BRIDGE FORMATION AND CYTOPLASMIC FLOW BETWEEN PHAGOCYTIC CELLS*

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Stained preparations of histiocytes maintained *in vitro* and infected with *Listeria monocytogenes* occasionally show cytoplasmic bridges; in some of these bridges, bacteria have been observed as if in passage from one cell to another (1). Preparations were obtained after modifying the conventional fixation technique. The essential feature of the new technique consisted in keeping the cells warm (37°C) during fixation.

In order to verify the supposed passage of bacteria *via* such bridges, a simple chamber for observation of cells at 37°C and under a high magnification was constructed. It soon became apparent that even non-infected cells very often interacted to form cytoplasmic bridges, and that this activity was continuous.

The purpose of this communication is to describe the behavior of several types of phagocytic cells (mainly that of peritoneal histiocytes and lung macrophages), both in the infected and the non-infected state. This study was supplemented by autoradiography in order to demonstrate the passage of RNA from cell to cell.

Materials and Methods

(*a*) Rabbit peritoneal histiocytes were elicited, harvested, and maintained according to Fong *et al.* (2).

(*b*) Lung macrophages were obtained according to a technique developed by De Mello (3): Rabbits were killed by air injection, the trachea was exposed and clamped with a hemostat. 50 ml of Tyrode's solution was injected into the trachea and then withdrawn. Yields ranged from 2 to 10 $\times$ 10^6 macrophages, with almost no contamination by other cells.

(*c*) BCG was grown on Löwenstein's medium. After 2 weeks the slants were gently washed with 10 ml of Tyrode's solution. The suspension, which contained many clumps of bacteria, was shaken with glass beads (3 to 5 mm) for 1 hour and left overnight to settle in a test tube. On the next day, the upper part of the suspension was aspirated, and washed three times with Tyrode's solution. The final preparation consisted mainly of single cells and very small aggregates.

(*d*) The observation chamber (Fig. 1) was devised by D. Yassky and I. Wolf of this institute. A "slide" was cut from a pyrex Petri dish, and a U-shaped glass tube welded to it. The chamber was then closed by welding a short glass rod between the two arms of the U. The

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chamber was cleaned, dried, covered with a layer of silicone grease (Edwards High Vacuum, Ltd., Crawley, Sussex, England) along the tubing, and then autoclaved inside a large test tube. The roof of the chamber consisted of a thin coverglass, 22 × 40. The coverglasses were washed, dried, lined with silicone grease along the edges, inserted into small Petri dishes, and autoclaved. The Petri dishes were kept in a 37°C incubator. 1 ml of a suspension containing 5×10^5 cells was transferred onto each coverglass and allowed to settle for 1 hour. The chamber was then filled to capacity with maintenance medium, and the coverglass taken up with thin forceps, inverted, and placed on top of the chamber. Some pressure was applied to seal off the chamber and to remove air bubbles so as to prevent the drying out of the cells. The chamber was heated by connecting the U-shaped arms to a circulating pump (thermomix II Braun, Melsungen, Germany).

The chambers remained sterile even without addition of antibiotics.

(*e*) Autoradiography was done as described in reference 1, with the following RNA precursors:

H^3 uridine, Schwarz BioResearch, Inc., Orangeburg, New York; Specific activity 0,310 C/mM
H^3 cytidine, Schwarz BioResearch, Inc.; Specific activity 1,25 C/mM

RESULTS

Non-Infected Peritoneal Histiocytes.—Observations were started immediately after mounting of the chambers. During the first 2 to 3 hours, most of the cells appear rounded and do not show much activity. Later, they begin to form pseudopodia and to move in every direction. At a higher magnification (× 750), undulatory membranes can be discerned moving very swiftly. Soon afterwards, cytoplasmic bridges between histiocytes can be seen (Fig. 2). These bridges may last from a few seconds to a few minutes, although a 30 minute duration in older preparations (2 to 3 days) is not uncommon. Besides these bridges, pseudopodia can often be seen emerging from one cell and lying over, or underneath, another cell. The genuine bridge, on the other hand, is characterized by a flow of cytoplasm within the bridge and by the manner in which it terminates, namely: after tearing apart, each cell withdraws its loose end. The now disconnected cells will continue almost immediately to move towards neighboring cells, and in many cases new bridges will be formed. Quite often, the cells come too close together for clear observation or may even "ride" one on top of the other; but the following describes a typical formation of a bridge: One cell sends out a pseudopodium, which may meet a similar pseudopodium from another cell, or may be withdrawn. Sometimes one cell will circle around another until they eventually form a connection. Occasionally, a "wave" of cytoplasm can be seen moving very rapidly from one of the cells into the bridge, and immediately being pulled back so forcefully that the bridge snaps as a result. This behavior will then continue as long as the cells are alive,—it may slow down, but it does not stop. After 3 days, the cells generally become quite sluggish, but their activity can be renewed by opening the chamber and replacing the medium.

Some histiocytes show more activity than others, forming up to 3 to 4 bridges,

each with a different partner cell. Occasionally also, a bridge may broaden in the middle to 3 to 4 times its normal width.

Connections between histiocytes and polymorphonuclears or fibroblasts, which invariably contaminate all the preparations, were never observed. The histiocytes are easily distinguishable: they are originally elicited in the peritoneal cavity by means of mineral oil irritation, and the oil is then phagocytosed and appears as a highly refractile vacuole.

Besides the oil vacuole, one can on occasion see the nucleus, mitochondria, and lipochondria[1] (4).

Lipochondria are not easily stained by conventional differential techniques. Their number increases remarkably after the 1st day, to such an extent that some cells seem to be completely "loaded" with them; however, they do not merge into fat vacuoles. Lipochondria can very often be observed in the bridges; they move from one cell in the direction of the other, and then back again. Occasionally, they pass from cell to cell, but this does not happen frequently. In dying cells, they are sometimes seen rotating very rapidly.

Non-Infected Lung Macrophages.—At the beginning, lung macrophages appear quite different from peritoneal histiocytes. Their undulatory membranes are not flat and do not adhere to the glass, and in their constant movement resemble cilia. In their behavior, however, the macrophages strongly resemble histiocytes: pseudopodia and bridges are formed, torn apart, reformed, etc. When lung macrophages and peritoneal histiocytes are mixed in the same chamber, "hybrid" bridges between the two types of cells can be observed. This finding is interesting in view of the differences in lysozyme content and in respiratory metabolism which are known to exist between the two types of cells (5, 6).

Infected Peritoneal Histiocytes.—Attempts to demonstrate passage of *L. monocytogenes* or of *Escherichia coli* between histiocytes were not successful, since the bacteria could not be discerned. Possibly, the streptomycin used to check external growth of the bacteria destroyed them upon penetrating into the already damaged histiocytes.

These experiments were then repeated with BCG, which was chosen because of the intracellular location of *Mycobacterium tuberculosis*, and because of its slow rate of growth, which made the use of streptomycin unnecessary. Experiments were conducted in the following way: The histiocytes were parasitized on the coverglass by mixing 10^8 bacteria and $2,5 \times 10^5$ histiocytes, and allowing them to settle. After 1 hour, the coverglass was rinsed in two changes of sterile Tyrode's solution, and was again inserted into a small Petri dish; then an additional $2,5 \times 10^5$ histiocytes were pipetted on top of the already infected cells and were likewise allowed to settle. Thus, a population made up of heavily

[1] Identified as such by Dr. Shahar of this laboratory.

infected cells mixed with non-infected ones was obtained. The bacteria could be easily detected, particularly after 24 hours, when they had grown in size; but even after only a few hours they could already be seen in the bridges, passing from one cell to another (Figs. 3 to 6).

Sometimes a series of 5 to 8 bacteria was seen crossing a bridge one after the other (Figs. 7 to 30).

Exchange of RNA.—After cytoplasmic flow between histiocytes had been observed, the possibility of a transfer of RNA between the cells was investigated. Normal or BCG-infected histiocytes were incubated in Leighton tubes for 3 to 24 hours in presence of 1 μc/ml H^3 uridine or cytidine. The coverglasses were then rinsed aseptically in six changes of warm Tyrode's solution, and eventually inserted into new Leighton tubes containing a suspension of $2,5 \times 10^5$ non-labeled histiocytes. (These tubes were previously kept in the cold to prevent the histiocytes from adhering to the glass.)

The temperature was then raised to 37°C, and the tubes incubated for 5, 8, or 24 hours, after which periods the coverslips were fixed according to reference 1. The preparations were exposed for autoradiography for 2 to 4 weeks, and then developed and stained. Now the slides were scanned for radioactive material in the bridges, which would indicate the transfer of labeled RNA from a labeled cell to an unlabeled one. Figs. 31 and 32 show two pairs of histiocytes in which RNA may thus be presumed to be crossing between the cells. Whereas several such pairs were found, the possibility of artifacts cannot yet be excluded, for which reason these results should not be accepted as final without additional independent evidence.

DISCUSSION

To demonstrate the occurrence *in vivo* of cytoplasmic bridges and of cytoplasmic flow between histiocytes is likely to be a most difficult undertaking. Nevertheless, in our system, this phenomenon appeared in every experiment,—which suggests that, under certain conditions, histiocytes regularly manifest such behavior. The following remarks are based on the assumption that histiocytes do transfer and exchange ingested materials and RNA *in vivo*, and deal with the possible significance of such an exchange in a number of diverse situations related to immunity:

1. This mechanism may be visualized as a safety device operating in cases of histiocytes heavily parasitized by intracellular bacteria.

It may be that at the early stage of some chronic diseases, such as brucellosis and tuberculosis, one of the tasks of histiocytes, monocytes, and macrophages, etc., is to contain the bacteria and to prevent their dissemination until immunity, whether cellular (*via* immune histiocytes), humoral, or both, is developed. Dying or "loaded" histiocytes might then transfer bacteria to other

host cells, thus preventing them from escaping into the blood stream and from invading new tissues.

2. Still within this framework, another observation may be explained, namely, that experimental animals inoculated with *Brucella*, and then treated with bactericidal concentrations of streptomycin for months without interruption, will not become clear of bacteria. Since streptomycin kills bacteria within seconds (but does not affect them intracellularly), one should expect the bacteria liberated from dying phagocytic cells to be killed before they are phagocytosed anew. One of the possible explanations for their survival may be the passage of bacteria from cell to cell.[2]

3. Several pertinent questions can be asked about the nature and origin of "immune phagocytic cells" (2), whether monocytes, histiocytes, or macrophages. It is not known whether the process of "immunity" develops in normal cells after their having been exposed to certain antigens, or whether "immune" cells arise from specific "immune clones." At any rate it should be remembered that: (*a*) not all histiocytes come into contact with antigens; (*b*) in the body of the immune animal new generations of histiocytes keep forming; therefore, regardless of what the origin of the immune cell will ultimately be found to be, one must ask for an explanation of the *continuity of the immune state.*

In case immunity develops through the exposure of normal cells to antigens, the additional question poses itself: "how do the unexposed cells acquire immunity?" It is our belief that cytoplasmic transfer may explain how antigenic material, or its equivalent chemical "information" (7), is shared among cells and transmitted to new generations.

The author expresses his gratitude to A. Kaplun, D. Yassky, and A. Shahar, without whose generous help this work would not have been possible.

Some of the topics discussed were initially evolved during the author's stay at Dr. S. Elberg's laboratory at the University of California, Berkeley.

SUMMARY

A simple chamber was constructed for observation of cells at 37°C and under high magnification. Rabbit peritoneal histiocytes observed in this chamber manifested a constant interaction, which lasted as long as the preparation itself. This interaction consisted of formation of cytoplasmic bridges and flow of cytoplasm between the cells.

Lung macrophages formed bridges both between themselves, and with peritoneal histiocytes. On the other hand, no bridges were noticed between histiocytes and polymorphonuclears or fibroblasts.

BCG bacteria were observed passing from one histiocyte to another.

[2] The author is indebted to K. Faunce, of the Department of Bacteriology, University of California, Berkeley, for this idea.

Autoradiographic studies suggest that RNA may be transferred from one cell to another.

The possible significance of these observations is discussed.

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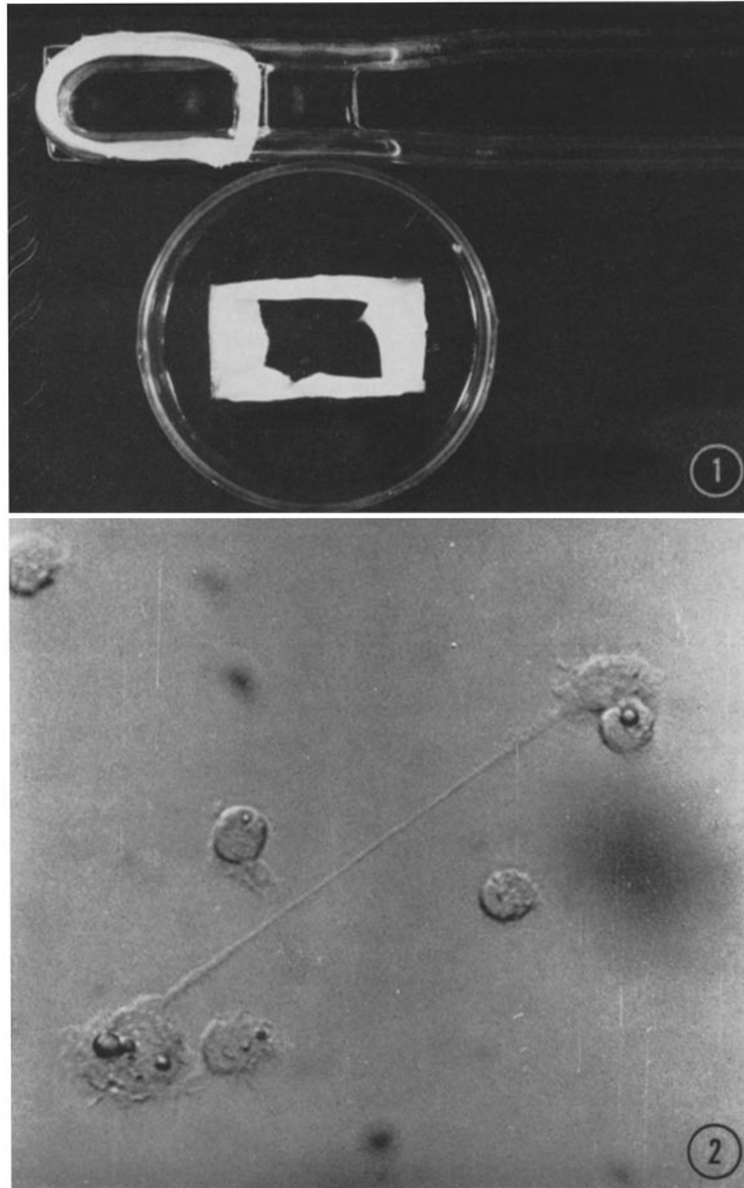
EXPLANATION OF PLATES

PLATE 94

FIG. 1. Unassembled observation "chamber" (¾ natural size); details in text.

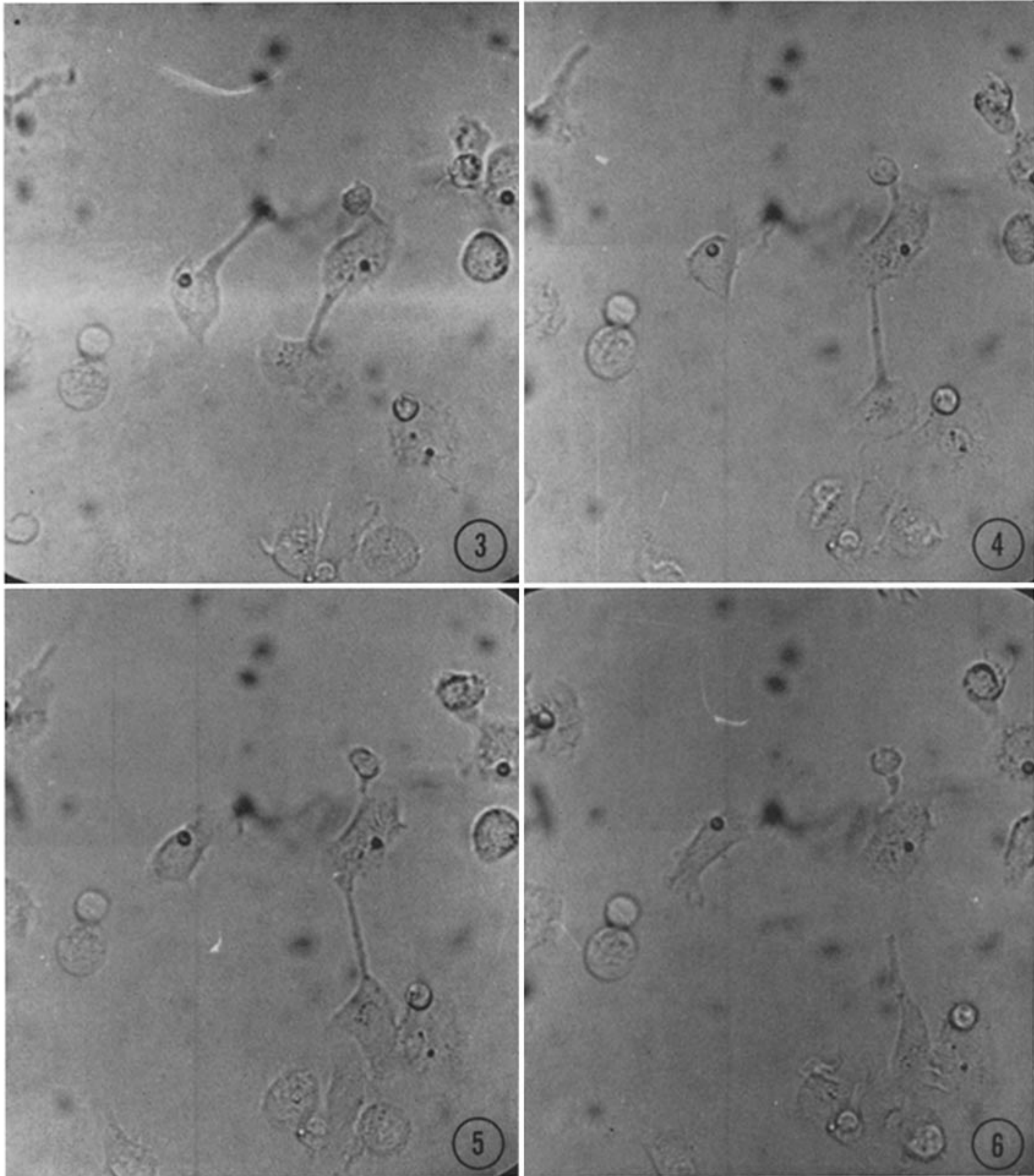
FIG. 2. A long bridge between two peritoneal histiocytes. × 1000.



(Aronson: Phagocytic cells)

Plate 95

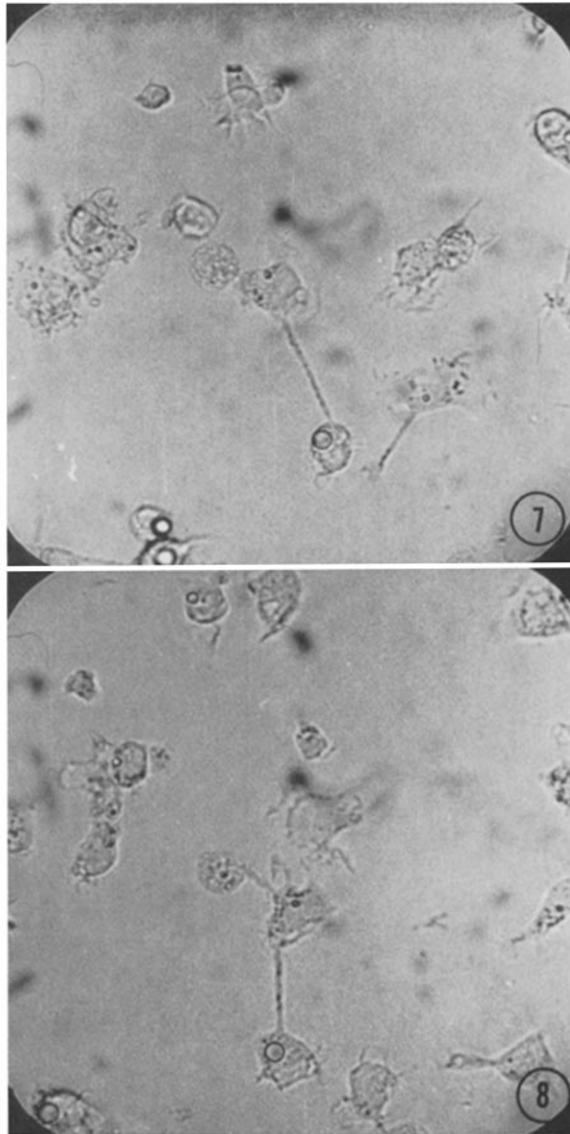
Figs. 3 to 6. Passage of BCG bacteria from one histiocyte to another *via* a cytoplasmic bridge. In Fig. 3, a bacterium is just at the entrance to the bridge; in Fig. 4, it is in the middle of the bridge; in Fig. 5, it has arrived at the opposite end; in Fig. 6, after the cells have disengaged, the bacterium remains in the recipient cell. The entire sequence lasted 2 to 3 minutes. × 500.



(Aronson: Phagocytic cells)

PLATE 96

FIGS. 7 and 8. Passage of BCG bacteria from one histiocyte to another via a cytoplasmic bridge. In Fig. 7, 8 BCG bacteria can be seen in the bridge; in Fig. 8, there are only 5, the remaining 3 having moved over into one of the cells. The cytoplasmic "wave" which pushed these 3 bacteria was very sudden and lasted a few seconds. $\times$ 500.



(Aronson: Phagocytic cells)

PLATES 97 AND 98

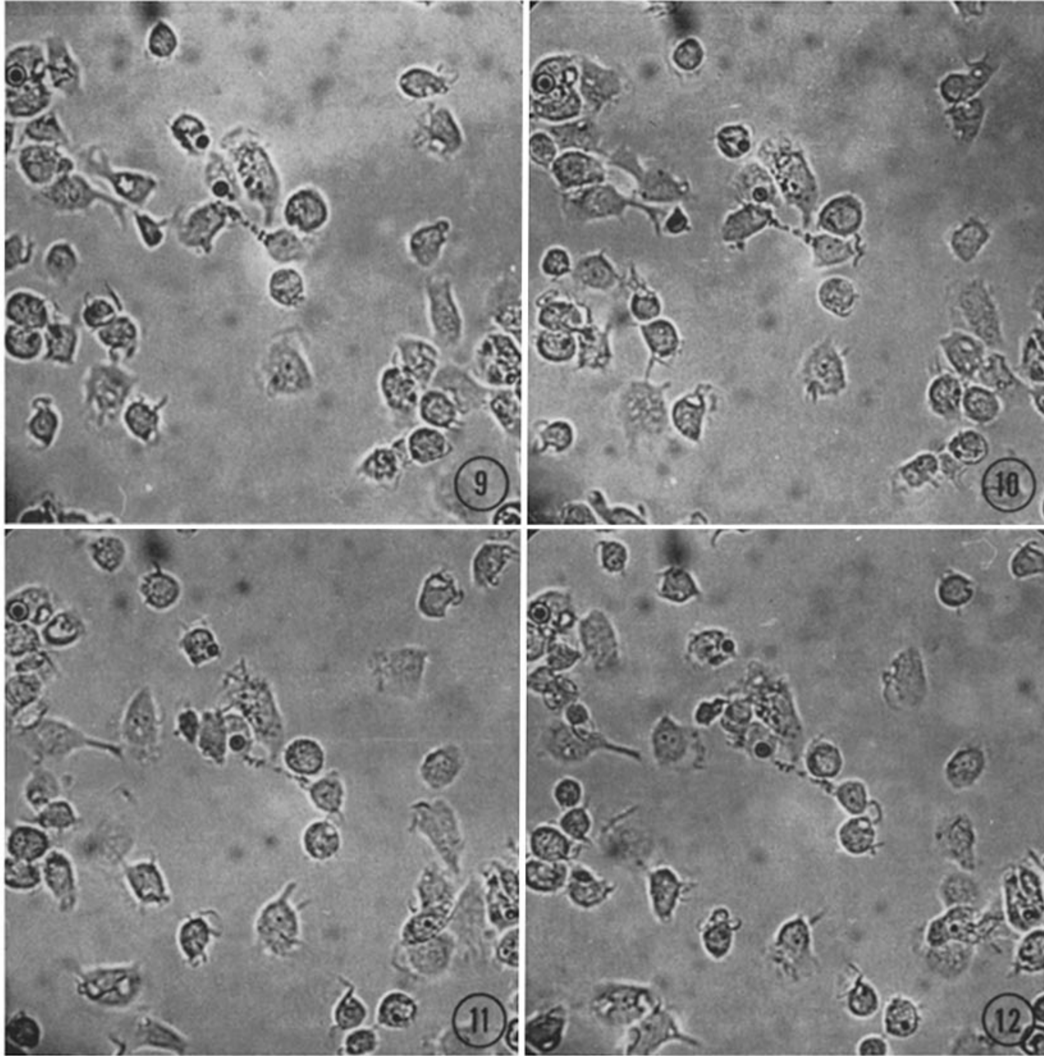
FIGS. 9 to 17. Passage of BCG bacteria from one histiocyte to another *via* a cytoplasmic bridge. In Figs. 9 and 10, a pseudopodium full of bacteria is seen resting on another histiocyte. In Fig. 11, as a result of cell movement, the pseudopodium is attaching itself to a different cell. In Figs. 12 to 15, the bacteria are seen moving towards this latter cell. In Figs. 16 and 17, the bridge terminates, with the bacteria over on the other side. The entire sequence lasted 10 minutes. × 500.

PLATES 99 AND 100

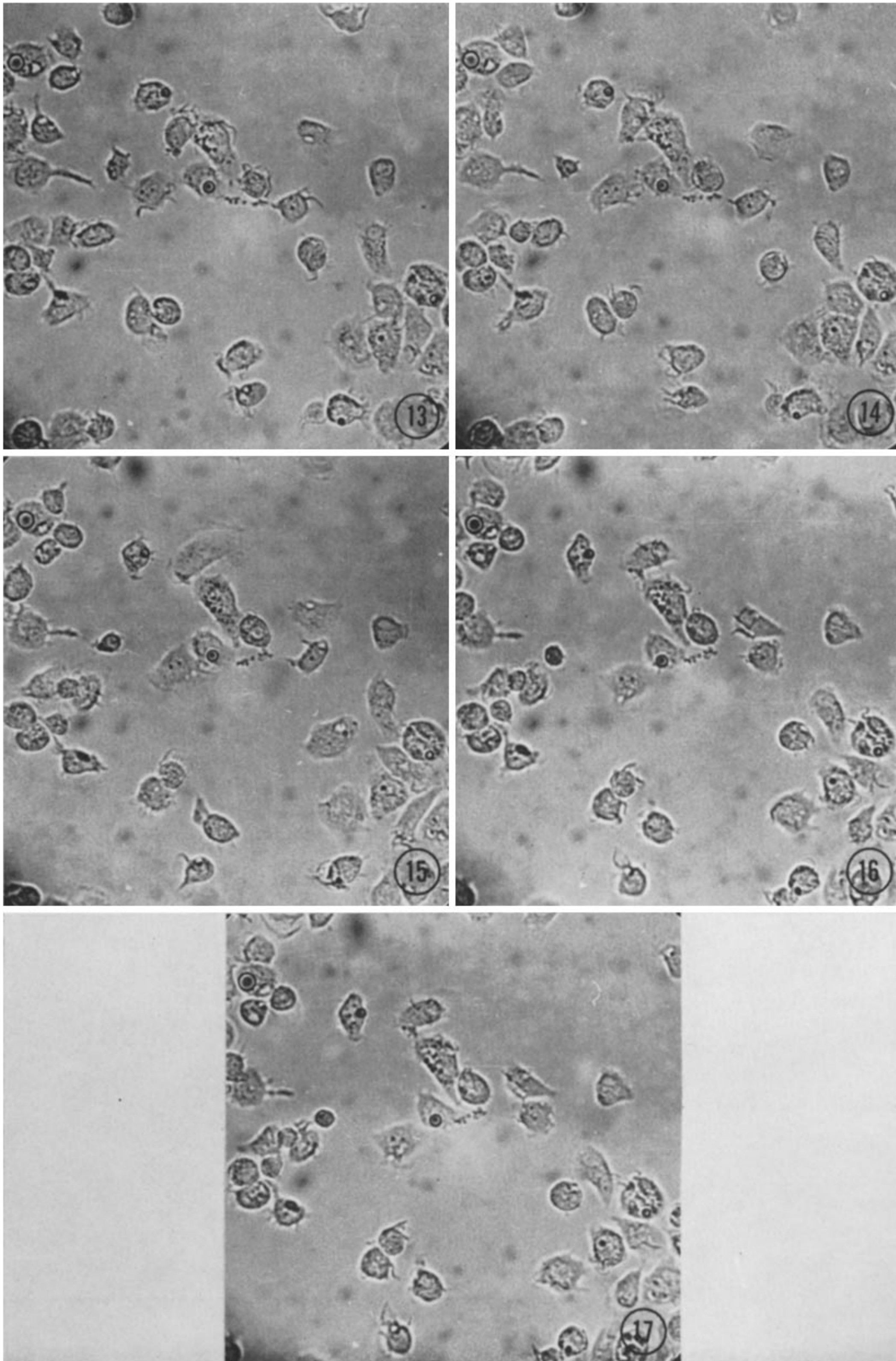
FIGS. 18 to 25. Passage of BCG bacteria from one histiocyte to another *via* a cytoplasmic bridge. The bacteria are marked by arrows. Duration of this sequence: 15 minutes. × 500.

PLATE 101

FIGS. 26 to 30. Passage of BCG bacteria from one histiocyte to another *via* a cytoplasmic bridge. × 500.



(Aronson: Phagocytic cells)



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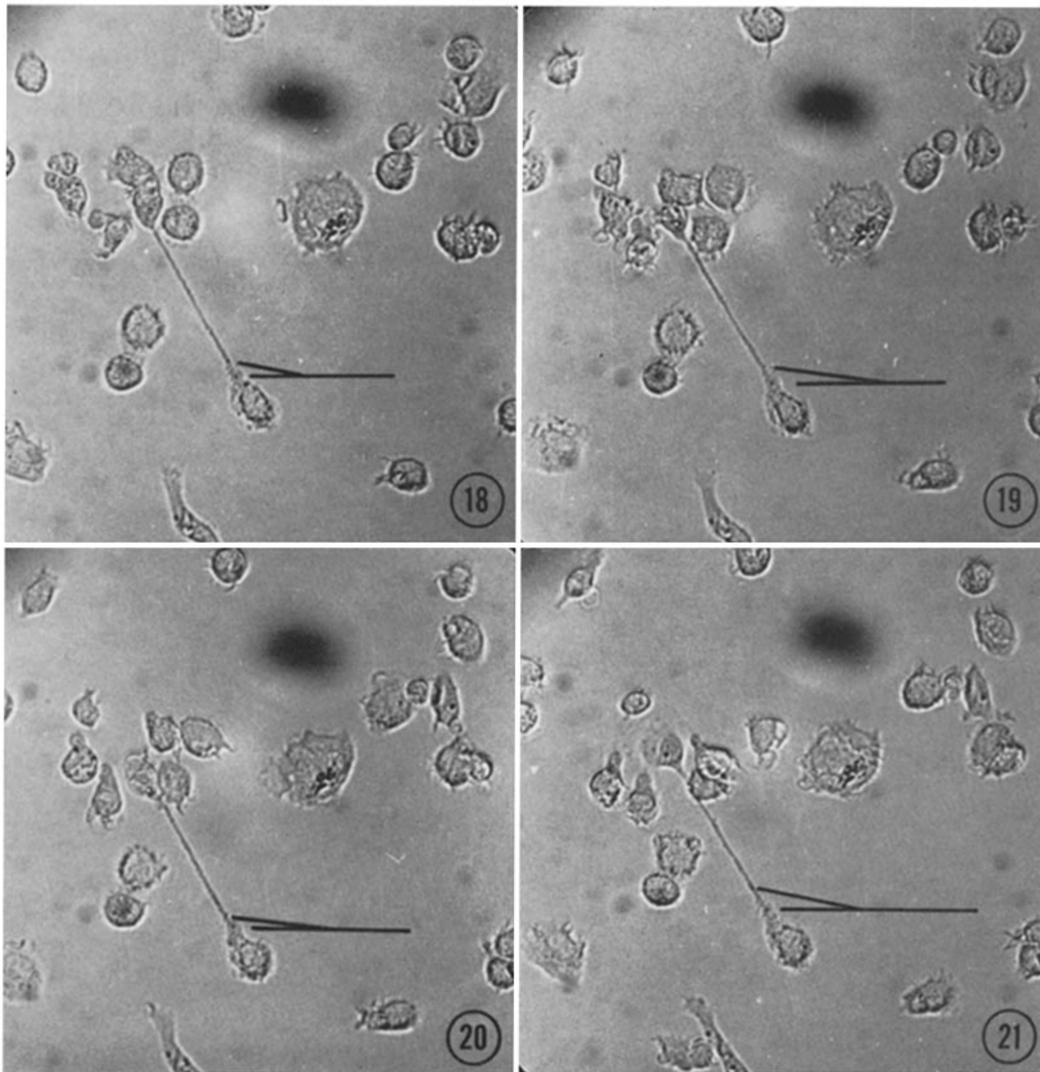

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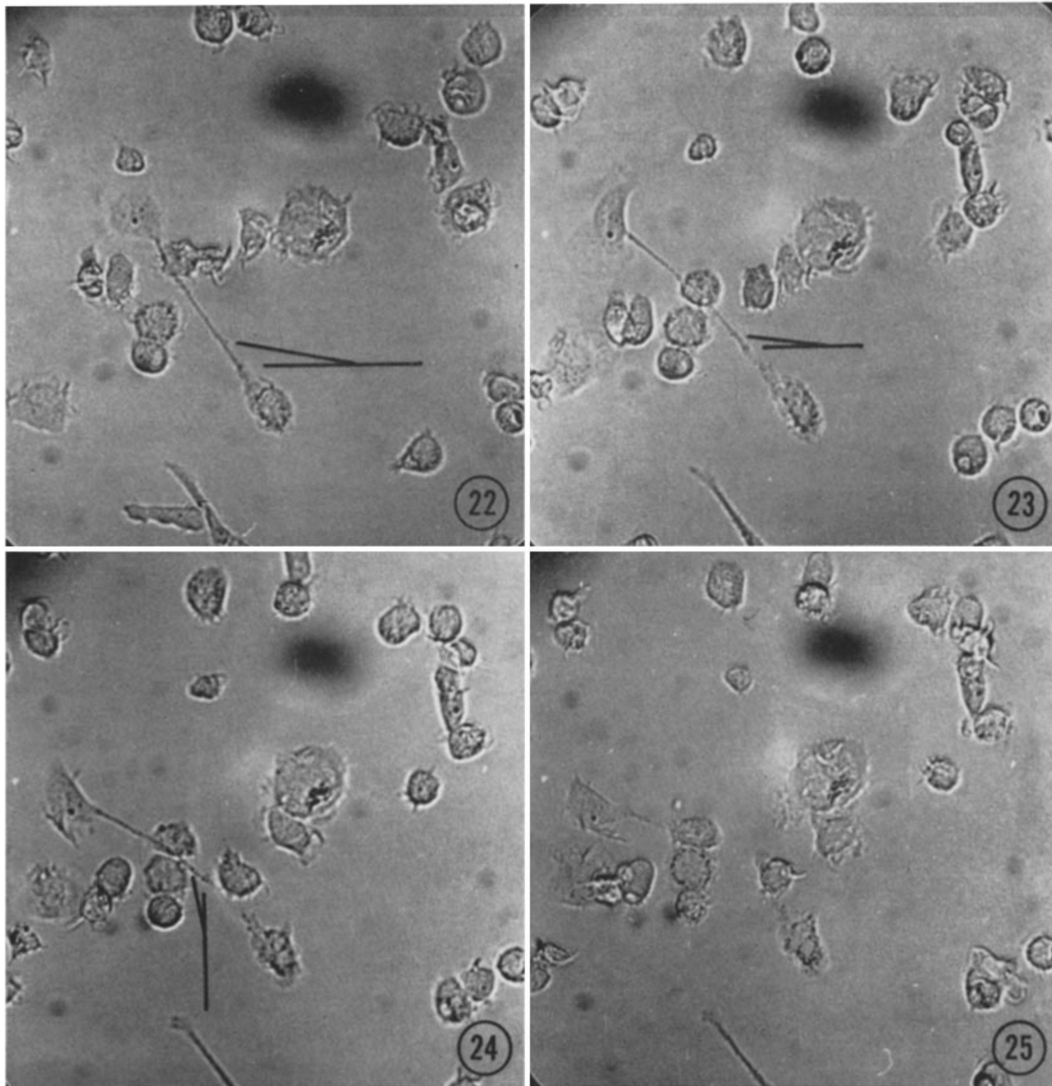
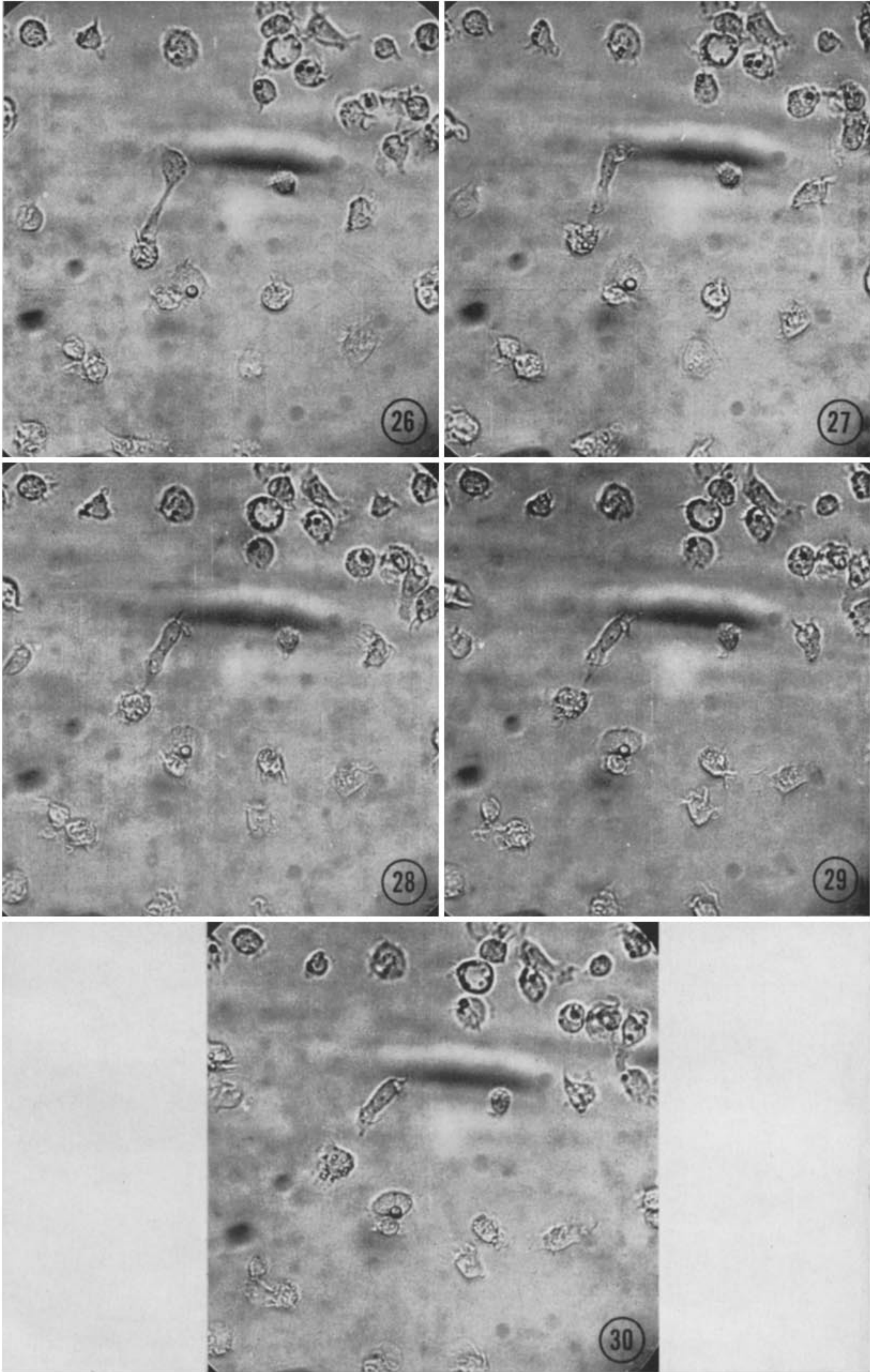


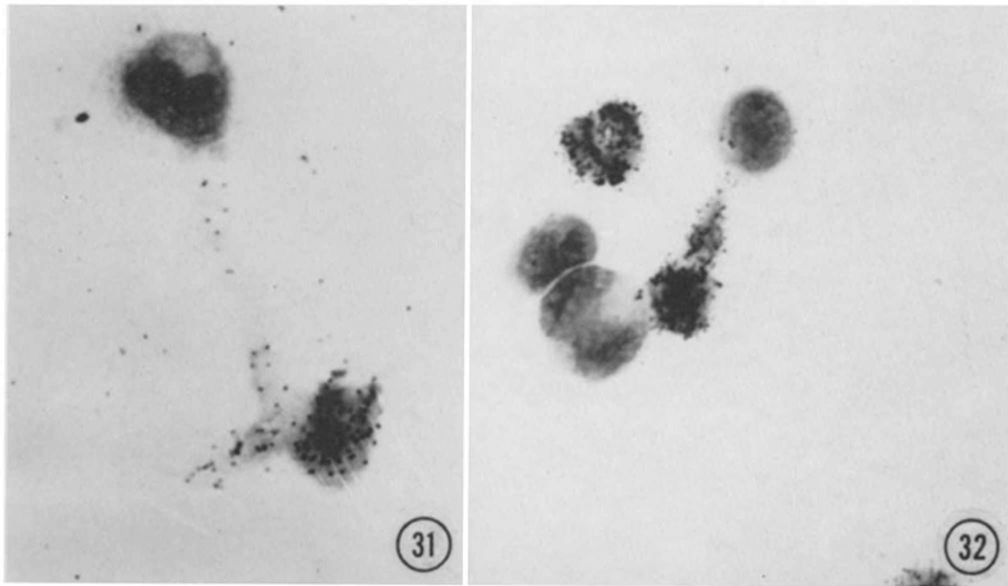
(Aronson: Phagocytic cells)



(Aronson: Phagocytic cells)

PLATE 102

FIGS. 31 and 32. Transfer of RNA between histiocytes. × 1000.



(Aronson: Phagocytic cells)