JOURNAL OF BACTERIOLOGY, Nov. 1970, p. 966-980

Vol. 104, No. 2
Printed in U.S.A.

Action of Penicillin G on Endosymbiote *Lambda* Particles of *Paramecium aurelia*

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Received for publication 3 August 1970

The kinetics of loss from the cytoplasm and changes in ultrastructure of symbiont *lambda* particles after treatment of axenically cultivated *lambda*-bearing *Paramecium aurelia* with penicillin G was investigated. Low concentrations (1 to 2 unit/ml) of the antibiotic caused many particles within the cell to become filamentous; high concentrations (2,000 unit/ml) caused lysis of the particles without noticeably affecting the protozoan. The ED_{50} value (2 to 3 unit/ml) was within the range of values found to cause lysis of many gram-negative bacteria. Rapidly dividing *lambda* were more vulnerable to the action of the antibiotic than slowly dividing particles. Nondividing particles were not affected by exposure to the antibiotic. Ultrastructural changes observed in *lambda* during lysis by penicillin G were consistent with the view that penicillin interferes with the synthesis of a vital component of the cell envelope of the particle, possibly a peptidoglycan similar to that found in the cell walls of bacteria. The deoxyribonucleic acid of *lambda* was dispersed throughout the particle as electron dense fibers enclosed within electron transparent areas. The cell envelope appeared to consist of at least two morphologically distinguishable layers, an inner layer homologous to the plasma membrane of bacteria and an outer layer homologous to the bacterial cell wall. *Lambda* may be regarded as a randomly distributed population of bacteria growing and dividing synchronously within the collective cytoplasm of its protozoan host.

Lambda is one of a genre of genetically autonomous particles of unknown origin that inhabit the cytoplasm of certain strains of the ciliate *Paramecium aurelia* (17). The precise nature of these and related particles, *kappa*, *mu*, *pi*, and *sigma*, has been a matter of controversy. *Kappa* particles, for example, were considered to be plasmagenes or viruses, a view that was later abandoned when visualization of the particles with nuclear dyes was achieved, and the particles were found to be comparable to bacteria in size (10).

Recent studies suggest the particles may be bacterial endosymbiotes. *Lambda* particles produced quantities of folic acid, an essential nutrilite for *P. aurelia*, sufficient to support the growth of the ciliates in a medium devoid of this vitamin (14). They have been maintained extracellularly for long periods in a complex medium (23). *Lambda* deoxyribonucleic acid (DNA), in a manner characteristic of many bacterial DNA species, undergoes almost complete renaturation after reannealing at 60 C (16). The DNA of the *kappa* and *mu* particle has been examined and reported to be membrane bound (12, 19). Other evidence, derived primarily from studies with the electron microscope, suggest that the particles are a diverse group of bacteria that live within the cytoplasm of the host *Paramecium* (2).

This concept is further strengthened by the fact that the particles are destroyed by the action of certain antibiotics, notably penicillin, under conditions in which the protozoan remains unaffected (14, 17). Because the mode of action of this antibiotic is better understood now than in the past, detailed studies on the effect of penicillin may serve to provide an insight into the relationship between the particles and its protozoan host. This paper describes the kinetics of loss and changes observed in the ultrastructure of *lambda* resulting from treatment of axenically cultivated, *lambda*-bearing *P. aurelia* with the antibiotic.

MATERIALS AND METHODS

Culture methods. *Paramecium aurelia*, stock 299, containing *lambda* particles was cultured axenically and maintained at 27 C in the dark (15). The medium consisted of a mixture of proteose peptone (10 mg/ml), Trypticase (5 mg/ml), yeast nucleic acid (1 mg/ml), $MgSO_4 \cdot 7H_2O$ (0.5 mg/ml), stigmasterol (5 μg/ml), calcium pantothenate (5 μg/ml), pyridoxal hydrochloride (5 μg/ml), pyridoxamine hydrochloride

(2.5 μg/ml), riboflavine (5 μg/ml), folic acid (2.5 μg/ml), thiamine hydrochloride (15 μg/ml), biotin (0.00125 μg/ml), DL-thioctic acid (0.05 μg/ml) and tartaric acid esters of beef tallow monoglycerides (TEM/4T, 100 μg/ml). Subcultures were made by inoculating 5 ml of fresh medium with 0.1 ml of a previous 7-day culture. Under these conditions, maximum populations were reached in 7 days and ranged from 12,000 to 15,000 per ml. The number of *lambda* particles present in the protozoans was approximately 2,000 per cell.

Counting procedures. The population of ciliates was determined by direct count of a suitable dilution of the culture medium. Quantitative determinations of *lambda* particles were made as described previously (15). In some cases, a variation of the procedure was used, especially when the expected number of *lambda* particles was low. Animals from 5 or 10 ml of culture were collected by centrifugation at 600 × *g* for 1 min, suspended in a small volume of buffered salt solution (mg/liter; NaCl, 30; $CaCl_2 \cdot 2H_2O$, 300; $MgCl_2 \cdot 6H_2O$, 200; KH_2PO_4, 30; $K_2HPO_4 \cdot 3H_2O$, 45; *p*H 7.0) and, after determination of the number of ciliates present, disrupted in a Potter Elvehjem-type homogenizer equipped with a Teflon pestle. This treatment released the particles quantitatively from the ciliates.

Penicillin treatment. Freshly prepared solutions of penicillin G at the desired concentration were added to 50- or 100-ml quantities of cultures of *lambda*-bearing *P. aurelia* contained in Erlenmeyer flasks. The flasks were incubated at 27 C in the dark. Samples were removed at intervals. The ciliates in each sample were collected by centrifugation and suspended in a small volume of buffered salt solution. Quantitative determinations on the number of ciliates and *lambda* particles were made as described above.

Phase microscopy. After concentration of the *lambda*-bearing organisms by centrifugation, the ciliates were picked up in a micropipette and injected into a small drop of aceto-orcein dye on a glass slide (13). This procedure revealed the particles within the cell. Prefixing the ciliates suspended in a small drop of culture fluid on a glass slide by exposure to OsO_4 vapors for 15 to 20 sec before staining with aceto-orcein aided in preserving the structural integrity of the protozoans. Stained preparations were observed in a Leitz microscope under oil by using phase optics.

Electron microscopy. The ciliates were fixed in 1.33% (w/v) OsO_4 buffered with either 0.2 M γ-collidine, *p*H 7.4, or 6% (w/v) glutaraldehyde in 0.15 M phosphate, *p*H 7.2, for 1 hr at 0 C. Both buffers gave satisfactory results. After fixation, the ciliates were allowed to stand in buffer overnight at 0 C. They were dehydrated through a graded series of alcohol, suspended in propylene oxide, and embedded in araldite (Durcapan). Ultrathin sections were cut with a glass or diamond knife, doubly stained with alcohol-uranyl acetate and lead citrate (11, 21), and viewed with a Phillips EM-300 electron microscope.

Enzyme treatments. After fixation in osmium-phosphate buffer solution for 1 hr at 0 C, the ciliates were washed free of fixative and suspended in buffer solution containing 1 mg/ml of either deoxyribonuclease or Pronase. The deoxyribonuclease solution was supplemented with 5 mM $MgCl_2$. After digestion at 37 C for 1 hr, the ciliates were washed with buffer solution, dehydrated, and embedded in araldite.

Materials. Vitamins were obtained from Nutritional Biochemicals, Inc. Yeast nucleic acid (Grade C), stigmasterol, and Pronase were products of Calbiochemicals, Inc. Proteose peptone was purchased from Difco; Trypticase was from BBL. Orcein was obtained from Matheson Co., Inc., and penicillin G (K salt) was from the Pfizer Co. TEM/4T was a gift of H. Birnbaum of the Hachmeister Co. Deoxyribonuclease was purchased from Worthington Biochemical Corp.

RESULTS

Axenically cultivated *lambda*-bearing *P. aurelia* undergo complete and irreversible loss of particles resulting from a single treatment with 2,000 units/ml of penicillin G. We have maintained such *lambda*-free organisms for several years through hundreds of subcultures without observing recurrence of the particles. Figures 1 and 2 contrast the appearance of a portion of the cytoplasm of one of these animals with a portion of the cytoplasm of a *lambda*-bearing *Paramecium* carried through an equal number of transfers. *Lambda* particles appear as numerous rod-shaped bodies distributed at random throughout the cytoplasm (Fig. 1). No such rod-shaped bodies are seen in the treated protozoans (Fig. 2). The minimum concentration of antibiotic required to produce this effect was approximately 25 units/ml (Fig. 3); the ED_{50} value was approximately 2.5 units/ml. The antibiotic destroyed *lambda* particles selectively; ciliate populations remained unaffected at concentrations of penicillin G as high as 10,000 units/ml.

Effect of high concentrations of penicillin on lambda-bearing Paramecium. In the presence of high concentrations of penicillin G (e.g. 2,000 units/ml), loss of *lambda* from the cytoplasm of ciliates in the logarithmic phase of growth proceeded without a lag (Fig. 4) and was complete in less than 36 hr after administration of the antibiotic. Loss of *lambda* from animals treated with penicillin G during stationary phase of growth was accompanied by a lag period of approximately 5 or 6 hr (Fig. 5). Similarly, loss of *lambda* from the cytoplasm of ciliates taken from the logarithmic phase of growth, suspended in buffered salt solution, and treated with antibiotic ("resting" cells) was accompanied by an even more pronounced lag period which lasted approximately 10 to 12 hr. The time required for complete loss of particles was 60 hr.

In a variation of this experiment, *lambda*-bearers were taken from the logarithmic phase of growth and allowed to remain at 27 C in sterile buffered salt solution for a period of 2 days before administration of the antibiotic. Under these con-

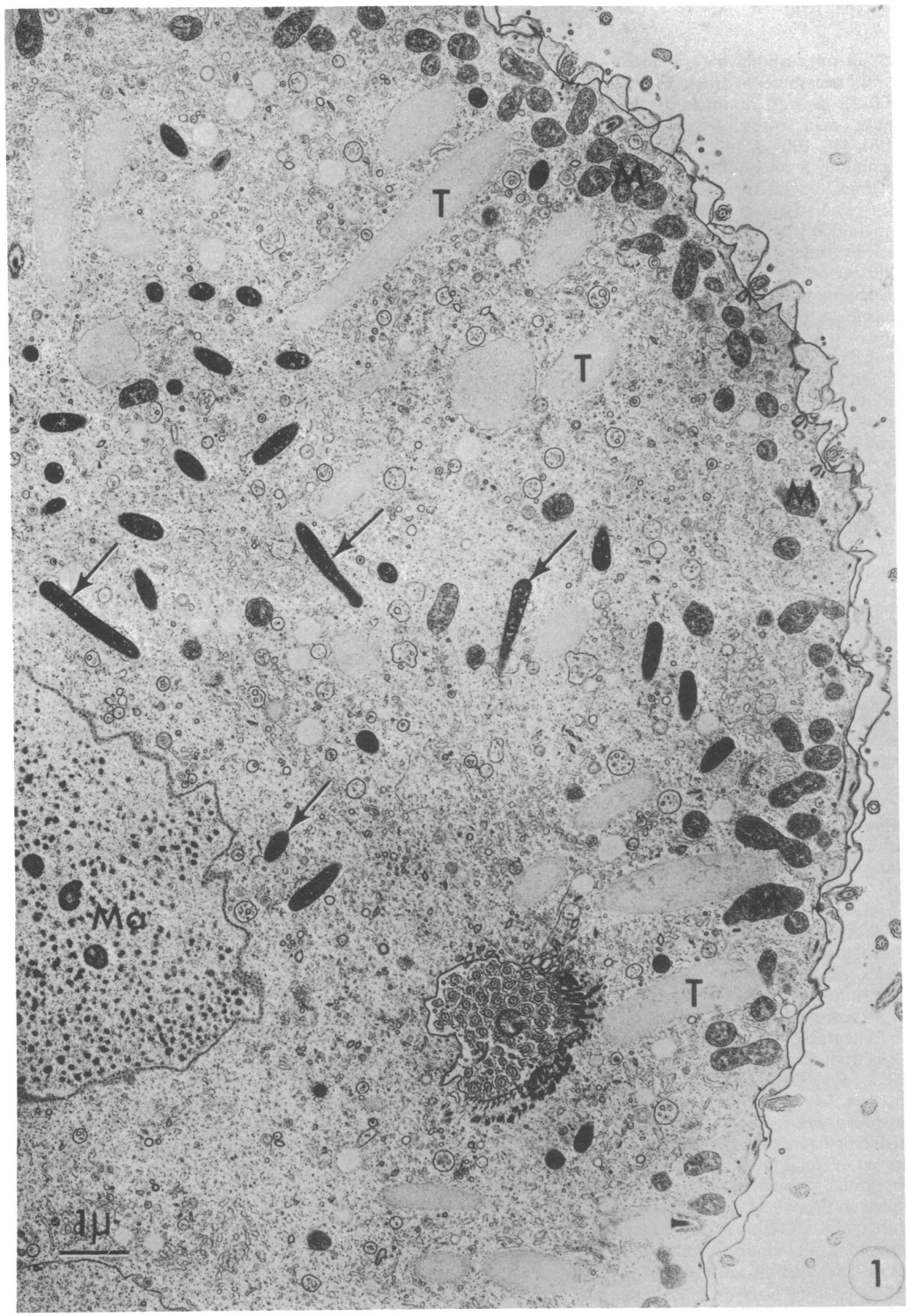


FIG. 1. *Low-power electron micrograph of a portion of the cytoplasm of an untreated lambda-bearing Paramecium. Symbols: arrows indicate lambda particles; (G) gullet, (M) mitochondrion; (Ma) macronucleus; (T) trichocyst.* ×*9,883.*

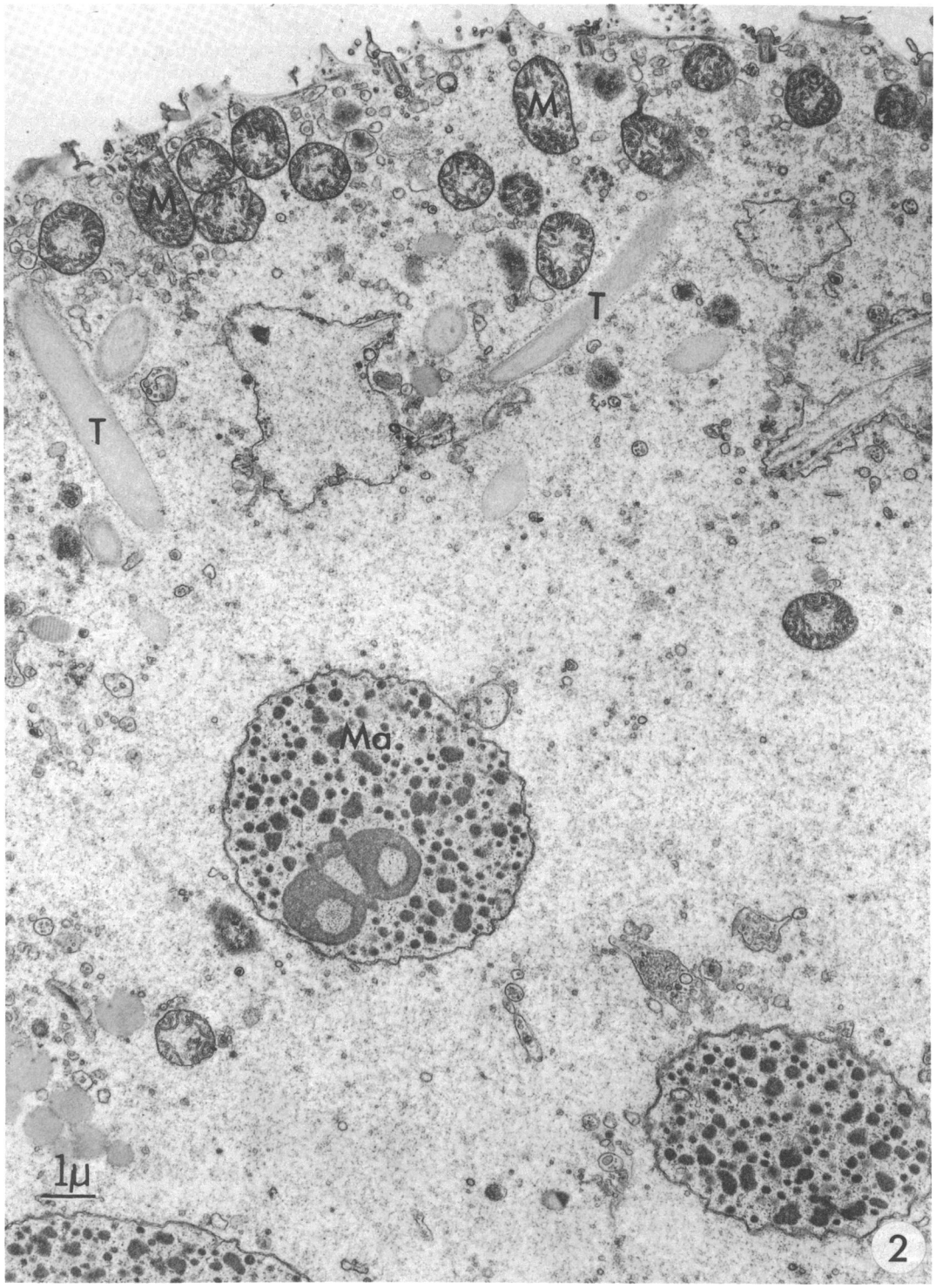


FIG. 2. *Low-power electron micrograph of a portion of the cytoplasm of a lambda-bearing Paramecium after treatment with 2000 units of penicillin G per ml to remove the particles. Symbols: (M) mitochondrion; (Ma) macronucleus; (T) trichocyst.* ×7,707.

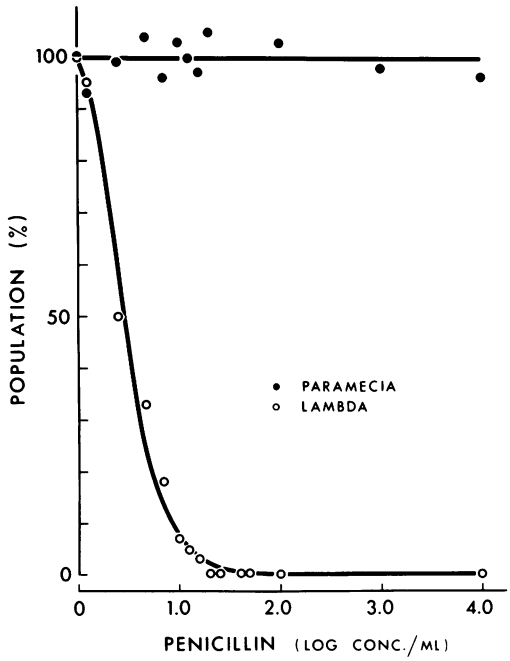


FIG. 3. *Selective destruction of lambda particles by penicillin G. Various concentrations of penicillin G were added to cultures of lambda-bearing P. aurelia at time of inoculation into fresh medium. After 7 days of growth at 27 C in the dark the population of paramecia (●) and lambda (○) was determined. Data are expressed as per cent of paramecia or lambda remaining in the treated cultures compared to an untreated control. The population of P. aurelia in the untreated culture averaged 13,600/ml; the average number of lambda per cell in the untreated culture was 2,180.*

ditions, destruction of *lambda* was incomplete. The particle population was reduced to about 10 to 15% of its original number in about 2 days and remained at that level for an indefinite period. Microscopic examination of the aceto-orcein stained animals revealed that large numbers of protozoans contained an average of 20 to 30 *lambda* particles per cell. An occasional ciliate was observed that contained as many as 2,000 *lambda* particles.

Suspensions of *lambda* particles in buffered salt solutions, freshly released from the protozoans by homogenizing animals taken from the logarithmic phase of growth, were treated with penicillin G and incubated at 0 C and 28 C (Fig. 6). The number of *lambda* particles decreased significantly with time of exposure when the suspensions were incubated at 28 C but this was unrelated to the presence of penicillin. By way of contrast, the decrease in the number of these particles held at 0 C was small. No effect of the antibiotic could be detected under either set of conditions.

During the course of treatment of *lambda*-bearers with penicillin G, some individual members of the population contained *lambda* particles undergoing characteristic morphological changes. In addition to normal rod-shaped particles, the cytoplasm of these individuals contained some *lambda* which were elongate, often with a swollen end, and others which had become spherical in shape. *Paramecium* containing these "mixed" populations of both normal and abnormal particles were readily identified in the phase microscope after staining with aceto-orcein. Figure 7 (a-c) compares the appearance of the cytoplasm of one of these animals (Fig. 7b) with the appearance of the cytoplasm of both a normal, untreated *lambda*-bearer (Fig. 7a) and a particle-free animal (Fig. 7c) treated with the antibiotic for removal of the particles. After the addition of 2,000 units of penicillin G per ml to a culture of *lambda*-bearers in the logarithmic phase of growth, there is a rapid decrease in the number of *lambda*-bearers containing normal particles. This is accompanied by an equally rapid increase in the number of protozoa whose cytoplasm contains a mixture of both normal and abnormal *lambda* particles (Fig. 8). This number reaches a peak after 10 hr and is followed

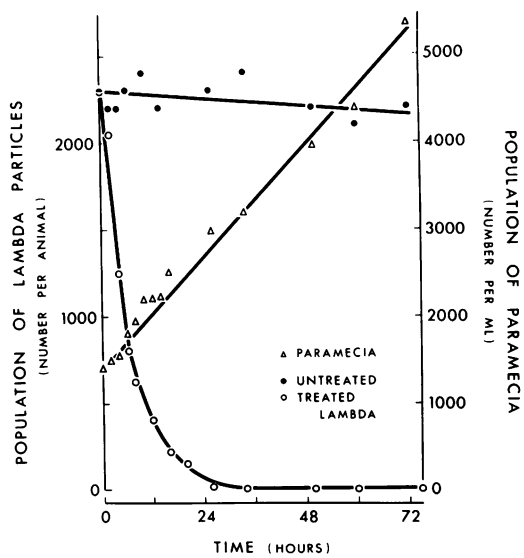


FIG. 4. *Kinetics of loss of lambda particles from the cytoplasm of lambda-bearing P. aurelia in the logarithmic phase of growth treated with penicillin G. Penicillin G was added to a 3-day old culture of lambda-bearing P. aurelia at a concentration of 2,000 unit/ml. The population of protozoan cells (△) and the number of lambda particles per protozoan (●) were determined at intervals after administration of the antibiotic. The number of lambda particles per protozoan (○) of an untreated control was included for comparison.*

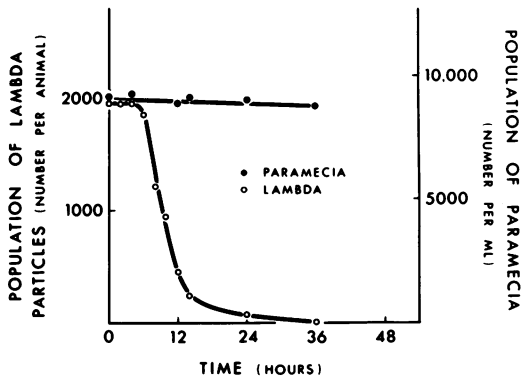


FIG. 5. *Kinetics of loss of lambda particles from the cytoplasm of lambda-bearing P. aurelia treated with penicillin while in the stationary phase of growth. Conditions of the experiment were similar to those described in Fig. 4 except that penicillin was added to a 7-day old culture of lambda-bearing P. aurelia. Paramecia* (●); *lambda particles* (○). *Ordinate scale is linear.*

during the next 24 hr by an increase to almost 100% the number of cells without *lambda* particles.

To determine whether animals still containing normal *lambda* particles during penicillin treatment were capable of retaining their particles, we ran the following experiment. Individual *lambda*-bearers, after exposure to the antibiotic for various periods, were removed to fresh, penicillin-free culture medium contained in the wells of depression slides and permitted to divide and form clones. When the clones reached populations numbering approximately 1,000 (9 or 10 days at 27 C in the dark), 20 to 30 members of each were stained with aceto-orcein and examined in the phase microscope for the presence or absence of *lambda* particles. Some clones were found in which all members contained the full complement of *lambda* particles (e.g. approximately 2,000 particles/protozoan). Others were completely free of the particles. We never observed a clone that contained a mixture of *lambda*-bearing and *lambda*-free animals. When the percentage of clones containing *lambda* particles was compared to the percentage of *lambda*-bearers in the culture from which these clones were derived, an approximate one to one correspondence was observed (Fig. 9).

Effect of low concentrations of penicillin G on lambda-bearers. Exposure of *lambda*-bearing *P. aurelia* to low concentrations (0.5 to 2.0 units/ml) of penicillin G for prolonged periods (i) causes a decrease in the average number of *lambda* particles maintained in the cytoplasm and (ii) induces the formation of abnormal *lambda* particles in proportion to the amount of penicillin added to the culture (Table 1). Abnormal *lambda* particles take the form of filamentous rods that are as wide but often several fold longer than normal *lambda* (Fig. 10). Some of these forms have been observed to extend the entire length of the protozoan itself. Removal of these penicillin-treated animals to penicillin-free medium, even after several weeks of exposure, restores to normal both the number and morphological appearance of *lambda* particles within the cytoplasm.

Electron microscopy. *Lambda* particles of axenically cultivated *P. aurelia* may normally be seen in the cytoplasm enclosed within a vacuole surrounded by a smooth-walled membranous sheath (Fig. 11). Usually, one or two particles (Fig. 11, 12) occupy a single vacuole, but this number may vary considerably. We have observed as many as 20 *lambda* contained within a single vacuole in ciliates harboring a large population of particles (2,500/cell). Contained within the vacuoles, in addition to the *lambda* particle itself, are numerous filamentous rod-shaped bodies which Jurand and Preer (6) described as flagella (Fig. 13).

The particles themselves resemble bacteria in size and shape and are surrounded by a cell enve-

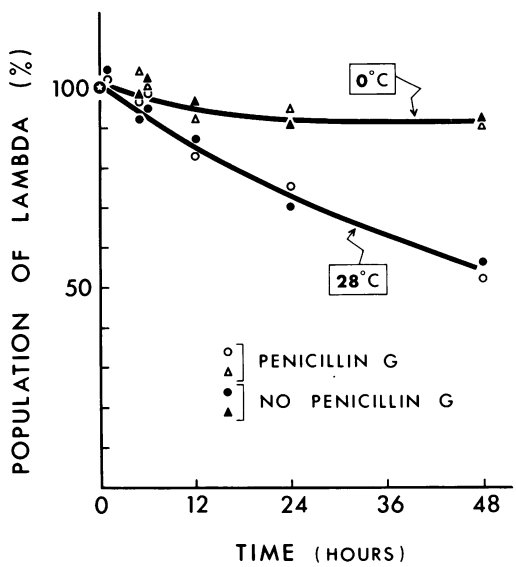


FIG. 6. *Effect of penicillin G on freshly prepared suspensions of lambda particles. Lambda were quantitatively released from lambda-bearing P. aurelia in a culture taken from the logarithmic phase of growth (3-day old culture) by homogenizing in buffered salt solution in a Potter Elvehjem-type homogenizer equipped with a Teflon pestle under aseptic conditions. The homogenates were incubated at 0 and 28 C in the presence and in the absence of the antibiotic. No penicillin* (○, △); *penicillin G, 2,000 units/ml* (●, ▲). *Ordinate scale is linear.*

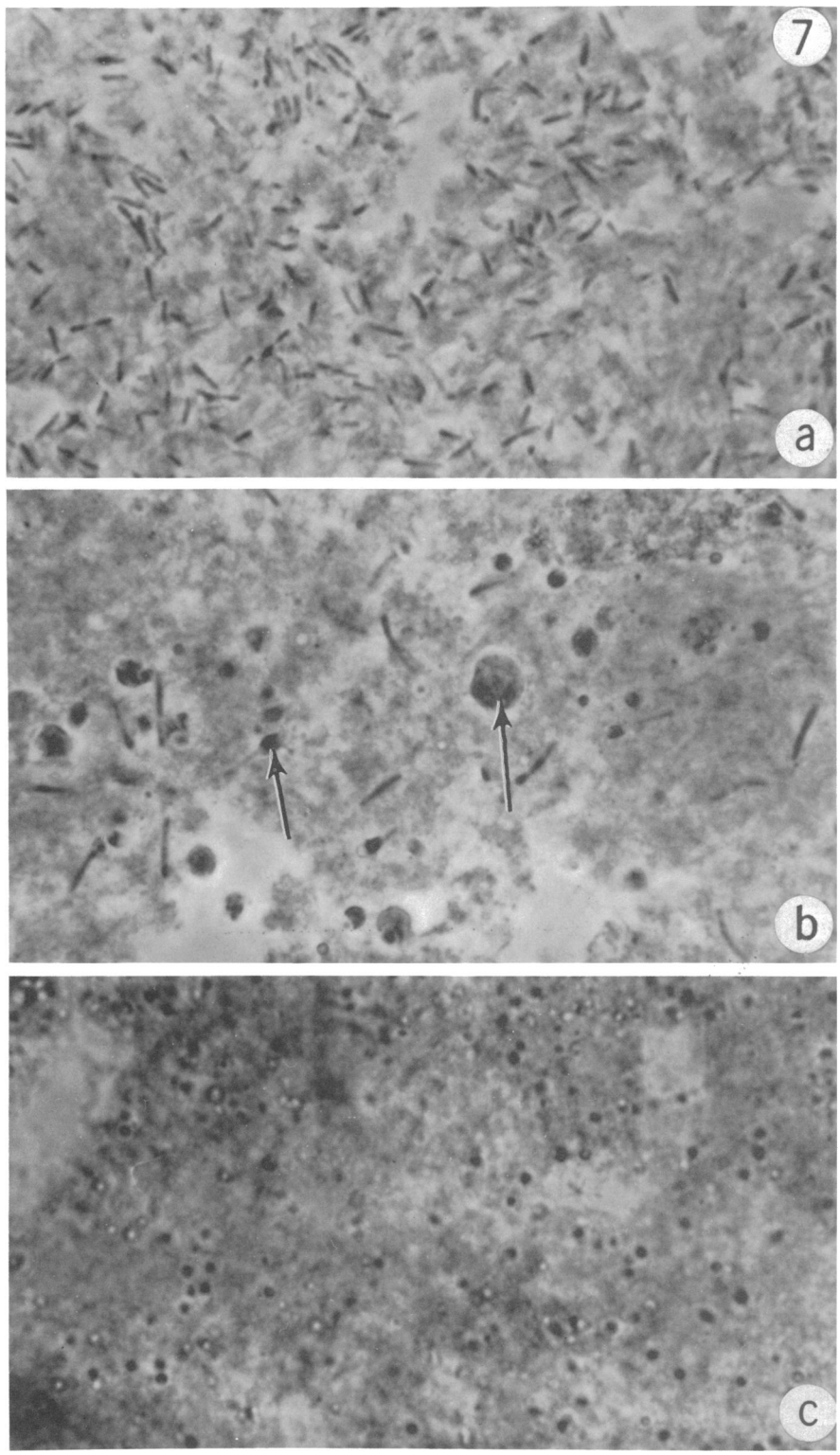


FIG. 7. *Phase micrographs of lambda-bearing P. aurelia. Aceto-orcein stain. Appearance of cytoplasm (a) before penicillin treatment showing a normal population of lambda, (b) during pencillin treatment showing a "mixed" population of normal and abnormal (arrows) lambda, and (c) after penicillin treatment showing loss of lambda.* ×*1,300.*

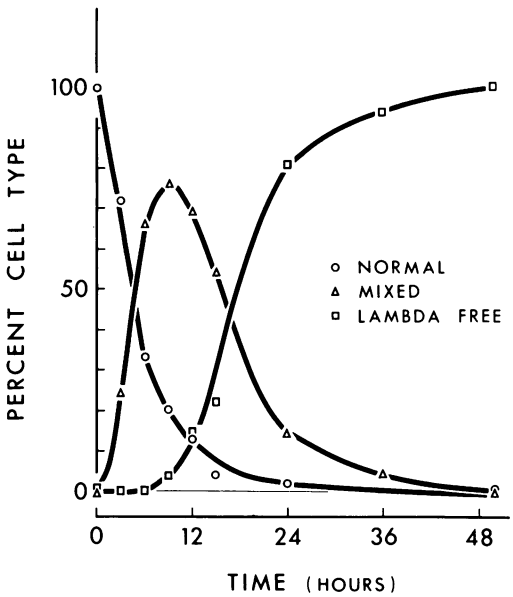


FIG. 8. *Change in the distribution of paramecia containing normal and "mixed" lambda populations after treatment of lambda-bearing P. aurelia with penicillin G. The antibiotic was added at a concentration of 2,000 units/ml to a culture of lambda-bearers in the logarithmic phase of growth (3-day old culture). Samples were removed at intervals and the relative numbers of P. aurelia containing, normal lambda (○), "mixed" normal, and abnormal lambda (△), no lambda (□) determined.*

lope consisting of at least two membrane layers (Fig. 14a). The outermost layer produces a wrinkled profile in contrast to the taut appearance of the innermost layer. The characteristic appearance of these layers is more readily seen after Pronase treatment (Fig. 14b). Dispersed throughout the cytoplasm are areas of low density that contain fibrous material. The fibers disappear after treatment with deoxyribonuclease and are presumed to be DNA (Fig. 14c).

High concentrations of penicillin G cause the particles to lyse. The process appears to be a random one and occurs independently of the number of particles occupying a vacuole (Fig. 15).

During the early stages of lysis by penicillin there is a loss of definition of internal structure, particularly the fibrous material. Often, the internal contents of the particle are seen to be pulled away from the outer membrane (Fig. 16a) which creates the appearance of vacuolization, sometimes seen when the particles are viewed in the phase microscope. Breaks in the outer membrane may also occur. In other instances, both the inner and outer membranes rupture causing the release of the internal contents of the particle into the vacuolar space (Fig. 16b). It is the filling of the vacuole with the ruptured contents of the particle that produces the spherical bodies that may be

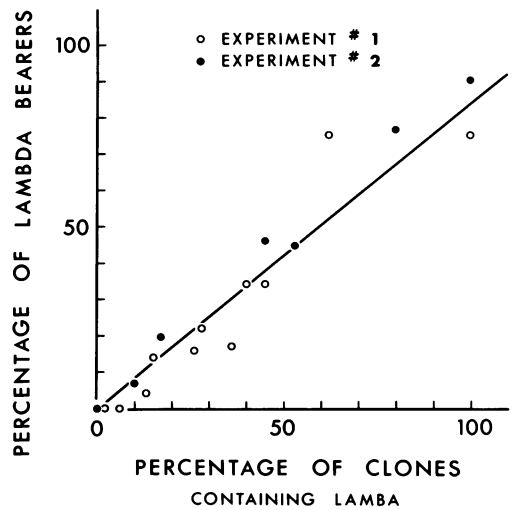


FIG. 9. *Correspondence between the percentage of lambda-bearing P. aurelia containing normal particles and the percentage of clones containing lambda-bearers during treatment with penicillin G. Penicillin G at a concentration of 2,000 units/ml was added to a 3-day old culture of lambda-bearing P. aurelia. At intervals after administration of the antibiotic, samples were removed and the number of animals containing normal lambda particles determined. Individual animals (25 to 50) were taken from each sample and inoculated into 0.3-ml portions of freshly prepared penicillin-free culture medium contained in the wells of depression slides. These were incubated in humidifying chambers for a period of 10 days at 27 C in the dark. Each clone was then examined for the presence of lambda within the protozoan. Under these conditions, almost 100% of the individual paramecia produced a viable clone. Ordinate scale is linear.*

TABLE 1. *Effect of prolonged exposure of lambda-bearing P. aurelia to low concentrations of penicillin G*

Concn of penicillin G (units/ml)	No. of *lambda* particles per protozoan			
	0[a]	1	2	4
None	1,600	1,550	1,570	1,700
	(0)[b]	(0)	(0)	(0)
0.5		1,380	1,530	1,500
		(14)	(16)	(5)
1.0		1,370	1,280	1,300
		(17)	(38)	(12)
2.0		1,220	1,140	1,100
		(72)	(70)	(60)

[a] Week.

[b] Numbers in parentheses indicate the per cent of *lambda*-bearers containing abnormal particles.

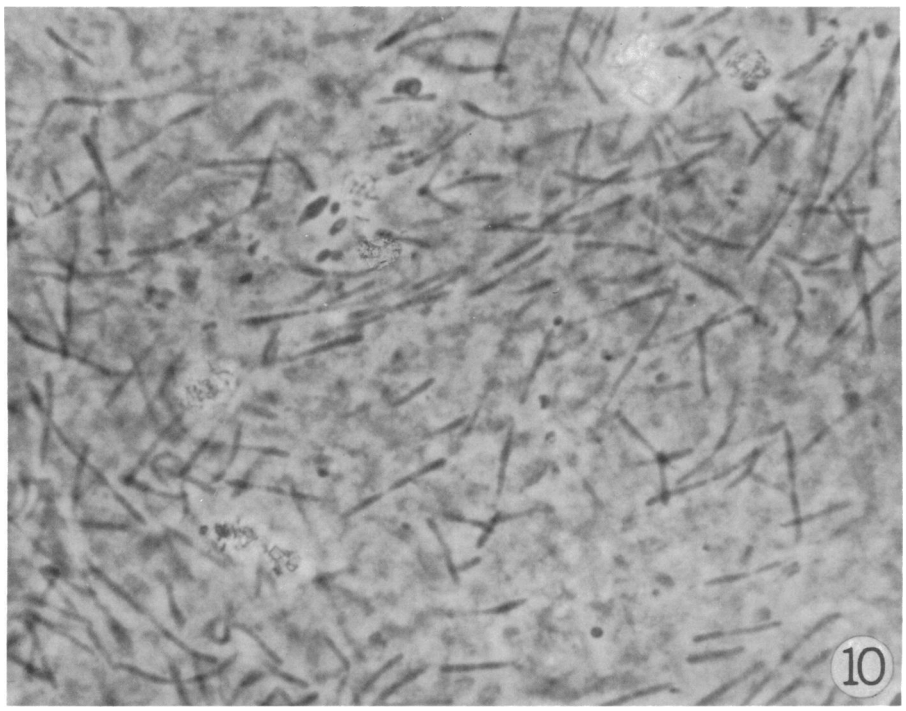


FIG. 10. *Phase micrograph of a lambda-bearer after exposure to 2 units/ml of penicillin G. Note filamentous appearance of the lambda particles.* ×*1,300.*

seen in the phase microscope as abnormal particles after staining with aceto-orcein dye (Fig. 7b). Often, the final stages of lysis are characterized by the rupture of the vacuolar sheath (Fig. 16c). Occasionally, the outer membrane of the particle itself and the vacuolar sheath persist for several hours before they eventually disappear (Fig. 16d).

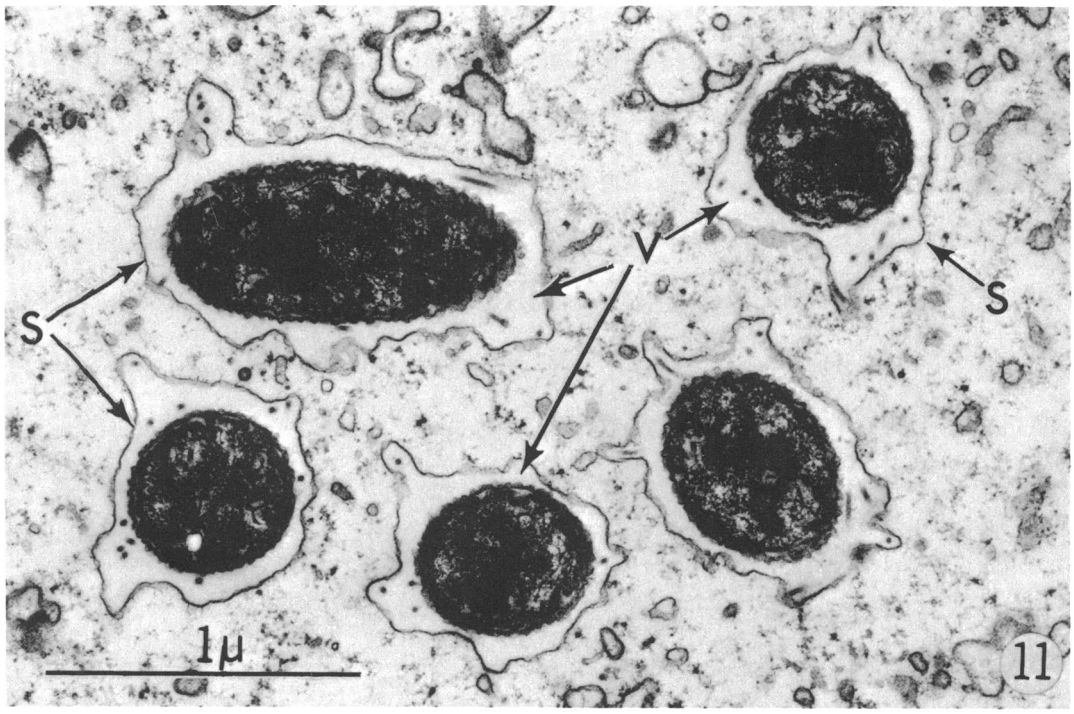
DISCUSSION

The salient features of the action of penicillin on *lambda* particles are these: low concentrations of the antibiotic cause many *lambda* particles within the cell to become filamentous; the number of particles within the protozoan is reduced in proportion to the amount of antibiotic present in the culture; high concentrations of the antibiotic cause lysis of *lambda* particles within the cytoplasm without noticeable effect on the protozoan; the ED_{50} value is approximately 2 to 3 units/ml; *lambda* in animals taken from the logarithmic phase of growth (rapidly dividing cells) are more vulnerable to the action of the antibiotic than *lambda* in animals taken from the stationary phase of growth or "resting cells" (slowly dividing cells); the antibiotic is without measurable effect on suspensions of *lambda* particles freshly released from protozoans by gentle homogenization (nondividing cells); and destruction of *lambda* particles within the cytoplasm takes place at random.

In summary, *lambda* particles respond to penicillin as if they were a randomly distributed bacterial population growing and dividing within the collective cytoplasm of protozoan cells.

To understand this argument more fully, it is useful to review certain features of the division of *lambda* within the protozoan host as well as the specific action of penicillin on bacteria.

Obviously, *lambda* must divide at a rate comparable to that of the protozoan, otherwise the particles would not be maintained. Even a small difference in the growth rate of *lambda* as contrasted to that of the protozoan would result eventually in either the loss or overpopulation of particles. Quantitative measurements of the growth rate of *lambda* particles within the cytoplasm of axenically cultivated *P. aurelia* point to the remarkable synchronism that exists between the particles and the protozoan (14). The synchronism is evident at temperatures and *p*H values that permit growth and survival of the protozoan (15). Even removal of actively dividing *lambda*-bearing *P. aurelia* to "resting media" (salt solutions isotonic for the protozoan) causes immediate and simultaneous cessation of division of both the protozoan and *lambda* particle.



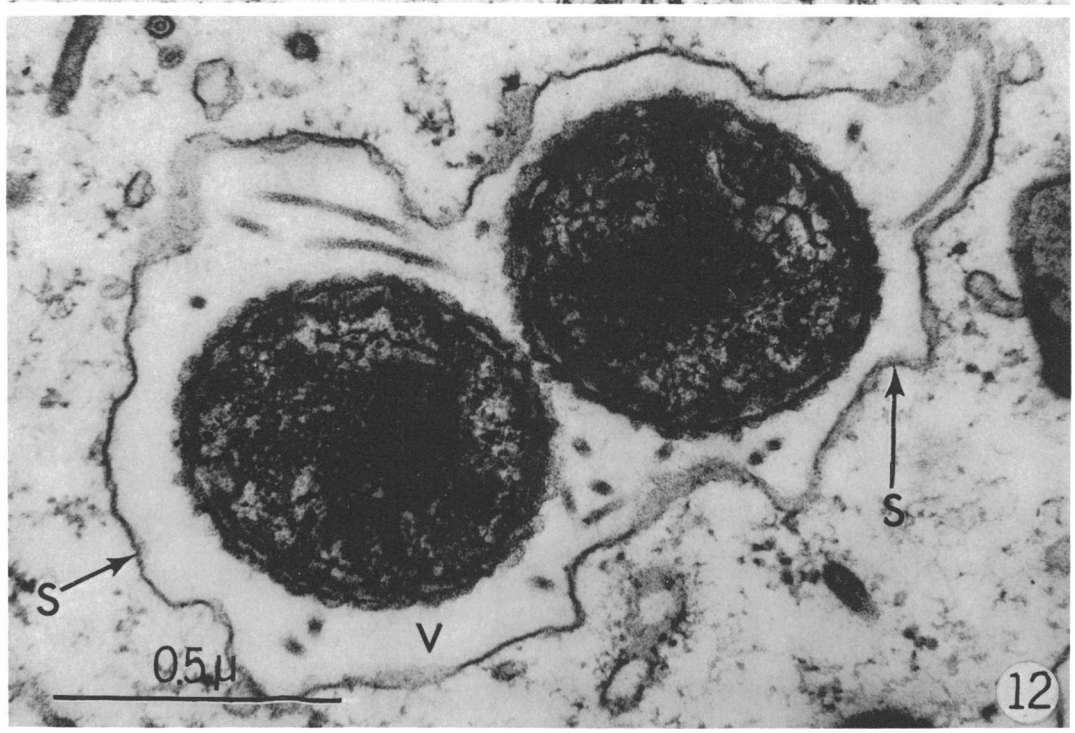


FIG. 11. *High-power electron micrograph showing lambda enclosed within individual vacuoles (V) surrounded by a smooth-walled membranous sheath (S).* ×*41,040.*

FIG. 12. *Electron micrograph showing two lambda enclosed within a single vacuole (V). (S) sheath.* ×*73,416.*

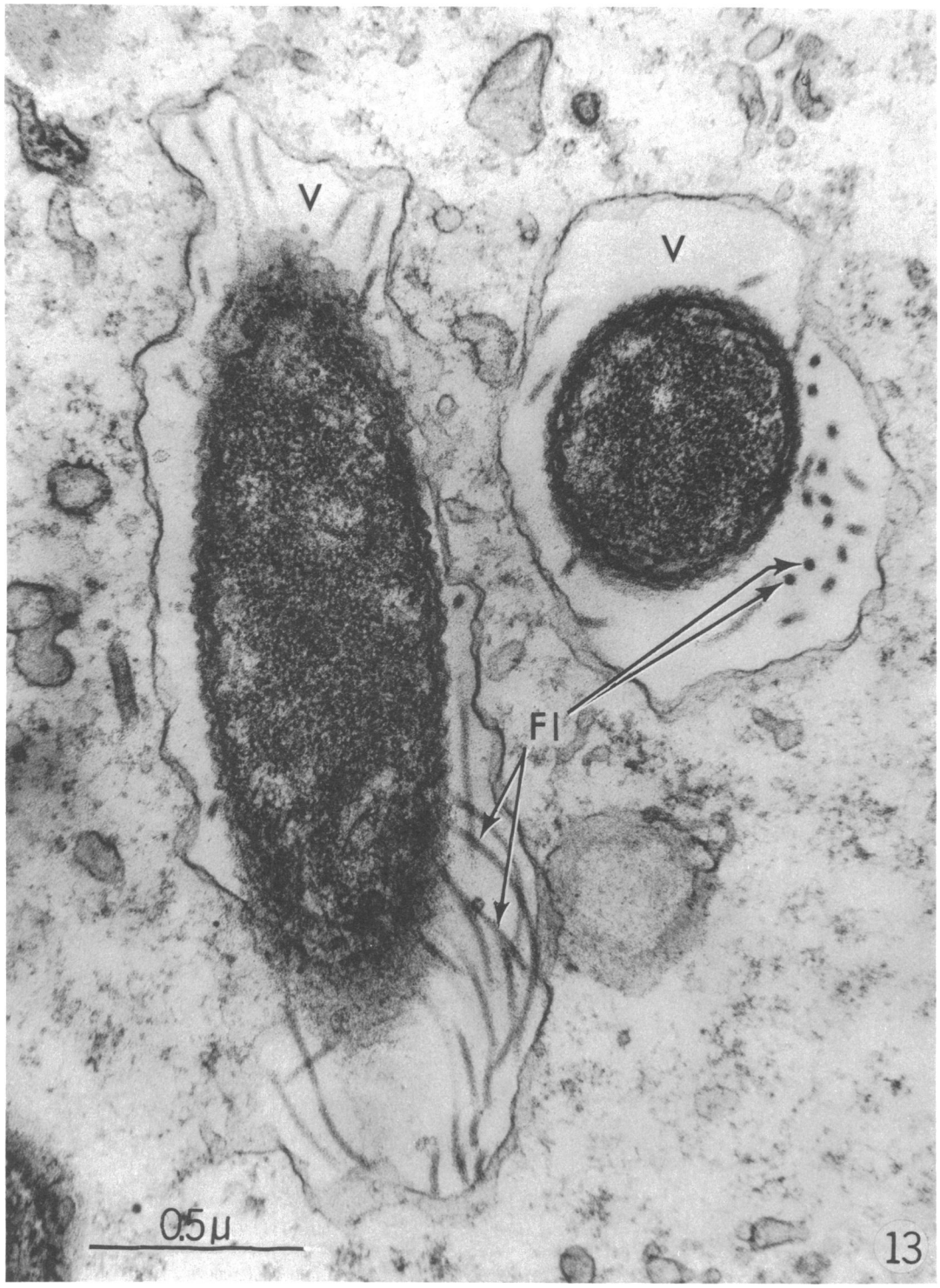


FIG. 13. *Electron micrograph of two lambda particles, each enclosed within a vacuole (V) showing flagella (Fl).* ×*65,208.*

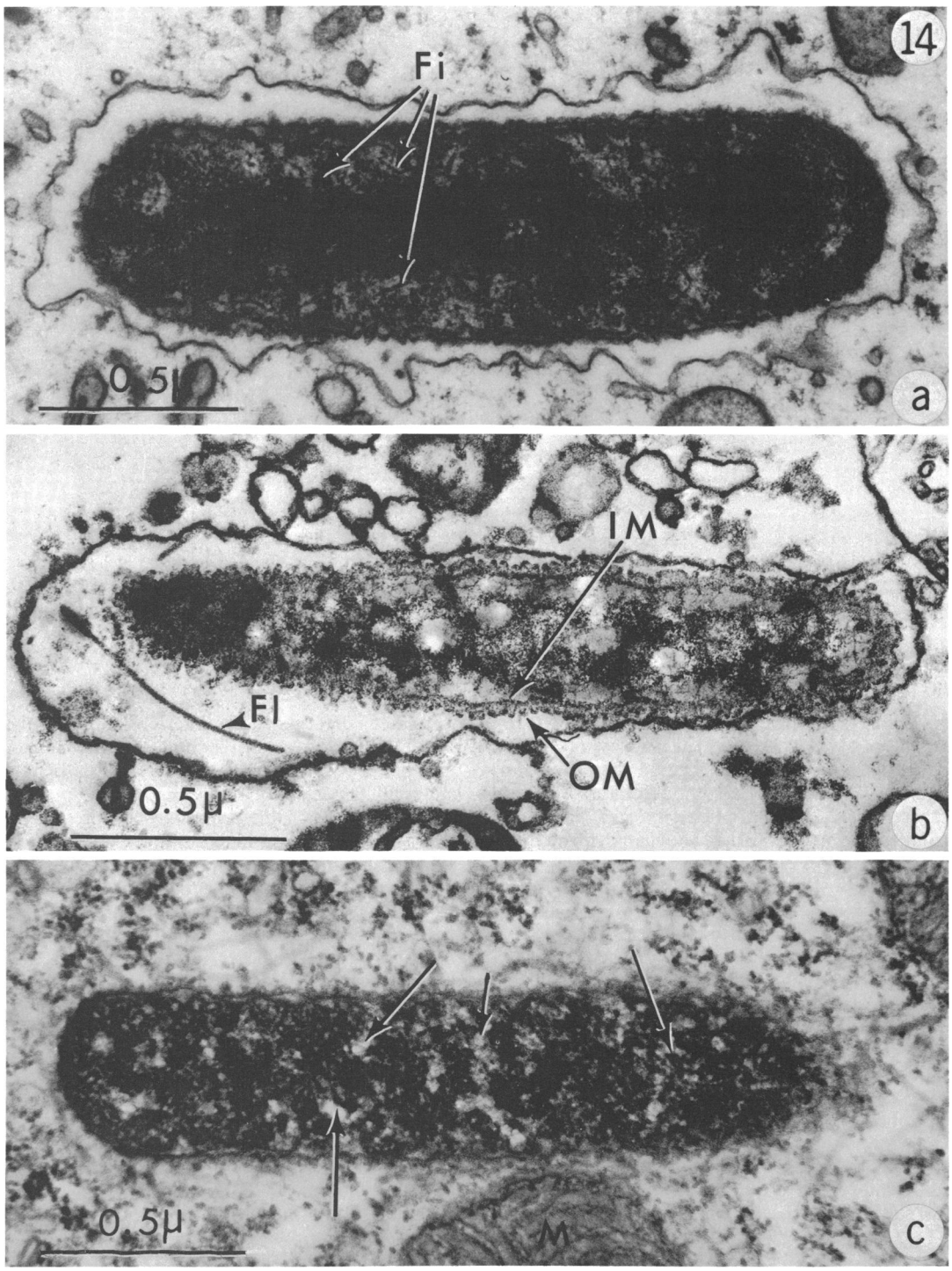


FIG. 14. *Longitudinal sections of lambda (a) normal (×61,500), (b) Pronase-treated (×64,000), (c) deoxyribonuclease-treated (×65,000). Note loss of fibers (arrows). (Fi) fibers, (Fl) flagellum, (IM) inner membrane, (OM) outer membrane, (M) mitochondrion.*

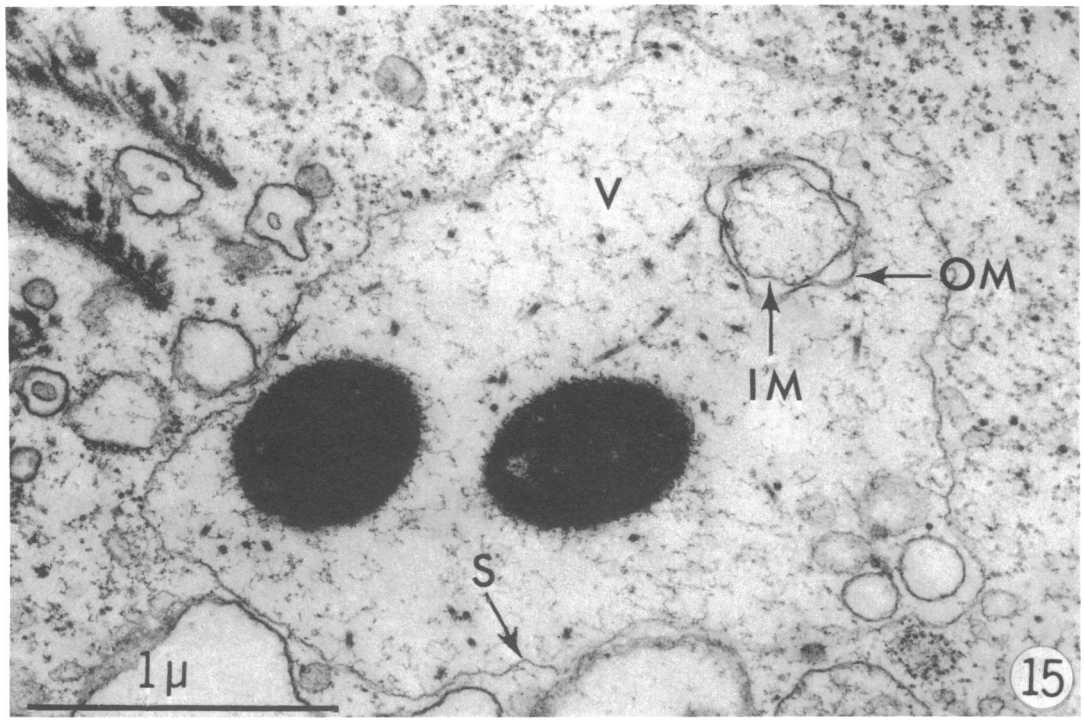


FIG. 15. *Penicillin-treated lambda showing separation of inner (IM) and outer (OM) membranes. (V) vacuole (S) sheath. ×41,000.*

With respect to the action of penicillin G on bacteria, early work revealed that low concentrations of the antibiotic caused morphological changes in intestinal bacilli. Inhibitory, but not bacteriocidal, concentrations induced the formation of involution forms, elongated filamentous rods, in certain gram-negative bacteria (22). ED_{50} values against gram-negative bacteria were in the range of 1 to 5 units/ml. Eriksen (5), in a study of the action of penicillin upon various representative gram-positive and gram-negative bacilli, showed that high concentrations of penicillin G were most marked upon gram-negative bacilli and caused lysis without preceding growth or swelling. Further, it was established that penicillin acted optimally on dividing cells (20). These observations led to the view (4) that penicillin had a specific action on bacteria and that the locus of action was the cell wall itself.

Recent evidence indicates that penicillin blocks the synthesis of a mucopeptide essential for the formation of the cell wall (7, 9, 24). It has been postulated that penicillin interferes specifically with a transpeptidation reaction involved in the final or cross-linking step in the synthesis of the bacterial cell wall peptidoglycan (24).

The structure of this peptidoglycan, present in both gram-negative and gram-positive bacteria, consists of latticework of polysaccharide chains, composed of alternating units of the sugars, *N*-acetyl glucosamine and *N*-acetyl muramic acid, covalently linked through peptide bonds. Cross linking involves the carboxyl end of D-alanine and the free amino group of a diamino acid, usually L-lysine (in gram-positive organisms) of a neighboring peptide. Diaminopimelic acid, diaminobutyric acid, and ornithine have also been found in the peptidoglycan of certain gram-negative bacteria.

Presumably, a component of the cell wall of the *lamba* particle is composed of a similar or related structure and may contain *N*-acetyl glucosamine, muramic acid, and possibly diaminopimelic acid. In a study of the chemical composition of the *mu*

FIG. 16. *(a) Longitudinal section of lambda showing an early stage of lysis by penicillin G. Note breaks (arrows) in the outer membrane. ×43,000. (b) Lambda particles undergoing lysis by penicillin G. Both the inner and outer membranes (area outlined) have ruptured causing the release of the internal contents of the particle into the vacuolar space. ×45,000. (c) A late stage of lysis by penicillin G. Note rupture of vacuolar sheath (arrows). ×19,000. (d) Final stages of lysis of lambda by penicillin G. (Fl) flagellum, (OM) outer membrane, (S) sheath, (V) vacuole. ×29,000.*

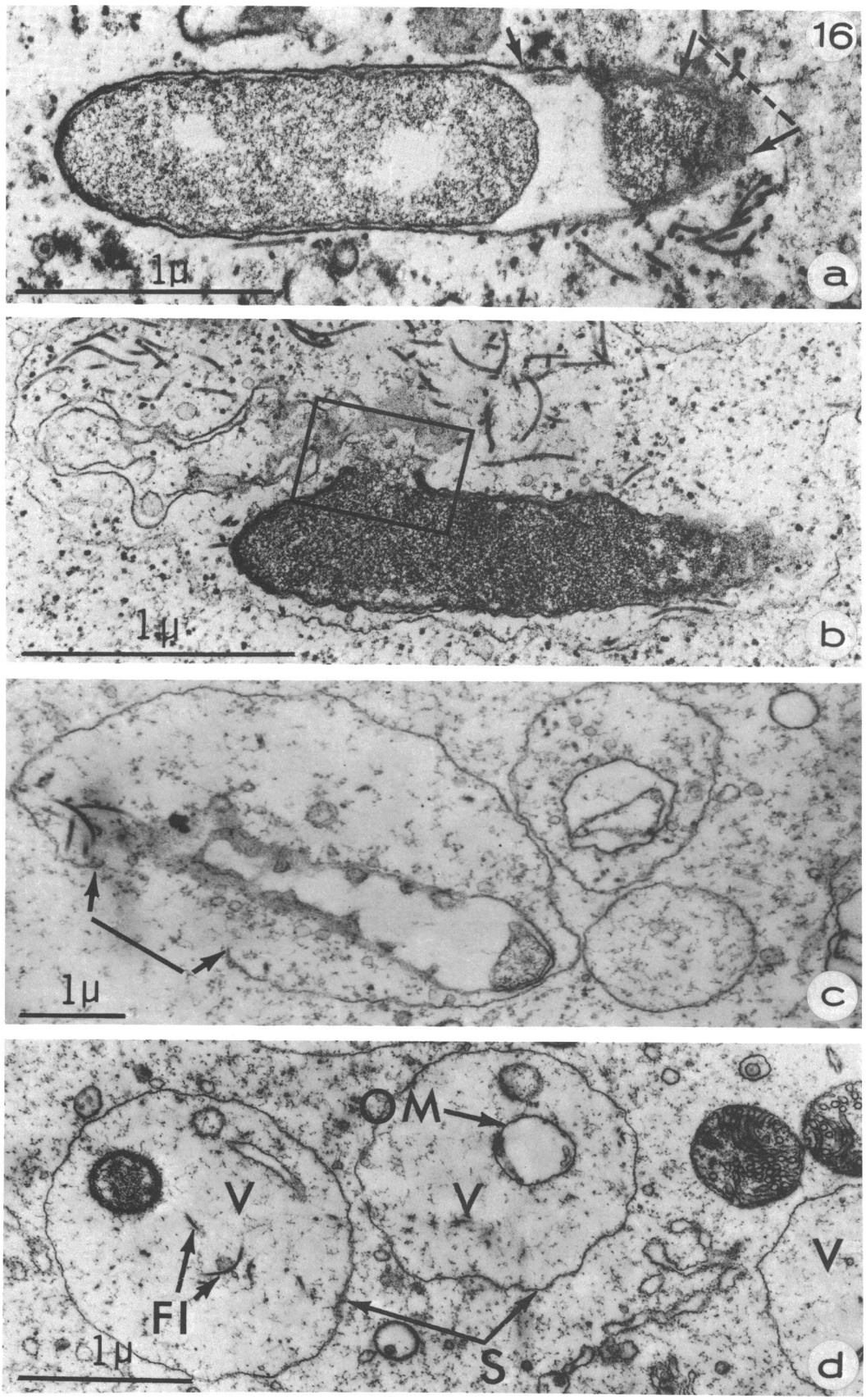


FIG. 16.

particle, a related symbiont of *P. aurelia*, Stevenson (18) found diaminopimelic acid in acid hydrolysates. Two hexoseamines, one identified as glucosamine, the other thought to be muramic acid but not positively identified as such, were also found in hydrolysates. This work was carried out on *mu* particles isolated from *P. aurelia* grown in the presence of living bacteria as a food source.

Morphological and ultrastructural changes observed in *lambda* particles are consistent with the view that penicillin interferes with the synthesis of a vital component of the cell wall of the particle. Low concentrations of the antibiotic permit growth of the particles without cell division. Lysis of *lambda* by high concentrations of penicillin G appears to be due to a weakening of a component of the cell envelope resulting in both the separation of the inner and outer membranes (Fig. 15). This is accompanied by rupture of the membranes and release of the internal contents of the particle into the vacuolar space (Fig. 16b). Loss of definition of the internal contents of the particles, particularly the fibers, may be due to the action of protozoan nucleases infiltrating the particles through the damaged membranes.

Observations of ultrathin sections of axenically cultivated *lambda*-bearers confirm earlier findings of Jurand and Preer (6) that the particles possess flagella and are enclosed within vacuoles. Like *kappa* and *mu* particles (1, 3), the DNA of *lambda* appears to be dispersed throughout the particle and not localized in "nucleoids" or "nuclear bodies" such as those found in many bacteria. Pronase treatment (Fig. 14b) of fixed preparations revealed at least two morphologically distinguishable layers comprising the cell envelope. The inner layer is probably homologous to the plasma membrane of bacteria and the outer layer homologous to the cell wall (8) of gram-negative bacteria.

LITERATURE CITED

1. Beale, G. H., and A. Jurand. 1960. Structure of the mate-killer (Mu) particles in *Paramecium aurelia*, stock 540. J. Gen. Microbiol. 23:243–252.
2. Beale, G. H., A. Jurand, and J. R. Preer. 1969. The classes of endosymbiont of *Paramecium aurelia*. J. Cell Sci. 5:65–91.
3. Dippell, R. V. 1959. The distribution of DNA in *kappa* particles of *Paramecium* in relation to the problem of their bacterial affinities. Science 130:1415.
4. Duguid, J. P. 1946. Sensitivity of bacteria to action of penicillin. Edinburgh Med. J. 53:401–412.
5. Eriksen, K. R. 1946. Some studies on lytic action of penicillin on Staphylococci and pneumococci. Acta Pathol. Microbiol. Scand. 23:221–228.
6. Jurand, A., and L. B. Preer. 1968. Ultrastructure of flagellated *lambda* symbionts in *Paramecium aurelia*. J. Gen. Microbiol. 54:359–364.
7. Mandlestam, J., and H. J. Rogers. 1959. The incorporation of amino acids into the cell-wall mucopeptide of staphylococci and the effect of antibiotics on the process. Biochem. J. 72: 654–662.
8. Murray, R. G. E. 1968. Bacterial cell wall anatomy in relation to the formation of spheroplasts and protoplasts, p. 1–18. *In* L. B. Guze (ed.), Microbial protoplasts, spheroplasts and L-forms. Williams & Wilkins Co., Baltimore.
9. Park, J. T. 1964. Multiple effects of penicillin, p. 366–370. *In* J. C. Sylvester (ed.), Antimicrobial agents and chemotherapy—1963. American Society for Microbiology, Ann Arbor, Mich.
10. Preer, J. R. 1950. Microscopically visible bodies in the cytoplasm of the "killer" strains of *Paramecium aurelia*. Genetics (Princeton) 35:344–362.
11. Reynolds, E. S. 1963. The use of lead citrate at high *p*H as an electro-opaque stain in electron microscopy. J. Cell Biol. 17:208–212.
12. Smith-Sonneborn, J. E., and W. J. Van Wagtendonk, 1964. Purification and chemical characterization of *kappa* of stock 51 *Paramecium aurelia*. Exp. Cell Res. 33:50–59.
13. Soldo, A. T. 1960. Cultivation of two strains of killer *Paramecium aurelia* in axenic medium. Proc. Soc. Exp. Biol. Med. 105:612–615.
14. Soldo, A. T. 1963. Axenic culture of *Paramecium*. Some observations on the growth behavior and nutritional requirements of a particle-bearing strain of *Paramecium aurelia*, 299 *lambda*. Ann. N.Y. Acad. Sci. 108:380–388.
15. Soldo, A. T., G. A. Godoy, and W. J. Van Wagtendonk. 1966. Growth of particle-bearing and particle-free *Paramecium aurelia* in axenic culture. J. Protozool. 13:492–497.
16. Soldo, A. T., and S. J. Reid. 1968. Deoxyribonucleic acids of *lambda*-bearing *Paramecium aurelia*, stock 299. J. Protozool. 15:15.
17. Sonneborn, T. M. 1959. *Kappa* and related particles in *Paramecium*. Advan. Virus Res. 6:229–356.
18. Stevenson, I. 1967. Diaminopimelic acid in the *mu* particles of *Paramecium aurelia*. Nature (London) 215:434–435.
19. Stevenson, I. 1969. The biochemical status of μ particles in *Paramecium aurelia*. J. Gen. Microbiol. 57:61–75.
20. Stewart, G. T. 1945. Effect of penicillin on *Bacillus proteus*. Lancet 2:705–707.
21. Swift, H., and E. Rasch. 1958. Studies on electron microscope cytochemistry. Sci. Instr. News 3:1–4.
22. Thomas, A. R., Jr., and M. Levine. 1945. Some effects of penicillin on intestinal bacteria. J. Bacteriol. 49:623–627.
23. Wagtendonk, W. J. van, J. A. D. Clark, and G. A. Godoy. 1963. The biological status of *lambda* and related particles in *Paramecium aurelia*. Proc. Nat. Acad. Sci. U.S.A. 50: 835–838.
24. Wise, E. M., Jr., and J. T. Park. 1965. Penicillin: its basic site of action as an inhibitor of a peptide cross-linking reaction in cell wall mucopeptide synthesis. Proc. Nat. Acad. Sci. U.S.A. 54:75–81.