ADHESION PARTITIONING: INTRASOMATIC OBSERVATIONS ON NORMAL ESCHERICHIA COLI AND T2 BACTERIOPHAGE*

BY ROBERT C. BACKUS, Ph.D.

(From the Virus Laboratory, University of California, Berkeley)

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Our concepts of organized intracellular fine structures have been deduced largely from light microscopic observations on the physical properties of living cells supplemented by information obtained by micrurgical and cytological staining techniques (1, 2). Observations of functional cytoplasm have been limited for the most part to larger cells, extrapolations being made from these observations, with the aid of presumed structure-specific stains, to infer scaled down counterparts in smaller cells including bacteria (3, 4).

The electron microscope has sufficient resolving power for the direct observation of cytological macromolecular structures. However, because of the poor discrimination between contiguous organic structures and the limited penetration of the electron beam, it is usually necessary for making intracellular examinations to subdivide objects the size of bacteria and differentially impart contrast to them by heavy metal staining or, if internal structures are uncovered, by shadow casting.

Recent developments in the mechanics of ultrathin microtomy (5-8) have activated new enthusiasm for investigations of intracellular morphology. Microtomy is not without its limitations, however (9). The ambiguity which attends the interpretation of electron micrographs of difficult subjects is further complicated in sectioned material by orientation and reconstruction uncertainties. Furthermore, the usually involved procedure in the preparation of specimen material for sectioning including fixation, staining, dehydration in organic solvents, and plastic embedment, does not appear to lend itself readily to artifact evaluation or control and discourages major departures from accepted technique.

A substantially different approach to preparing material for intrasomatic observations has been developed in this laboratory and applied to the examination of Escherichia coli bacteria and the T2 coliphage. The method, termed adhesion partitioning, is relatively simple in concept and operation and results in the exposure of structural components that constitute biological entities.

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Two thin plastic films are brought into contact with the surfaces of specimen objects by drying a suspension of the specimen material between them. Upon separating the films along their common interface the specimen is partitioned, adherent parts being removed with each film.

Several techniques operationally related to adhesion partitioning have been reported. Fernandez-Morán in several noteworthy contributions (10, 11) developed and applied a "replica-adhesion" method for elaborating cytological components. Reed and Rudall (12) have described a method of "layer stripping" applied to the examination of the microstructure of earthworm cuticle. Laurell (13) adapted Hast's (14) particle fragmentation method for "surface sectioning" bacteria. Features which distinguish adhesion partitioning from the above mentioned techniques are: (a) the direct transfer of air-dry adhesions onto preformed films, (b) the ability to partition fresh material without subjecting it subsequently to aqueous or organic solvents or chemical fixatives unless desired, and (c) the relative ease with which the complete remains of small dissected objects can be observed.

The most informative work to date on the microstructure of *E. coli* bacteria is that reported by Birch-Andersen, Maaløe, and Sjöstrand (15). They interpret their micrographs of ultrathin sections of osmium-fixed, acrylate-embedded material as demonstrating the occurrence of a well defined, loosely attached cell mantle about 100 A thick in cross-section surrounding a "sponge-like" matrix with individual septa 50 to 100 A thick and an intersepta distance of 100 to 150 A. Vacuolated areas were observed in growing cells which contained dense central bodies composed of twisted opaque fibres. Cells of older cultures displayed a thread-like structure of about 250 A diameter running through a duct vacuole. The authors speculate on the possibility that the "vacuoles" may be vestiges of the long sought bacterial nuclear apparatus. No membranous structures, other than the cell mantle, were seen although several layers of septa near the surface of the matrix were found to be oriented parallel to the surface.

Fraser and Williams (16) in a very informative electron micrograph confirmed the finding by Herriott (17) that the bacteriophage membrane was separable from the nucleic acid portion of bacteriophage by osmotic shock. The micrograph was of a T6 coliphage which had been "shocked" on a supporting film preparatory to micrographing. Work reported here further confirms the separability of membranes and nucleic acid and provides additional evidence on the physical nature of the component parts.

**Materials and Methods**

_PrepARATION OF PLASTIC FILMS._—Films stripped from glass are preferred to films spread on the surface of water because of their greater strength. Clean glass slides are plastic-coated by dipping them into an approximately 0.5 per cent solution of collodion or formvar in suitable solvents and hanging them vertically until the film is free of solvent. The concentration of
plastic should be adjusted to give dry slides showing interference colors by reflection ranging from blue in the thickest part through yellow to clear. All but the thinnest areas can be used for partitioning.

Preparation of Grids.—In addition to their usual role, the grids serve to reinforce the films during partition, effectively dividing the film into a large number of small unit areas, each area being individually supported by adhesive material on the cross-wires of the grids. Athenê grids (18) which are thin and flexible and have matching patterns are used. The grids are mounted smoother side out on the adhesive side of pieces of Scotch cellulose tape.

Preparation of Specimen Material.—The E. coli bacteria were grown at 37°C. with aeration in 5 ml. quantities of a modified "F" medium (19) supplemented with 1 per cent glycerol and 1 per cent casein hydrolysate. The bacteria were washed by centrifugation in distilled water immediately before use. The T2 coliphage had been prepared by Dr. Dean Fraser of this laboratory from host bacteria grown in "glycerol medium" (20). The phages had been purified by differential centrifugation with 2 per cent ammonium acetate at neutral pH used as suspending medium. They were received in a concentration of $10^{12}$ particles per ml. and were diluted to a concentration of $10^{10}$ particles per ml. with ammonium acetate-suspending medium before use.

Partitioning.—Several methods, differing in operational details have been developed for partitioning. These methods have certain objectives in common, (a) to establish firm ad-
E. coli and T2 partitioning

Adhesive contact between the films and the specimen objects, (b) to be able to separate the plastic films conveniently, and (c) to have the films supported by grids for observation. An additional end is achieved if corresponding parts of partitioned objects can be readily observed.

One of the simpler procedures for the attainment of these objectives is as follows: A breath-moistened patch of plastic film is transferred by contact from one of the plastic-coated glass slides to an area of a piece of Scotch tape containing one of the prepared grids. Another film is transferred to a piece of Scotch tape from which a 6 mm diameter hole has been cut. A minute drop of a cloudy suspension of specimen material is placed on the center of the film spanning the hole. The film bearing the droplet is immediately laid on the surface of the film in place on the grid, causing the droplet to spread between the films. After water has evaporated from the interfilm space the piece of tape in which the hole had been cut is stripped away leaving both films in place over the grid. A second tape-mounted, unfilmed grid is now superimposed over the first grid bringing the adhesive in contact with the free surface of the top film. A dissecting microscope is helpful in the concentric and rectilinear alignment of the grid patterns. The complete ensemble including the two Scotch tape-mounted grids with the two films between them (Text-fig. 1) is compressed tightly between the thumb and the forefinger for several seconds to assure good grid-to-film contact. The Scotch tape pieces are peeled away from one another, each carrying with it one grid, one film, and the parts of partitioned objects. A second partition from either half of the first...
partition is done in a manner similar to the first with the important exception that the films are brought into contact in an air-dry condition.

Method of Locating Corresponding Parts.—In order to maintain the orientation of the grids for the purpose of conveniently locating corresponding areas in the electron microscope, the grid on which the films are first placed is circumscribed in an irregular pattern with a soft lead pencil before the second grid is brought into position. The pencilled pattern is partly transferred to provide a replicate pattern on the face of the opposing piece of Scotch tape when the tapes are pulled apart. The Scotch tape is trimmed to the pencilled pattern and the bits of tape with grids attached are arranged for shadow casting as indicated in Text-fig. 2. The shadow direction serves to complete the requirements for locating and identifying corresponding areas with the electron microscope if shadow casting is done at the same angle for corresponding points on each grid (Text-fig. 2). The use of the shadow direction as an orienting device is desirable because the Athene grids are made with dyad symmetry.

After shadow casting, each bit of Scotch tape bearing a grid is backed by another convenient length of Scotch tape for ease in handling and the grid is carefully pulled from its place. The length of Scotch tape to which the mounted grid has been transferred may be wrapped around a short piece of small diameter rod to provide a rigid convex support with the aid of which the grid can be released from the tape more easily. It has been found worthwhile to orient each grid in the specimen holder with the aid of a dissecting microscope to assure approximate alignment between specimen areas of sequential grids when viewed with the electron microscope.

RESULTS

The adhesion partition method provides information of several kinds on materials which have been prepared for examination. The retention of integrity of some structural units reflects the relative adhesive and cohesive bond strengths which oppose each other on partition. A single stage partition usually indicates the nature of outermost structures, whether capsular material is present, and the thickness and degree of complexity of the cell membrane. Membranes are seen from the inside when they are stripped from the body of an organism. After removal of the membrane further partitioning stages result in a fractionation of internal structures.

Bacteria are found to partition in a number of ways depending not only on the structures present but to a minor degree on their orientation to the direction of the pulling force that partitions them. Water appears to be present in freshly prepared young culture bacteria in sufficient quantity to disperse some small elements over an extended area around the bacterial remains. Also, bacteria in which rifts develop during partition display gum-like stranding across the interior of the rifts. From these observations it may be surmised that bacteria can be partitioned in a very fresh state indeed.

A pair of corresponding fields, representing the complete remains of once partitioned *E. coli* bacteria from a log-phase culture, is shown in Figs. 1 a and
Patches of material lying adjacent to the inside surface of the membrane have been removed from the matrix of the organism. The structure of bacteria on one film of a partition pair is seen replicated in detail in a corresponding position on the opposite film. The crowded appearance of the cells indicates the compression to which enveloped objects are subjected by interfacial tension which attends the final stages of evaporation of water from the space between the films.

Longitudinal fractures of bacteria, such as the one shown in corresponding partition in Figs. 4 a and 4 b, are occasionally observed. The organism shown was from a young culture; the bacterium was of abnormal length. The fine scale (10 to 50 mÅ) macromolecular composition of the submembranous area is clearly seen in the enlarged micrograph (Fig. 4 b). The cell membrane is judged to be about 10 mÅ thick from the edge-on view of it which appears as a serrated dividing line between the pebbly inside surface of the membrane and a margin which surrounds the bacterium between the bare collodion substrate and the cell wall. The margin is evidently produced by a tent-like enclosure formed during the enveloping process by the two films and the bacterium where the surfaces of all three converge but fail to meet. The enclosure is logically the place where the aqueous medium dries out last, depositing residual non-volatile extracellular materials.

It may be assumed that an elongated object of relatively homogenous composition when stressed at several points would probably break laterally. This is usually the case with bacteria subjected to partitioning and may be observed, for example, in the centermost bacterium shown in Figs. 1 a and 1 b. Conversely, it appears probable that some structural element running lengthwise of the bacterium would account for longitudinal breaks. There is a mere suggestion of such a structure in the form of a cord extending along the profile of the fracture face seen in Fig. 4 b. Inclusion bodies of various shapes and sizes are especially evident in the layer of matrix material adhering to the inner membrane surface in Fig. 4 a. Log-phase bacteria are usually more voluminous in matrix material than older culture bacteria.

A single stage partition of an old culture organism is shown in Fig. 2. The thin layer of matrix material is characteristic of organisms from nutrient-starved cultures. Second partitions of such organisms result in simple delamination, the layers of matrix material exhibiting no organized structure within easy resolvability of the electron microscope other than the opaque cottony white areas and occasional nodular structure. Young culture bacteria, on the other hand, usually suffer extensive disruptions (Fig. 3) which are accompanied

1 Opacity in this context refers to a relatively high degree of electron beam scattering ability indicating a deposit of material of higher average atomic number per unit volume than its surroundings.
by the distribution of a fine filamentous deposit, interspersed with microsomal particles 30 to 60 m\(\mu\) in diameter, over an extended area surrounding the larger bacterial fragments; this is interpreted as a release of a relatively copious quantity of fluid at the moment of rupture. The bacterial remnants frequently appear to be disorganized and the relationship of the various redistributed parts including striated discs, partition sections, etc., is rendered uncertain. It is believed that the observed difference in partition behavior between old and young cultures is due primarily to the state of aggregation of the protoplasm; young cultures contain a predominance of organisms with a gelatinous matrix, old cultures being more sol-like in composition.

The young culture bacterium shown in Figs. 4 a and 4 b is exceptional and may represent a third type, namely a sol-matrix condition in a young culture organism. The bulkiness and heterogeneity of the particulate composition of the protoplast clearly differentiate this organism from that obtained from old cultures.

Fig. 5 is a micrograph showing in one field various structures found on third film adhesions of young culture organisms. The filamentous fine scale residuum interspersed with larger spherical particulates covers the field. Two elliptical discs are seen, one of them overlaid by a thin, opaque, reticulate material, the other with a sector removed. A partition section with the dimensions of a cross-section through the mid-plane of a bacterium is shown in the center of the field. Irregular pieces of matrix material are dispersed about the field. The partition section is composed principally of filamentous macromolecules which appear in some places to be oriented and interwoven to produce a fabric-like effect. The electronically opaque whiter areas are seen to be diffuse. The two discs illustrate the wide variation observed in these structures. The removed sector of the larger disc may signify a predisposition for the disc to break along the radial striations. The smaller disc, showing a well marked center and numerous striae, is of particular interest because of the reticulate structure overlaying it. The reticulate structure is very thin, casting no apparent shadow. It possesses a high intrinsic opacity to the electron beam indicating the possible presence of a concentration of sequestered cations. It is believed that this structure may be intimately associated with the disc because of its position and the apparent tie-in at the center of the disc. However, such a “complete” structure has been observed only in this instance although fragments of material with similar properties have been seen on other occasions.

A single disc is shown in Fig. 6. The radial striae and a compact central body are particularly well defined structures in this micrograph. The striae are estimated to be less than 25 A in cross-section. The morphology of the discs’ centers is quite variable, suggesting that they represent a mobile, perhaps highly functional, structure within the bacterial cell.
Results obtained on subjecting T2 bacteriophage to two partitionings are shown in Figs. 7 and 8. Phage membranes are removed or broken in the first partition and second partitions withdraw a strand of material from the phage bodies.

**DISCUSSION**

Adhesion partitioning is a method which can be adapted to the microdissection of a variety of small biological objects. It is particularly well suited to electron microscopy because of the use of thin plastic films, the relative ease with which corresponding parts can be viewed, and its adaptability to shadow casting. An advantage of preparing biological subjects for viewing the complete remains is that results can be made more quantitatively significant. Partitioning may be done either with or without the intention of observing corresponding parts, however. Non-sequential partitioning may be done, of course, using almost any relatively smooth adherent surface in place of one of the films.

An extended study of the \textit{E. coli} organism by partitioning has not been completed. Correlations have appeared, however, that are believed to be significant. The observation that the bacterial matrix is found in at least two different physical conditions may offer a basis for relating protoplasmic behavior on this scale with the extensive observations which have been made on larger cells. An attractive hypothesis is that the discs found in young bacteria are at least part of a nuclear apparatus. Further work along these lines integrated with the findings with microtomy and other techniques should clarify this point. Of additional interest is the nature of the limiting surface of theoplast that remains after removal of the membrane on the initial partition. The bacteria retain their form and apparently hold a considerable percentage of mobile water until the second partitioning when they suffer extensive disruption. Results reported here support in general the observations of Birch-Andersen \textit{et al.}, in so far as their descriptions and interpretations permit. The "vacuoles" reported by them are somewhat difficult to reconstruct from our observations. The relatively copious amount of fluid released on disruption suggests that water-filled vacuoles do exist, however. The reticulated, electronically opaque structure occasionally found in partitions may also be anatomically related to the "...folded, highly scattering threads... in an empty vacuole." The high phosphorus content of nucleic acid, its affinity for magnesium and calcium ions, and its fibrous nature suggest a possible structure with the observed properties.

The 30 to 60 \textmu m diameter spherical particulates dispersed in the residuum of disrupted cells may occasion some concern when further work is conducted on intracellular host-phage studies. The nature of these "microsomes" remains
to be determined. Since they appear to be stable in aqueous media, a purified fraction containing them may be obtainable.

Analytical ultracentrifugation of extracts of a number of bacteria, including *E. coli*, reported by Schachman, Pardee, and Stanier (21) has shown the occurrence of three principal sedimenting fractions with coefficients of approximately 5, 29, and 40 S. The 40 S component was found to have a particle diameter of approximately 100 A. Assuming a spherical shape and comparable densities, the two smaller components would correspond to objects with diameters of approximately 50 and 90 A, and are thus seen to fall into the macromolecular category which probably characterizes the bulk of protein and other microparticulate protoplasmic material. One would be quite bold to attempt to draw correlations between the matrix particles seen in partitions and the ultracentrifugal components in view of the abundance of matrix particles of this approximate size and the variations in particle shapes. It should be noted that the bacteria used by these workers were in late logarithmic or early stationary phase of growth at the time of their harvest for extraction and they may reflect a general sol-matrix state for bacteria in this condition.

With regard to the structural features of the T2 bacteriophage which have been found in applying the adhesion partition technique, there is a clear differentiation between the enveloping membrane and the presumed nucleic acid. The withdrawing of a string of material from the phage body in an untangled state suggests two possibilities for aggregation of the nucleic acid. Either (a) the strands exist preformed and neatly coiled within the phage, or (b) the material is present as a viscous mass which forms a thread as it is withdrawn. The first possibility mentioned requires a very high degree of organization and orderliness in the deposit. The second possibility seems more likely, in so far as the stranding is concerned, in view of the different lengths of strands that are obtained and the fact that no strands have yet appeared in a knotted condition. An "orderly deposit" of the nucleic acid within the phage is not ruled out, however, because the nucleic acid acts as a viscous mass under the conditions indicated. The known viscous nature of nucleic acid would also support the second hypothesis. More important, perhaps, is the significance which might be ascribed to the complete evisceration which can be accomplished with a single motion. A mechanism for the transfer of nucleic acid from the inside of the phage to the inside of the bacterial host is suggested. The cytoplasm of the host need only be viscous and in motion with respect to a phage anchored to the membrane by its appendage to serve as a reeling device in making the transposition once the nucleic acid has made contact with the host matrix. The bacteriophage appendage assumes functional meaning both as a matrix-contacting device and as a duct. Fibrils extending from the terminal end of phage appendages are often seen in electron micrographs which could be con-
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cievably the ravelled ends of a nucleic acid strand. This hypothesis would obviate the assumption of any special physical action on the part of either participant in the consummation of infection.

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SUMMARY

Adhesion partitioning is a method for progressively dismantling small biological entities for observation of their internal structures. The method is particularly well suited to use with the electron microscope. Objects to be partitioned are air-dried between two preformed plastic films resulting in envelopment of the objects. On separating the films the objects are partitioned. Partitioned E. coli bacteria reveal a variety of structures which change markedly with culture age. Organisms from young cultures have a water-retaining gelatinous matrix in which radially striated discs, fabric-like structures, and microsomes are found. Older cultures are less anatomically complex. The T2 bacteriophage is shown to be composed of an outer limiting membrane and a cohesive semisolid fibrillar body substance, presumably nucleic acid, which can be drawn as a strand from the bacteriophage body.

BIBLIOGRAPHY

EXPLANATION OF PLATES

All micrographs shown were obtained with an RCA model EMU-2c electron microscope with compensated pole-piece, a 25 μ objective aperture, and a standard condenser aperture. Specimen materials were prepared on collodion films and shadow-cast at an approximately 1/4 angle with uranium metal. Magnification values are given in each figure legend. Micron or fractional micron marks are displayed on each micrograph.

PLATE 27

FIGS. 1 a and 1 b. A pair of corresponding fields showing the usual distribution of bacterial membranes and protoplasts resulting from a single partition of E. coli from a rapidly multiplying culture. Patches of material removed from the protoplast surface are seen over central areas of each membrane. Correspondence of particulate entities to positions from which they were extracted are to be seen even in small detail. The impressment of the bacteria into the plastic substrate film and the apparent crowding are due primarily to the interfacial tension force which draws the two films together on drying water from the interfilm space. The ring-like structure in the single bacterium is anomalous. × 20,000.

FIG. 2. A single partition of an E. coli bacterium from an old culture spent of nutrient material. The lack of any large scale organized structure and the fine granular layering of material are characteristic of aged cultures. The two cottony white areas are highly opaque to the electron beam in spite of their thinness. These areas correspond in position to the so called “polar bodies”; they must be quite compact and if differential structure is present it must be on a very fine scale. × 50,000.

FIG. 3. A twice partitioned young culture bacterium which has ruptured to reveal a gelatinous matrix and a radially striated central disc. The striae in this micrograph are quite fine and may be lost in reproduction. The area is covered by a thin filamentous residuum, also too fine for reproduction, in which a large number of “microsome” particles 30 to 60 μ are interspersed. × 42,000.
(Backus: *E. coli* and T2 partitioning)
Fig. 4 a and 4 b. Two magnifications of a single abnormally long *E. coli* bacterium from a log-phase culture are shown as corresponding halves of a single partition. Portions of the cell wall are seen in both halves as flat pebbly areas not unlike the structure of the collodion substrate. Between the collodion substrate and the area occupied by the bacterium is a margin representing an enclosed space produced during the envelopment stage by failure of the plastic films to meet completely in the interfilm plane around the bacterium. This area logically contains a disproportionate amount of non-volatile extracellular debris because the final stages of drying take place in the enclosure. An adherent layer of material in the central area of the bacterial membrane in Fig. 4 a and containing a variety of particles is represented by a corresponding "replicate" area in Fig. 4 b which has been removed from the surface of the protoplast. The protoplast is rather voluminous in macromolecular substance. Some significance may be ascribed to the longitudinal break especially in view of the structural continuity that appears to extend along the face of the break seen in Fig. 4 b. Lower magnification \( \times 18,000 \), higher magnification \( \times 110,000 \).
(Backus: E. coli and T2 partitioning)
PLATE 29

FIG. 5. A micrograph of an area containing debris from a second partition of several E. coli bacteria from a young culture. The entire area is covered by a fine filamentous residuum, several layers deep in some regions. The largest object is a partition section through the mid-plane of a bacterium. Varying degrees of opacity in this object without corresponding differences in thickness indicate concentrations of relatively high electron-scattering capacity. Two elliptical discs are seen. One of the discs, near the right end of the micron mark, appears to be overlaid with a web of material of high intrinsic electron-scattering quality. This material is so thin that it casts no apparent shadow. It appears to be tied into the center of the disc. Fragments of material like this are occasionally seen but this is the only presumably intact structure of this kind so far observed. The larger disc, to the right of the partition section, is particularly interesting because of the shape of the sector which has been removed. It would seem plausible from this observation that the striae are indicative of channels extending through the thickness of the disc.
(Backus: *E. coli* and T2 partitioning)
Fig. 6. A single striated disc showing a dense, fibrous central area and a texture gradient progressing from finer structure on the periphery to coarser structure toward the center. The striae are seen to terminate in fine fissures in the exposed edges of the disc. Several "microsomes" and chunks of matrix material are seen in the vicinity of the disc. \( \times 124,000 \).

Figs. 7 and 8. Micrographs of T2 coliphages which have been subjected to two partitionings. First partitions are required to remove half of the phage membranes; the second partitions often withdraw strands from the exposed nucleic acid deposits. Impressions of bacteriophage bodies and appendages remain in the films near areas from which the strands have been drawn. The strands are made up of much finer filaments. The shadowing was done at a right angle to the direction of the pulling force that partitioned the phages. An air-dried T2 bacteriophage is inserted in the lower left corner of Fig. 8 at the same magnification as that shown for both figures. Magnification for both figures \( \times 64,000 \)
(Backus: E. coli and T2 partitioning)