AN ELECTRON MICROSCOPIC STUDY OF THIN SECTIONS OF BACTERIA AND BACTERIOPHAGE GROWN ON AGAR PLATES

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SUMMARY

A method of preparing thin sections of bacteria and bacteriophage growing on conventional agar plates is described. Fixation using buffered osmium tetroxide solutions or permanganate must be followed by an immediate post-fixation in uranyl acetate solutions, which appear to stabilise the nucleic acid in the form of thin filaments (~ 50 Å).

The course of infection of *E. coli* by T2 phage is described. The phage appears to attach to the membrane of the bacterium by means of a small double layered disc at the end of the tail plug of the tubular tail sheath. Various aggregates within infected bacteria may represent stages in phage development. The nuclear core forms first and the head membrane later. Free tails have also been noted.

On lysis the cell membranes form large numbers of small vesicles to which phage also attach.

INTRODUCTION

The routine method of assaying bacteriophage, by mixing them with sensitive bacteria and plating on gels, yields a preparation that is convenient for electron microscopic examination by the method of thin sections, although the special conditions of infection prevailing on plates may lead to difficulties in interpretation. The fixative is poured over the surface of the plate and washed off after the required time. Small cubes containing the edge of plaques may then be cut out, dehydrated and embedded like a piece of tissue. Sections may then be cut of any chosen area.

Using *Escherichia coli* Strain B and T2 phage, a preliminary study showed that fixatives containing osmium tetroxide or potassium permanganate yielded a poor preservation of the nuclear structures. Better results followed the use of a post-fixation treatment with phosphotungstic acid (PTA) or uranyl acetate (UA)[1,2]. These substances also "stained" the protein components. Embedding in an epoxide resin ("Araldite") was found superior to methacrylate[3-5].

The appearance of *E. coli* in sections has been previously described[1,2,6,10]. In Fig. 1, the external membrane M is resolved into two or more dense layers and is separated from the protoplast membrane L by a gap probably enlarged during

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embedding. The protoplast membrane L becomes more dense as infection proceeds (Fig. 2 and Fig. 3) and is also seen to be doubled.

The cytoplasm (Fig. 1) contains dense particles R (diameter $<$ 100 Å — microsomes?) in an amorphous ground substance. A finer texture is found after permanganate fixation, suggesting a loss of the dense granules as occurs in mammalian cells[7].

No nuclear membrane was seen but a nuclear cavity (N) is well defined and contains fine threads (F) ($\sim$ 50 Å diameter) and a few denser aggregates.

The course of infection

Various stages in the course of infection could be recognised in sections which included the edge of a plaque and extended into the lysed zone. At the edge of the plaque many cells contain well formed phage heads (H, Fig. 2) without tails and the nuclear structure is already disorganised. Some details of phage development could be made out in sections, such as Fig. 3, but possibly owing to the peculiar conditions of plaque formation, the earliest stages of infection were not found. The cell shown in Fig. 3 is swollen and about to lyse. It contains many fine filaments (F), essentially similar to bacterial DNA, and well formed heads (H) with head membranes (m) and occasional unattached tails (T). In the meshwork of filaments are found many aggregates of various sizes (P). These may well be artefacts of preparation; but, on the other hand, if the phage heads are produced by a sort of winding-up of DNA filaments (as we are almost bound to assume), such appearances would be expected.

No empty head membranes were seen within the cells. On the face of it this finding seems to be contrary to certain evidence obtained by bursting cells during the latent period[8].

Just prior to lysis most cells have lost almost all their original cytoplasm and dozens of well defined phage have been counted in a single section. In the lysed region, free phage are numerous and typically large numbers of small vesicles (V, Fig. 4) are found. They seem to be formed by the rounding-up of fragments of lysed bacterial membranes. A similar formation of small vesicles from fragmented membranes results when mammalian cells are homogenised and may indicate a general property of biological membranes.

Phage structure

The appearance and dimensions of the phage in sections agree with the description given by FRASER AND WILLIAMS[11]. The contents of the head are always strongly electron scattering and require no added staining (Fig. 8). Occasional less dense areas were seen in the centre of the head (O in Fig. 8 and in Fig. 3). The head membrane and tail are not visible unless stained with PTA or UA. The membrane in mature phage is less than 50 Å thick and tightly invests the core (Fig. 9). Tail sheaths are clearly tubular (TS in Fig. 3) and their attachment to the head membrane is marked by a slight constriction (S, Fig. 5). These observations are in agreement with those made by BRENNER *et al.* on free phage and the nomenclature is that used by them[13]. Since free tails are to be seen both inside and outside the cells, it is possible that their attachment to the head membrane is relatively weak. Tail plugs (TP, Fig. 3 and Fig. 5) also occur free in cells and in the lysate. They seem to consist of a small rod bearing a double-layered disc (D, Figs. 5, 6 and 7) at one end. What seem to be tail fibres (TF) are attached to this disc and also to an opposing disc on the tail sheath.

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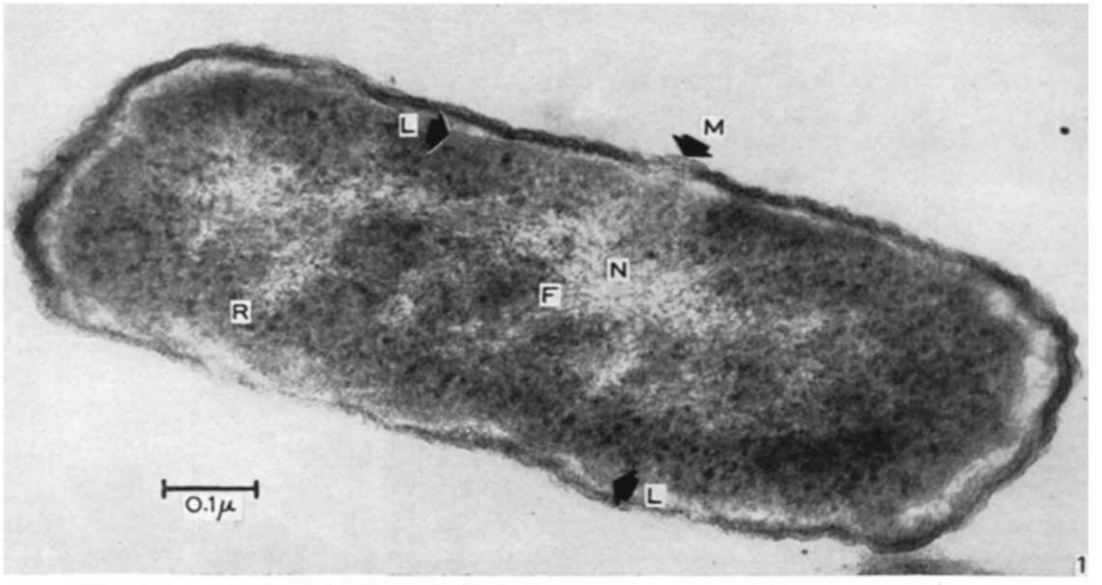

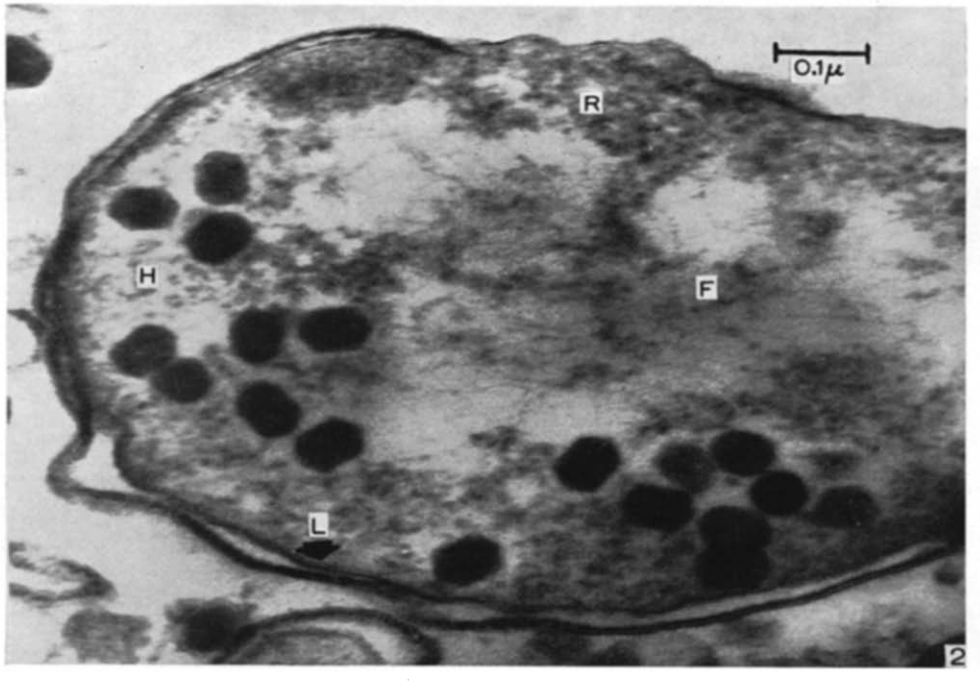

Fig. 1. Section of *E. coli* fixed 2 h in buffered (pH 7) OsO_4 and washed for 1 h in 1 % uranyl acetate. M external membrane, L protoplast membrane, N nuclear cavity, F filaments, probably DNA, R dense particles in cytoplasm. The gap between M and L was probably enlarged during embedding.

Fig. 2. Heads H of phage T2 appearing in an infected cell. Their size indicates that they have head membranes. Few tails are visible. Stain: uranyl acetate.

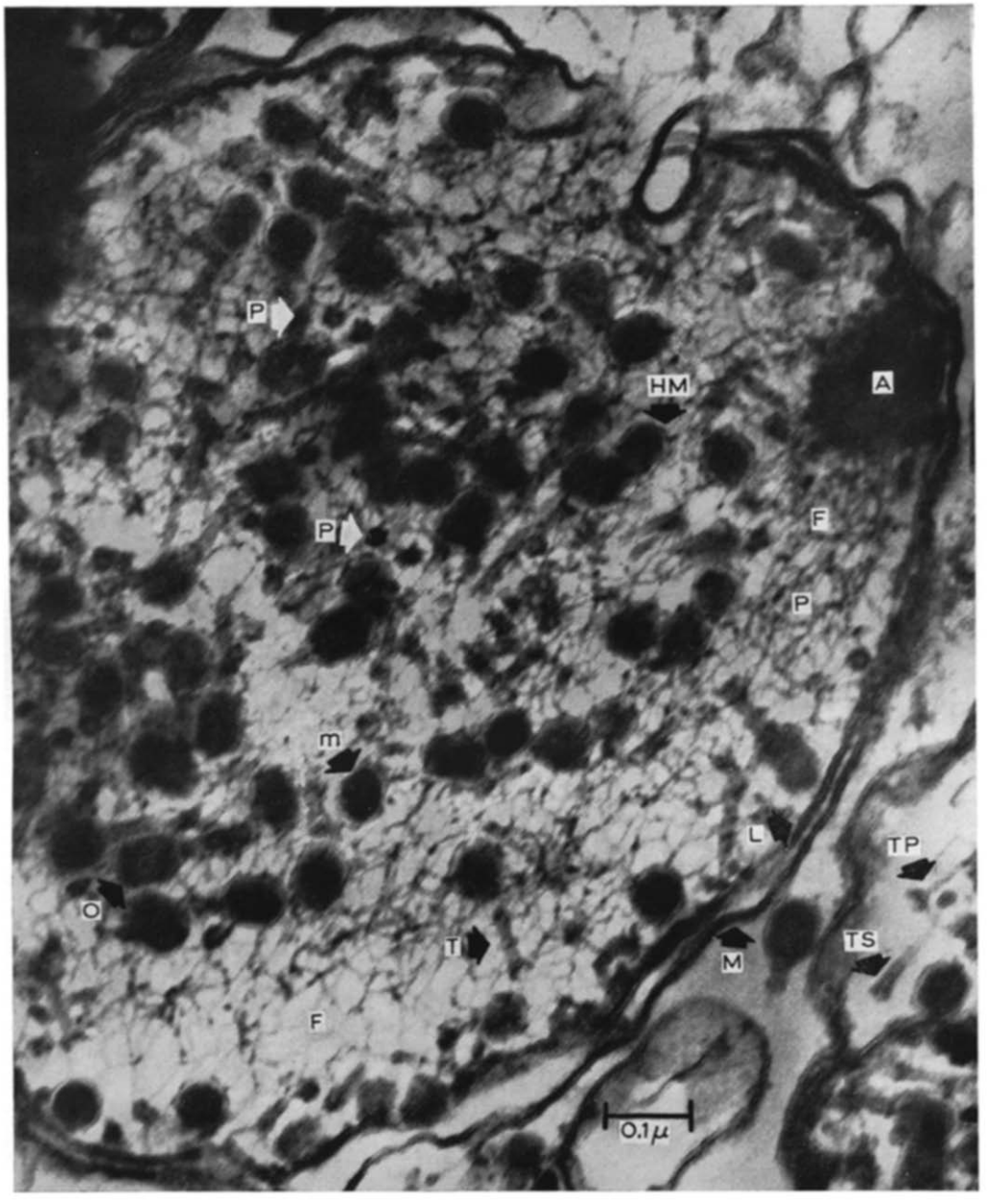

Fig. 3. A heavily infected and swollen cell probably approaching lysis containing many partly formed phage. T phage tails usually tubular, HM head membranes; A, resistant portion of bacterial cytoplasm which is often noted; at O may be seen a head showing signs of internal structure, *cf.* Fig. 8; P small clumps of DNA. Stain: uranyl acetate.

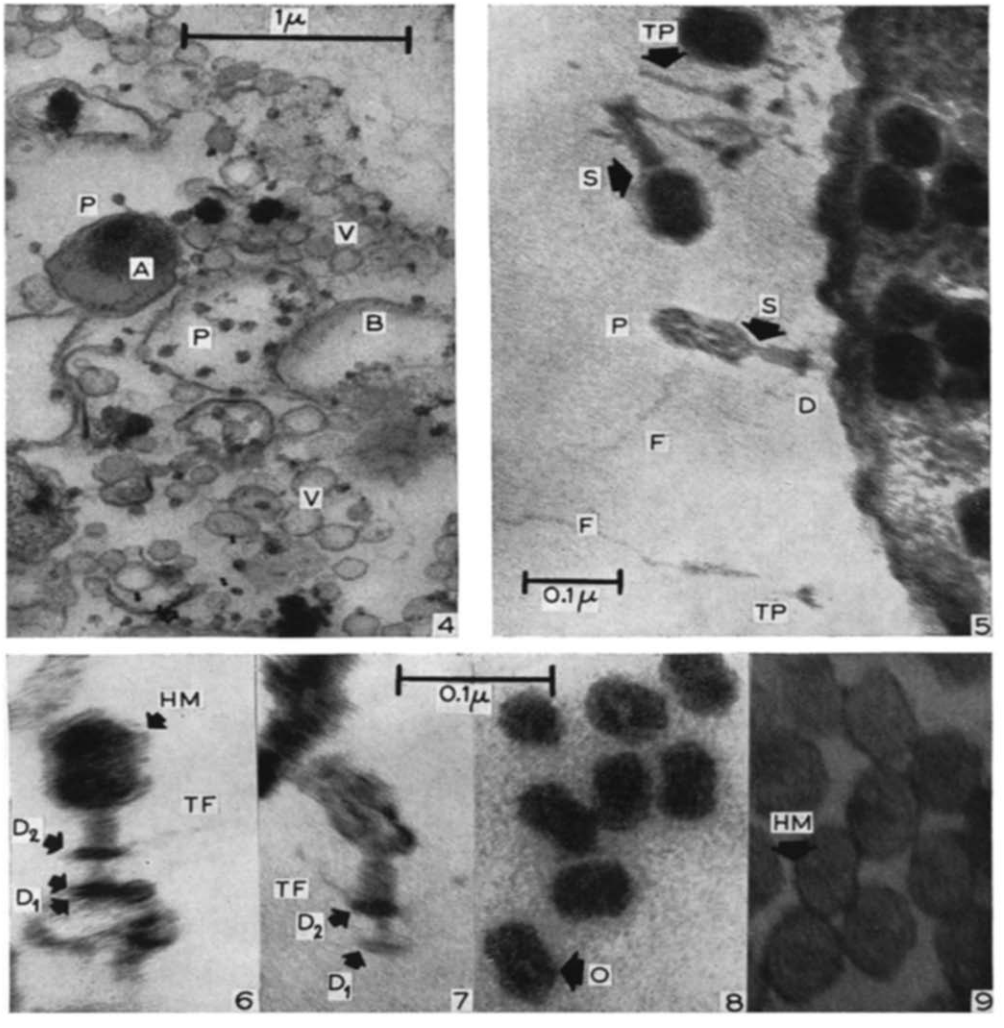

Fig. 4. Debris noted in zone of lysis. Fixed in potassium permanganate (1 %, pH 7, for 30 min) and stained with PTA (1 h in 1 %). P phage particles; V small vesicles produced by lysis of bacterial membranes; A resistant bacterial cytoplasm; B large fragments of bacterial membrane.

Fig. 5. The edge of an infected cell. An attached phage which has shed its DNA is seen at P. Note the constriction S, the tail sheath disc D and tail fibres. Free tail plugs are seen at TP. The long filaments F may be DNA.

Fig. 6. A phage attached to a small vesicle and showing the discs D_1 and D_2. Head membrane HM; tail fibres TF.

Fig. 7. An unattached phage which appears to have lost its DNA. TF tail fibres.

Fig. 8. DNA cores separated from phage during fixation with OsO_4. Note lightly stained areas O in some heads (see also Fig. 3).

Fig. 9. Phage heads in a very thin cross section. HM head membranes. There is a suggestion of structure in the DNA core.

In the zone of lysis, phage attach both to bacterial membranes and to the small vesicles resulting from lysis. Multiple infection seemed the rule and in no case could a primary infection be identified. When attached to a membrane the small disc of the tail plug seems to form the area of attachment and the tail sheath appears to unwind or retract[12,13] (Fig. 5 and Fig. 6), thus shortening and separating the two discs. It is possible that the cell membrane may contribute a receptor substance to the plug disc (see Fig. 6) and thicken it (*cf.* ref. [9]). The shaft of the plug remains in part within the tail sheath.

Although in many instances of attached phage the DNA has been shed, no hole was noted in the bacterial membrane nor had any part of the tail apparatus penetrated. This finding must perhaps be regarded cautiously since we have no way of knowing whether these particular contacts had really led to DNA transfer.

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