ELECTRON MICROSCOPY OF IN VITRO ENDOCYTOSIS OF T_2 PHAGE BY CELLS FROM RABBIT PERITONEAL EXUDATE*

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Current theories of antibody formation attribute important roles to two types of cells, namely, the macrophage which is involved in the uptake of the antigenic particles and the lymphoid cell which is responsible for the production of antibody. The production of antibody against T_2 phage by rat or rabbit lymphocytes incubated *in vitro* to which had been added the filtrate from homogenates of macrophages previously incubated with T_2 phage has been reported (1). This study pointed out the necessity for interaction between virus and macrophage as a prerequisite for the preparation of the immunogenic information for the subsequent antibody formation. The present study is an attempt to elucidate the mechanism of interaction between the phagocytic cell and the virus particle, and to follow the intracellular fate of the phage and the modifications that might occur in the cell during the period assumed to be necessary for the preparation of the immunogenic information.

The T_2 phage, which was found to be a choice model of virus antigen in immunologic studies (2), is also a convenient object for electron microscopy due to its size, electron density, and its characteristic hexagonal head.

It has already been demonstrated (3) that bacteriophage T_2 adheres to the cell membrane of guinea pig leucocytes in the peritoneal cavity and the electron micrographs of ghosts of such cells reveal the phage particles adsorbed in the membranes. Although the phage could not be visually demonstrated within these cells, counts of phage recovered from fractionated cells treated with antiphage serum before fractionation indicated its intracellular location (3).

It has also been shown that influenza virus incubated with phagocytic cells first adheres to the leucocytes, following which it is incorporated into the cells (4, 5). Under continued incubation, the virus undergoes structural disintegration (6) and in-

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activation (7). By means of fluorescent-bound antibodies, the virus antigen was found to be associated with granules isolated from fractionated cells (4).

Polioencephalitis virus was revealed by electron microscopy in polymorphonuclear leucocytes in the brain of animals previously infected with this virus (8). The virus particles were located in intracellular osmiophilic structures which were occasionally membrane-bound.

In the present study, T_2 particles incubated with macrophages are shown to be adsorbed to the cell membrane. The adsorption is followed by endocytosis. Within the cytoplasm, the phage particles are found confined within vacuoles as well as in membrane-bound ovoidal bodies displaying varying degrees of density.

Materials and Methods

T_2 Bacteriophage.—The bacteriophage was grown on *Escherichia coli* B in M-9 medium (9) and purified by differential centrifugation (10). Stocks of 10^{12} plaque-forming units/ml were maintained in sterile isotonic saline containing 0.001 M $MgSO_4$ at pH 7.0. Phage titer was determined according to the double agar layer method of Adams (9).

Peritoneal Exudate Cells.—Peritoneal exudate cells were elicited in rabbits by injecting 50 ml of mineral oil into the peritoneal cavity 5 days previous to harvesting of the exudate fluid according to Fong *et al.* (11). The cells were washed twice and resuspended in Tyrode's solution. Cell samples were maintained in the cold until commencing the incubation experiments. Incubation experiments did not start later than 3 hours after collection of the cells from the peritoneal cavity. The cell population obtained consisted of about 90 per cent macrophages and 10 per cent polymorphonuclear leucocytes.

Incubation Experiments.—Sterile, siliconized glassware was used in all experiments. The cells were concentrated and resuspended in 0.9 ml of fresh autologous or homologous rabbit serum to a final concentration of about 10^7 cells in narrow test tubes (8 mm in diameter and 10.0 cm long). In the later experiments, only fresh autologous serum was used. The test tubes were then placed in a 37°C water bath for 10 minutes, after which 0.1 ml of phage suspension (10^{11} plaque-forming units) was added. The incubation mixture was shaken several times during the incubation period. Incubations were carried out for varying periods, ranging from 10 (see Table I), to 120 minutes.

Control samples of the same concentration of cells, but without phage, were incubated under the same conditions.

Electron Microscopy.—After incubation, 1 ml of 2 per cent OsO_4 in veronal-acetate buffered saline at pH 7.4 was pipetted into the suspension in the test tube. After 2 minutes' centrifugation at 700 *g* the supernatant was discarded and the cells were resuspended in 1 ml of fresh 2 per cent OsO_4 and kept at 4°C for 1 hour, washed twice with isotonic buffered saline (pH 7.4), dehydrated through a graded series of acetone, and embedded in vestopal-W.[1] Thin sections were cut on either the Danon-Yeda[2] or the Sitte-Reichert[3] microtomes and mounted on formvar-coated copper grids reinforced with carbon. All sections were stained in a saturated solution of uranyl acetate in 10 per cent ethanol for about 3 hours. RCA-EMU 2A and EMU 3B electron microscopes were used.

[1] Purchased from Martin Jaeger, Versenaz/Geneva, Switzerland.

[2] YEDA, Research and Development Co., Ltd., at The Weizmann Institute of Science.

[3] C. Reichert Optische Werke, Aktiengesellschaft, Vienna, Austria.

TABLE I

The Incubation System of T_2 Phage and Rabbit Peritoneal Exudate Cells. Degree of Phage Incorporation

Sample	Concentration		Incubation			Incorporation*
	Cells	T_2 phage	Volume	H‡ or A‡	Time	
			ml		*min*	
1	5.0×10^7	—	5.0	H	30	−
2	5.0×10^7	2×10^6	5.0	H	30	−
3	4.2×10^7	2×10^5	5.0	H	30	−
4§	2.4×10^7	8×10^{11}	2.0	H	15	+++
5	7.5×10^7	4×10^{11}	2.5	H	20	−
6	2.5×10^7	4×10^{11}	1.0	A	10	+
7	2.5×10^7	4×10^{11}	1.0	A	10 w‖ T_2 10 wo‖ T_2	++
8	6.0×10^7	4×10^{11}	1.0	A	20	+++
9	1.8×10^7	—	1.0	A	30	−
10	1.8×10^7	4×10^{11}	1.0	A	30	+++
11	3.0×10^7	2×10^{11}	1.0	A	60	+++
12	3.0×10^7	2×10^{11}	1.0	A	75	+++
13	9.0×10^6	2×10^{11}	1.0	A	99	+++
14	2.4×10^7	—	1.0	A	60	−
15	2.4×10^7	2×10^{11}	1.0	A	60	+++
16	2.0×10^7	—	1.0	A	90	−
17	2.0×10^7	2×10^{11}	1.0	A	60 w T_2 30 wo T_2	+++
18	4.4×10^7	9×10^{11}	1.0	A	65	+++
19	2.8×10^7	—	1.0	A	120	−
20	2.8×10^7	9×10^{11}	1.0	A	120	+++

* Estimated degree of incorporation, based on scanning of a large number of sections from each sample. −, none; +, slight; ++, moderate; +++, high.

‡ Fresh homologous (H) or fresh autologous (A) rabbit serum.

§ Preservation of cells good except in sample 4, where a large number of disintegrated cells was encountered.

‖ w, with; wo, without.

RESULTS

Observations of Control Macrophages Incubated in Rabbit Serum.—The morphology of rabbit peritoneal macrophages (Fig. 1) agrees with the description of mammalian macrophages given by other authors (12–14). The bean-shaped nucleus sometimes showing a nucleolus and a thin layer of electron-opaque material adhering to the inner nuclear membrane is eccentrically located. Radiating out from the centrosomal region many small vesicles of the Golgi complex as well as membrane-bound electron-opaque ovoidal granules measuring from 50 to 400 mμ can be observed. These granules appear to be mostly

concentrated in the Golgi region near the indented part of the nucleus. Small dense granules are occasionally seen at the cell periphery. These granules can be distinguished from transverse sections of mitochondria by the lower electron density of the latter, as well as by the presence of cristae in them. Mitochondria are rare around the centrosomal region. Endoplasmic reticulum with attached ribosomes is present, as are free ribosomes. Lipid inclusions are occasionally seen. Pinocytic vacuoles of various sizes are generally seen at the periphery of the cell. Cytoplasmic filaments of the type reported by De Petris *et al.* (12) are rarely observed.

Observations of Rabbit Peritoneal Exudate Cells Incubated with T_2 Phage: Macrophages.—The main morphological difference between the control cells and the cells incubated with bacteriophage (apart from the presence of phage particles within the cells and adsorbed to cell membranes) is the reduction in number and the relative increase in size of the cytoplasmic-dense bodies of the experimental cells (Fig. 2).

The viruses are easily identified by their electron-opaque hexagonal head (Figs. 2–7). The measured size of the heads of these particles is in accordance with that reported in the literature (95 x 65 mμ) (15). Bacteriophage particles with attached tails are frequently seen outside the cells, adsorbed to the cell membrane, and inside the cell. When adsorbed to the cell membrane, no preferential orientation of the phage to the membrane could be noted. The phage particles adsorbed to the outer cell membrane or located in its proximity are surrounded by a diffuse electron-opaque material. When inside the cell, the phage particles are always found within membrane-bound structures of the cytoplasm (Figs. 2 to 8). Never were T_2 particles seen free in the cytoplasm.

The number of phage particles per vacuole varies considerably from cell to cell and from vacuole to vacuole within the same cell. As many as 30 particles have been counted in single vacuoles in sections about 50 to 60 mμ thick (16). Such loaded vacuoles are more frequently encountered deeper in the cytoplasm, whereas, at the periphery, the phage particles are often found singly or in small numbers within dense vacuoles.

The vacuoles containing the virus particles are generally ovoidal in form and display a variety of sizes (200 to 1500 mμ in diameter) and of densities. For convenience, we have classified them into three categories based on their density: the very dense vacuoles which are generally the smallest in diameter; the semidense vacuoles which are slightly larger and most frequently encountered and finally the clear vacuoles which are the largest and relatively rare.

All three types of vacuoles are present in cells exposed to the virus regardless of the incubation time. The electron-opaque vacuolar matrix in the dense vacuoles uniformly fills the membrane-bound area. Against this dense background, the even denser particles can sometimes hardly be discerned (this difficulty increased with thicker sections). Morphological analysis of the

dense vacuoles is practically impossible because of the opacity of their contents to the electron beam. Sometimes the hexagonal bodies of the virus are outlined by a thin less dense border between the dense virus body and the vacuolar matrix. No particular orientation of the phage within the vacuole could be noted. Clear spaces resembling the shape of a phage, though slightly larger in size are frequently noted in these vacuoles (Fig. 4). Such clearings are occasionally seen in dense vacuoles of control macrophages.

Phage particles are also found in semidense vacuoles, showing no preferential position within these bodies or towards one another. Here also, a thin clear border can sometimes be observed between the phage particle and the contents of the surrounding dense material. This space might result from the swelling of the protein coat, or it might be due to a preexisting electron-transparent layer between the dense nucleic core of the phage and its protein coat, since it can be seen in viruses outside the cell as well as in micrographs of thin sections of concentrated T_2 phage (17). Clear patches can frequently be distinguished in the vacuolar matrix (Figs. 2 and 7). The semidense vacuoles display a recognizable fibrillar content (Figs. 7 and 8).

In clear vacuoles, the phage particles are more widely dispersed among a rather loose filamentous content which looks like what might be a less concentrated semidense vacuole. Dense deposits, not observed in semidense vacuoles, line portions of the inner membrane of clear vacuoles (Fig. 8).

No significant correlation could be found between the type of vacuoles in a cell and the total incubation time of the cell with T_2 phage.

The vacuoles containing phage particles concentrate in the Golgi region. The dense vacuoles containing phage resemble in form, density, and distribution the dense granules of the Golgi region noted in the control macrophages; however, the former are generally larger.

In the preparations fixed following incubations of 10 up to 120 minutes, the phage particles are seen within the cells, adhering to cell membranes, trapped between adjacent cells, and free between cells. Single phage particles can be seen in small vacuoles in the peripheral cytoplasm. Some of them are still connected to the outside by a channel (Fig. 5). Occasionally the cell surface on which two or more phage particles are adsorbed shows invagination carrying the particles along with some of the surrounding medium (Fig. 3). Phage particles are also taken up by phagocytosis; large vacuoles containing cell debris and phage particles have been noted. Relatively fewer phage particles could be seen in cells incubated with the virus for 10 minutes than in those incubated for longer periods.

In an incubation experiment in which the phage particles were removed from the medium by washing after 60 minutes and the cells reincubated in autologous serum free of phage for another 30 minutes, no phage particles could be found outside the cells or adhering to the membranes; this is in contrast to experi-

ments in which cells fixed immediately after the same total incubation period still show phage particles bound to the outer cell membranes. Vacuoles of similar morphology and distribution are found in both types of experiments.

Observations of Polymorphonuclear Leucocytes in Control Samples.—The polymorphonuclear leucocytes found in rabbit peritoneal exudate cells contain a lobed nucleus having a thick electron-opaque edge along the inner membrane (Fig. 9). Unfortunately, preservation of the cells is rather poor as shown in the electron micrographs. This may be related to the conditions of fixation which were adapted for optimal preservation of macrophages, or possibly the granulocyte, which is a short-lived cell, may have undergone damage during the incubation procedure.

Several types of membrane-delimited granules have been described in rabbit polymorphonuclear cells (18, 19) and are found dispersed in the cytoplasm. These granules either display a uniform density, or show a grainy osmiophilic content of varying textures while others show a granular content with a still more darkly staining central component corresponding to the description of target-like granules (19). Vacuoles, which for the most part contain fragments of diffuse material are often encountered. Mitochondria are rarely seen; endoplasmic reticulum can only occasionally be identified; however, it is not profuse or well organized.

Observations of Rabbit Peritoneal Exudate Cells Incubated with T_2 Phage: Polymorphonuclear Leucocytes.—Uptake of phage particles by the granulocyte follows the same sequence of events as described for the macrophage; adsorption to the cell membrane and subsequent endocytosis. Experimental cells display a much higher degree of vacuolization than control cells.

In the shorter incubation periods (up to 30 minutes), phage particles are found surrounded by osmiophilic material within cytoplasmic vacuoles. Such vacuoles are usually partially empty and the dark staining material surrounding the phage is found towards the center of the vacuole (Fig. 10). Larger vacuoles appeared to have been formed by the fusing of several vacuoles or by the fusing of vacuoles with granules.

After an incubation period of 120 minutes, the leucocytes show fewer granules; however, the cytoplasm of the cell is filled with extremely large vacuoles in which phage surrounded by osmiophilic material is situated in the center (Fig. 11). The density of the material surrounding the phage is similar to that of the granules seen in control cells.

Disintegrated phage can be found in polymorphonuclears after 15 minutes' incubation (Fig. 12); however, even after 120 minutes' incubation, seemingly intact phage particles can still be seen in the leucocyte vacuoles.

DISCUSSION

The electron micrographs obtained in the present study show that the incorporation of phage particles by phagocytic cells may take place in various

ways. The general process of phage uptake can be termed endocytosis (20–22). This term covers the various engulfing mechanisms in which the incorporated material is surrounded by a membrane which originally was a part of the cell membrane. Phage particles attached to the cell membrane can be seen drawn into the cell by invagination of the membrane (Fig. 5) conforming to the description of rhopheocytosis (23, 24). Larger vacuoles in formation and intracellular vacuoles containing phage particles surrounded by clear material are visible at the cell periphery (Fig. 3). These seemed to have been formed by veils or projections extending from the cell membrane which enclosed the particles together with the surrounding medium suggesting cell "drinking" or pinocytosis (25); this process when below the resolution of the optic microscope is then termed micropinocytosis (21). Phagocytosed debris containing phage particles can occasionally be seen within vacuoles of the cytoplasm.

From these results and other studies (26), it appears that uptake of phage particles by phagocytic cells *in vitro* under the described conditions is initiated after about 7 minutes' incubation, and maximum incorporation occurs between 15 and 30 minutes.

Fishman has shown that at least 30 minutes' incubation of macrophage and phage are required to prepare the "information" necessary for antibody-forming cells to produce specific antibody. Ten minutes' incubation was shown to be insufficient; however, if after washing away the phage, the cells were allowed to incubate for another 20 minutes and then fractionated, addition of this filtrate resulted in antibody production in lymph node cells (1).

Our electron micrographs show low incorporation of the phage by the macrophage after 10 minutes' incubation. If after this period, the phage particles are washed away from the suspending medium and incubation is resumed for another 10 minutes before fixation, phage particles can still be seen adsorbed to the outer cell membranes and trapped between cells as after the first 10 minutes of incubation; however, the number of the intracellular phage particles has increased. It seems that washing removes only the free phage but not the phage particles adhering to the outer cell surface or trapped between cells; apparently the cells continue engulfing these phage upon additional incubation.

It should be emphasized that in the present experiments, the conditions as used by Fishman (1) were not exactly repeated. When electron microscopic preparations using ratios of phage to macrophage approaching those employed by Fishman (see Table I) were examined, no phage particles were found in the sectioned cells and the morphology of the latter resembled that of the control cells. Attempting to find phage particles by means of electron microscopy, while using such low concentrations of phage to macrophage could be equated to the proverbial "searching for a needle in a haystack." Previous electron microscopic studies of human buffy coat incubated with T_2 phage at a concentration of 10,000 plaque-forming units per cell at 37°C for 15 to 30 minutes presented sections with phage particles easily recognizable free in the

surrounding medium, adsorbed to leucocyte membranes, in the process of being engulfed, and within cytoplasmic bodies (26). Therefore to increase the probability of visualizing the virus particles in electron microscopic preparations of the incubation systems, high concentrations of phage particles were used. Further, it should be mentioned that these preparations were not tested for immunogenic activity. In a previous report, Fishman found that a ratio of phage to macrophage higher than 1:250 inhibited antibody formation; however, in recent experiments, the same author found that a higher multiplicity of phage to macrophage (100:1) does not interfere with antibody formation (27).

The events occurring after the uptake of the bacteriophage by the macrophage appear to be similar to those described for other endocytosed material in macrophage and in other cell types (22, 25, 28–32). The ingested material is segregated into cytoplasmic or digestive vacuoles. With time, these inclusions concentrate towards the Golgi region. Such endocytic bodies have been described as having varying degrees of density which increase with time apparently due to a concentration of their contents (28, 29, 31).

The vacuoles found at the periphery of the cytoplasm usually contain a single phage or small numbers of phage particles, whereas the bodies deeper in the cell contain larger numbers of phage surrounded by denser material; therefore it appears reasonable to assume that the particles are accumulated within larger bodies by fusion of smaller vacuoles (Figs. 6 and 7). The different densities of the endocytic vacuoles may either be due to fusion with preexisting dense bodies of the cell (Fig. 7; references 14, 19, 20, 33), or to a concentration of the material within the fused vacuoles (28, 31), or be the result of both processes.

The dense vacuoles of the macrophage cytoplasm seem to correspond to the digestive vacuoles of various phagocytic cells in which hydrolytic activity has been demonstrated (34). In addition to demonstrating the presence of hydrolytic enzymes associated with lysosomes (20) from fractionated rabbit alveolar macrophages by histochemical methods, Cohn *et al.* (34) showed acid phosphatase activity to be localized in granular elements of the cytoplasm of these cells. Following ingestion of yeast cell walls, the acid phosphatase activity was demonstrated in close proximity to the ingested material suggesting "release of acid phosphatase into the phagocytic vacuoles, perhaps by a process of membrane fusion." Acid phosphatase activity was shown by histochemical methods in rabbit macrophages obtained from the peritoneal exudate (35) pointing to the presence of lysosomes in these cells.

In other cell types, cytoplasmic granules have been found associated with pinocytic or phagocytic vacuoles and such dense bodies have either been shown by histochemical methods or assumed to be related to lysosomes (28, 33, 36). Recently, dense bodies of fibroblasts in tissue cultures were identified as lyso-

somes and shown to be involved in the digestion of phagocytosed nucleoprotein particles (37). Immunogenic activity has been localized within the lysosomal fraction of spleen cells from mice previously injected with antigen (38). It seems likely that the membrane-bound dense cytoplasmic granules seen in the macrophages in the present study correspond to lysosomes.

No obvious evidence of phage disintegration within vacuoles of the macrophage could be observed following incubation of up to 2 hours. Occasionally fragments of phage appeared to be present in vacuoles but such appearances were noted in all samples regardless of the length of the incubation period and may have been the result of the engulfment of an incomplete particle or due to the incidence of the section through the virus. Such fragments could occasionally be observed outside the cell. Whole phage particles with tails were noted in the cells at about the same frequency as seen outside the cells.

Disintegrated phage particles were seen within vacuoles of polymorphonuclear cells (Fig. 12) as early as after 15 minutes' incubation. These cells appeared as a contaminating minor population of the peritoneal exudate cells, and were very active in the uptake of phage particles. Such leucocytes have been demonstrated to possess primary lysosomes (39) and these granules have been shown to discharge their contents into phagosomes (39, 40). It is interesting, that the antigenic determinants of *E. coli* were shown to be rapidly destroyed within the rabbit polymorphonuclear cell, whereas antigenicity was retained for at least 2 hours within the macrophage (41).

If the production of antibody requires as a preliminary step a partial degradation of the protein (42) of the antigenic particle within the macrophage, then such degradation may not necessarily be associated with marked structural alterations and the methods employed in the present study may be inadequate to resolve such an effect. Yet, it is significant that following the time of incubation found necessary by Fishman to obtain an active preparation from the macrophage, the phage particles in our micrographs are found restricted within vacuoles containing electron-opaque material assumed to be rich in hydrolytic enzymes. Such an environment might allow for at least a partial digestion of the protein components of the phage. Further studies using phage with radioactively labeled protein coats are being carried on.

SUMMARY

Macrophages from rabbit peritoneal exudate cells incubated *in vitro* with T_2 bacteriophage from 10 up to 120 minutes show phage particles adsorbed to cell membranes, in the process of being engulfed by means of rhopheocytosis, micropinocytosis, and phagocytosis, and localized within dense vacuoles, semidense vacuoles, and clear vacuoles of the cytoplasm.

The electronmicrographs suggest that newly formed endocytic vacuoles containing phage particles fuse with one another and also fuse with dense bodies

of the cytoplasm as they migrate towards the cell interior, thus yielding larger vacuoles of varying densities containing higher concentrations of phage.

The polymorphonuclear cells present in a small proportion in the peritoneal exudate cells also endocytosed phage particles. The T_2 particles are found in large cytoplasmic vacuoles surrounded by an electron-opaque material presumably derived from cytoplasmic granules.

No disintegration of T_2 phage within the macrophage following incubations up to 120 minutes could be demonstrated; however, disrupted phage particles were noted within cytoplasmic vacuoles of polymorphonuclear leucocytes after 15 minutes' incubation.

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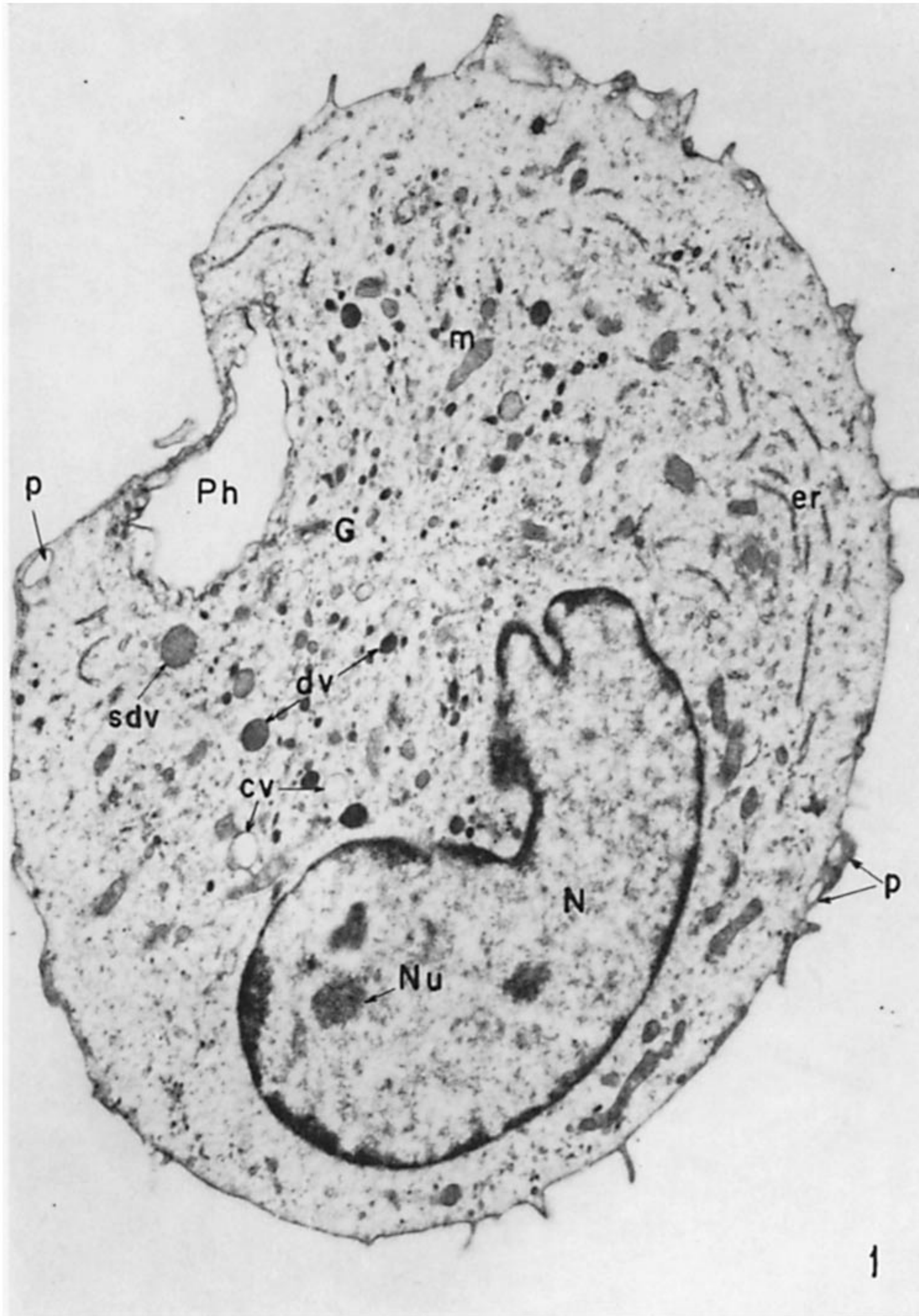
EXPLANATION OF PLATES

PLATE 87

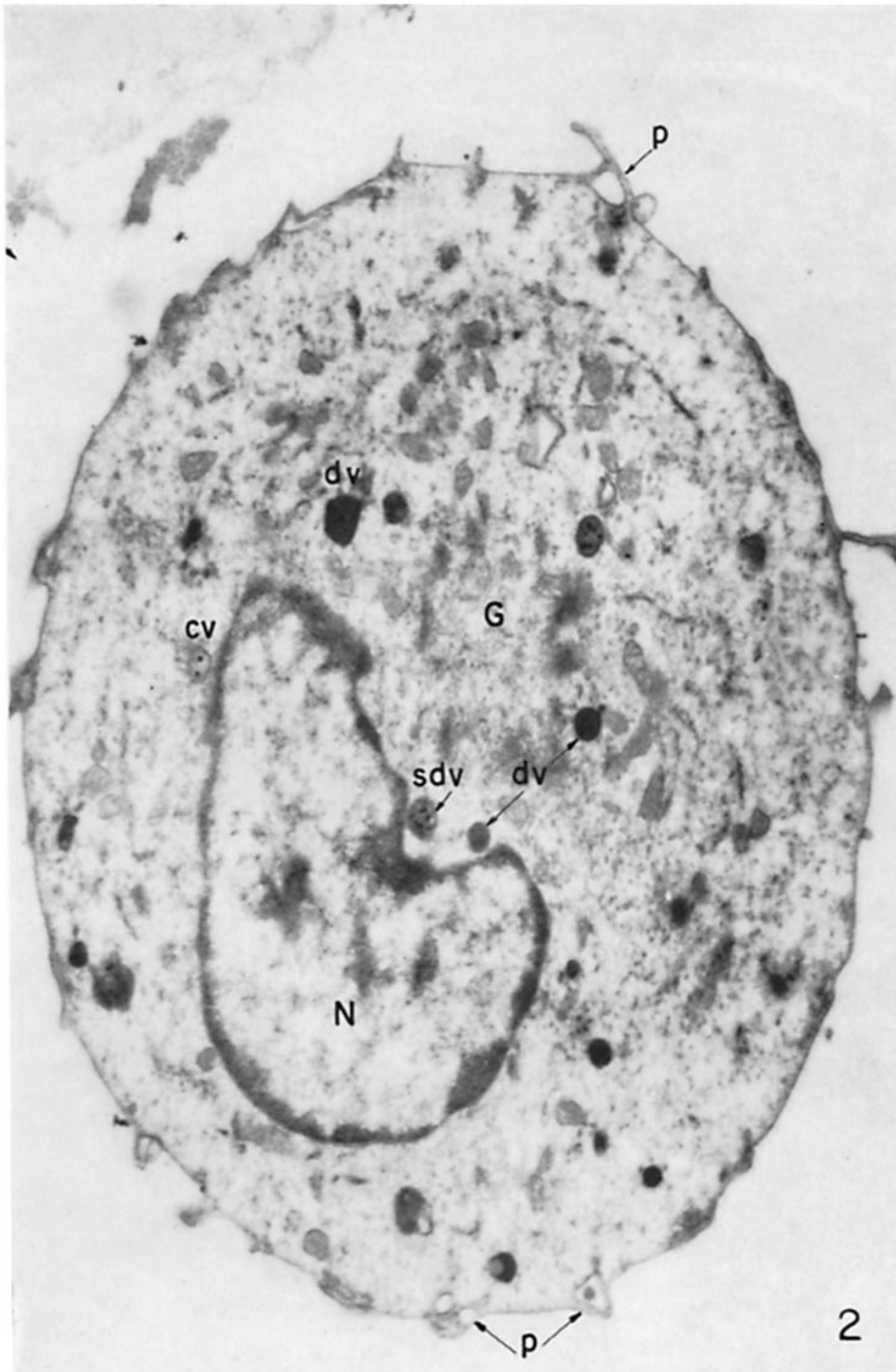
FIG. 1. Macrophage from control preparation incubated for 120 minutes at 37°C. Note the pinocytic extensions from the cell surface, pinocytic vacuoles (*p*), phagocytic vacuole (*Ph*), mitochondria (*m*), endoplasmic reticulum (*er*), membranes and vesicles of the Golgi system (*G*), nucleus (*N*), nucleolus (*Nu*). Many small vacuoles and granules showing varying densities can be seen radiating out from the centrosomal region: dense vacuoles (*dv*), semidense vacuoles (*sdv*), and clear vacuoles (*cv*). Uranyl acetate stain. × 14,000.



(Aronow *et al.*: *In vitro* endocytosis of T_2 phage)

Plate 88

Fig. 2. Macrophage incubated with T_2 phage for 120 minutes. Note pinocytic vacuoles (*p*) at the periphery of the cell. Phage particles can be seen within dense vacuoles (*dv*), semidense vacuoles (*sdv*), and a clear vacuole (*cv*). Golgi system (*G*). Uranyl acetate stain. × 16,000.



(Aronow *et al.*: *In vitro* endocytosis of T_2 phage)

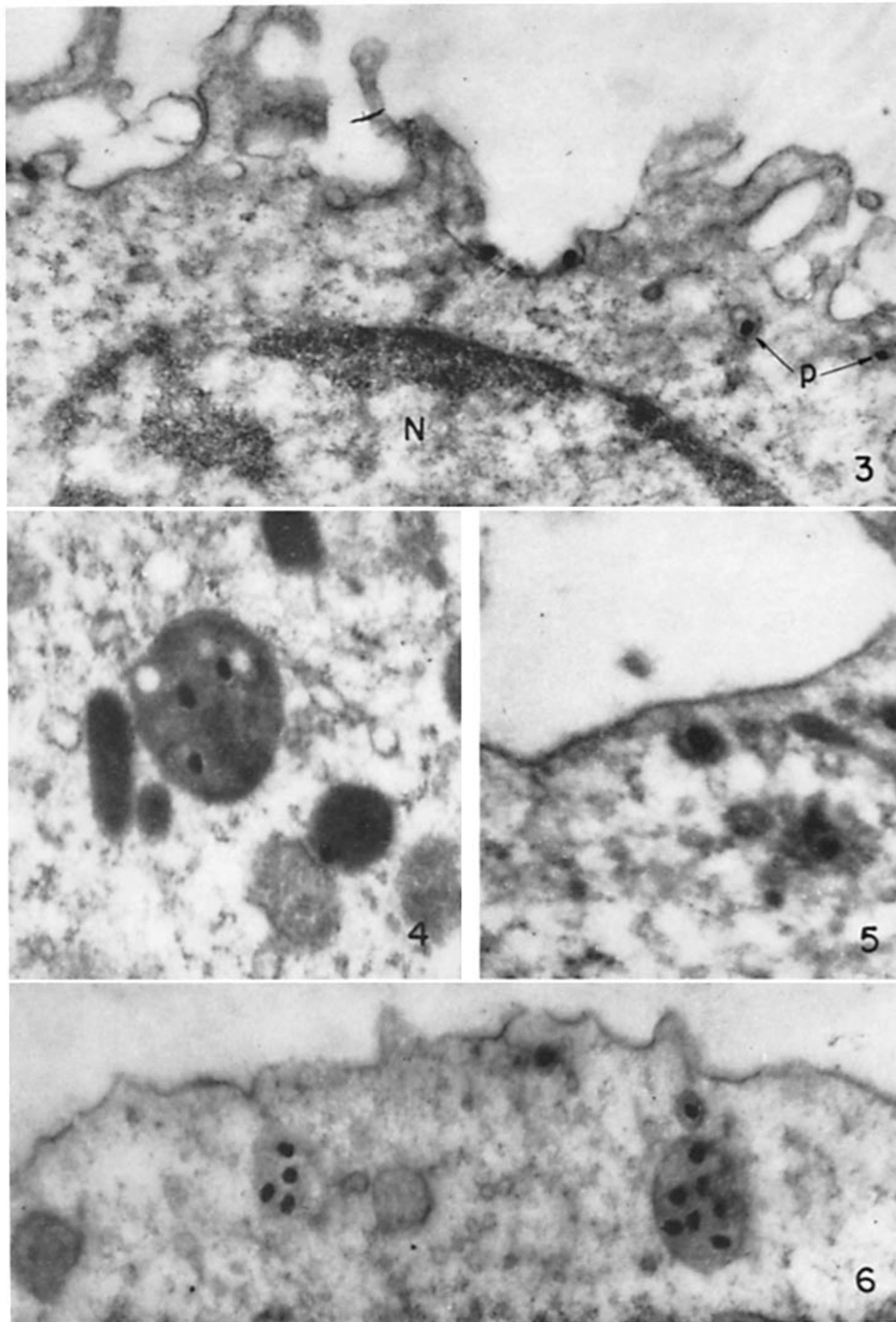
PLATE 89

FIG. 3. Macrophage incubated with T_2 phage for 30 minutes. Phage particles partially enveloped by a diffuse electron-opaque material are adsorbed to the cell membrane and pinocytic vacuoles are being formed. Two endocytic vacuoles (*p*) containing single phage particles are present at the right. Uranyl acetate stain. × 33,000.

FIG. 4. Dense vacuole containing phage particles which also shows clear areas within its dense matrix. Uranyl acetate stain. × 45,000.

FIG. 5. A portion of the cell membrane of a macrophage from a sample of cells incubated with T_2 phage for 10 minutes showing rhopheocytosis. Note channel connecting with the outer cell membrane. Uranyl acetate stain. × 50,000.

FIG. 6. Macrophage incubated with T_2 phage for 30 minutes. At right is a vacuole containing a single phage in close proximity to a larger semidense vacuole containing 7 phage particles. A tangential section through a semidense vacuole can be seen on the left. Uranyl acetate stain. × 45,000.

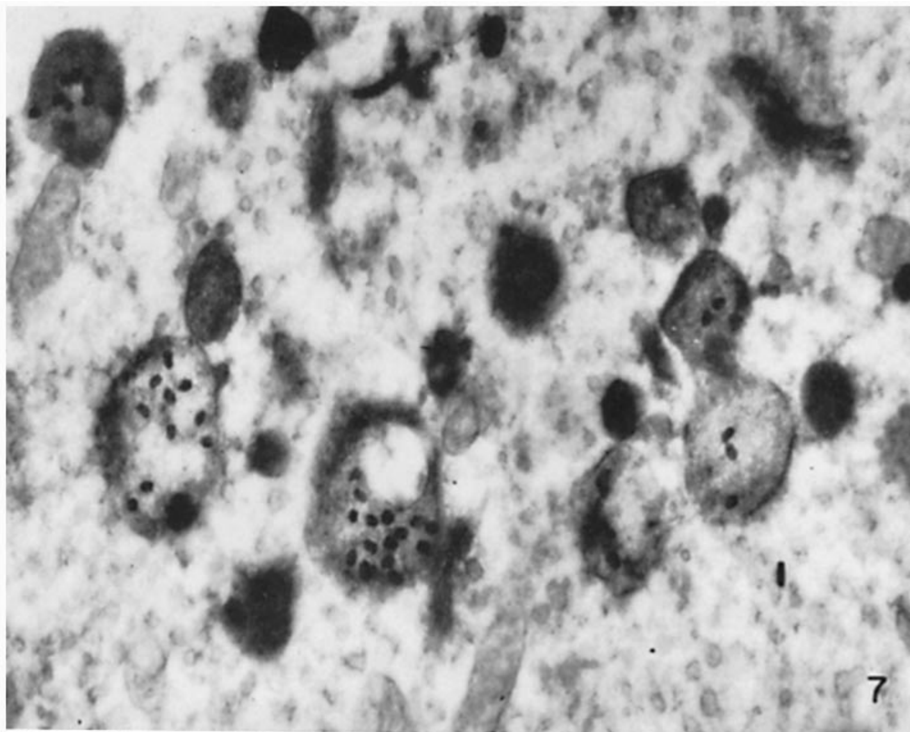


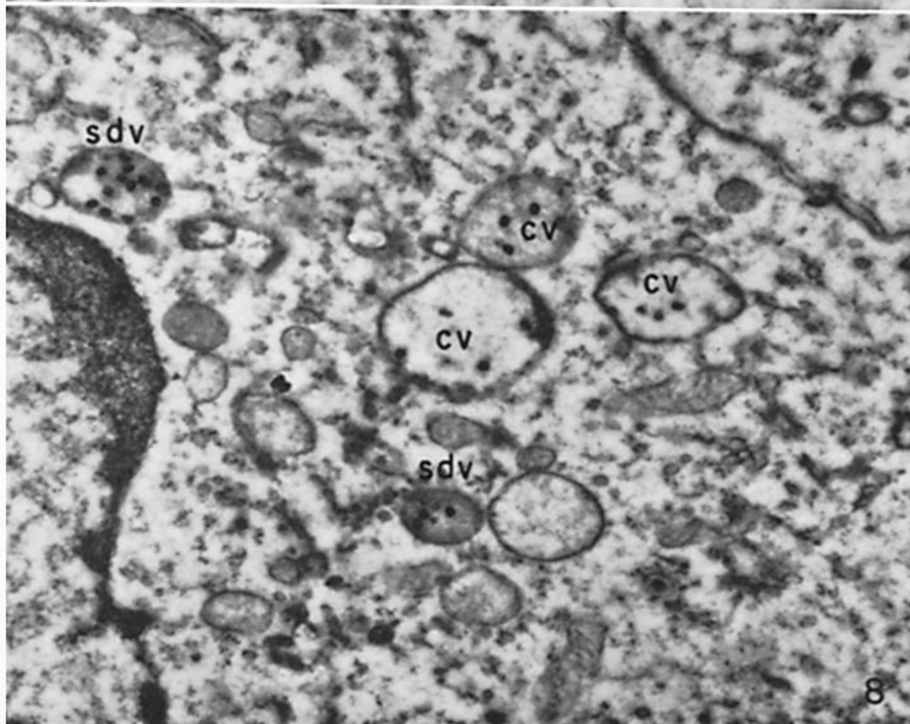
(Aronow *et al.*: *In vitro* endocytosis of T_2 phage)

PLATE 90

FIG. 7. Macrophage incubated with T_2 phage for 30 minutes showing dense and semidense vacuoles containing phage particles. At left, a dense body appears to be fusing with a semidense vacuole containing phage. On the right, two semidense vacuoles containing phage and one dense body appear to be in the process of fusing. Note clear area in semidense vacuole at center and the proximity of the Golgi membranes to vacuoles. Uranyl acetate stain. × 35,000.

FIG. 8. Macrophage incubated with T_2 phage for 30 minutes showing semidense (*sdv*) and clear vacuoles (*cv*) containing phage particles in the Golgi region. Note dense deposits along inner membrane of clear vacuoles. At top right, the edge of an adjoining cell can be seen. Uranyl acetate stain. × 25,000.



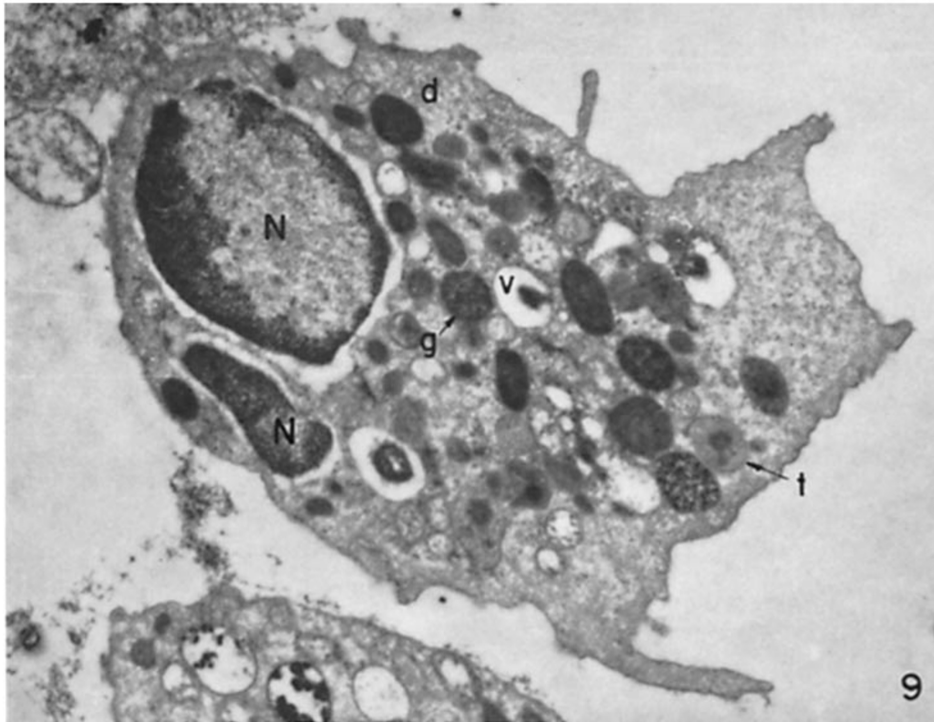


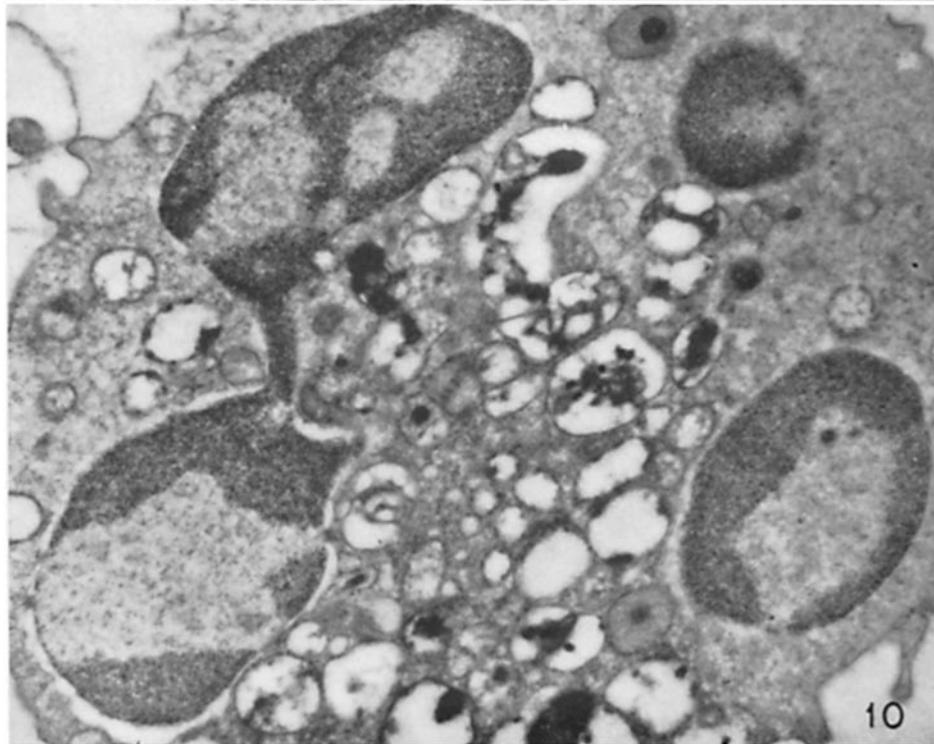
(Aronow *et al.*: *In vitro* endocytosis of T_2 phage)

Plate 91

Fig. 9. Polymorphonuclear leucocyte from a control sample of peritoneal exudate cells incubated for 35 minutes. Note the uniformly dense granules (*d*), granules with an osmiophilic grainy content (*g*), vacuoles containing fragments of dense material (*v*), and the target granules (*t*). There is a shrinkage artefact visible between nucleus (*N*) and cytoplasm. Uranyl acetate stain. × 16,000.

Fig. 10. Polymorphonuclear leucocyte from peritoneal exudate cells incubated with T_2 phage for 15 minutes. Note the increased number and size of vacuoles present in the cytoplasm. Phage particles are present in the center of vacuoles surrounded by electron-opaque material. At top center, a large vacuole appears to have been formed by the joining of several smaller ones. Uranyl acetate stain. × 20,000.



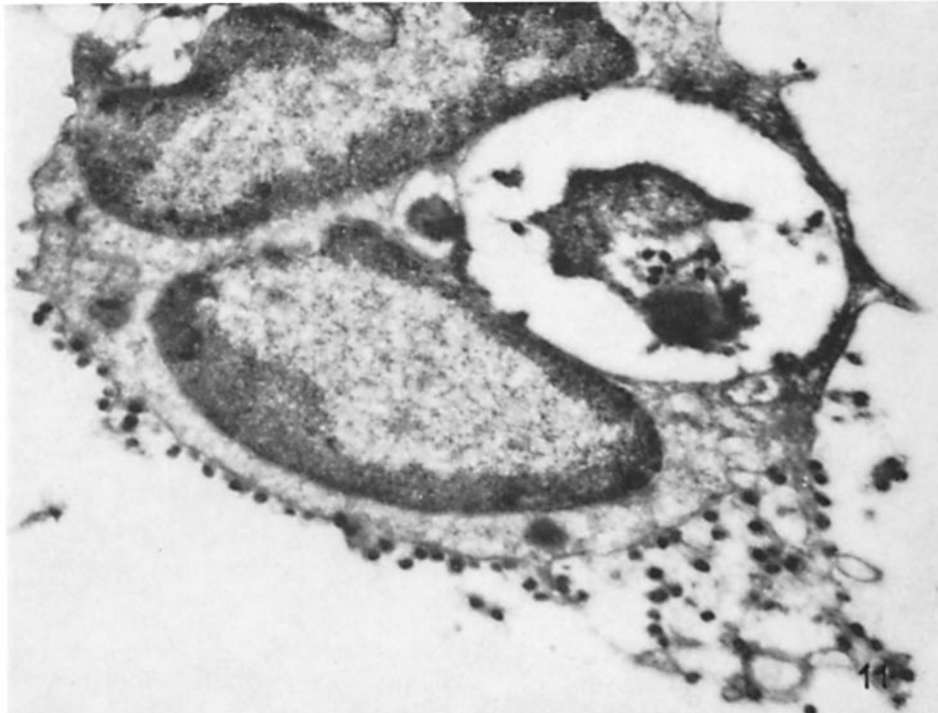


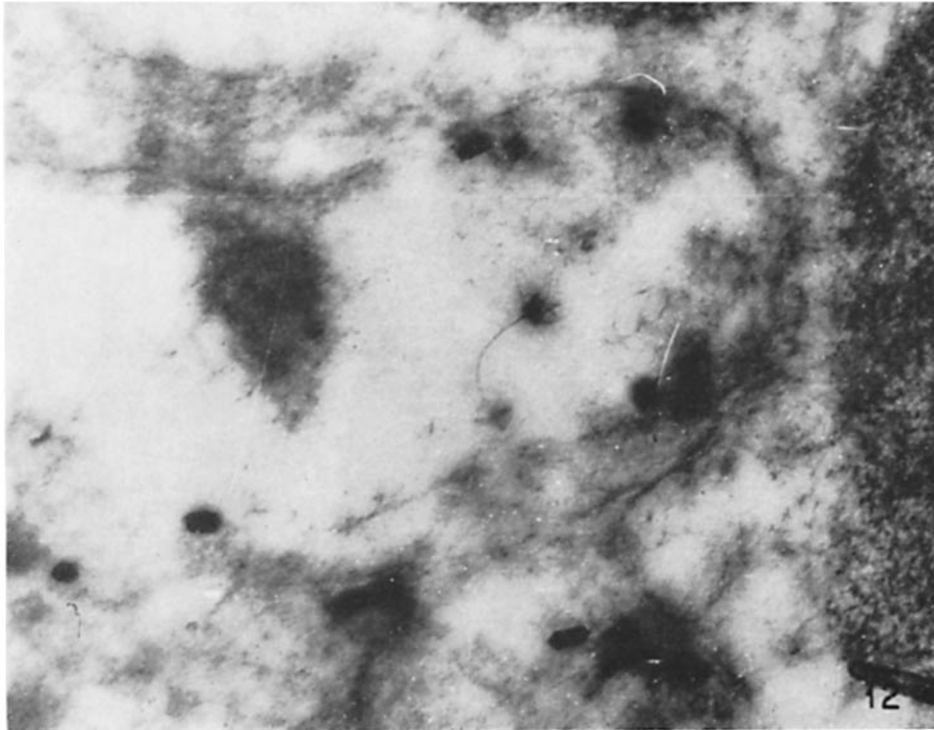
(Aronow *et al.*: *In vitro* endocytosis of T_2 phage)

PLATE 92

FIG. 11. Polymorphonuclear leucocyte incubated with T_2 phage for 120 minutes. Note a single large vacuole containing phage particles surrounded by electron-opaque material. Large numbers of phage particles are adsorbed to the cell membrane. Uranyl acetate stain. × 16,000.

FIG. 12. Disintegrated T_2 phage in vacuole of polymorphonuclear leucocyte incubated with T_2 particles for 15 minutes. Uranyl acetate stain. × 95,000.





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