intensively. Samples taken 30 min after the intraperitoneal injection indicated that a large fraction of the bacterial population was found inside the macrophages, whereas only a few bacteria remained outside the phagocytes. Macrophage ingesting bacteria was most conspicuously marked by exclusive formation of a tight type of phagocytic vacuoles (Fig. 6). The bacteria located therein, most of which appeared unaltered, were

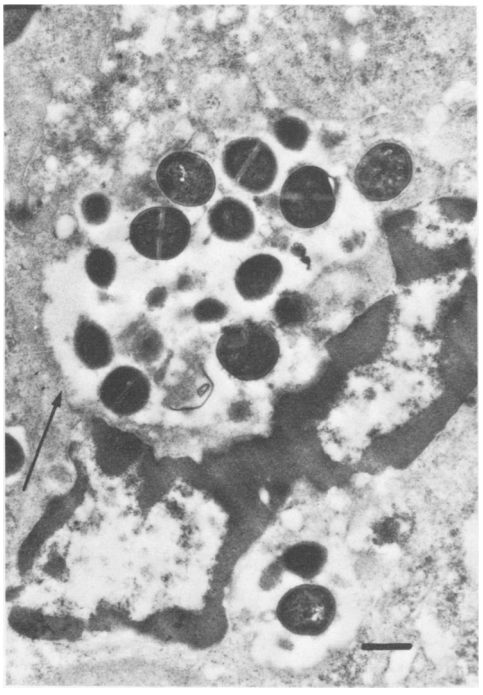


FIG. 4. *At 4 h after injection of low-virulent cocci. A huge phagocytic vacuole (arrow) develops in the cytoplasm, which contains numerous staphylococci. They appear to be morphologically intact. The cytoplasmic area surrounding the vacuole shows appreciable degeneration.*

surrounded by a halo of amorphous materials of moderate density that was similar in appearance to the contents of cytoplasmic vesicles. This suggests that such amorphous materials deposited in the phagocytic vacuoles may be derived from the cytoplasmic vesicles as the result of their fusion with the phagocytic vacuoles, although no cytological evidence was available in this study. After 3 h, several morphological changes were observed, suggesting severe degradation of the ingested bacteria (Fig. 7); such changes included a marked decrease in the density of endoplasmic substances and a loss in the configuration and the integrity of cell walls along with their partial dissolution.

Samples taken after 24 h showed residues of the digestive process, approximating final degradation, which appeared to consist mainly of residues of cell walls and endoplasmic materials of the bacteria intermingled with the contents of phagosomes (Fig. 8). At this stage, there was no intracellular bacterium with the gross configuration that remained intact. In the infection with avirulent staphylococcus, no degenerative macrophage, whether it had phagocytized bacteria or not, was observed over a 24-h period.

Infection with the high-virulent strain. Consistent with our bacteriological and photomicroscopic data reported previously (12, 27), the present electron microscopic study confirmed that the high-virulent strain was more resistant to phagocytosis than the other two strains of staphylococci. Upon injection of the high-virulent bacteria, few were present in a limited fraction of the peritoneal macrophage population after 30 min. However, once initiated, the engulfment process for this strain of bacteria was as rapid as that observed with the other two strains. Samples taken 30 min after infection indicated that large phagocytic vacuoles of the loose type as well as smaller ones of the tight type developed abundantly in the cytoplasmic area of macrophages phagocytizing bacteria (Fig. 9). Most of the bacteria within the vacuoles showed the unaltered profile. In some vacuoles, the peribacterial space appeared empty, but in others it contained relatively small amounts of amorphous materials. Samples taken at 4 h had essentially the same cytology. At this time, no

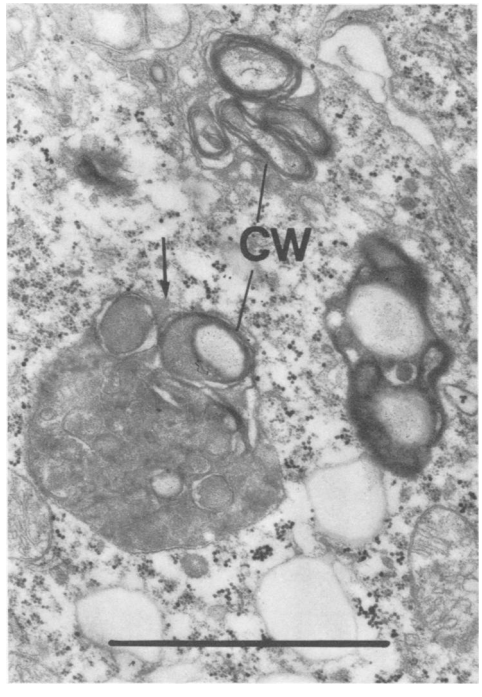


FIG. 5. *At 24 h after injection of avirulent bacteria. Portion of the cytoplasm of a macrophage containing almost completely digested bacteria, which retain only cell walls (CW). They are circumscribed by the phagocytic membrane (arrow) and surrounded by dense material. Normal appearance of the fine structure of the cytoplasm (cf. Fig. 1) suggests that the macrophage has completely recovered from the toxic effect of the ingested bacteria.*

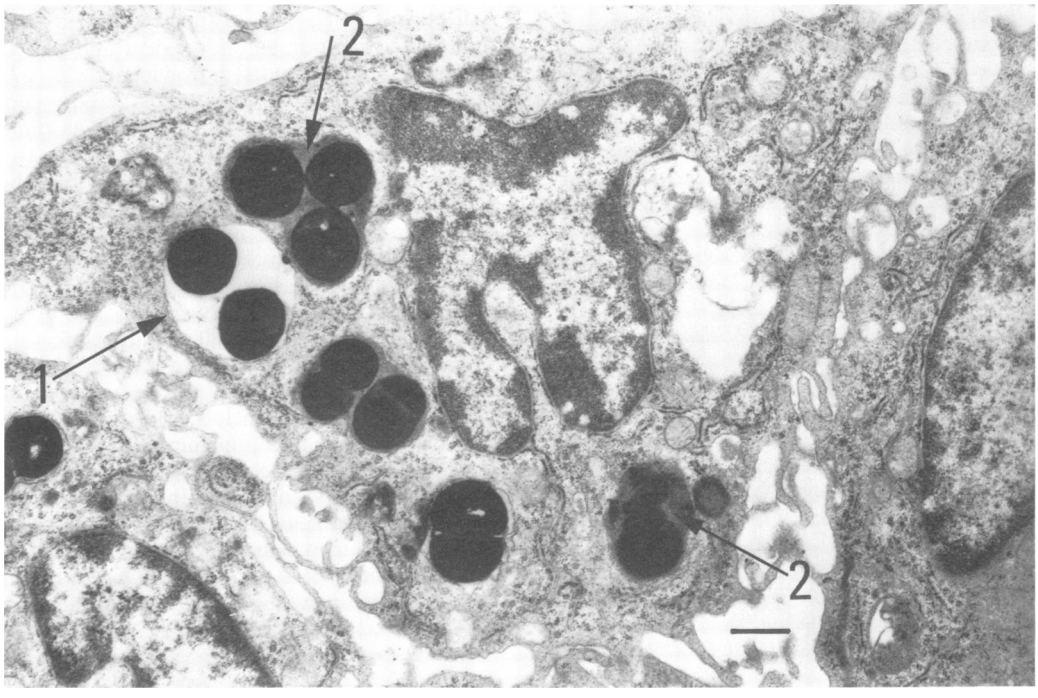


FIG. 6. *Portion of macrophage fixed 30 min after injection of avirulent bacteria. All phagocytic vacuoles are of a tight type, with the exception of one, which is of a loose type (arrow 1). Dense material (arrow 2) is accumulated in most of the former type vacuoles around an ingested bacteria.*

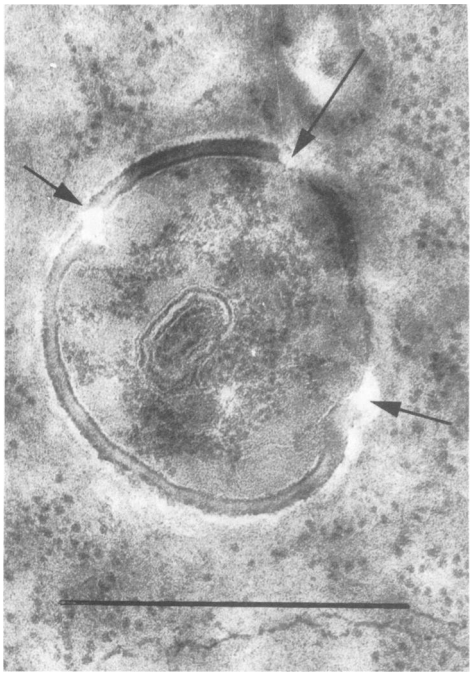
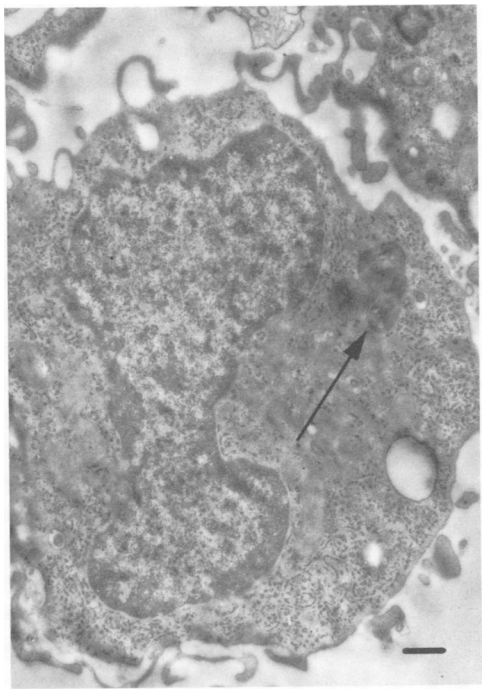


FIG. 7. *Staphylococcal cell of the avirulent strain seen in the cytoplasm of macrophage fixed 3 h after injection. The bacterium appears to be within the phagocytic vacuole, but the phagocytic membrane is not visible. Note the remarkable dissolution of the bacterial cell wall at several points as shown by arrows. Moreover, the intracellular area of the bacterium is filled with materials similar to the surrounding cytoplasmic materials of macrophage, suggesting that a large portion of the bacterial cellular constituents has been digested and absorbed.*

degenerative change was produced in the ingesting phagocytes.

After 6 h of infection, since phagocytic vacuoles showed a marked decrease in size, their limited membranes became directly apposed over the cell wall of the bacteria lodged therein (Fig. 10). The majority of bacteria within the vacuoles appeared to remain intact, but a few others showed several morphological changes, including deformity in the cell wall contour and a decrease in the density of the endoplasm of bacterial cells, which suggest their degradation. In such bacteria, however, the gross configuration was fairly well preserved. Many macrophages containing bacteria manifested some degree of cytoplasmic degeneration as revealed by disintegration of intracellular constituents. Occasionally, these macrophages, together with the bacteria present in their cytoplasms, were phagocytized by another macrophage (Fig. 11). After



24 h, such a degenerative change became more prominent (Fig. 12). On the other hand, no additional morphological change was noted with the bacteria ingested, suggesting their resistance to a further degradation.

DISCUSSION

In this laboratory, recent studies on experimental staphylococcosis have centered on the high-virulent strain (E46) because of its unique biological properties distinct from those of typical *S. aureus* and *S. epidermidis* strains (10, 13): its high-virulence for mice (11, 13), its resistance to phagocytosis in vivo and in vitro (12, 27), and its ability to produce specific cytotoxic substances (27). The present electron microscopic study provides additional information on the morphological aspect of the interaction be-

FIG. 8. *Macrophage fixed 24 h after injection of avirulent bacteria. No phagocytic vacuole or well-defined bacterial cell is seen in any macrophage. Only irregularly contoured structures of moderate density (arrow) occasionally occur intracellularly, probably a mixture of bacterial residues and some cytoplasmic materials of the host cell.*

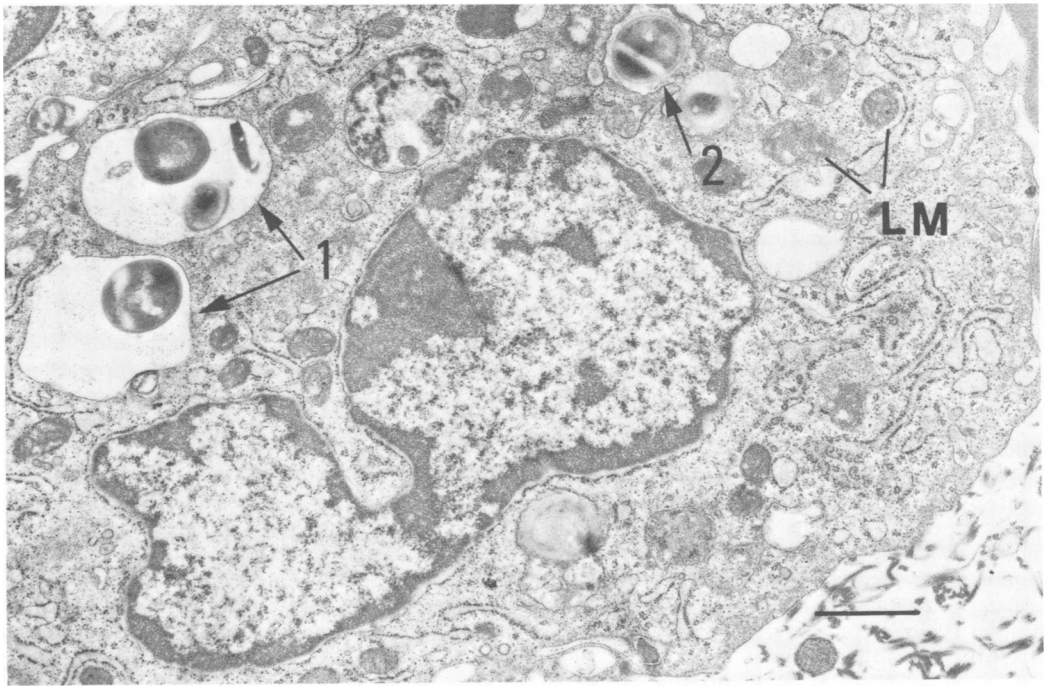


FIG. 9. *Macrophage fixed 30 min after injection of high-virulent bacteria. Phagocytic vacuoles of the loose type (arrow 1) and of the tight type (arrow 2), both containing bacteria, are seen. Peribacterial space is almost vacant. Numerous cytoplasmic vesicles (LM) remain in cytoplasmic loci near or distal to vacuoles, but no diffuse electron-dense material is evident in the vacuoles.*

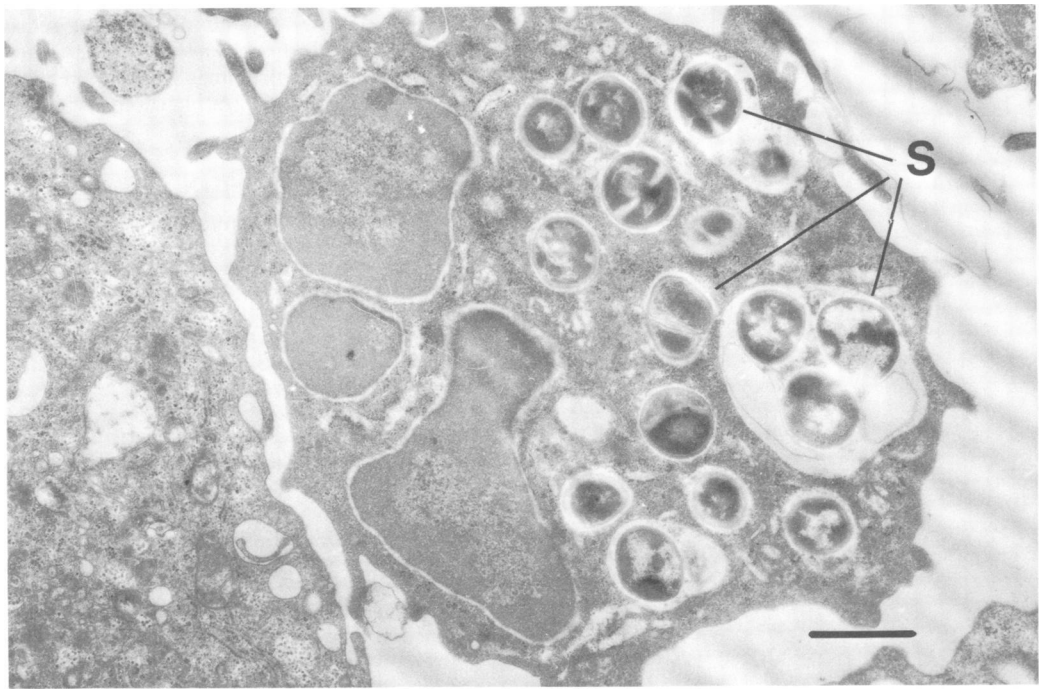


FIG. 10. *Macrophage fixed 6 h after injection of high-virulent bacteria. In some macrophages, some staphylococcal cells (S) are encountered within the well-defined phagocytic vacuoles. Most of these bacteria appear to be morphologically intact, with no sign of disintegration. On the other hand, the residual portion of the cytoplasm of ingesting macrophage shows several signs of degeneration, particularly disappearance of most cytoplasmic constituents, including cytoplasmic vesicles, mitochondria, endoplasmic reticulum, and other membranous organelles. Compare this degeneration with the cytology of the macrophage on the left side, which contains no bacterium.*

tween phagocytes and invading staphylococci that may be correlated with the outcome of the infection.

There is a general notion that the first stage of the intracellular digestion of phagocytized particles is the formation of phagocytic vacuoles in the cytoplasm of the phagocytes. Findings by electron microscopic techniques employed in various laboratories in the study of phagocytic processes occurring in peritoneal macrophages have indicated that distinct phagocytic vacuoles, which are larger in volume than the ingested particles, are usually formed after the engulfment of various pathogenic microorganisms (4, 8, 15, 19, 20). Dumont and Robert (4), who undertook a precise electron microscopic study on phagocytosis of *Histoplasma capsulatum* by hamster peritoneal macrophages, reported that the two types of phagocytic vacuoles are concurrently formed early in the infection: a "tight type" in which the limited membrane is directly apposed over the cell wall of the ingested microorganism and a "loose type" in which a wide space is present between the limited membrane and the cell wall of the organism. The results obtained in the present study show that phagocytic vacuoles of both the loose and tight types formed after ingestion of high-virulent staphylococci were characterized by the specific nature of peribacterial space that was occasionally empty or, otherwise, contained a relatively small quantity of amorphous, floccular, or membranous materials. In contrast, phagocytic vacuoles formed after ingestion of avirulent staphylococci were mostly of a smaller tight type, and peribacterial space occurring therein was most often surrounded by a halo of amorphous or fine granular materials of moderate density. Such materials appear compatible with the contents of cytoplasmic vesicles. Esner and Novikoff (5) obtained evidence by means of histochemical electron microscopy that most of the cytoplasmic vesicles revealed by electron microscopy are lysosomes. This suggests the possibility that the electron-dense, amorphous materials deposited around some of the ingested bacteria as observed in the present study may represent concentrated digestive enzymes of lysosomal origin. Thus, it follows that avirulent staphylococci are generally exposed to a greater concentration of lysosomal enzymes than are virulent staphylococci. This assumption can well account for our other

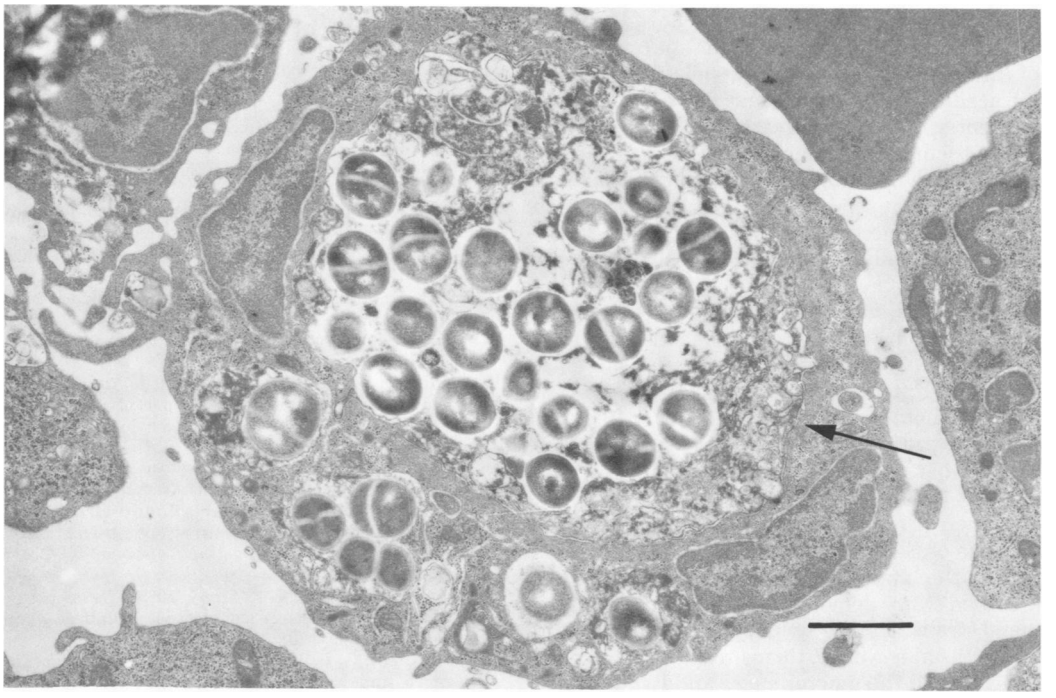


FIG. 11. *At 8 h after injection of virulent bacteria. A degenerated but complete phagocyte (macrophage) containing multiple unaltered staphylococcal cells has been phagocytized by another macrophage (arrow). In addition, several bacteria were ingested by the macrophage and are present within phagocytic vacuoles.*

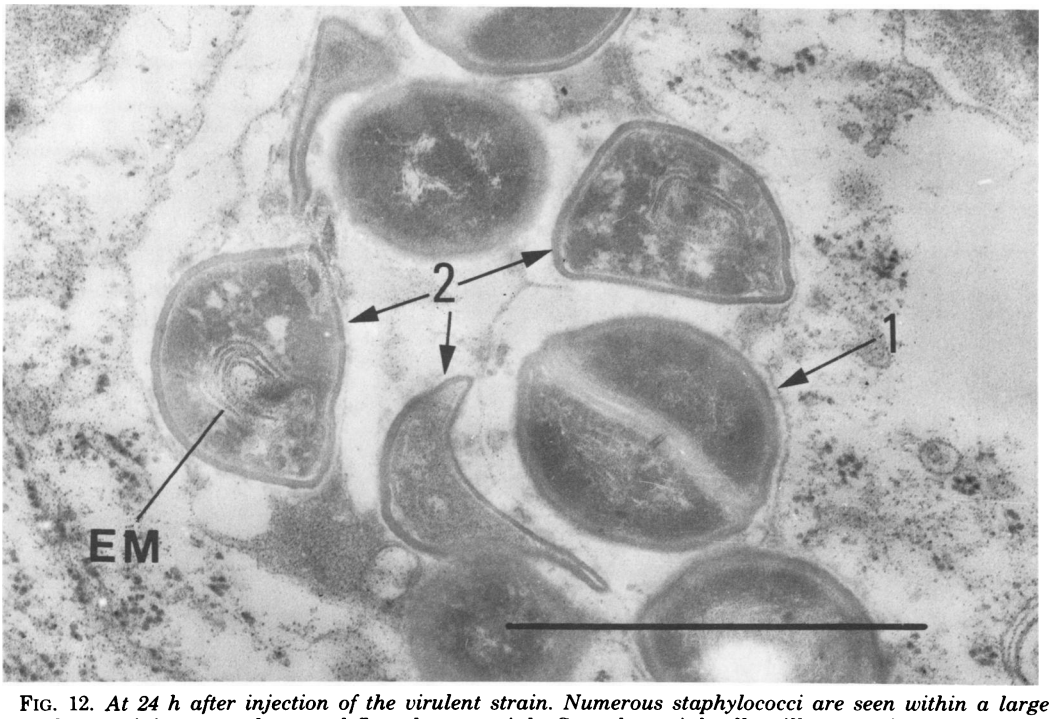


FIG. 12. *At 24 h after injection of the virulent strain. Numerous staphylococci are seen within a large vacuole containing amorphous and floccular materials. Some bacterial cells still appear intact (arrow 1), but others show marked deformity in cell contour (arrow 2). Even in the latter, however, the configuration of cell walls and the intracellular organization are fairly well preserved, with prominent appearance of endoplasmic membrane systems (EM).*

electron microscopical findings that of the three strains of *Staphylococcus* under study, bacteria of the avirulent strain are the most liable to the intracellular degradation and those of the high-virulent strain are the least, and there is a frequent occurrence of bacterial cells with the figure of cell division upon infection with the high- or low-virulent strains but not so upon infection with the avirulent strain. Virulent bacteria persisting intracellularly may adversely affect the macrophages that have phagocytized them. All these electron microscopical data are favorably consistent with our preceding bacteriological as well as photomicroscopical data, showing that the high-virulent strain (E46) is more resistant to phagocytosis and intracellular killing by mouse peritoneal macrophages than are the avirulent strain of *S. epidermidis* and the low-virulent Smith-type strain of *S. aureus* (27). However, it remains to be determined how the bacteria of E46 withstand degradation processes within the macrophages. In this respect, our previous study demonstrates that this high-virulent strain has the ability to produce certain low-molecular-weight toxins that are selectively toxic toward the macrophages and lymphocytes of mice and probably other animals (27). The chemical and biological characterization of these products is currently in progress.

LITERATURE CITED

1. **Cohn, Z. A.** 1963. Determinants of infection in the peritoneal cavity. 1. Response to and fate of *Staphylococcus aureus* and *Staphylococcus albus* in the mouse. Yale J. Biol. Med. **35**:12-28.
2. **Cohn, Z. A., J. G. Hirsch, and M. E. Fedorko.** 1966. The *in vitro* differentiation of mononuclear phagocytes. IV. The ultrastructure of macrophage differentiation in the peritoneal cavity and in culture. J. Exp. Med. **123**:747-766.
3. **Diengdoh, J. V., and J. L. Turk.** 1965. Immunological significance of lysosomes within lymphocytes *in vitro*. Nature (London) **207**:1405-1406.
4. **Dumont, A., and A. Robert.** 1970. Electron microscopic study of phagocytosis of *Histoplasma capsulatum* by hamster peritoneal macrophages. Lab. Invest. **23**:278-286.
5. **Essner, E., and A. B. Novikoff.** 1962. Cytological studies on two functional hepatomas. Interrelations of endoplasmic reticulum, Golgi apparatus, and lysosomes. J. Cell Biol. **15**:289-312.
6. **Goodman, J. R., and R. E. Moore.** 1956. Electron microscopic study of phagocytosis of staphylococcus by human leukocytes. J. Bacteriol. **71**:547-556.
7. **Goodman, J. R., R. E. Moore, and R. F. Baker.** 1957. Electron microscopic study of Phagocytosis of staphylococcus by human leukocytes. II. Virulent and nonvirulent staphylococci. J. Bacteriol. **72**:736-745.
8. **Hard, G. C.** 1969. Electron microscopic study of the differentiation of mouse peritoneal macrophages stimulated by *Corynebacterium ovis* infection. Lab. Invest. **21**:309-315.
9. **Horn, R. G., M. G. Koenig, J. S. Goodman, and R. D. Collins.** 1969. Phagocytosis of *Staphylococcus aureus* by hepatic reticuloendothelial cells. Lab. Invest. **21**:406-414.
10. **Iwata, K., and T. Eda.** 1968. Studies on unique staphylococcal strains exhibiting high virulence for mice by intraperitoneal inoculation. 1. Morphological and biological properties of the strains. Jpn. J. Bacteriol. **23**:165-171.
11. **Iwata, K., and T. Eda.** 1968. Studies on unique staphylococcal strains exhibiting high virulence for mice by intraperitoneal inoculation. 2. Virulence of the strains. Jpn. J. Bacteriol. **23**:348-354.
12. **Iwata, K., and T. Eda.** 1968. Studies on unique staphylococcal strains exhibiting high virulence for mice by intraperitoneal inoculation. 3. Experimental analyses on virulence. Jpn. J. Bacteriol. **23**:392-400.
13. **Iwata, K., T. Eda, S. Kimura, and I. Aoyama.** 1967. Studies on the extracellular enzymes of staphylococci. I. Identification of pathogenic staphylococci by use of the deoxyribonuclease test. Jpn. J. Bacteriol. **22**:577-582.
14. **Kapral, F. A.** 1965. The phagocytosis and intracellular fate of staphylococci. Ann. N.Y. Acad. Sci. **182**:285-300.
15. **Karlsbad, G., R. W. I. Kessel, S. de Petris, and L. Monaco.** 1964. Electron microscope observations of *Brucella abortus* grown within monocytes *in vitro*. J. Gen. Microbiol. **35**:383-390.
16. **Koenig, M. G., and M. A. Melly.** 1965. The importance of surface antigens in staphylococcal virulence. Ann. N.Y. Acad. Sci. **128**:231-250.
17. **Mackaness, G. B.** 1960. The phagocytosis and inactivation of staphylococci by macrophages of normal rabbits. J. Exp. Med. **112**:35-53.
18. **Melly, M. A., J. B. Thomison, and D. E. Rogers.** 1960. Fate of staphylococci within human leukocytes. J. Exp. Med. **112**:533-558.
19. **North, R. J., and G. B. Mackaness.** 1963. Electronmicroscopical observations on the peritoneal macrophages of normal mice and mice immunized with *Listeria monocytogenes*. I. Structure of normal macrophages and the early cytoplasmic response to the presence of ingested bacteria. Br. J. Exp. Pathol. **44**:601-611.
20. **Pearson, G. R., B. A. Freeman, and W. D. Hines.** 1963. Thin section electron micrographs of monocytes infected with *Brucella suis*. J. Bacteriol. **86**:1123-1125.
21. **Rogers, D. E., and M. A. Melly.** 1960. Further observations on the behavior of staphylococci within human leukocytes. J. Exp. Med. **111**:533-558.
22. **Rogers, D. E., and R. Tompsett.** 1952. The survival of staphylococci within human leukocytes. J. Exp. Med. **95**:209-230.
23. **Shayegani, M. G., and F. A. Kapral.** 1962. The eventual intracellular destruction of staphylococci by mononuclear cells. J. Gen. Microbiol. **29**:637-644.
24. **Suganuma, A., Y. Yokota, K. Oishi, and H. Morioka.** 1976. The cell envelope of staphylococci—electron microscopy, p. 451-456. *In* J. Jeljaszewicz (ed.), Staphylococci and staphylococcal diseases. Gustav Fischer Verlag, Stuttgart, Germany.
25. **Suter, E.** 1956. Interaction between phagocytes and pathogenic microorganisms. Bacteriol. Rev. **20**:94-132.
26. **Suter, E., and H. Ramseier.** 1964. Cellular reactions in infections. Adv. Immunol. **4**:117-173.
27. **Yamaguchi, H., Y. Nozu, Y. Kanda, K. Iwata, T. Sato, and H. Seto.** 1975. Interaction of staphylococci with mouse peritoneal macrophage *in vivo* and *in vitro*, p. 203-211. *In* T. Hasegawa (ed.), Proceedings of the 1st Intersectional Congress of the International Association of Microbiological Societies, Tokyo, 1974, vol. 3. Science Council of Japan, Tokyo.