An Analysis of the Phagocytic Potential of Multinucleate Foreign Body Giant Cells

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Multinucleate giant cells were collected by subcutaneous implantation of plastic films into mice. The attached cells were challenged *in vitro* with staphylococci, yeasts and sheep erythrocytes treated with either glutaraldehyde or isologous or heterologous antiserum. Cells containing more than seven nuclei rarely phagocytized yeasts or staphylococci, and the uptake and ingestion of sheep erythrocytes treated with heterologous antiserum was equally infrequent. Many sheep erythrocytes treated with isologous antiserum or glutaraldehyde attached to giant cells. When the adherent erythrocytes were related to the increased size of the multinucleate cell by dividing the number attaching by the number of nuclei in the giant cell, a progressive relative reduction was demonstrated as the nuclear content increased. It is suggested that these phenomena are due to the loss of surface receptors subsequent to fusion during the formation of multinucleate cells. (Am J Pathol 78:343–358, 1975)

Multinucleate giant cells are observed in a wide variety of pathologic conditions, especially those in which inflammation is provoked. Virchow [1] and Langhans [2] have both discussed their significance, Langhans [2] also describing in some detail their participation in tuberculous and foreign-body reactions.

The origin of multinucleate giant cells from mononuclear precursors was first observed by Lambert [3] and later by the Lewises.[4,5] More recently, several investigators provided further evidence for their formation from mononuclear phagocytes.[6–9]

Such a derivation from mononuclear phagocytes suggests that multinucleate giant cells may be implicated in phagocytic phenomena. Faber [10] in 1893 injected colored agar into rabbits and concluded that phagocytosis by giant cells was feasible on finding the injected dye inside multinucleate cells surrounding the agar. Since these early investigations, no detailed analysis of the phagocytic potential of multinucleate foreign-body giant cells has been attempted. In this study,

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these cells were induced in mice by the subcutaneous implantation of plastic films,[11-14] and their phagocytic behavior was examined.

Materials and Methods

Mice

Young adult mice (weighing 18 to 25 g,) of the C57 Be/6 or Prince Henry strain were used.

Production of Giant Cells

Giant cell production was induced by implanting pieces of Melenex plastic film into the dorsum of mice.[11-14] The implants were removed 2 weeks after insertion, washed in Hanks' balanced salt solution and then maintained in Leighton tubes containing a medium composed of 85% Hanks' balanced salt solution, 10% fresh horse serum and 5% fetal calf serum, with penicillin (120 units/ml) and streptomycin (1 mg/ml). All specimens were used within 2 hours of explantation.

Phagocytic Tests

The following organisms were used: a) *Staphylococcus aureus,* and b) brewer's yeast *(Saccharomyces cervisiae).* In both instances a final concentration of 10^3/ml of each microorganism was prepared in nutrient medium, and the resulting suspensions were added to the Leighton tubes containing the explants. They were then incubated for 2 hours at 37 C.

Sheep red blood cells (SRBC) treated either with heterologous (rabbit) or isologous (mouse) SRBC antisera were added to the explants to produce a final concentration of 3%. These were incubated for 1 hour at 37 C.

Sheep red blood cells treated with glutaraldehyde and washed several times in normal saline were added to the explants to produce a final concentration of 3%. These were incubated for 1 hour at 37 C.

After incubation all preparations were vigorously washed in culture medium and then fixed in 4% buffered formaldehyde. Specimens were stained with a modified Gram-Twort technic and examined under oil immersion optics. Two parameters were assessed: a) the number of nuclei within a cell, and b) the number of ingested particles.

Samples from 6 to 10 mice were pooled for each agent tested. A total of 5 to 7 $\times 10^3$ cells were examined for each individual experiment, and the results were assessed statistically.

Electron Microscopy

Specimens were fixed in 2.5% glutaraldehyde in 0.05 M cacodylate buffer (pH 7.4) for 4 hours, postfixed in 1% osmium tetroxide in 0.1 M cacodylate buffer (pH 7.4), dehydrated in graded solutions of ethanol, and embedded in araldite. Sections were cut on an LKB ultramicrotome, stained with lead citrate, and examined with a Philips 301 electron microscope.

Results

The ingestion of microorganisms and the uptake or attachment of some of the variously treated SRBC occurred much less frequently in multinucleate giant cells containing more than ten nuclei (Table 1).

Table 1—Phagocytosis of Various Agents by Multinucleate Giant Cells

No. of nuclei within cells	No. of yeasts ingested/cell	No. of staphylococci ingested/cell	No. of SRBC sensitized with isologous antiserum attached/cell	No. of SRBC sensitized with heterologous antiserum attached/cell	No. of SRBC treated with glutaraldehyde attached/cell
1	7.7 ± 3.2	59.8 ± 20.5	10.6 ± 3.6	4.1 ± 1.4	7.9 ± 2.9
2	7.3 ± 3.4	61.4 ± 12.6	14.6 ± 4.2	3.6 ± 1.2	11.76 ± 3.5
3	6.7 ± 2.8	51.2 ± 12.1	17.6 ± 4.8	2.9 ± 1.3	15.6 ± 3.7
4	6.6 ± 2.2	46.1 ± 16.5	20.7 ± 6.9	2.6 ± 1.2	20.9 ± 5.2
5	6.9 ± 2.4	47.3 ± 14.3	22.3 ± 7.7	1.5 ± 1.2	21.5 ± 6.5
6	6.7 ± 2.7	48.3 ± 13.4	25.4 ± 8.4	1.1 ± 1.1	23.0 ± 4.2
7+	0.04 ± 0.32	3.2 ± 1.2	19.52 ± 10.9	0.3 ± 0.8	27.8 ± 23.3

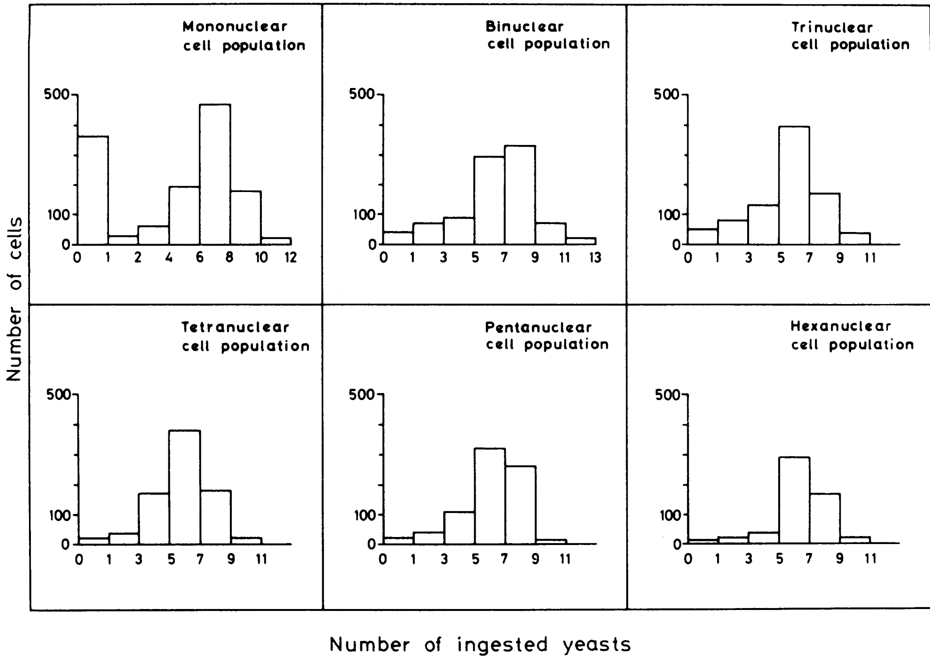
Syncytia with dozens of nuclei usually displayed either no or very few ingested organisms; their relative affinity for the SRBC preparations, especially those sensitized with heterologous antibody, was also diminished. As the number of multinucleate giant cells with more than six nuclei was small, a detailed statistical analysis of the results was not possible on multinucleate cells possessing seven or more nuclei. Consequently, the data for multinucleate cells with seven or more nuclei were pooled.

Twenty to forty percent of the mononuclear cell population attached to an implant failed to ingest microorganisms or to show attachment to any of the variously treated SRBC (Text-figures 1–5). Although this suggests the presence of a distinct subpopulation among the adherent mononuclear cells, the data for all mononuclear cells were nevertheless pooled, since the only definitive feature distinguishing this population was the single nuclei.

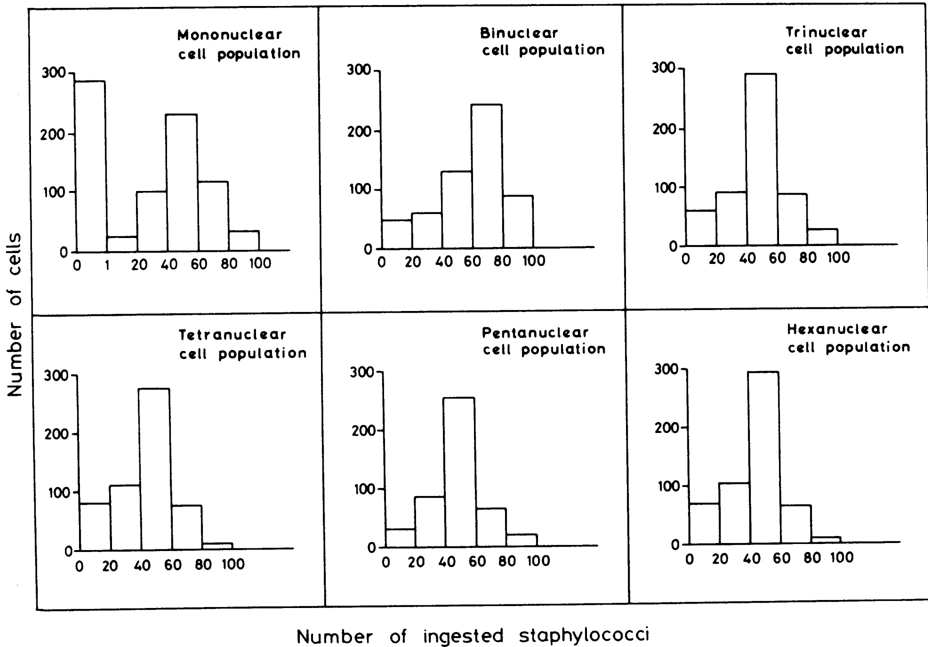
The results obtained are shown in Text-figures 1–5, and these were compared and contrasted with those from the multinucleate giant cell population.

Since the size of multinucleate giant cells increases with the nuclear number, the ratio of ingested microorganisms to this number was calculated to provide an index of phagocytic potential relative to size (Text-figures 6 and 7). In experiments with SRBC a similar index was calculated, but, as ingestion or attachment could not be differentiated, the total number of erythrocytes surrounding a giant cell was used in the mathematical assessment (Text-figure 8).

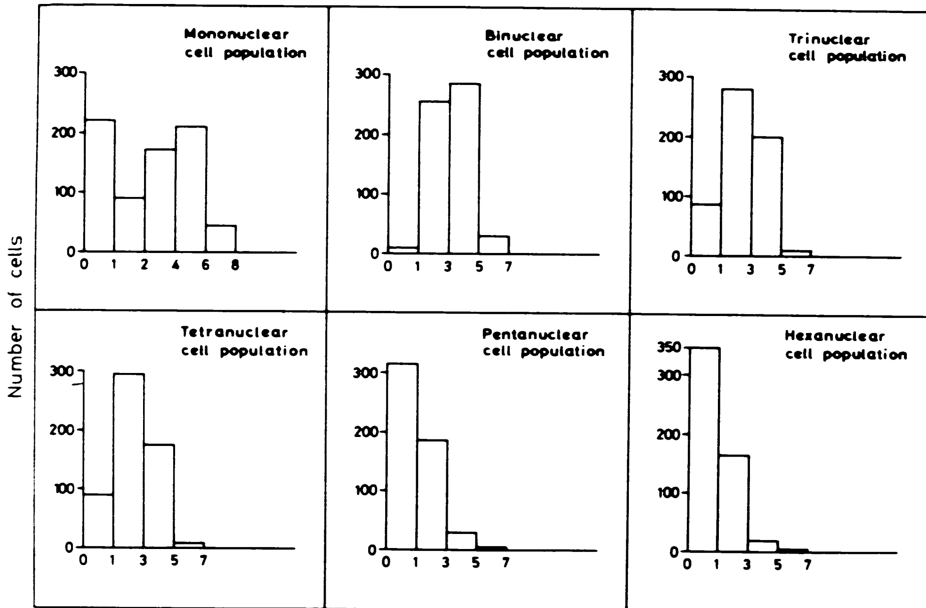
As shown in Table 1 and Text-figures 1 and 2, the total number of ingested microorganisms in cells containing two to six nuclei is similar, but a precipitious twentyfold decrease occurs when the nuclear number



TEXT-FIG 1—The distribution of ingested yeasts in cells possessing different numbers of nuclei.

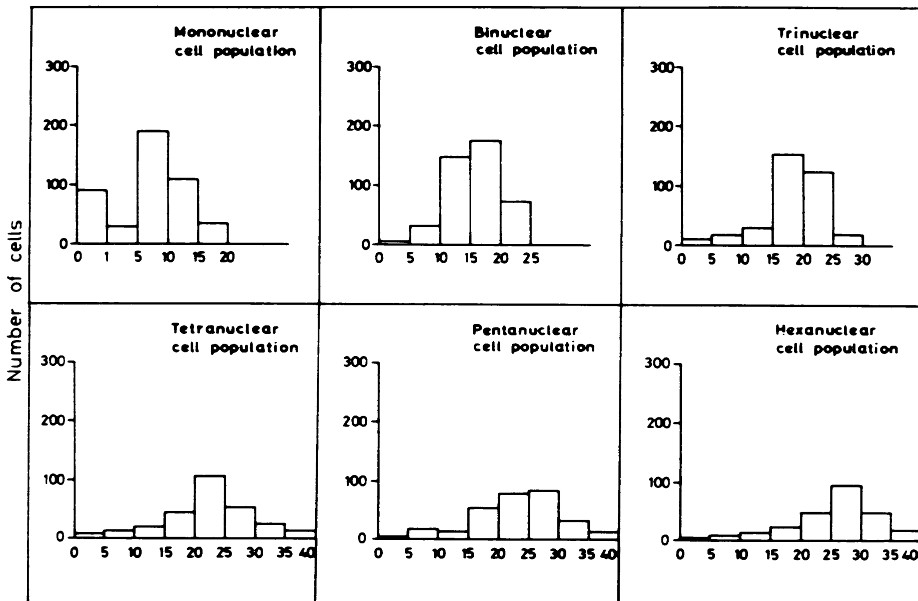


TEXT-FIG 2—The distribution of ingested staphylococci in cells possessing different numbers of nuclei.



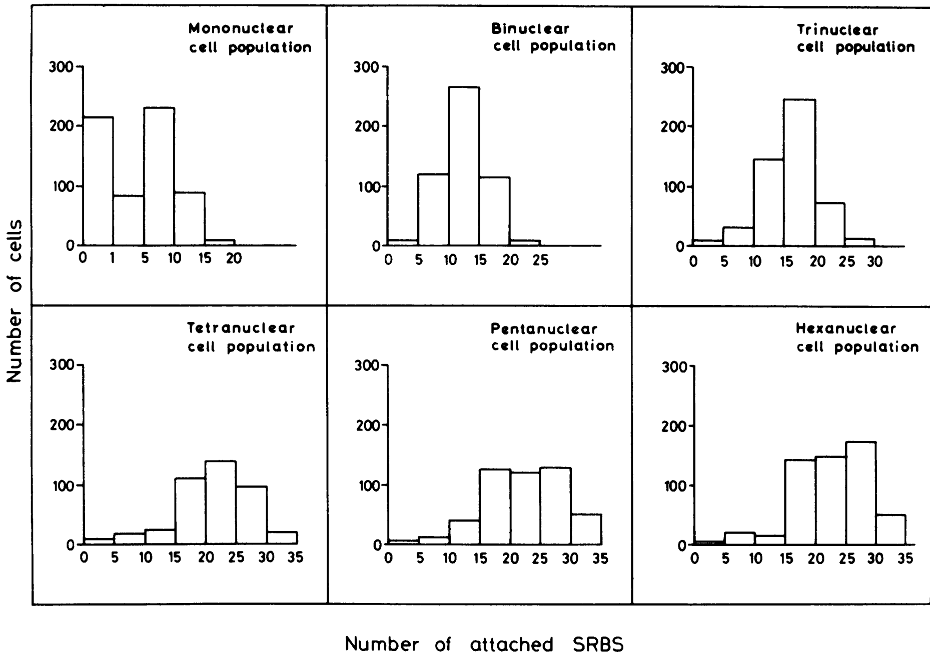
Number of attached SRBS

TEXT-FIG 3—The distribution of SRBC sensitized with heterologous antiserum in cells possessing different numbers of nuclei.



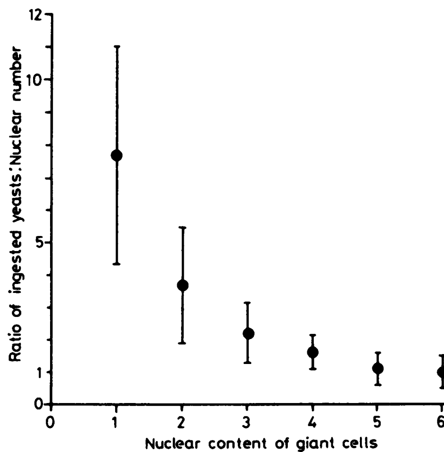
Number of attached SRBS

TEXT-FIG 4—The distribution of SRBC sensitized with isologous antiserum on cells possessing different numbers of nuclei.

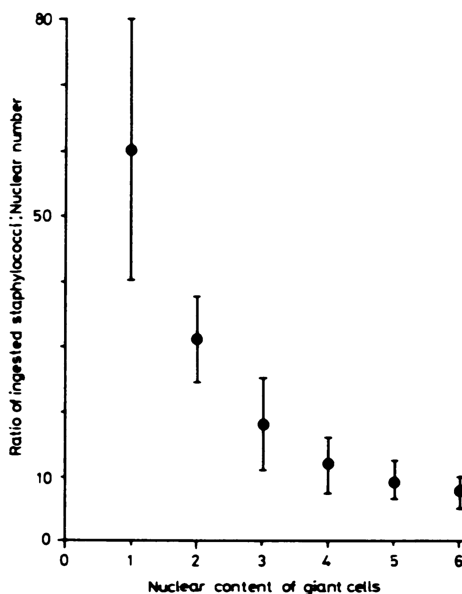


TEXT-FIG 5—The distribution of SRBC treated with glutaraldehyde on cells possessing different numbers of nuclei.

exceeds six. Should the number of ingested organisms be related to the size of the giant cells (by dividing the number of ingested organisms by the nuclear number), then this index indicates an exponential reduction in phagocytic potential relative to size (Text-figures 6 and 7). In both instances (phagocytosis of yeasts and staphylococci), a seven-



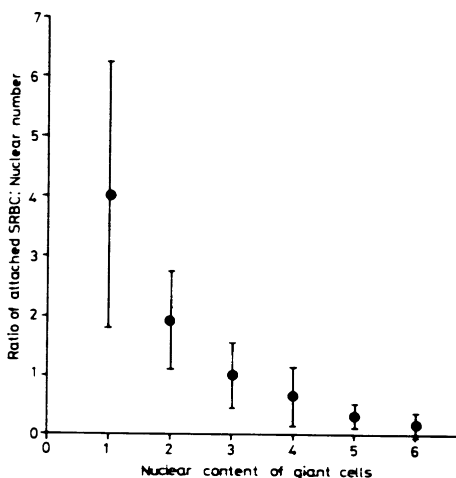
TEXT-FIG 6—Progressive decrease in the ratio of ingested yeasts:nuclear number as the number of nuclei within a cell increases.



Text-fig 7—Progressive decrease in the ratio of ingested staphylococci:nuclear number as the number of nuclei within a cell increases.

to eightfold decrease in phagocytic potential is found between the mononuclear and hexanuclear cell populations (Text-figures 6 and 7).

Even though similar concentrations of SRBC were used in different experiments, the numbers attaching after treatment with heterologous antiserum were consistently smaller than when the SRBC were pretreated with either isologous antibody or glutaraldehyde (Table 1, Text-figures 3 to 5). Moreover, the characteristics of attachment to giant cells by SRBC sensitized with heterologous antibody differed from those treated otherwise.



Text-fig 8—Progressive decrease in the ratio of attached SRBC sensitized with heterologous antiserum:nuclear number as the number of nuclei within a cell increases.

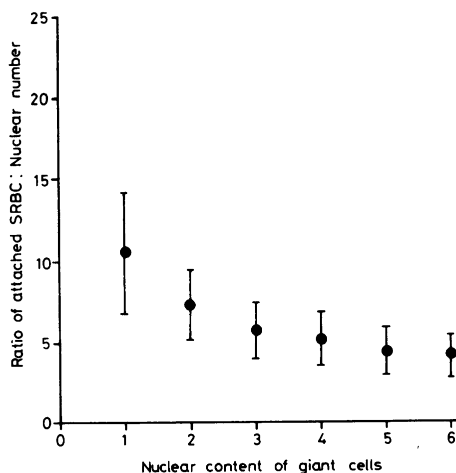
The absolute number of attached SRBC sensitized with heterologous antiserum diminished as the nuclei within giant cells increased (Table 1, Text-figure 3). When this "phagocytic potential" was related to cell size (Text-figure 8), an exponential decrease in relative performance was seen as the cells enlarged, and an eight- to tenfold difference was found between the mononuclear and the hexanuclear cell populations (Text-figure 8).

On the other hand, the absolute number of SRBC treated with isologous antiserum or fixed in glutaraldehyde increased with the number of nuclei within giant cells (Table 1, Text-figures 4 and 5). There is still, however, a decrease in phagocytic performance relative to size when mononuclear and hexanuclear cell populations are compared (Text-figures 9 and 10). The decrease between these two populations is only twofold, and the relationship in this instance appears to be linear.

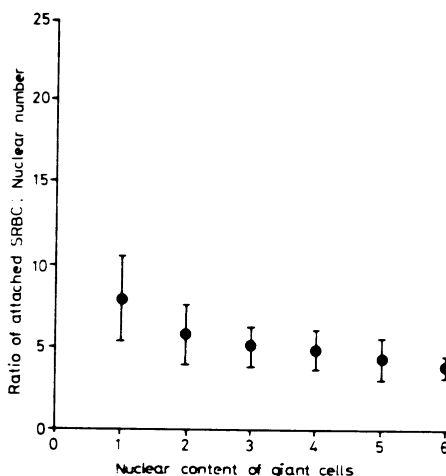
Microorganisms were occasionally found within the few phagocytic vacuoles of multinucleate giant cells (Figure 1). Similarly, SRBC sensitized with heterologous antiserum were rarely found within giant cells.

On the other hand, SRBC sensitized with isologous antiserum or fixed in glutaraldehyde were frequently seen within multinucleate giant cells (Figure 2) or attached to their surface (Figure 3). In the latter case, thin cytoplasmic projections or flaps adhered to the surface of the erythrocyte. Small portions of the attached erythrocyte were sometimes found within small surface indentations of the multinucleate cell (Figure 4).

Ingested erythrocytes were contained within membrane-bounded



TEXT-FIG 9—A decrease in the ratio of attached SRBC sensitized with isologous antiserum:nuclear number as the number of nuclei within a cell reaches six.



TEXT-FIG 10—A decrease in the ratio of attached SRBC treated with glutaraldehyde:nuclear number as the number of nuclei within a cell reaches six.

phagocytic vacuoles (Figure 5). The cytoplasm in the immediate vicinity of the phagosome consisted of a layer of fine, fibrillar material; surrounding this, many lysosomal dense bodies were present (Figure 5). Small vesicles opened into the phagocytic vacuoles, and narrow tubular structures traversed the periphagosomal, fibrillar zone to abut against the phagocytic vacuole (Figure 5). Continuous, closely apposed membranous lamellae, which communicated with myelin figures, also surrounded the phagocytic vacuole.

Discussion

The results indicate that 20 to 40% of the mononuclear population on the implanted plastic completely fail to ingest any of the two microorganisms tested. The latter were present in excess, and the failure of mononuclear phagocytosis is difficult to explain. It is possible that this subpopulation of mononuclear leukocytes represents the nonphagocytic "epithelioid" cell population described in similar implants by Papadimitriou and Spector,[12] or else these cells might be related to the A cell, a nonphagocytic, glass-adhering mononuclear, described by Allison [15] in peritoneal exudates. Although a somewhat similar proportion of mononuclear cells failed to ingest or attach SRBC pretreated with heterologous antiserum, isologous antiserum or glutaraldehyde, it is uncertain from the results whether the same nonphagocytic, mononuclear subpopulation is being tested. Nonetheless, the data suggest the presence of a nonphagocytic subpopulation of mononuclear cells among the adherent cells.

Ingestion of microorganisms by giant cells possessing more than seven nuclei is much reduced when compared with other cell popula-

tions on the implant. Even smaller multinucleate cells with two to six nuclei have a diminished phagocytic potential when the number of ingested microorganisms is corrected for the increased cell size. The loss of phagocytic potential is exponential when related to nuclear content. Likewise, the loss of the surface receptors for heterologous immunoglobulin [16] shown by the progressive lack of attachment of SRBC sensitized with heterologous antiserum shows a similar exponential pattern when related to nuclear content and also a similar reduction in magnitude. These phenomena may be due to loss of membrane material during the process of mononuclear fusion and multinucleate cell formation.

The total number of SRBC sensitized with isologous antiserum or treated with glutaraldehyde which attached to giant cells increases with the number of nuclei. If this figure, however, is corrected for the increase in size by dividing by the number of nuclei, then a progressive, linear decrease is seen as the number of nuclei within a cell increases. This indicates a progressive loss of some receptor site occurring as a result of cell fusion.

It appears that the proportional loss of heterologous and isologous immunoglobulin receptor sites is different, a greater proportion of the former being lost during giant cell formation. The receptor site for heterologous immunoglobulin is possibly situated closer to that region of the membrane involved in fusion and may, therefore, be degraded in the process. Complete reconstitution of lost plasmalemmal components may not occur because of the short life-span of the murine multinucleate giant cell.[14] The receptor for glutaraldehyde-fixed SRBC behaves similarly to that for isologous immunoglobulin and suggests that its situation on the plasmalemma is also at a greater distance from the sites of fusion than the receptor for heterologous immunoglobulin.

Electron microscopic examination confirmed the paucity of microorganisms within giant cells. Mononuclear cells without ingested microorganisms resembled the nonphagocytic "epithelioid" cells described by Papadimitriou and Spector.[12]

Phagocytosis of SRBC treated with isologous antiserum or glutaraldehyde occurred most frequently. This finding indicates that the phagocytic mechanism is still efficient in these giant cells, and the failure to ingest many microorganisms may be due to loss of some surface receptor site(s).

A prominent accompaniment of phagocytosis is the reaction of the subplasmalemmal cytoplasm. A distinct zone of finely fibrillar and granular material forms; this may be related to the various events of phagocytic vacuolar formation and the subsequent discharge of lyso-

somal material into it. The formation of tubules and lamellae around the phagocytic vacuoles are probably associated with this secretion of lysosomal enzymes into the phagocytic vacuole.

In conclusion, it can be stated that the phagocytic performance of multinucleate giant cells decreases with the increase in the number of nuclei. The diminished performance is accompanied by loss of surface receptors for isologous and heterologous immunoglobulins as well as those for attachment of glutaraldehyde-treated SRBC. Loss of these receptor sites may be due to plasmalemmal loss after fusion of the mononuclear precursors.

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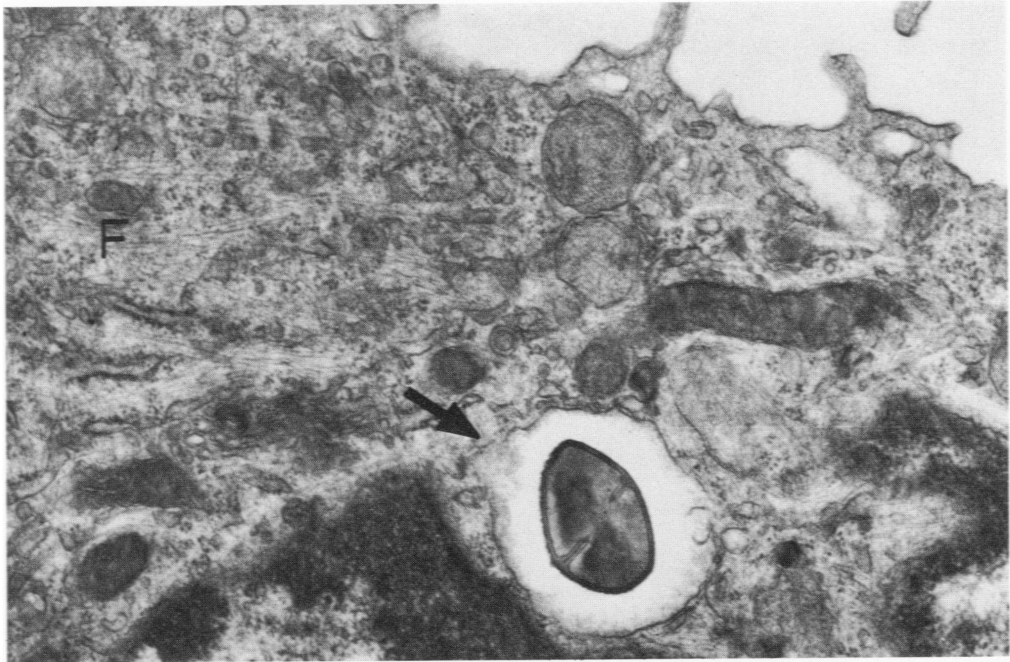
[*Illustrations follow*]



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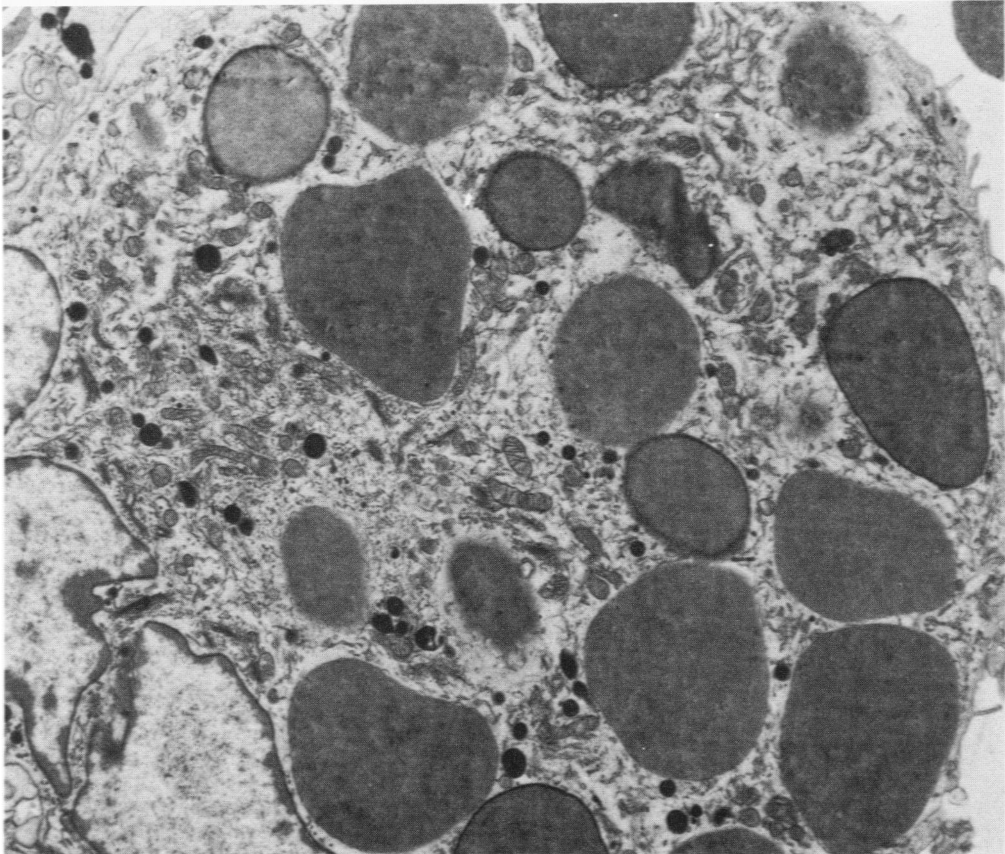

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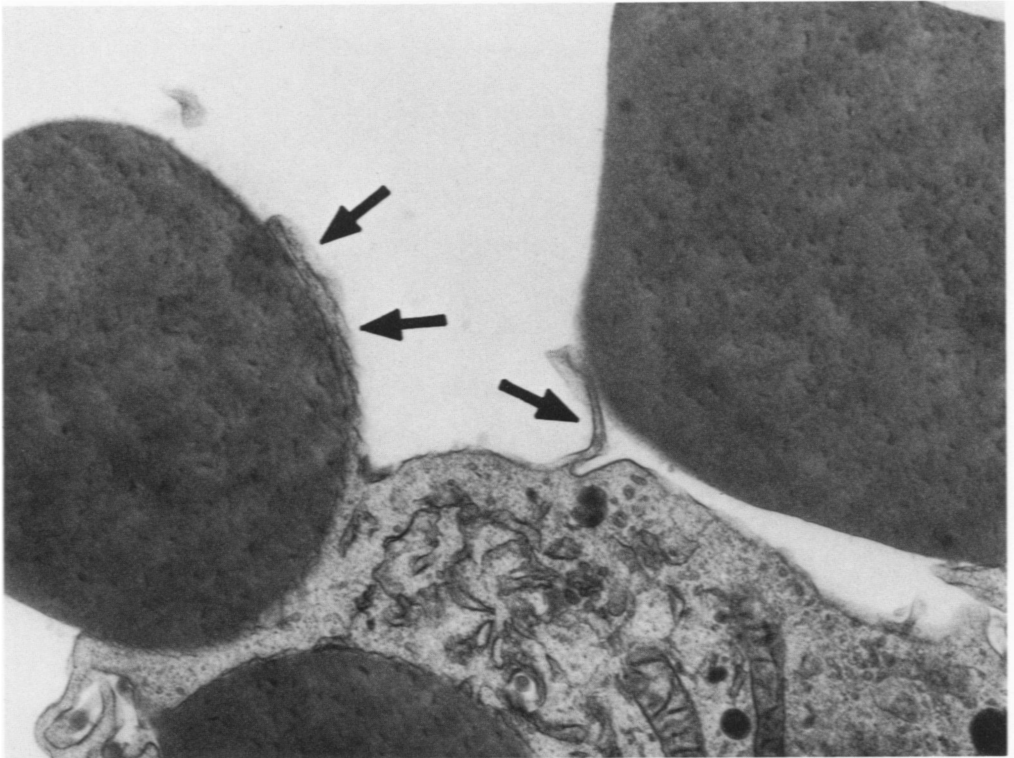
Fig 1—An ingested dividing staphylococcus is seen within a phagocytic vacuole (*arrow*) of a multinucleate cell. Aggregates of filaments (*F*) traverse the cytoplasm (× 34,500).
Fig 2—A multinucleate giant cell exhibiting many ingested SRBC treated with glutaraldehyde (× 15,000).

Fig 3—SRBC sensitized with isologous antiserum are attached to the cell surface of a multinucleate cell. Thin cytoplasmic flaps (*arrow*) adhere to the erythrocytic surface (× 23,000).

Fig 4—Piecemeal ingestion of an attached SRBC treated with glutaraldehyde (*arrows*) (× 71,000).



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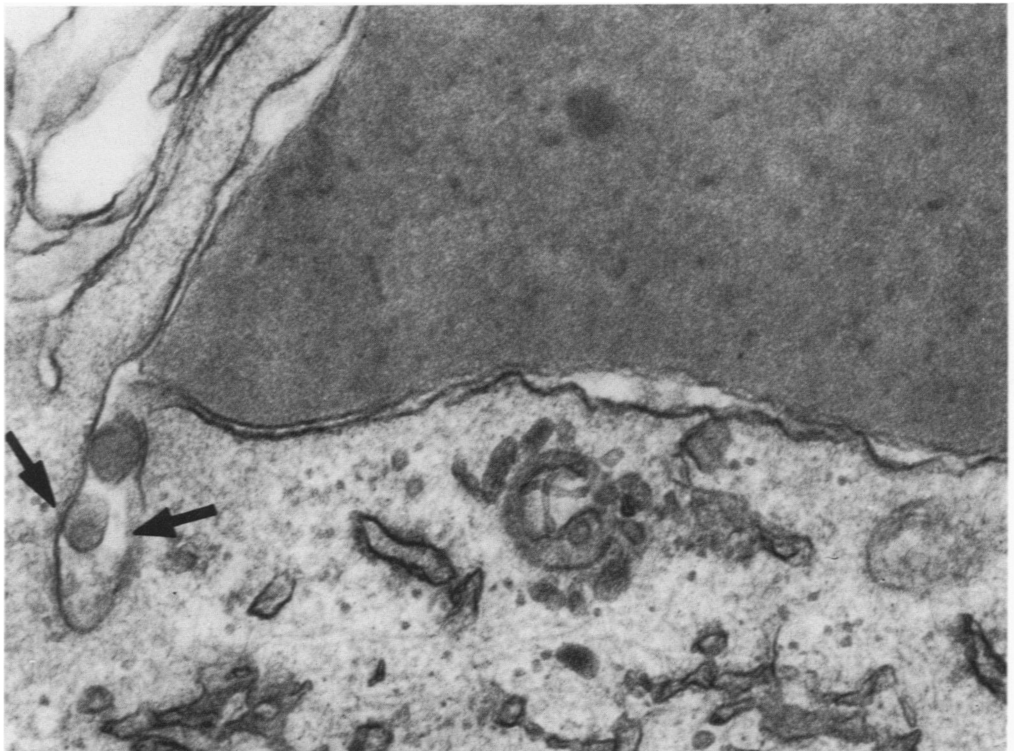

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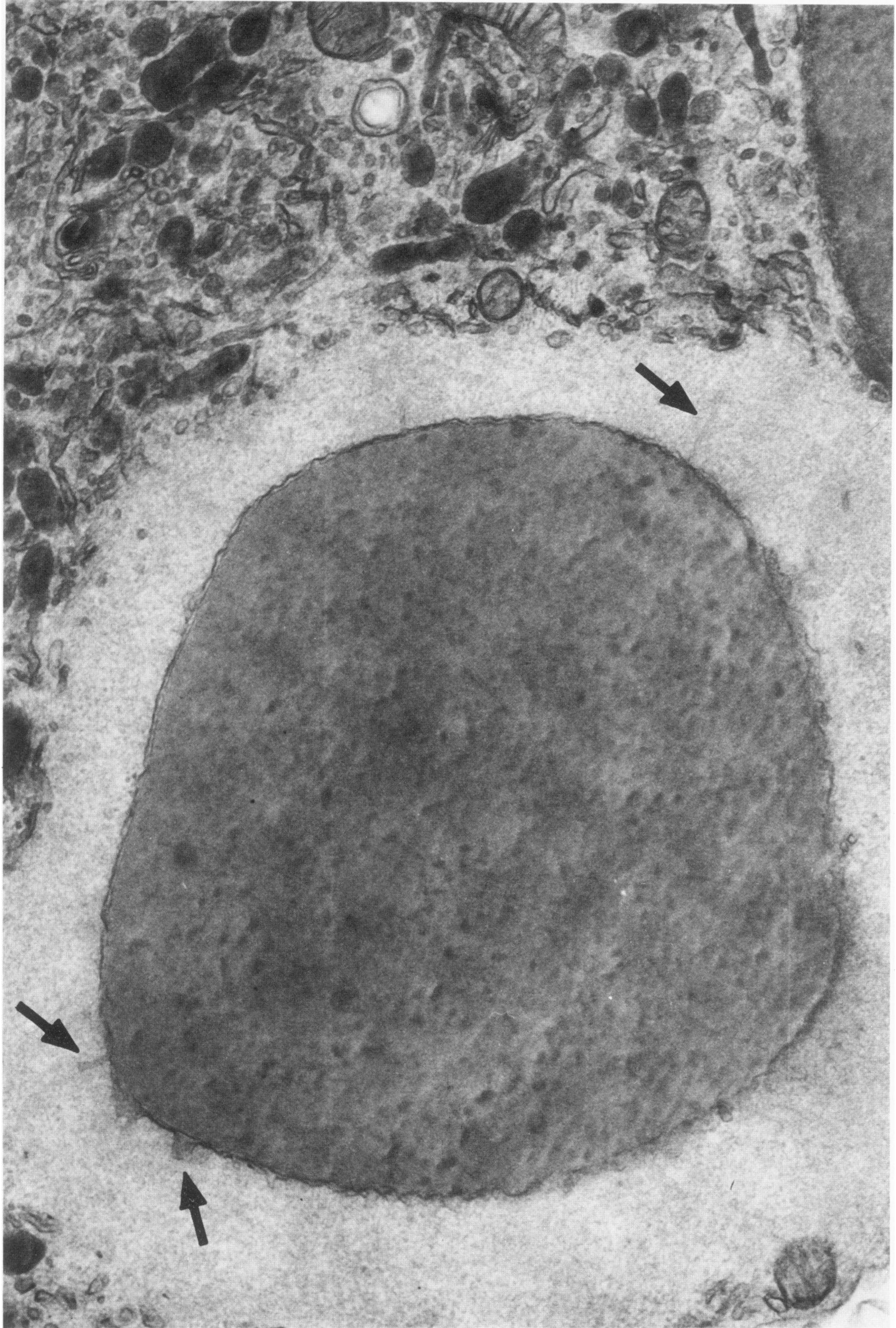

Fig 5—A finely granular halo surrounds a phagocytic vacuole containing a SRBC treated with glutaraldehyde. Tubules and vesicles (*arrows*) are found in this zone (× 37,800).