ELECTRON MICROSCOPIC STUDY OF PHAGOCYTOSIS OF STAPHYLOCOCCUS BY HUMAN LEUKOCYTES

II. Virulent and Non-Virulent Staphylococci

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Many studies have been published on the relationship between the infective agents and the host cells. Studies on phagocytosis have reported an increase of viable bacteria from virulent strains and a decrease in non-virulent strains (Rogers and Tompsett, 1952). Only recently has it been possible to "visualize" the interaction of bacteria and host leukocytes at high magnifications and compare the resulting action to virulence and *in vitro* tests.

Work in this laboratory has shown the relationship between leukocytes and ingested bacteria in electron micrographs (Goodman and Moore, 1956). The same techniques have now been used to observe the contrast between a virulent coagulase and mannitol positive hemolytic *Staphylococcus aureus* strain and an attentuated non-virulent coagulase and mannitol-negative nonhemolytic strain.

The relationship of these bacterial strains of contrasting virulence to the leukocytes that have phagocytized them were examined at 20,000 to 30,000 magnification in the electron microscope using thin sectioning techniques.

MATERIALS AND METHODS

Leukocytes were obtained by venipuncture from an individual who was afebrile and had not recently received antibiotics. The sterile specimen was heparinized and centrifuged at 800 rpm for 15 min. The plasma containing the leukocytes, platelets and a few erythrocytes was transferred with a pipette into a sterile glass tube. An aliquot (0.5 ml) for a control specimen was removed and incubated along with inoculated suspensions of cells. The remaining suspension of leukocytes was divided into two equal volumes. The first of these was inoculated with a concentrated suspension (0.3 ml of a bacterial suspension) from a rapidly growing culture of virulent *S. aureus*. This culture had been transferred once from a colony freshly isolated on blood agar from a patient with a deep subcutaneous abscess. The second transfer was grown for 5 hr in nutrient broth at 37 C. This organism produced α-hemolysin and was coagulase and mannitol positive.[1]

The second portion of the suspension of leukocytes was inoculated with a 5-hr-old concentrated suspension of a rapidly growing non-virulent *S. aureus* and incubated at 37 C. This culture was a stock laboratory strain of long lineage on artificial media. This organism was nonhemolytic, coagulase and mannitol negative.[1] Control tubes of each bacterial and control suspension of leukocytes were also incubated with the inoculated suspensions of leukocytes.

Aliquots of 0.25 ml of the two inoculated suspensions of leukocytes were removed at 5, 15, 30 min, 1 and 2 hr intervals. These were fixed immediately in 1 per cent isotonic buffered (pH 7.4) osmium tetroxide. The leukocyte control and the two bacterial suspension controls were also fixed with osmium tetroxide after the 2-hr incubation period. All of these preparations were processed for examination on the electron microscope as described by Goodman and Moore (1956). Before sampling, care was taken to break up the clumps of cells that developed in the coagulase positive preparation after 30 min of incubation.

RESULTS

Virulent strain. Thin sections of neutrophils consistently showed excellent detail in the cytoplasm and nucleus (figure 1). Typical granules and an occasional rod-like mitochondrion were seen. These have been described previously by

[1] These cultures were characterized and supplied by Dr. Dexter Howard, Department of Infectious Diseases, UCLA School of Medicine.

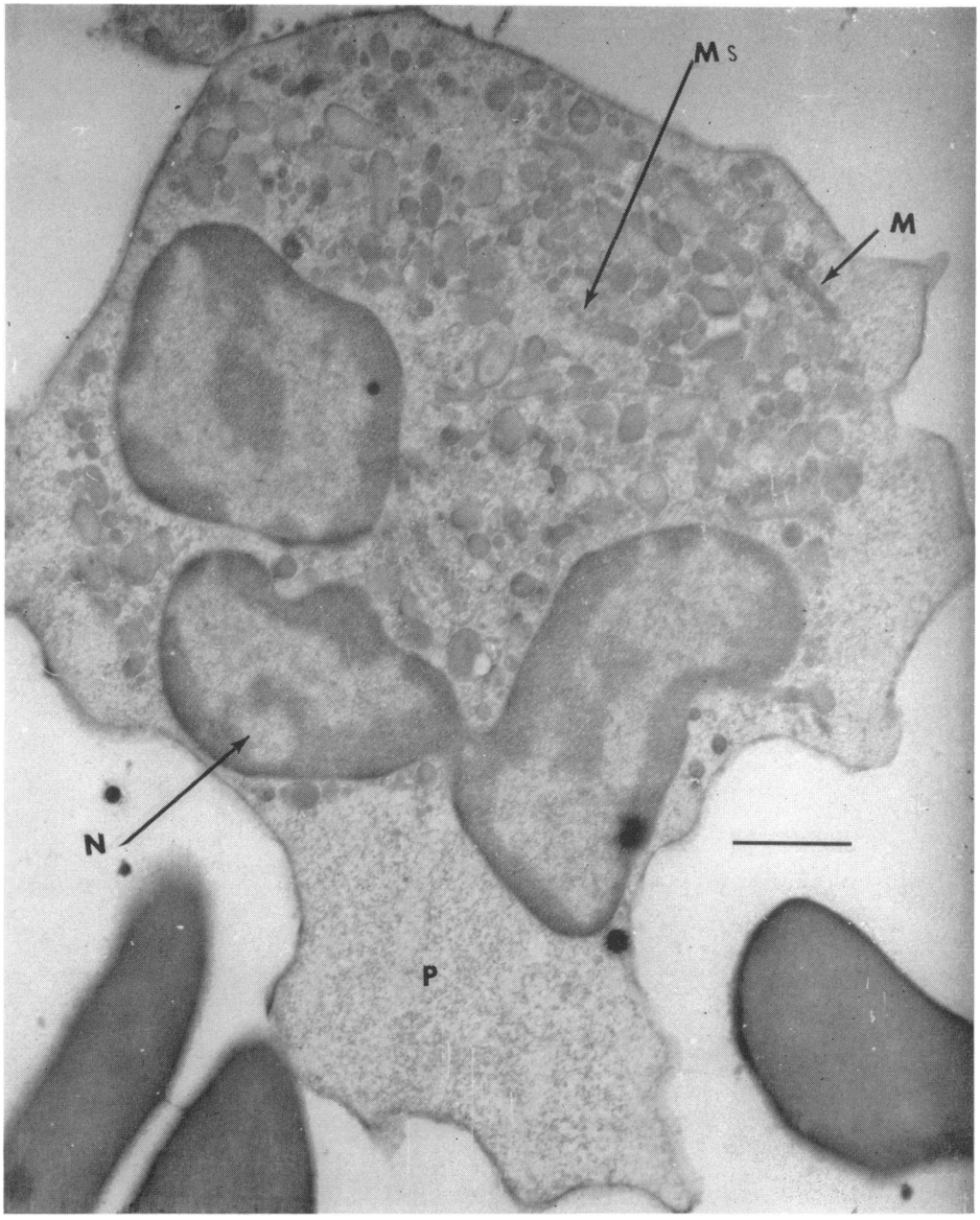

Figure 1. This is a normal neutrophil from the control specimen after 2 hr of incubation. The cell appears to have been unaffected by the incubation at 37 C. A normal pseudopod is present and the lobes of the nucleus have been transected in three places (*N*). Three red cells are seen at the top of the picture. An occasional rod type mitochondrion (*M*) is seen along with many microsomes (*Ms*). The two dense black dots in the nucleus and one in the pseudopod near the nucleus are artifacts. (Black line at bottom of this and following figures represents one micron.)

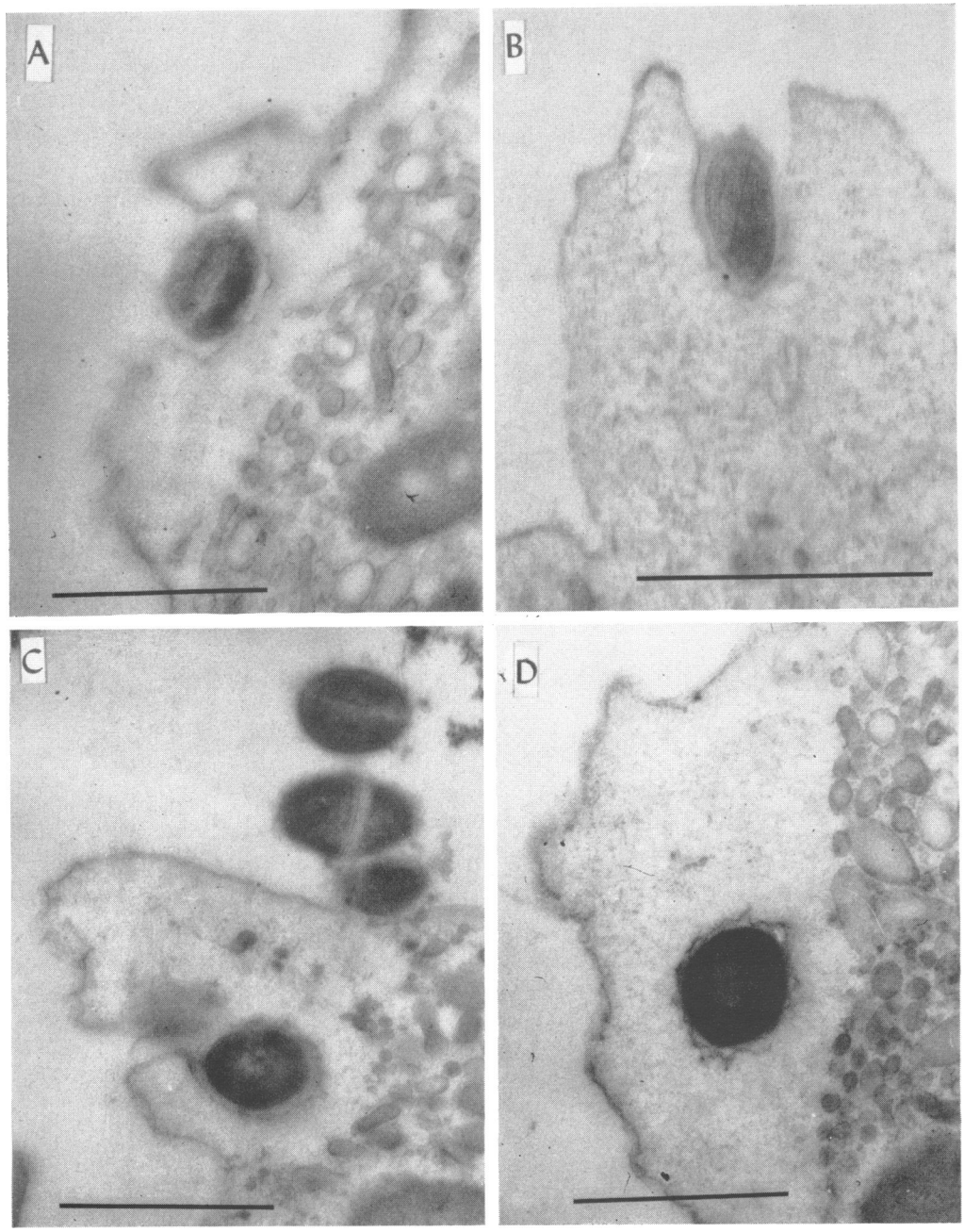

Figure 2. This panel shows four stages in the engulfing of the bacteria. *A:* A small pseudopod has been put out by the neutrophil and upon contact with the bacterium has indented to start the engulfing process. *B:* Here the two sides of the pseudopod have been extended further and the bacterium well settled within the invagination of the leukemic cell wall. *C:* Numerous bacteria are still outside this cell while the one at the bottom is nearly completely enclosed. The two parts of the pseudopod are about to coalesce. *D:* This bacterium is completely within the pseudopod with the invagination completely healed.

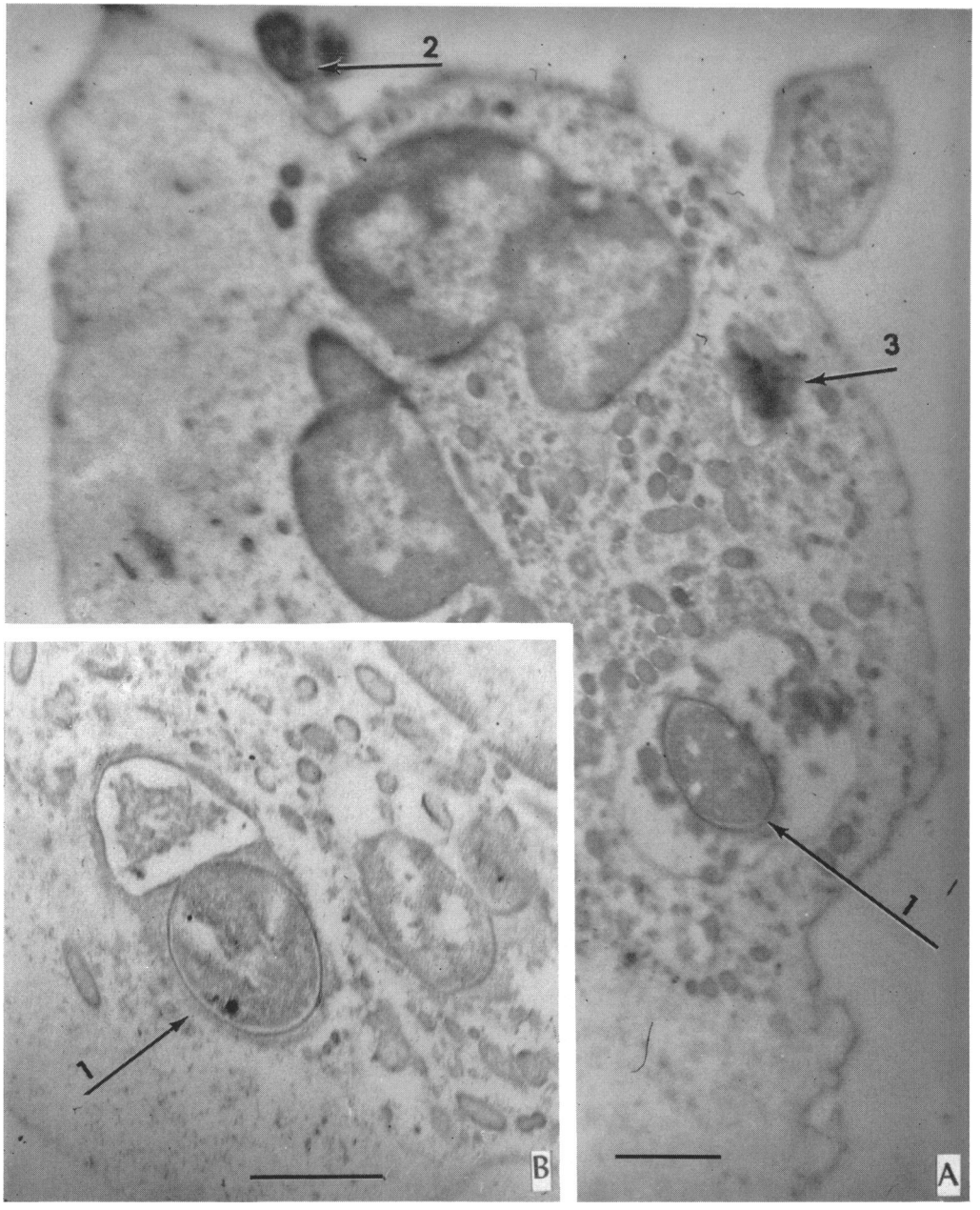

Figure 3. A: This neutrophil was incubated for 30 min with the non-virulent bacterial suspension. A disintegrating bacterium is seen at arrow *1*. A convenient comparison for size and density can be made to the free bacteria seen at arrow *2*. Here a bacterium is seen just starting to contact the surface of the neutrophil, even though they had been mixed for 30 min. A recently ingested bacterium is seen at *3*, although a poor cut has made it a little indistinct. *B:* This is a thinner section of another neutrophil showing bacterial degradation within 30 min. Greatly distended pale remains of bacteria are seen at arrow *1*. The bacterial cell wall is still discernible although vacuolation of the bacteria is well developed.

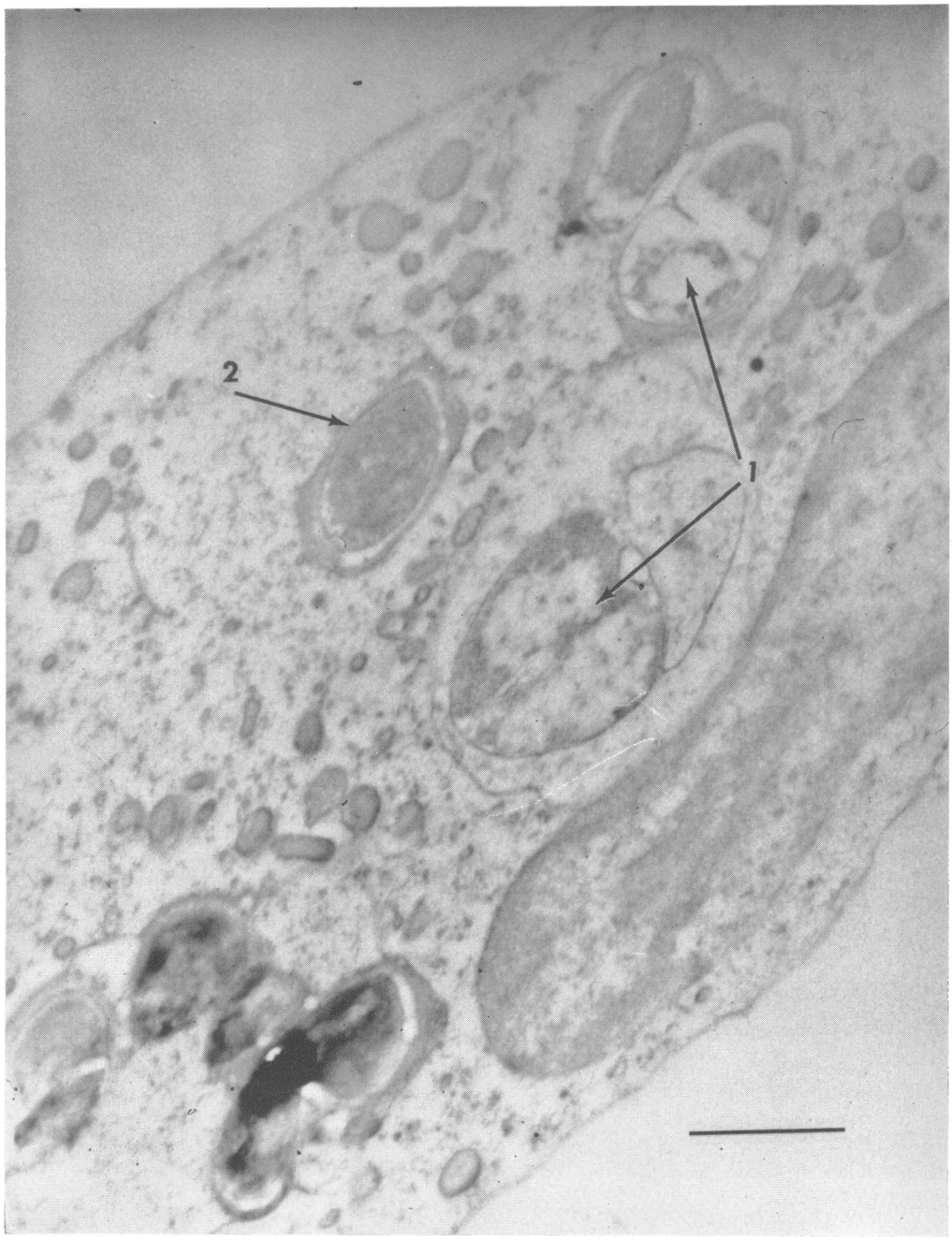

Figure 4. A neutrophil after 1 hr of incubation with the non-virulent strain of staphylococcus. Several stages of bacterial disintegration are evident. Greatly vacuolated and distended areas are shown by arrow *1*. Less advanced destruction is seen at *2*. An increased thickening of dense material is seen around some of the degenerating bacteria.

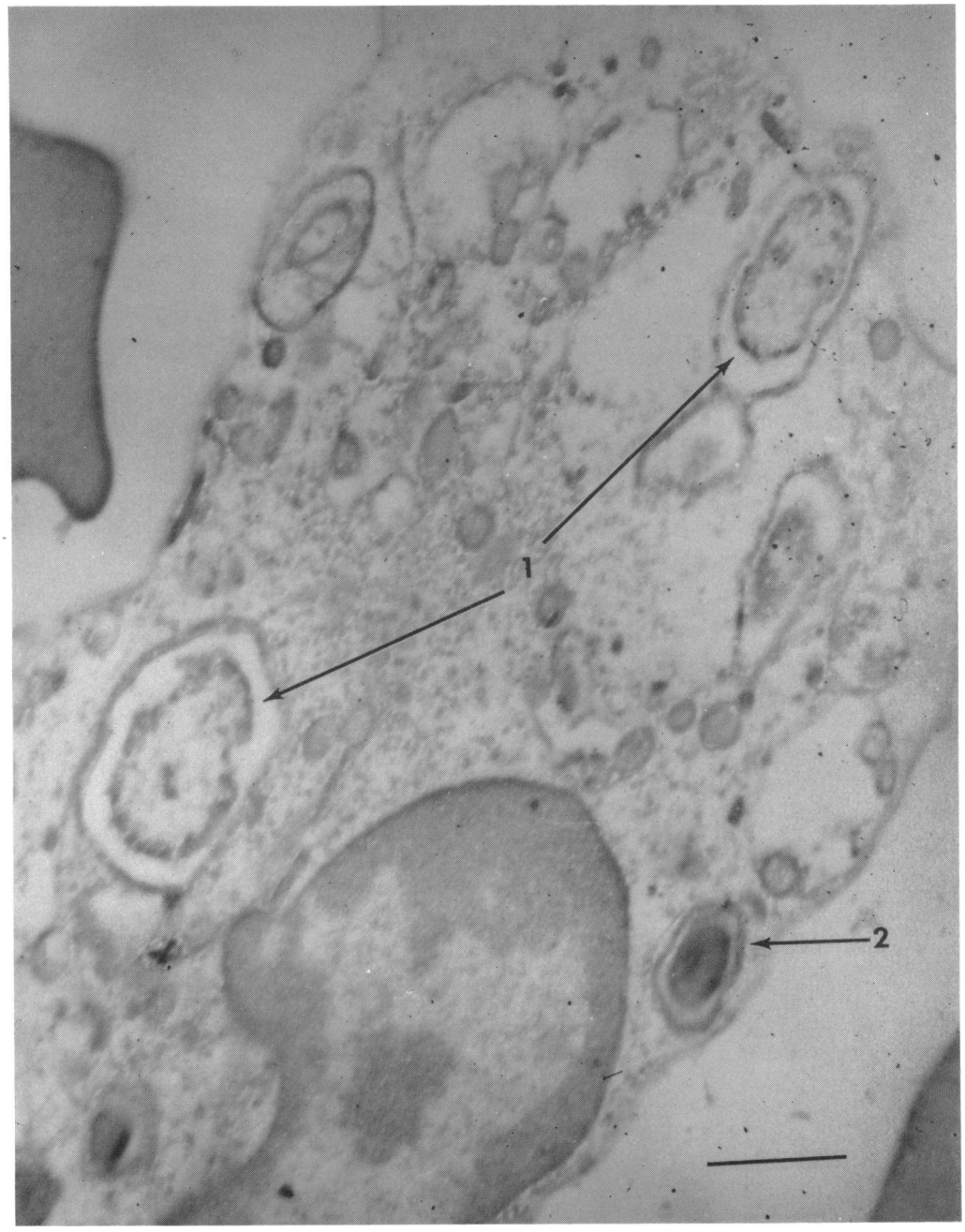

Figure 5. A neutrophil with ingested non-virulent organisms. Destruction is nearly complete in several areas (arrow *1*). The thick shell about the degraded bacteria is well developed in this neutrophil. A partially digested bacterium is seen at arrow *2*.

the authors. The action between the leukocytes and the virulent organisms was very similar to that presented in the previous series (Goodman and Moore, 1956). The bacterial organisms were readily ingested by the leukocytes as shown in figure 2. This virulent strain also multiplied within the neutrophils after they were ingested and the bacteria were able to destroy the white cells. The interaction between the white cell and the bacteria after ingestion will depend on the virulence of the bacteria and the ability of the neutrophil to resist either the bacterial growth or toxins. In the previously reported series neutrophils were rapidly destroyed. Either the strain of organism used for this study was less toxic or the person who supplied the neutrophils was more resistant to this strain. There were many neutrophils destroyed by the virulent organism and a great abundance of free bacteria were present after the 2-hr incubation. An occasional neutrophil was seen to be still intact with bacteria present in the cytoplasm. This can be contrasted with the almost complete absence of intact neutrophils and a great excess of free bacteria after 1 hr of incubation with the strain of *Staphylococcus aureus* reported in the previous publication.

Non-virulent strain. The non-virulent laboratory stock strain of *S. aureus* was readily ingested by the neutrophils. Sections from the 5- and 15-min incubation were nearly identical with the corresponding specimens of the virulent strain. Free bacteria were frequently seen in the "5-min" specimens and occasionally in the "15-min" preparation. There was an increased number of bacteria in the cytoplasm of the neutrophils with increased time of incubation.

Sections through leukocytes that had been incubated with non-virulent bacteria for 30 min showed numerous bacteria within their cytoplasm. By this time the leukocytes had started to destroy the ingested bacteria (figure 3). The first clearly demonstrable change was a swelling of the bacterium. Along with this increase in size there was a decrease in osmiophilic or dense material comprising most of the bacterial cytoplasm. Some bacteria continued to enlarge and become quite electron-transparent. Other individuals developed vacuoles within the bacterium as the swelling proceeded. In either case the bacterial cell wall retained its relatively dense appearance and was apparently the last portion of the bacterium to lose its identity, although it too had been greatly extended in the swelling process (figure 4). The loss of density by the bacterial cytoplasm may be a manifestation of the swelling and may not represent loss of material. The loss of density may also be caused by a change in the chemical nature of the material due to the digestive action of the leukocyte. This latter concept seems more tenable in view of the retention of density by the bacterial wall.

Distention of the bacteria may be fairly well advanced in the "30-min" specimen. However, in the 1- and 2-hr specimens nearly all stages of this process could be seen. Many of the areas surrounding disintegrating bacteria had an increasingly thick dense shell of fine grain material (figures 4 and 5). This was apparently built up by the leukocyte as the bacterium was destroyed. It was too thick to be considered a vacuolar membrane as these were usually quite thin. This dense material was not seen in our preparations except in the area surrounding disintegrating bacterium. The chemical nature and physiological function of this ring are not known.

DISCUSSION

These preparations have shown the two contrasting processes that follow the phagocytosis of a bacterium. In the case of the virulent organism the bacterium was seen to multiply and destroy the leukocyte (figure 6). The non-virulent bacterium on the other hand was destroyed by the white cells from the same human suspension that was demolished by the virulent strain. This was consistent with the light microscopic and bacteriological data of Rogers and Tompsett (1952). It was possible, upon re-examination of the electron micrographs, to observe in the virulent study some defense action of the leukocytes against the bacteria. In this series the action of the leukocyte against the virulent bacteria could be seen in figure 7. Here the decrease in density and swollen nature of the bacteria were similar to the early stages of the action of the leukocytes on the non-virulent organisms. The difference in density could not be attributed to a difference in thickness of the section as adjacent bacteria were seen to be much darker. The affected bacterium was also larger than any cross section of other individual bacterium from this staphylococci strain as seen in this and other pictures. This state of bacterial

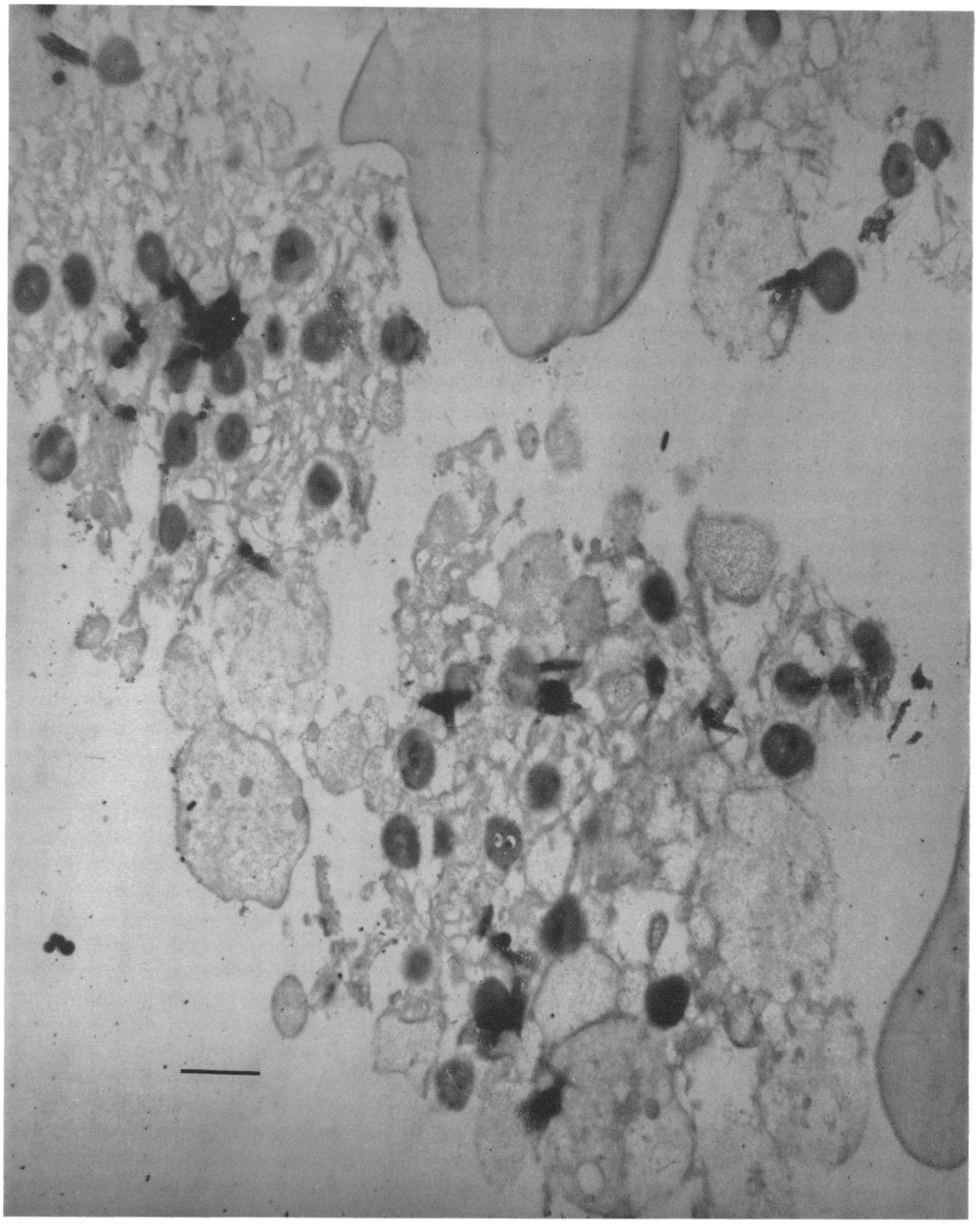

Figure 6. The destructive action of the virulent organism is clearly demonstrated. There are many intact dense bacteria scattered throughout the fragmentary remains of neutrophils. This is a common finding in the specimen that has been incubated for 2 hr and is in contrast to figure 1 which is a control neutrophil incubated 2 hr without either bacterial strain.

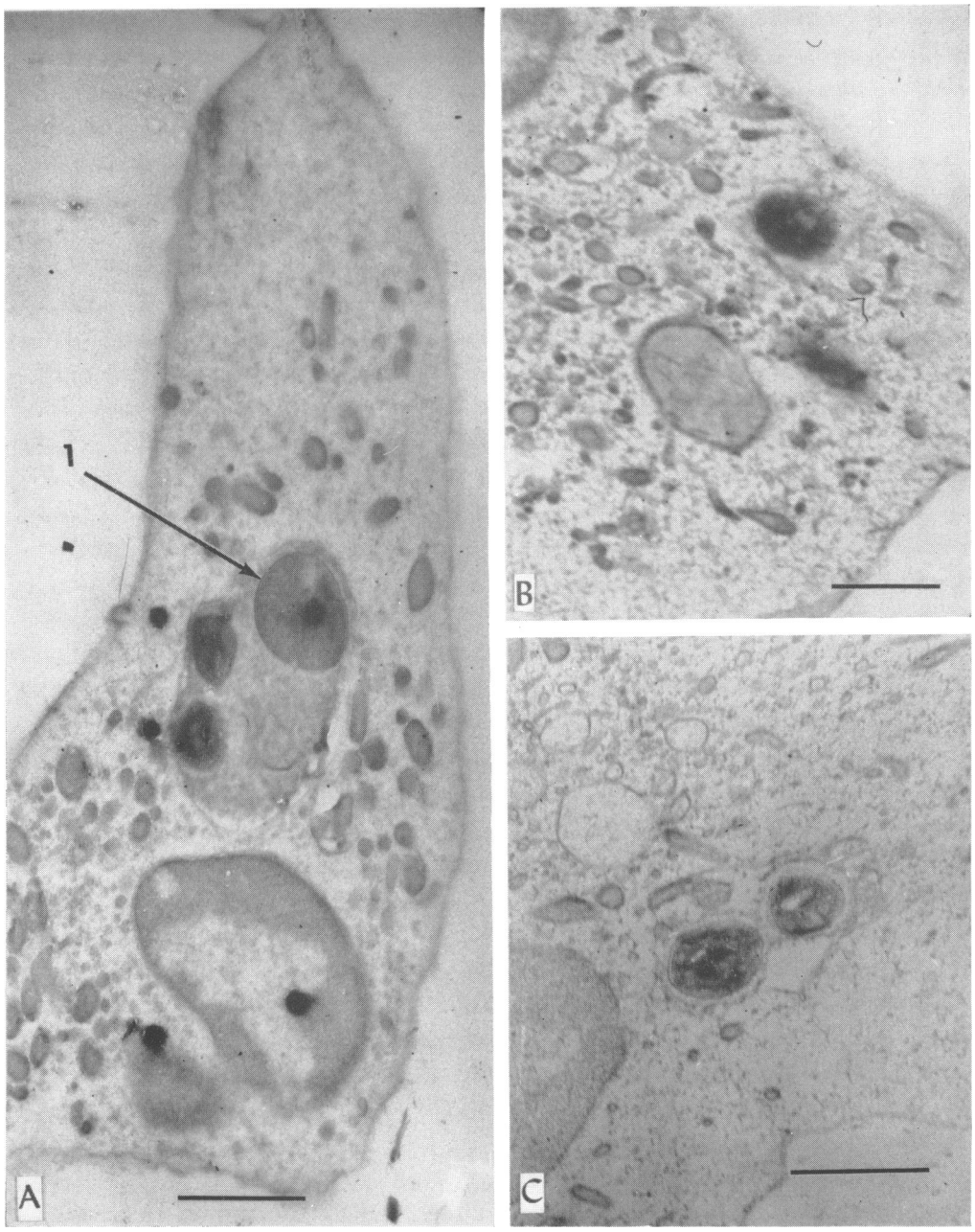

Figure 7. This panel shows evidence of the neutrophil's reaction against the virulent staphylococcus strain after 30 min incubation. *A:* The bacterium at arrow *1* appears swollen and relatively pale, resembling the non-virulent strain when the neutrophil has started to act upon it. Adjacent to this organism are two bacteria that as yet show little evidence of neutrophil action. *B* and *C:* These also show the effects of the neutrophil's action with increase in size and some evidence of vacuolation in the bacteria. Examples of virulent bacteria degradation by the neutrophils were not very common.

degradation was only occasionally seen and almost no virulent bacteria were observed in the last stages of disintegration as depicted in the non-virulent series.

The effort of the non-virulent organisms to survive within the cytoplasm of the leukocyte was not as easily evaluated. There were frequent examples of bacterial cells in the cytoplasm of the leukocyte clearly showing fission activity and apparent growth.

One can not be certain whether this fission process had preceded the act of ingestion or had developed after the bacterium was within the leukocyte. The close apposition of four bacteria that might be interpreted as two generations was not seen in any sections of the non-virulent series. This was seen occasionally in the virulent series where the bacteria were rapidly multiplying *in situ*.

The function of the morphologically identifiable components of the leukocyte in the process of bacterial digestion was not clearly demonstrated. Apparently the nucleus is not visually affected by either the destruction of the white cell by the virulent organisms or the leukocyte's digestion of the bacterium. In some few instances microsomes seemed to have increased about a vacuole. This concentration of microsomes was sufficiently infrequent to suggest that it was probably chance displacement caused by the presence of the vacuole. The cytoplasmic protein material is apparently the source of activity against the bacterium within the leukocyte. The development of an increasingly wide and dense shell about disintegrating bacteria seems to be due to the activity of the cytoplasmic protein substance. The shell may be derived from the leukocyte cytoplasm or from the interaction of the cytoplasmic protein and the disintegrated bacterial products. This thick shell-like structure was not seen in normal cells from the control suspension of leukocytes. Numerous other normal leukocytes not involved in this study of phagocytosis have not produced structures similar to those that develop about a destroyed bacterium. The lack of physical orientation of the granular components about the disintegrating bacteria does not necessarily imply that they do not have a functional relationship to this process. Other approaches to the functional relationship of the various cellular components to the cytological degradation of bacteria may be more productive than this present study.

The role of specific antibodies in the morphology of phagocytosis and the fate of leukocytes or bacteria were not examined in this study.

SUMMARY

An electron micrographic study was made of the interaction of virulent and non-virulent strains of *Staphylococcus aureus* with human leukocytes. After phagocytosis the virulent strains multiplied within the neutrophils and destroyed them. The non-virulent strain was destroyed by the neutrophil in some manner which involved a process of swelling and vacuolation of the bacterium. These processes and the role of the leukocyte cytoplasmic protein are discussed.

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