BERYLLIUM FILMS AS OBJECT SUPPORTS IN THE ELECTRON MICROSCOPY OF BIOLOGICAL SPECIMENS

by

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I. INTRODUCTION

The film used to support a specimen for electron microscopy can confuse the image in two ways: by possessing a structure of its own, and by diffusely scattering the electron beam passing through it. It is not difficult to find materials which form films of the order of 10 millimicrons thickness which are structureless, or at least have structural details smaller than the present limit of resolution. But electron scattering occurs in every substance, and the most that can be done is to reduce it to a minimum. The degree of scattering depends on the physical thickness of the film, the density and the atomic number of the material composing it. It is therefore advantageous to keep all these factors low.

The practical limit to the thickness of the supporting film is set by its mechanical strength. In the case of collodion and similar films of an organic nature, this is in the region of 10 millimicrons; in practice it is usual to work with a thickness of 15 to 20 millimicrons, in order to reduce the risk of breakage. Such films have low physical density, and are mainly composed of carbon, oxygen, nitrogen, and hydrogen, all of reasonably low atomic number. Therefore they prove to be excellent as object supports for most purposes.

However, when the objects under investigation, or structural details of them, are comparable with the film in thickness or mass density, a collodion film can obscure important features in the image. In order to overcome this limitation, it has been proposed to use aluminium oxide supporting films[1]. These can be prepared with reasonable ease in a sufficiently thin and structureless form; they are also much more resistant to the heating effect of the electron beam than films of organic materials. On the other hand, the mass density is greater than for the latter and there is no reduction in scattering power, — in fact the reverse.

Beryllium, on the other hand, has a very low density (1.93) and atomic number (5), so that its scattering power is about the same as for organic materials. As it can be prepared in much thinner films than the latter, it has obvious attractions as a specimen support. Its use was first suggested by RÜDIGER[2] as a replica method for investigating metal surfaces. Later it was used by HAST[3] for mounting stripped particles of clay. During the examination of plant viruses, it occurred to us that it should give more informative pictures than the conventional specimen supports. This has proved to be the case, not only with plant viruses, but also with the larger animal viruses and even with bacteria.

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2. PREPARATION OF FILMS

Suitable beryllium films may be prepared in two ways: by deposition on the specimen itself, supported on collodion or other film, or by separate deposition on a backing material. In either case the latter is subsequently dissolved away with organic solvents.

In the first method, the specimen is mounted on a collodion film on a specimen grid in the usual way. This is inserted in a holder carried on a support within a bell-jar, which is then evacuated to a residual pressure of the order of 10^{-5} mm of mercury. Beryllium is then evaporated perpendicularly on to the specimen surface from a boat or strip of tungsten or tantalum, heated by the passage of a current of the order of 20 amp. The melting point of beryllium is 1450° C, but at this temperature it already has a high vapour pressure, its boiling point being given as 1500° C. It can therefore be evaporated in the usual gold-shadowing apparatus. In a typical case, the evaporation of 0.3 mg of beryllium filings is enough to form a film 2.5 millimicrons thick on a surface at a distance of 10 cm from the source. The grid is then removed from its holder, transferred to the specimen holder of the microscope, and immersed in amyl acetate or acetone. The collodion support is dissolved away in twenty minutes or so, after which the holder is removed from the solvent and left to dry. The drying process may be assisted with filter-paper, but great care must be used not to rupture the film, which readily occurs on over-rapid drying.

It is also possible to treat several specimen grids at once, mounting them on a rougher and larger gauze for immersion in solvent and subsequent drying. But such a procedure involves more manipulation, and also the final transfer of the grid to the specimen holder, all of which is attended by further risk of breakage. We find it preferable to mount the grid in the microscope holder before immersion in the solvent. In any case, it is advisable to examine the grid finally under the optical microscope before insertion in the electron microscope, to ensure that it is still intact. Even at a thickness of 2 millimicrons the film is still quite visible in white light. Thinner films than this are difficult to prepare, although HAST claims to have made them.

The alternative method of preparation is to coat a clean glass slide with a collodion film, mount it under a bell-jar, and evaporate beryllium on to the surface in the required thickness, in the same way as described above. The surface of the slide is then divided into squares by scratching lines with a fine needle, and the slide immersed in acetone or amyl acetate. As the underlying collodion dissolves, the squares of beryllium film float up to the surface. They are collected on specimen grids, which are immersed on a piece of coarse gauze; by draining away (or siphoning off) the solvent the films are settled on to the grids. These are then removed, dried, and stored ready for use. Again it is preferable to mount the grid plus beryllium film directly in the specimen holder, before drying off. If the specimen can be exposed to the solvent, it is also desirable to deposit it on the film whilst still moist, so that the beryllium is exposed only once to the mechanical strain of drying out. The films are remarkably strong, even when as thin as 2 millimicrons, and can readily be steered about in the solvent with the point of a fine needle, so that it is not difficult to settle them on the supporting grids as the solvent is removed.

3. RESULTS

Beryllium films have been used by us for a variety of biological specimens. The

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clearest images are always obtained when the beryllium is deposited on the mounted specimen, according to the first method of preparation. The outline is then much sharper and the resolution of detail better than when the specimen is deposited on a pre-formed beryllium film. Fig. 1 shows tomato aucuba mosaic virus rods deposited in this way on beryllium, and Fig. 2 shows the result of evaporating beryllium on to them. Fig. 3 shows turnip yellow mosaic virus crystals with a deposited beryllium film[4]. The detail of Figs 2 and 3 is much sharper than in Fig. 1. Indeed, it has proved difficult to obtain good photographs of plant viruses by mounting them on pre-formed films of beryllium. The accentuation of contrast when the beryllium is evaporated on to the specimen is probably due to a type of replica effect. If the metal forms a layer of uniform thickness all over the surface, this layer will present a greater thickness to the beam in places where it slopes with respect to the plane of the whole film, as will especially be the case at the edge of objects. This is the same effect as produces contrast in silica replicas. In the present case, of course, the object remains in the "replica" film so that the contrast is even better than in a silica replica. It is also possible that the deposited beryllium molecules have a certain degree of mobility on the receiving surface, and aggregate preferentially along edges and at corners of objects. That something of the sort occurs is evidenced by the lack of sharp shadows on the bell-jar after the evaporation, in contrast to gold-shadowing. The higher temperature of evaporation of the beryllium would also endow the molecules with greater energy, and favour mobility on the surface.

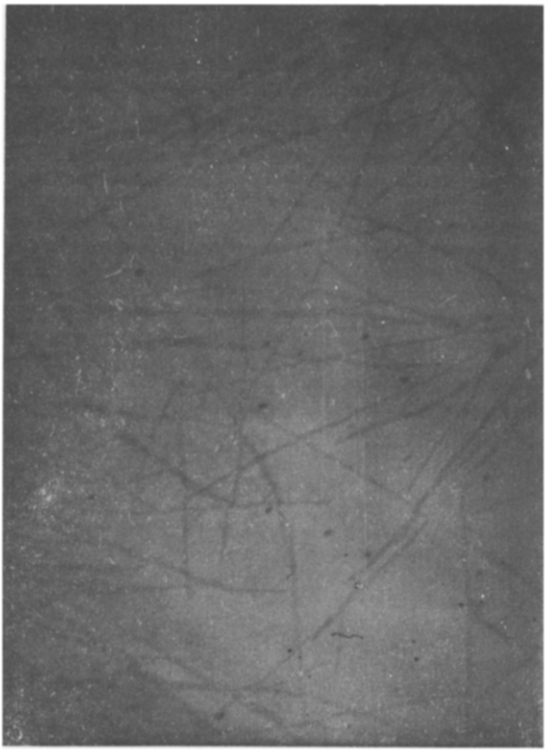

Fig. 1. Tomato Aucuba Mosaic Virus, deposited on preformed Beryllium film (× 36 000)

Both Figs 2 and 3 show much more detail than the conventional specimen prepared on collodion. In the case of crystalline fragments of turnip virus, the lattice character can sometimes just be discerned in the latter type of preparation, but it is very much sharper on an evaporated beryllium support. It is observed, however, that the field of view becomes clouded over with continued exposure in the electron beam, probably owing to disintegration of the virus particles. It is therefore essential to operate with as low a beam current as practicable for viewing, and to select and photograph the required area as quickly as possible. The same difficulty occurs whether collodion or beryllium supports are employed, but it is much more readily appreciated in the latter case, owing to the initially higher picture contrast.

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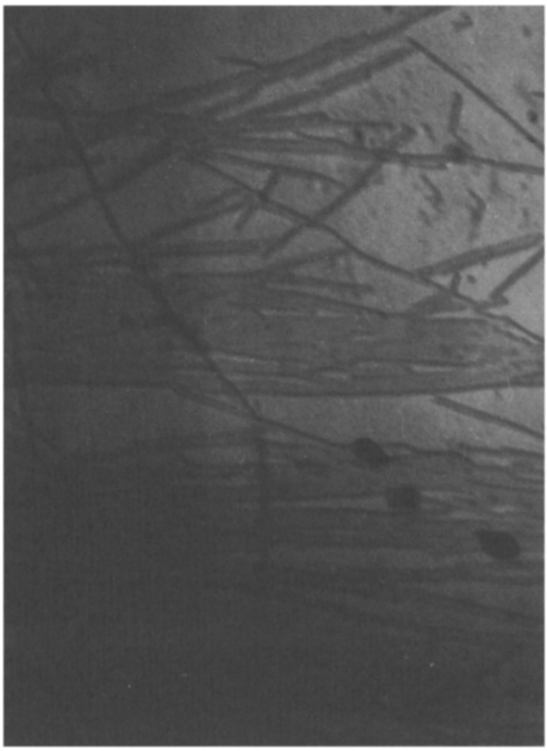

Fig. 2. Tomato Aucuba Mosaic Virus, upon which a Beryllium film has been formed by evaporation (× 45 000)

In the case of animal viruses also, additional information is obtained by using beryllium films. Fig. 4 shows pig pox elementary bodies photographed in this way in the electron microscope. The central dense region, which is only vaguely to be seen in collodion-supported specimens, now shows up very sharply. The appearance of the elementary bodies is similar to that observed by MACFARLANE AND DAWSON[5] in the case of vaccinia virus after treatment with pepsin.

Where bacteria are concerned, the use of a beryllium support is calculated to reveal finer structural details than the conventional collodion. Fig. 5 shows a type of *Pseudomonas* when mounted in a deposited beryllium film. Not only is the outline of the cell extremely sharp, but a bundle of minor flagella are rendered visible. In the conventionally prepared bacterium, only a single major flagellum is normally to be seen, — hence the classification of this organism as a *pseudomonas* type. Such minor flagella have been reported by HOFER[6] to occur on some other bacteria (*azotobacter*), but they are extremely fragile and difficult to photograph in the electron microscope. They are frequently to be found in preparations of *pseudomonas* on beryllium.

The use of beryllium supports can obviously be extended with advantage to other organisms and tissues, where fine detail is being sought. It does not commend itself where the specimen is very thick and has no marginal processes, such as flagella, nor when sections are under examination.

SUMMARY

In the electron microscopy of objects of the order of 100 Angstrom units in size, especially of a biological nature, the thickness of the supporting film has an important effect on the contrast and resolution in the image. Beryllium films are preferable to collodion in such circumstances, as they can be made thinner for the same limiting mechanical strength, and have approximately the same scattering power per unit thickness. A technique is described for forming beryllium films directly on the specimen. An alternative method is to pre-form a film and then deposit the specimen on it, but this gives less good contrast. Films as thin as 25 Angstrom units can be prepared and manipulated without undue difficulty. Examples are shown of the value of such supporting films in the investigation of plant and animal viruses, and in the examination of the fine structure of bacteria, such as flagella.

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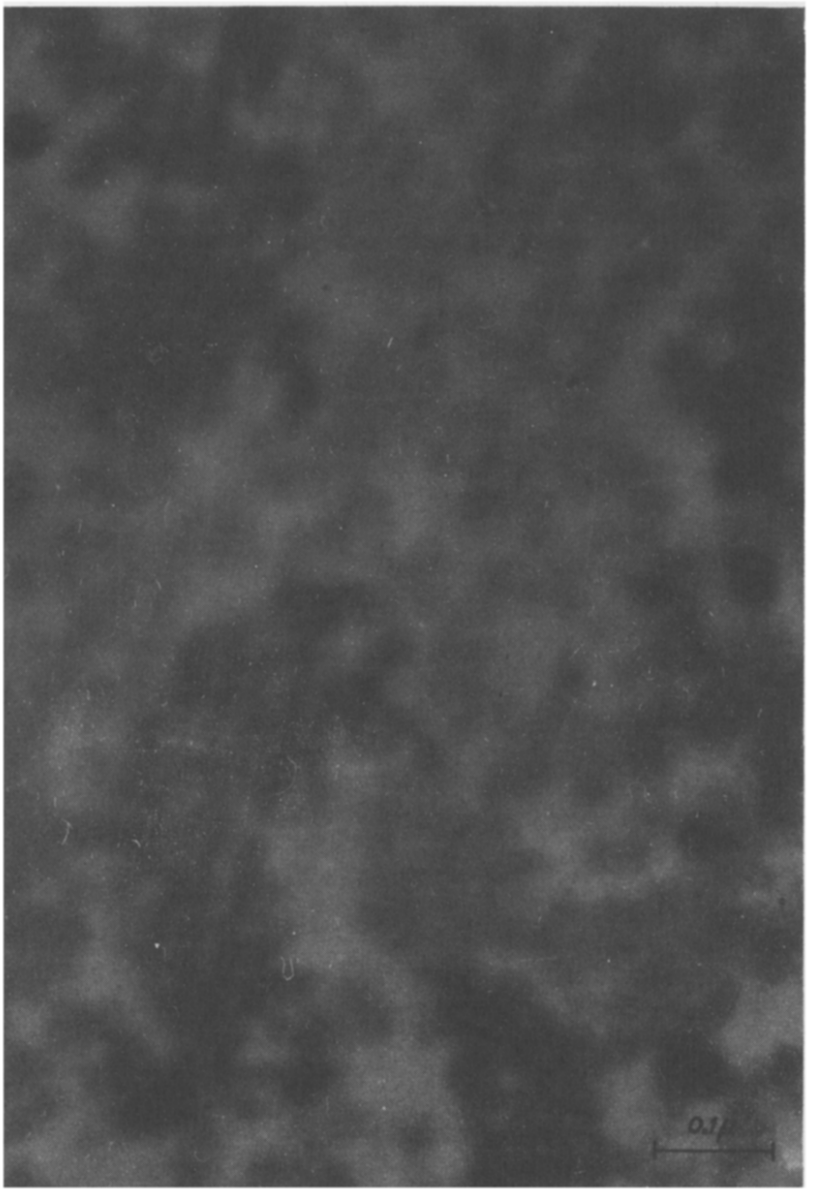

Fig. 3. Crystalline fragments of Turnip Yellow Mosaic Virus, in an evaporated Beryllium film (× 165 000)

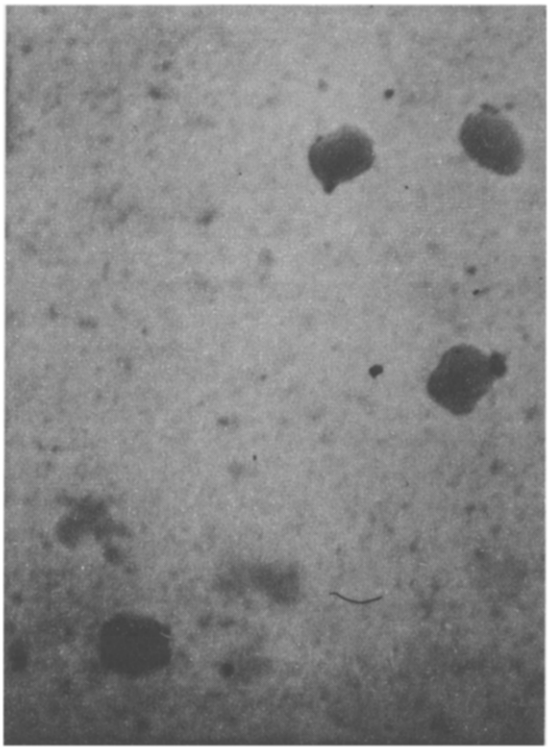

Fig. 4. Elementary bodies of Pig Pox, in an evaporated film of Beryllium (× 29 000)

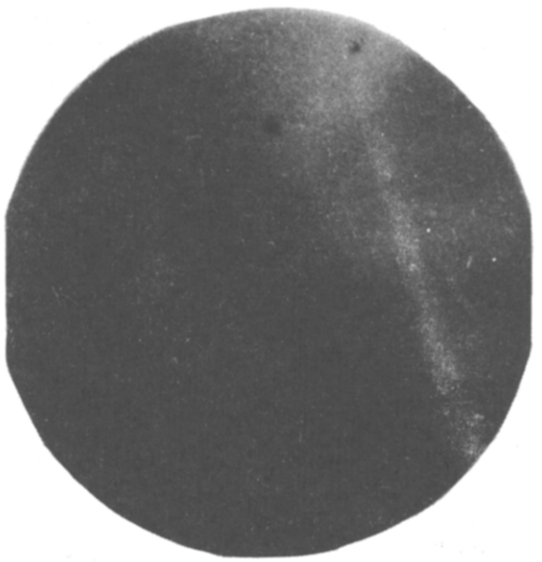

Fig. 5. A *Pseudomonas* type of bacterium, in an evaporated film of Beryllium, showing the presence of minor flagella in addition to the major one (× 39 000)

RÉSUMÉ

Dans l'étude au microscope électronique d'objets d'une taille d'environ 100 Å, en particulier d'objets de nature biologique, l'épaisseur du film support a une importance considérable sur le contraste et la résolution de l'image. Les films de béryllium se montrent, dans ces conditions, préférables aux films de collodion, car à résistance égale, ils sont beaucoup plus fins et possèdent à peu près le même pouvoir de diffraction par unité d'épaisseur. Une technique est décrite, permettant de former des films de béryllium, directement sur l'échantillon. Une autre méthode consiste à préparer un film à l'avance, puis à déposer l'échantillon sur lui, mais cette méthode donne un contraste moins bon. On peut préparer et manipuler sans difficulté des films dont l'épaisseur ne dépasse pas 25 Å. Des exemples sont donnés de l'intérêt de tels films comme supports, dans des recherches sur des virus animaux et végétaux, et dans l'examen de la structure fine de bactéries telles que les flagelles.

ZUSAMMENFASSUNG

Bei der Elektronenmikroskopie von Objekten von der Grössenordnung von 100 Å, insbesondere bei Objekten von biologischem Ursprung, hat die Dicke des tragenden Films einen bedeutenden Effekt auf den Kontrast und die Auflösung im Bild. Berylliumfilme sind unter diesen Umständen Kollodiumfilmen vorzuziehen, da sie bei gleicher mechanischer Stärkegrenze viel dünner gemacht werden können, und ungefähr dasselbe Streuvermögen per Dicke-Einheit haben. Ein Verfahren, um Berylliumfilme direkt auf dem Objekt zu bilden, wird beschrieben. Eine alternative Methode besteht darin, dass erst der Film geformt wird und dann das Objekt darauf deponiert wird; sie ergibt aber einen weniger guten Kontrast. Filme, die nur 25 Å dünn sind können ohne besondere Schwierigkeiten bereitet und hantiert werden. Beispiele, die den Wert derartiger Trägerfilme bei der Untersuchung von Pflanzen- und Tierviren, und bei der Erforschung der Feinstruktur von Bakterien, wie z.B. Flagella, zeigen, werden angegeben.

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Received April 26th, 1948