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High Voltage Electron Microscopy and its Application in Biology

V. E. Cosslett

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High voltage electron microscopy and its application in biology

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[Plates 3 to 5]

The advantages and limitations of electron microscopy at voltages up to 1 MV are outlined. Greater thickness of specimen can be examined, the increase being almost linear with applied voltage for carbonaceous material. Alternatively, a much improved image resolution is obtained from a specimen of given thickness. For such a specimen, radiation damage and temperature rise is less than at 100 kV, but these effects probably set a limit to the maximum thickness of specimen which can be examined at 1 MV. The main disadvantage is that contrast decreases with increasing voltage, as also does the response of the fluorescent screen and of photographic emulsions. The prospective slight gain in ultimate resolving power, which might make possible the imaging of atoms, is largely offset by difficulties in maintaining electrical and mechanical stability.

Examples are shown of the usefulness of high voltage microscopy for examining whole chromosomes and thick sections (up to 2 μm). Stereomicrography is necessary if the three-dimensional structure of such relatively thick specimens is to be properly evaluated. The further possibilities for investigating wet samples in special environmental cells are outlined. It is concluded that the prospects for observing living material are remote.

1. Introduction

Biologists have lagged behind their metallurgical colleagues in appreciating the advantages of using much higher voltages for electron microscopy than the 80 to 100 kV provided in the standard instrument. Yet the original impetus in the late 1940s towards high voltage operation came from a desire to examine whole cells, at a time when techniques of thin sectioning had not been developed. Several projects for microscopes with voltages up to the order of 500 kV were started, but to the best of my knowledge only one of them reached the point of successful operation. In 1947 van Dorsten, Oosterkamp & Le Poole published a description of a 400 kV microscope and some micrographs of yeast cells taken at 350 kV. Their paper discusses the advantages and limitations of high voltage operation very thoroughly, except the problem of observing cells in the living state. Further development of the subject was temporarily halted by the discovery of techniques for preparing specimens thin enough for transmission microscopy at normal working voltages: in metallurgy by carefully controlled etching of foils and in biology by refining the advance mechanism of the standard microtome.

The revival of interest in high voltages in the past decade has come primarily from the metallurgist, worried by the validity for bulk metals of observations and conclusions obtained from thin films of the order of 100 nm in thickness. A secondary cause, however, and an important one because it was behind the building of the first very high voltage microscope (1.5 MV) at Toulouse, was biological: the hope of examining living matter. The prospects of doing so had been looked into at the Cavendish immediately after the publication by van Dorsten *et al.* (1947) of their results, since we had the possibility of taking over a 2 MV Cockcroft–Walton generator from the nuclear section. In the light of available evidence, however, it seemed that even the most resistant organisms could not survive the dose required to focus and photograph them at a resolution better than that of the ultraviolet microscope, even by operating at the minimum of the radiation damage–voltage curve (about 1.5 MV). Although this conclusion was reported at a conference in Paris in 1950 (Cosslett 1953), Dupouy set

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on foot a few years later the Toulouse project, showing a healthy disregard for theoretical pessimism. The first micrographs of bacteria in the wet state were published in 1960 (Dupouy, Perrier & Durrieu 1960), taken at 650 kV, and argument has continued since then as to whether they survived exposure without lethal damage. Similar micrographs have since been obtained at 1 MV (Dupouy 1968).

Meanwhile, several high-voltage electron microscopes had been built in Britain, Japan and the United States (see Dupouy 1968). The results of their application to problems of metal physics and metallurgy were such that commercial models for voltages up to 1 MV were soon made available. The three Japanese manufacturers have now described their prototype instruments (Kobayashi *et al.* 1964; Watanabe *et al.* 1968; Katagiri, Kimura, Ozasa & Shiraishi 1969). The AEI 1 MV microscope, based on a 750 kV experimental model designed and built at the Cavendish Laboratory (Smith, Considine & Cosslett 1966; Cosslett 1969*a*), has so far been described only in the maker's brochure. The recent exploratory studies of various biological applications, reviewed below, have been carried out mainly on the AEI, Cavendish, Hitachi and Japan Electron Optics Laboratory (J.E.O.L) instruments, although some work of this nature has been done on a 1 MV microscope at the United States Steel Corporation Research Center.

2. Advantages and limitations of high-voltage microscopy

The chief reason for using high voltages is the greater thickness of specimen that can be imaged, roughly in proportion to the applied voltage in the case of carbonaceous material. At the same time, the image resolution does not deteriorate with thickness so badly as used to be assumed. As the other side of this same coin, the practical resolution obtained from a specimen of given thickness is found to improve rapidly as the voltage is raised. On the other hand, the ultimate resolving power of an electron microscope improves only slowly with voltage, since the decrease in electron wavelength is partially offset by an increase in spherical aberration.

Radiation damage to the specimen also has two aspects that vary in opposite senses with voltage. The total amount of energy transferred by the beam to a given thickness of material decreases as voltage is raised, reaching a flat minimum between 1 and 2 MV. So ionization, the main cause of injury to living matter, decreases with voltage and so also does temperature rise. The ejection of carbon (and other) atoms from a specimen obeys quite different rules, however. The effect does not occur below a threshold, which appears to be at about 30 kV for carbon. Above that voltage the probability of an atom being removed, by head-on impact of an electron, increases rapidly (Cosslett 1970). The resulting damage, however, is still on too small a scale to be observable in normal conditions of microscopy, except in metals in which the aggregation of vacancies leads to visible effects.

The chief limitation of high-voltage microscopy lies in the decrease in image contrast, which is the concomitant of increased penetration. Again this is not so serious for biology as it is for metallurgy, since it can be counteracted by heavier staining of most specimens. It becomes a limiting factor on the ultimate resolution attainable from very thin specimens, when staining would mask the fine detail it is desired to image. Similarly, the response of the viewing screen of the microscope and of photographic emulsions decreases as the voltage is raised. Fortunately the increase in exposure is not so large as was initially predicted, so that the usual types of photographic material can still be used. The problem of the viewing screen is more difficult to solve, however, as the resolution of the visible image deteriorates as well as its brightness. At present

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the expedient is adopted of having a thickly coated screen for general purpose viewing, and a much thinner one on a transparent backing for use when focusing the image.

These main aspects of practical microscopy at high voltages are further discussed below. Since they have been covered in full detail in recent survey articles (Dupouy 1968; Cosslett 1969*a*), the theoretical background will be largely omitted and emphasis laid on the present state of experimental evidence. In most respects the subject is in a state of active development, especially as regards the special conditions of biological applications on the one hand and of high resolution imaging on the other hand, so that many conclusions have to remain tentative. A brief outline is also given of the main differences in construction and operation between high-voltage microscopes and the standard type of instrument.

3. Specimen penetration

The maximum thickness of specimen observable in an electron microscope may be defined either in terms of (*a*) the fraction of the incident beam (I/I_0) collected in the objective aperture and thus determining the overall image brightness, or (*b*) the energy lost by the beam in traversing the specimen, which limits the resolution obtained in the image owing to the chromatic aberration of the objective lens (see § 4). For the range of thickness observable in practice, the fractional transmission obeys an exponential law

$$I/I_0 = \mathrm{e}^{-\mu t}. \tag{1}$$

Here μ is the equivalent of an absorption coefficient, but it should be noted that the specimen is in fact highly transparent ($> 90\,\%$) to the electron beam; μ is concerned with the part of this transmitted beam that falls within the very small aperture of the lens, α_0 ($10^{-3} < \alpha_0 < 10^{-2}$ rad).

Multiple scattering theory then allows us to calculate the thickness of specimen which will result in a given attenuation of the imaging beam with respect to the incident beam, at a given accelerating voltage V_0 and aperture α_0. For convenience, we take the maximum observable specimen thickness as that for which $I/I_0 = \mathrm{e}^{-4}$, so that the imaging beam intensity is slightly less than $2\,\%$ of the incident intensity. With the high incident intensity of $1\ \mathrm{A\ cm^{-2}}$ on the specimen, this would allow an image of adequate brightness to be formed on the viewing screen at a magnification of 20 000. If a lower magnification is acceptable, images could be formed at a lower transmission than e^{-4}.

On this basis and using the multiple scattering theory due to Lenz (1954), we find that the maximum observable thickness of carbon rises only slowly with beam voltage if $\alpha_0 = 10^{-3}$, but that it increases almost linearly with voltage for $\alpha_0 = 5 \times 10^{-3}$, the size of angular aperture usual in electron microscopy (see Cosslett 1969*a*). For heavier elements, such as gold, the increase with voltage is much slower even with a large aperture, because of the variation of the ratio of inelastic to elastic scattering with atomic number. If, alternatively, we calculate the thickness of specimen that will cause a certain energy loss and hence a prescribed chromatic aberration in the image (from (2) below), it turns out that the thickness–voltage curve is again almost a straight line for carbon (and for gold also, now).

Direct measurements of fractional transmission through carbon films of measured mass thickness, for various apertures and voltages, have largely confirmed the predictions of the Lenz treatment (Curtis 1968; Marais 1970). The alternative multiple scattering theory of Smith & Burge (1963) in general gives an appreciably lower transmission than the experimental values.

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The requirements of practical electron microscopists are more complex, of course, involving contrast and resolution as well as image brightness. Some recent observations of thick sections at high voltages, however, appear to accord fairly well with theory. For 500 kV and an aperture of 5×10^{-3} the mass thickness of carbon giving an attenuation of e^{-4} is calculated to be 0.5 mg cm^{-2}, a thickness of 2.5 μm, and with an image resolution of about 10 nm (Cosslett 1969*a*, Fig. 3). Micrographs published by Hama & Porter (1969), Nagata, Hama & Porter (1969) and Ris (1969), as well as our own experience, indicate that 1 μm sections (even though heavily stained) give clear images at 500 kV and 2 to 3 μm sections equally so at 1 MV. The limitation on examining still thicker sections is set by temperature rise and radiation damage, rather than by inadequate transmission or resolution. The resolution is frequently better than predicted, for reasons discussed in § 4.

4. Energy loss in the specimen and chromatic aberration

For specimens thicker than about one-tenth of the maximum observable thickness the image resolution is determined by chromatic aberration. Electrons lose energy by inelastic collisions with atoms in the specimen, so that the monochromaticity of the incident beam is destroyed and the chromatic aberration of the objective lens gives rise to overlapping images formed by electrons of differing energy. The radius of the disk of least confusion in the paraxial image plane δ_c is given by

$$\delta_c = \alpha_0 C_c \, (\Delta V / V_0)_r, \tag{2}$$

where α_0 is the angular semi-aperture of the objective, C_c the chromatic coefficient of that lens and ΔV a measure of the energy loss in a beam of initial voltage V_0; the subscript r indicates that a relativistic correction must be included. When the film is thin, it is not easy to decide what value to assign to ΔV, as the imaging beam still contains a proportion of electrons which have suffered no energy loss. With thick films, however, this is no longer so. The energy distribution now has a well-defined shape, characterized by the peak (or most probable) energy E_p ($= eV_p$) and the width at half-height, ΔE_w.

Direct measurements of such distributions have now been made for the range of thicknesses and the angular apertures usual in electron microscopy: on magnesium oxide at 100, 190 and 480 kV by Kamiya (1969), and on aluminium and collodion at 200 and 500 kV by Considine (1969). The results for three thicknesses of collodion at 500 kV, shown in figure 1, have a typical Poissonian form. If the position of the peak $(E_0 - E_p)$ is assumed to be given by a simple multiple of the main characteristic energy loss of carbon (25 eV), the mean free path for this loss process proves to be almost identical with that calculated relativistically for aluminium (Hirsch & Humphreys 1968). The width of the distribution ΔE_w, however, is appreciably greater than predicted on that basis, especially for the thickest films. On the other hand, it is in close agreement with the values predicted by the treatment of energy loss due to Landau (1944), which takes all types of excitation into account (Considine, Smith & Cosslett 1970).

If we now take ΔV in equation (2) as given by ΔE_w, the measured values of Considine would predict an image resolution of 11 nm for 1 μm of collodion at 200 kV and 2.7 nm at 500 kV (with $\alpha_0 = 5 \times 10^{-3}$ and $C_c = 4$ mm). This surprisingly good resolution is in rough accord with the general experience of observing thick sections at high voltages, and with the particular statement that a lattice period of 0.457 nm could be resolved through a 0.3 μm thick section at

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800 kV (Nagata & Hama 1969). Furthermore, it can be shown that the asymmetric form of the energy distribution should lead to an appreciably smaller chromatic disk of confusion than that given by (2), by as much as 50 % (Cosslett 1969*b*). In any case there is a sound theoretical basis for the experimental observation that image resolution in thick sections at very high voltages is much better than was expected from experience with thin sections at 80 to 100 kV.

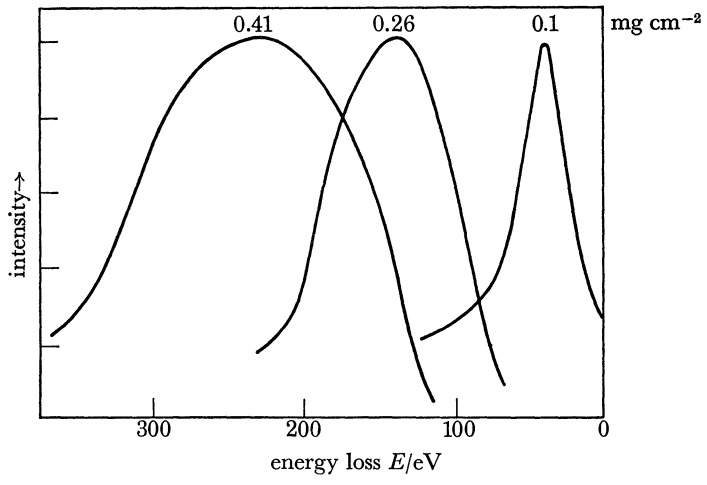

FIGURE 1. Energy-loss distribution in collodion films at 500 kV. Curves are for mass thicknesses of 0.1, 0.26 and 0.41 mg cm^{-2}, respectively (from Considine 1969).

5. ULTIMATE RESOLVING POWER

The limit to the resolving power of an electron microscope, on the classical definition of the separation of two neighbouring point objects δ_s, is set by the wavelength λ of the electron beam and the spherical aberration coefficient C_s of the objective lens:

$$\delta_s = a\,(C_s\lambda^3)^{\frac{1}{4}}. \tag{3}$$

The value of the factor a lies between 0.4 and 1, depending on the assumptions made about the illuminating conditions (coherent or incoherent), the nature of the points imaged (amplitude or phase objects), the plane of observation (Gaussian or of least confusion) and the minimum contrast difference detectable. Although originally derived by geometrical optics, equation (3) remains the same (apart from the value of a) in a full treatment of the imaging process by contrast transfer theory (see Hanszen (1970) for a review).

With increasing beam voltage λ decreases, but C_s increases, in a given microscope objective operated at maximum magnetic induction. The net result is that δ_s varies as $\lambda^{\frac{1}{2}}$ and hence with $V_r^{\frac{1}{4}}$, where V_r is the accelerating voltage corrected for relativistic effects. So an increase in operating voltage from 100 kV to 1 MV gives a prospective improvement in resolving power by a factor of about 2. This gain, however, is largely offset by increased problems of mechanical and electrical instability, apart from the matter of cost, which rises almost linearly with the maximum voltage provided. At the same time image contrast diminishes, owing to the fall in the scattering cross-section. The best resolution so far obtained at 1 MV is about a factor of two below that from a 100 kV microscope.

Since the prospects of imaging the lighter atoms at 100 kV is marginal (see Eisenhandler & Siegel 1966, 1967; Reimer 1966, 1969; Niehrs 1969), there is a case for a moderate rise in

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operating voltage to 300 or 500 kV, in order to gain some advantage from decreased wavelength without running into severe problems of cost and stability. For the strong condenser-objective lens, this would give a resolving power of 0.123 nm at 500 kV in place of 0.229 nm at 100 kV, taking $a = 0.56$ in (3) (Ruska 1965). Several projects aiming at atomic resolution are going ahead on this basis. The use of zone plates or of image deconvolution procedures, however, may provide a solution valid at 100 kV or an even lower operating voltage (see contributions to this Discussion by Erickson, Hoppe, Siegel and Unwin).

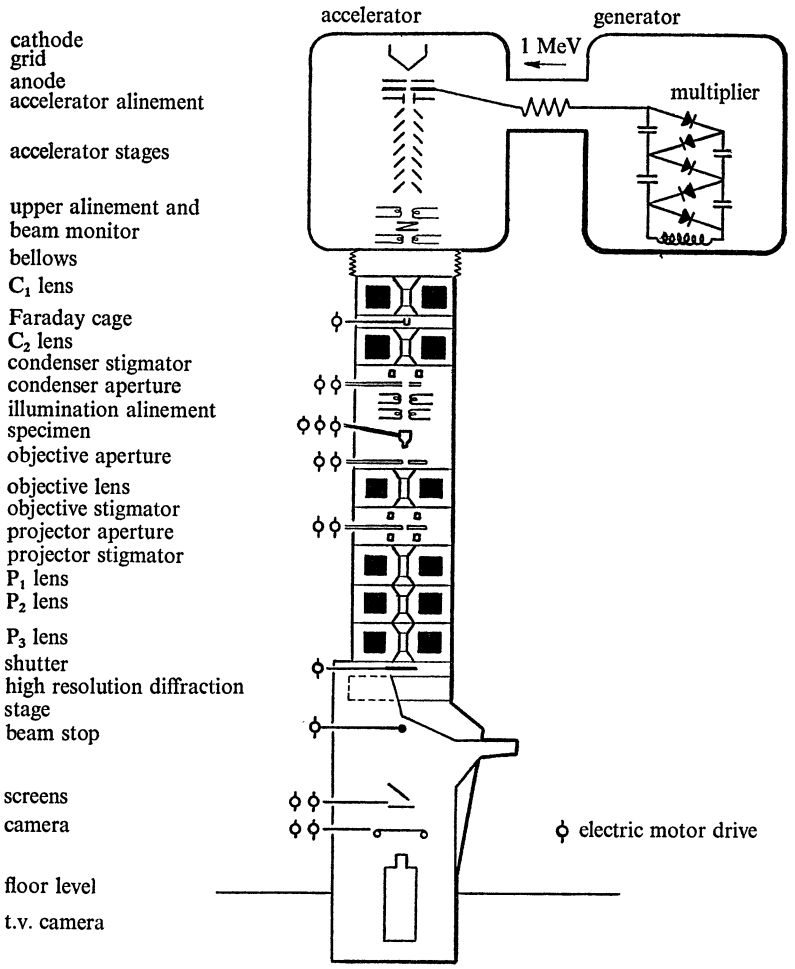

FIGURE 2. Schematic diagram of a 1 MV electron microscope (Associated Electrical Industries, Ltd, Harlow).

6. CONSTRUCTION AND OPERATION OF A HIGH-VOLTAGE ELECTRON MICROSCOPE

The design of a 1 MV microscope is in principle identical with that of a 100 kV instrument; it is mainly a matter of scaling up the electron lenses. The chief differences concern the electron source and associated generator, and the provision of protection against X-radiation.

The single-stage electron gun of the conventional microscope is replaced by a gun plus a multi-stage accelerator column (figure 2). To reduce the clearance distances and hence the overall size, the accelerator is surrounded by a gas of high dielectric constant (freon or sulphur hexafluoride) in a pressure tank. Similarly, the high-voltage generator, a scaled-up version of the

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Cockcroft–Walton set used for 100 kV, is placed in the same tank (as in two Japanese models) or in a parallel tank with an insulated feed-through (as in the A.E.I. and another Japanese model). In either case the installation becomes of considerable size and weight, requiring a steel gantry to support it and a travelling overhead crane for handling the upper shells of the tanks.

The microscope column also becomes many times heavier than that of a 100 kV instrument, mainly because of the need to build in massive lead shielding to reduce X-radiation to the permitted level. The column of the AEI 1 MV microscope (figure 3) weighs about 6 tonnes. With the generator and accelerator tanks and the superstructure the total weight is over 20 tonnes,

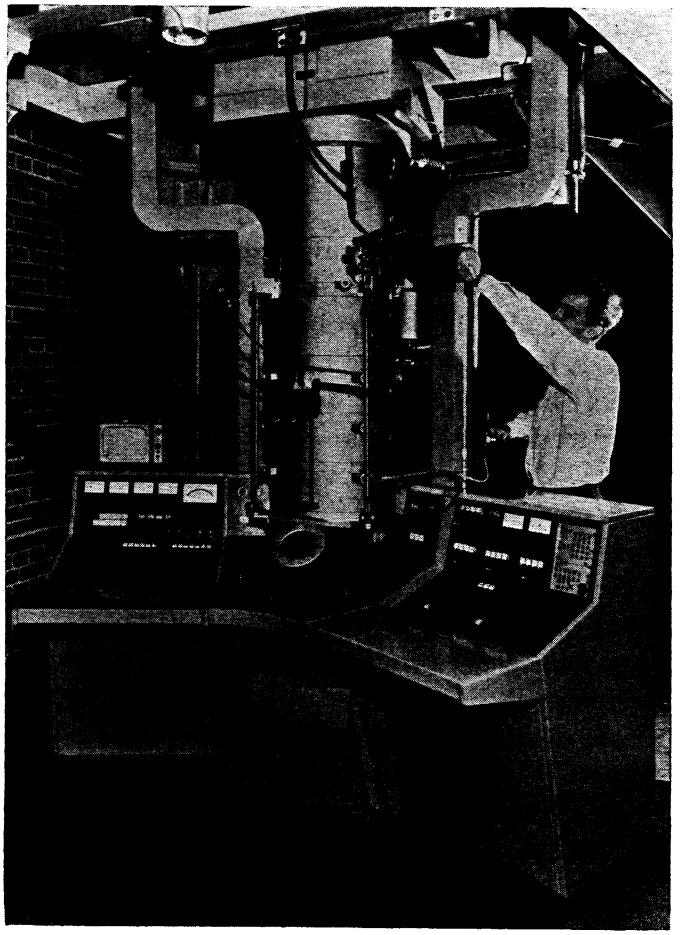

FIGURE 3. Column and control desk of the AEI 1 MV electron microscope.

requiring a specially designed foundation block, suitably sprung to minimize mechanical vibration. If a radiation wall is provided for protection, as in the Cavendish and Toulouse microscopes, the column can be made much lighter and the foundations simpler and less costly.

Although in externals a high-voltage microscope seems very different from the conventional instrument, in operation little difference will be found. The controls are identical in most respects, except that remote operation of apertures and specimen movement by servo-motors is necessitated by the height of the column. The chief difference is the need for a prescribed routine for raising the voltage, since it cannot be switched direct to its maximum value without

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grave risk of flash-over in the accelerator and breakdown in the supply circuits. The image is less sharp on the viewing screen, as already noted, making difficult the correction of astigmatism by the Fresnel fringe method. But the photographic image is perfectly sharp, with an exposure time little different from that needed at 100 kV at the same magnification. Fortunately the brightness of the electron gun increases in proportion to the overall applied voltage, so that very high magnifications are possible in high voltage microscopy.

7. Applications in biology

For the practical biologist, high voltage microscopy offers two main advantages. First, it will allow much greater thicknesses of specimen to be examined (at given resolution), approximately in proportion to the operating voltage. Secondly, it will give clear images with much improved resolution of a given specimen, say 200 to 300 nm thick, that is only just visible at 100 kV. In this latter case, radiation damage is reduced also as voltage is increased, a consideration of great importance for radiation-sensitive materials such as polymers (Kobayashi & Sakaoku 1965).

These prospective advantages have only recently begun to be exploited. Ris (1969) has shown some impressive images of whole chromosomes, both in squash preparations and in sectioned material (figure 4, plate 3), and of intact fibroblasts (figure 5, plate 3). A variety of tissues, in sections up to 2 μm thick, have been studied by Porter and his colleagues in several different high voltage microscopes (Hama & Porter 1969, Nagata *et al.* 1969). Figure 6, plate 3, is an example of a thick section, taken on the AEI 1 MV instrument, and figure 7, plate 4, of a thinner section at 700 kV on the Hitachi microscope. At the Cavendish Laboratory, the 750 kV microscope has been chiefly employed in physical and metallurgical investigations, but lately we have turned our attention to its biological applications. Figure 8, plate 4, is a typical instance of the resolution obtainable in a section about 200 nm thick at 500 kV. Figures 9 and 10, plate 5, show the amount of detail imaged in muscle sections.

It is an advantage, and sometimes an embarrassment, that detail all through the thickness of a section is in focus in the image, owing to the great depth of focus provided by the electron microscope. So it is usually desirable to record stereo-pairs of micrographs, by tilting the specimen through a small angle (5° to 10°) between two exposures. The corresponding positives, when mounted in correct orientation in a stereo-viewer, show clearly the three-dimensional structure of a thick section (as in figure 4). The relative position of different features is at once apparent, and can be quantitatively evaluated if a stereogrammeter is available. The

Description of plate 3

Figure 4. Section 1 μm thick of Gyrodinium cohnii nucleus during division. Fixation: Karnovsky's paraformaldehyde-glutaraldehyde, followed by 2% OsO_4 in 0.1 mol l^{-1} phosphate buffer, pH 7. Embedded in eponaraldite. Stained in uranyl–magnesium–acetate and lead citrate. Stereopair (tilt ± 10°) taken at 1 MV in the AEI EM7. (Magn. × 16000; H. Ris, unpublished.)

Figure 5. Human skin fibroblast, intact and unsectioned, as grown on formvar-carbon film on titanium grid. Fixation and staining, see legend to figure 4; dried by Anderson's critical point method. One of a stereopair (tilt ± 5°) taken at 1 MV in the Toulouse 1.5 MV microscope. (Magn. × 13500; H. Ris, unpublished.)

Figure 6. 1 μm section of retina of dog fish. Fixed in glutaraldehyde, embedded in Epon, sections stained with uranyl acetate and lead citrate. One of a stereopair (tilt ± 10°) taken at 1 MV in the AEI EM7. (Magn. × 13000; K. R. Porter, unpublished.)

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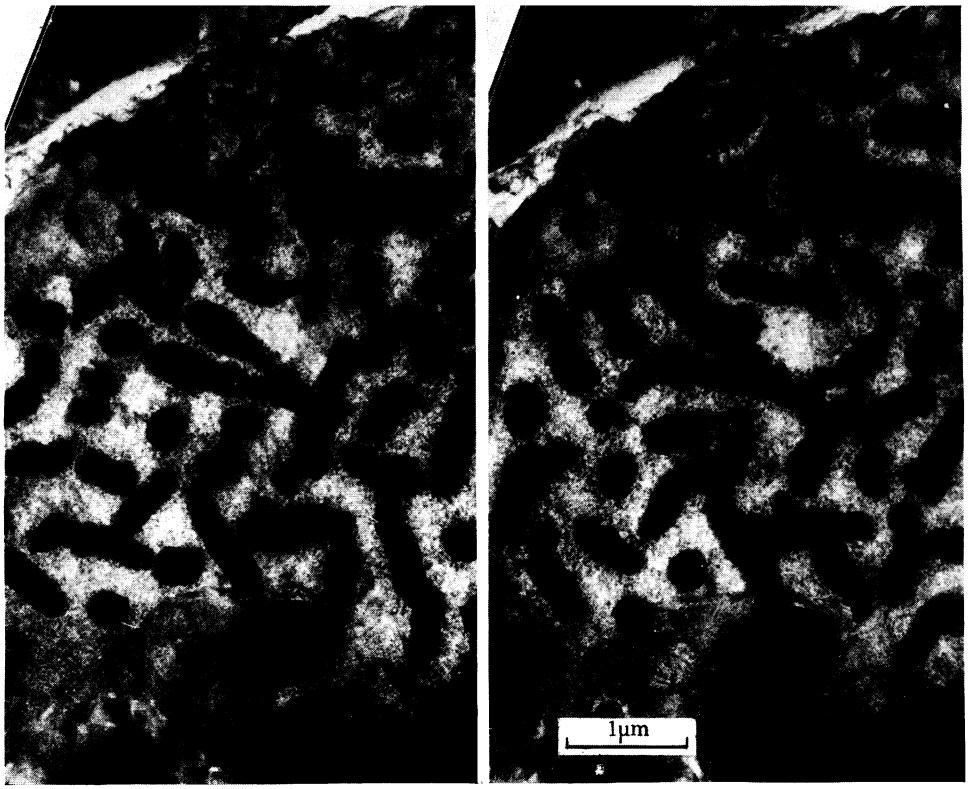

FIGURE 4

FIGURE 5

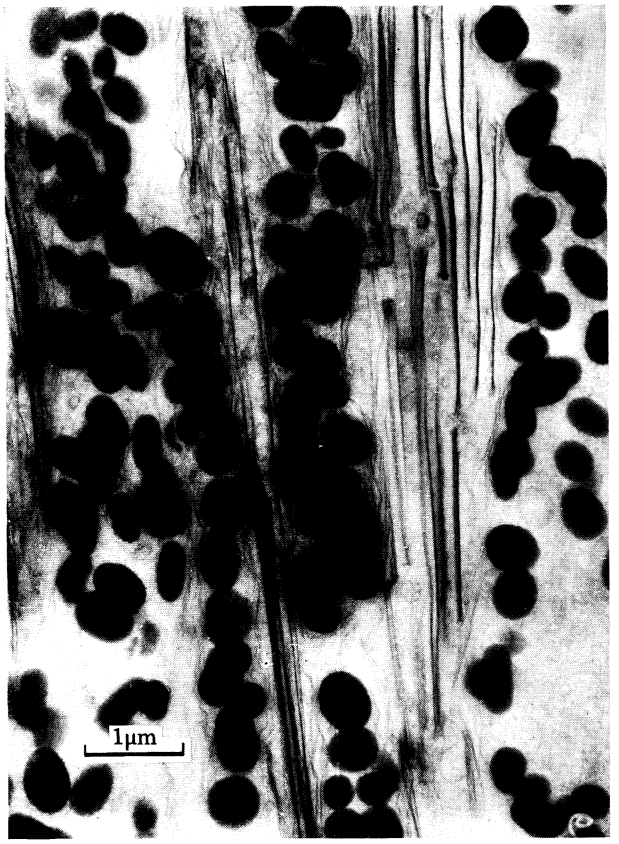

FIGURE 6

FIGURES 4 to 6. For legends see facing page

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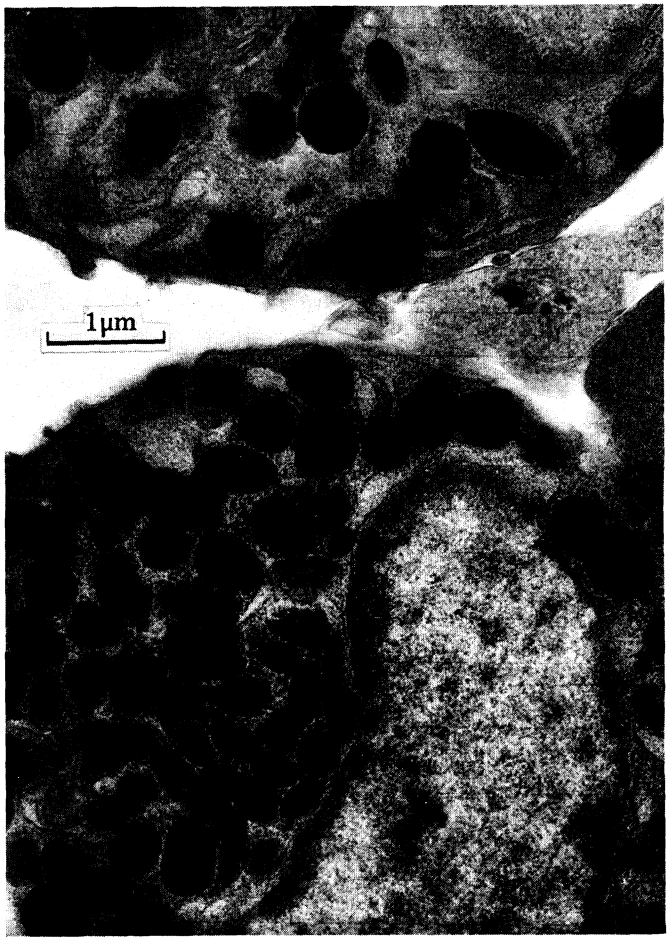

FIGURE 7. 0.5 μm section of bone marrow. Preparation as legend to figure 6. Taken at 700 kV in the Hitachi 1 MV microscope. (Magn. × 15000; K. R. Porter, unpublished.)

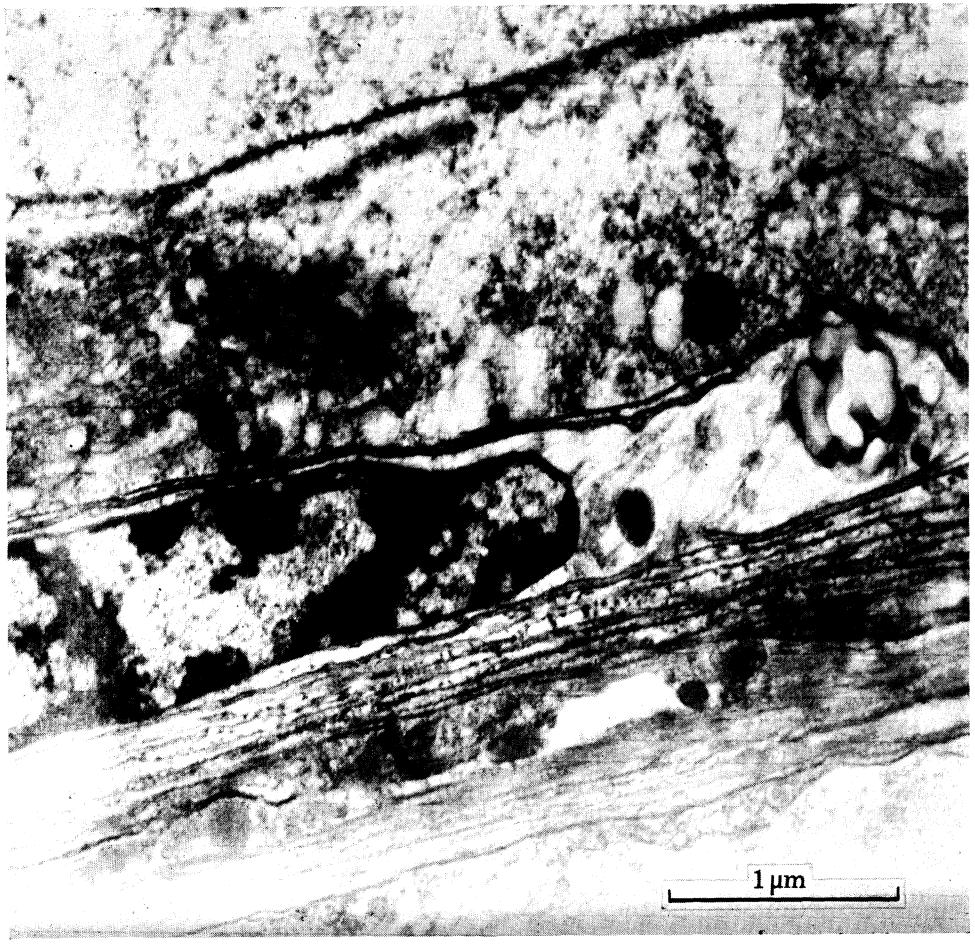

FIGURE 8. Boundary tissue of testis follicle of *Schistocerca gregaria* (after treatment with cyclophosphamide for 3 days). Section about 0.2 μm thick. Fixed in glutaraldehyde and post-fixed in OsO_4, stained with uranyl acetate, embedded in Epon. Section stained in uranyl acetate and lead citrate (30 min). Taken at 500 kV in the Cavendish 750 kV microscope. (Magn. × 30000; F. M. O. Hawkes, unpublished.)

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FIGURES 9 and 10. For legends see facing page.

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appearance of depth is especially striking in Ris's images of whole chromosomes (seen in a hand-viewer passed around the audience), and in some of the micrographs recently published by Nagata *et al.* (1969).

The high-voltage microscope thus opens up a considerable area of research for biologists. Apart from thick sections, single-layer tissue cultures as well as whole bacterial cells, or cells isolated from bulk tissues, can now be examined with a resolution of the order of 10 nm, so long as they are in the dry state. The next stage must be the development of a reliable technique for mounting wet material in a double-walled cell. The way has been pioneered by Dupouy (1968), with a cell in which the pressure of saturated water vapour can be maintained against the vacuum of the electron microscope, and progress is being made towards a type that will allow the specimen to be kept at atmospheric pressure, by using sapphire windows (Hale & Henderson-Brown 1969). It cannot be expected that high definition micrographs will be obtainable from wet specimens, but we might hope for a resolution better than 100 nm, which would be adequate for many studies of desiccation, radiation damage and perhaps some biochemical reactions. As already stressed, it is most unlikely that living processes can be observed by electron microscopy even in controlled environment cells.

A final comment is perhaps necessary regarding preparative methods. It would be wrong to assume that the techniques developed for cutting ultra-thin sections are those best suited for sections 1 μm or more in thickness. There is scope here for experimenting with new (or perhaps very old) embedding media, variations in the details of sectioning, and probably also in staining methods. It is already clear that very heavy staining is required if details near the practical limit of resolution are to show enough contrast in electron microscopy at 1 MV.

I am grateful to the Paul Instrument Fund of the Royal Society for the grant which made possible the design and construction of the 750 kV microscope at the Cavendish Laboratory, experience with which has provided the basis for this review of the subject. For the ready provision of illustrations I am indebted to Professor H. Ris of the University of Wisconsin, Professor K. R. Porter of Harvard University, and to Dr R. H. Warren (N.I.H. Fellow) and Dr Françoise Hawkes (Wellcome Trust Fellow) of the Cavendish Laboratory.

Description of plate 5

Figure 9. Section 0.25 μm thick of 2-week-old culture of cells from embryonic chick breast muscle. Myogenesis is advanced in the culture, and myotubes with thick and thin myofilaments are present within the cells. Collagen fibres appear in the extracellular spaces. Preparation as legend to figure 8. Taken at 500 kV on the Cavendish 750 kV microscope. (Magn. $\times$ 40 000; R. H. Warren, unpublished; culture prepared by H. Holtzer, M.R.C. Laboratory of Molecular Biology, Cambridge.)

Figure 10. Section 0.25 μm thick of 2-week-old culture of cells from embryonic chick breast muscle, treated with 5-bromodeoxyuridine (BUdR). Myogenesis, but not mitosis, is blocked in the culture. Thick and thin myofilaments are not present intracellularly, and no obvious collagen fibres are present extracellularly. Preparation as legend to figure 8. Taken at 500 kV on the Cavendish 750 kV microscope. (Magn. $\times$ 40 000; R. H. Warren, unpublished; culture prepared by Howard Holtzer, M.R.C. Laboratory of Molecular Biology, Cambridge).

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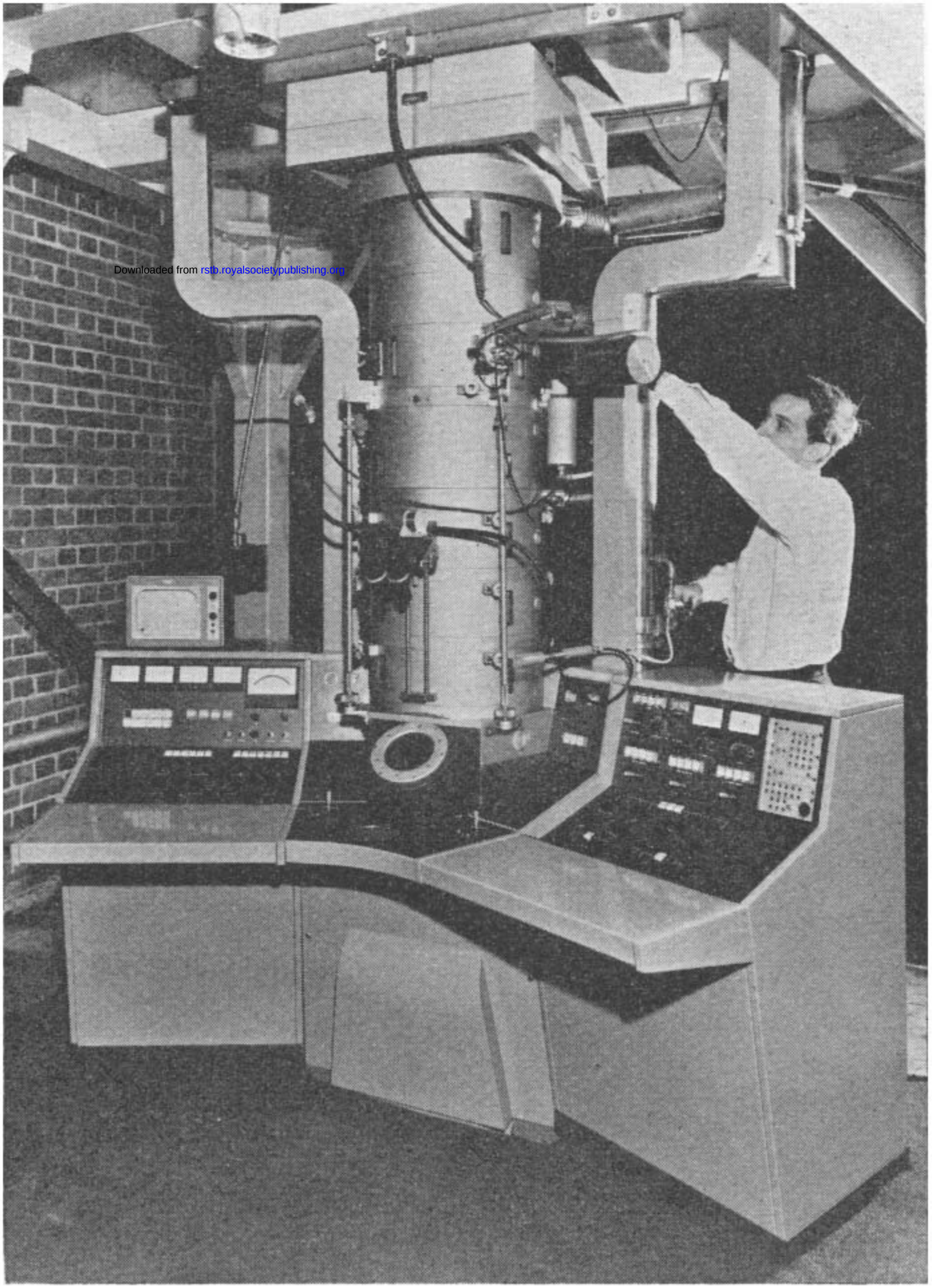

FIGURE 3. Column and control desk of the AEI 1 MV electron microscope.

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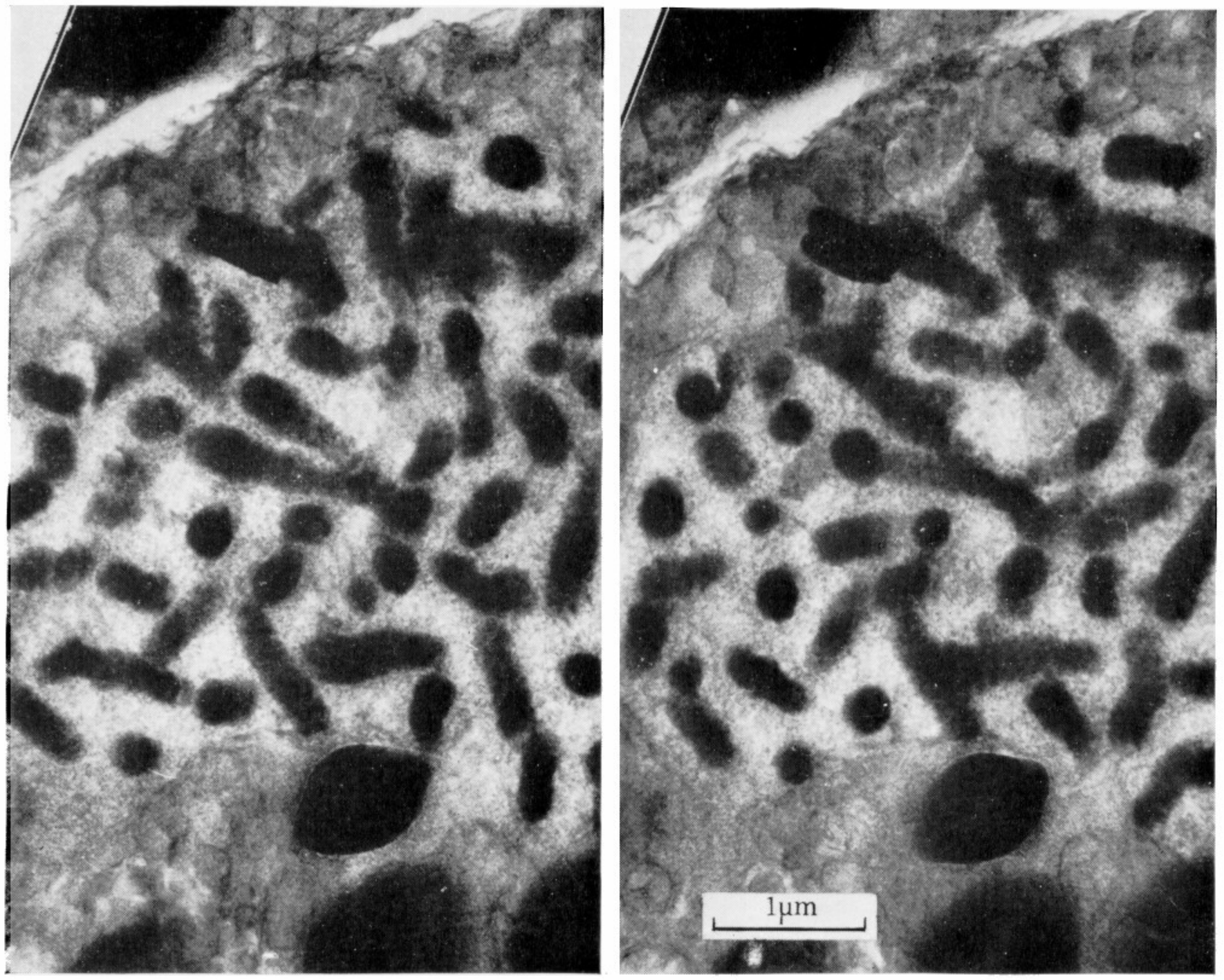

FIGURE 4

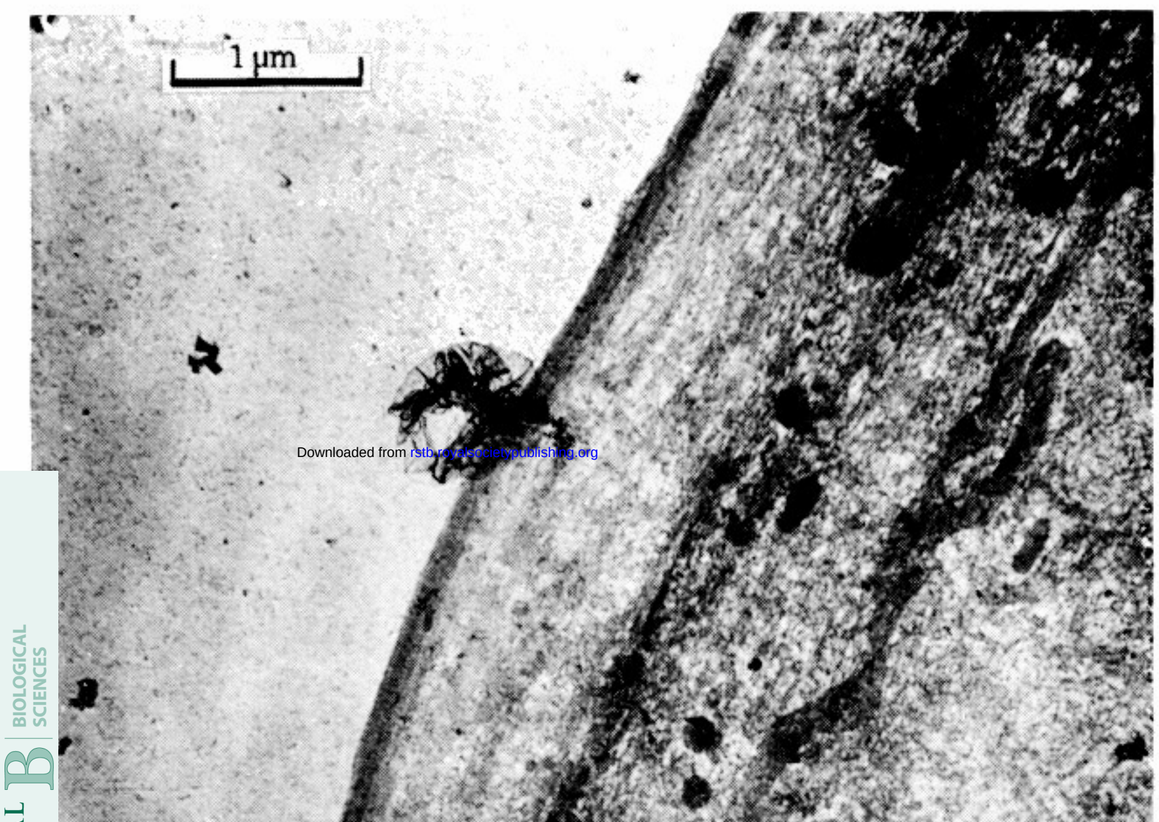

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FIGURE 5

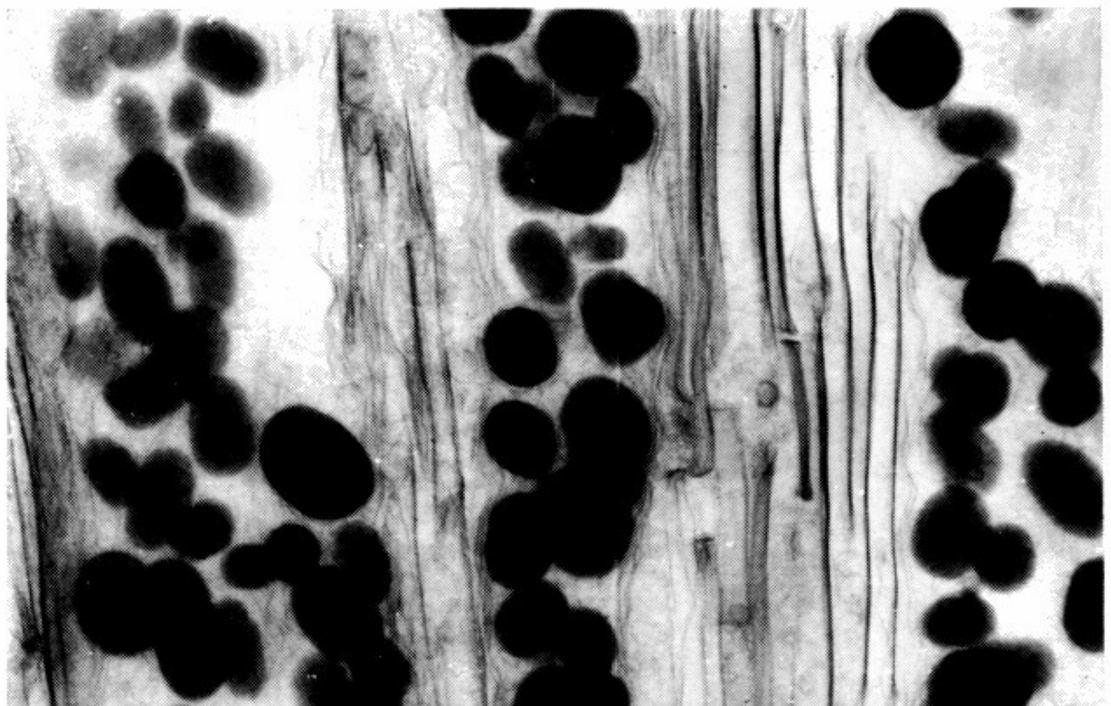

FIGURE 6

FIGURES 4 to 6. For legends see facing page

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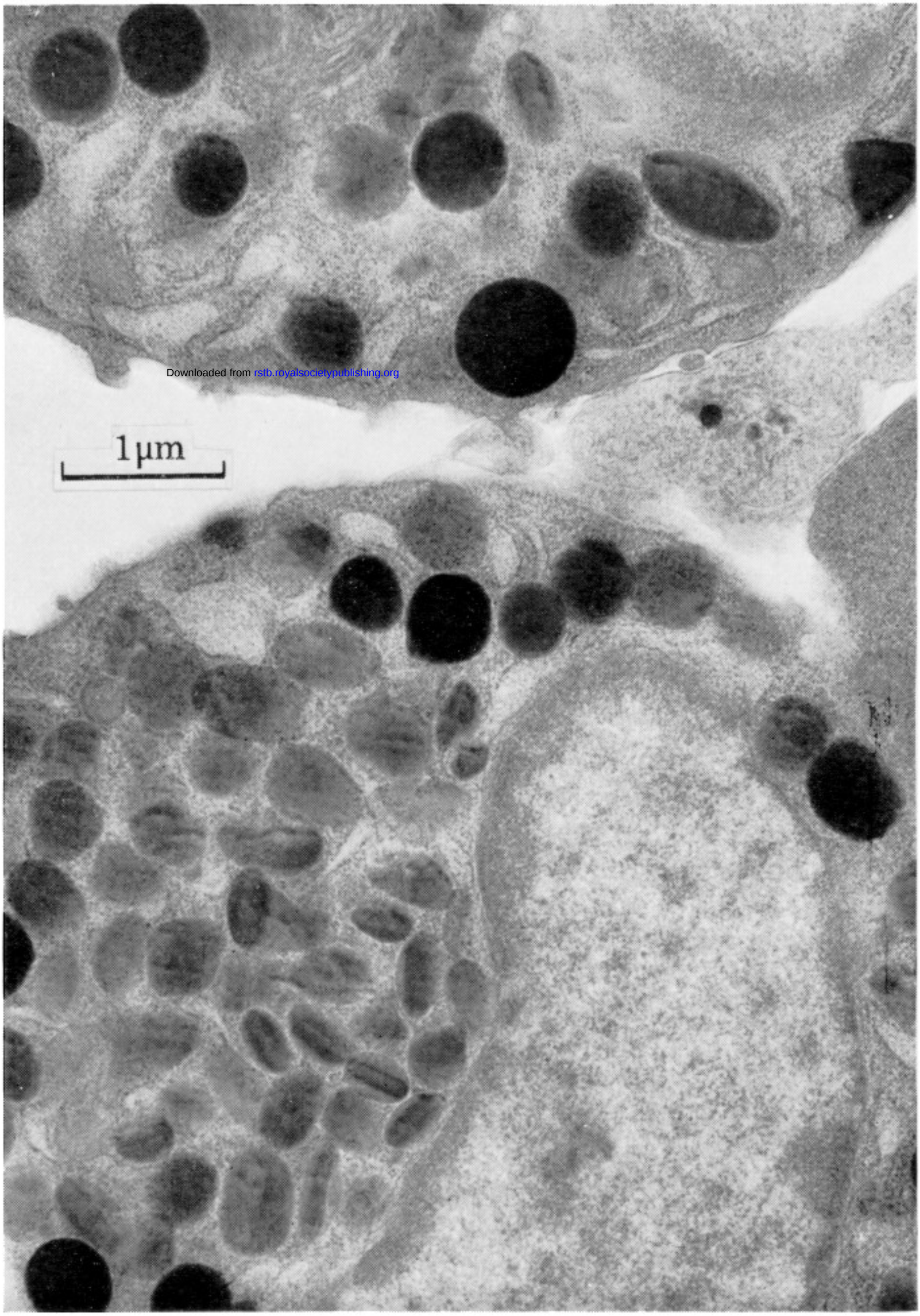

FIGURE 7. 0.5 μm section of bone marrow. Preparation as legend to figure 6. Taken at 700 kV in the Hitachi 1 MV microscope. (Magn. $\times$ 15 000; K. R. Porter, unpublished.)

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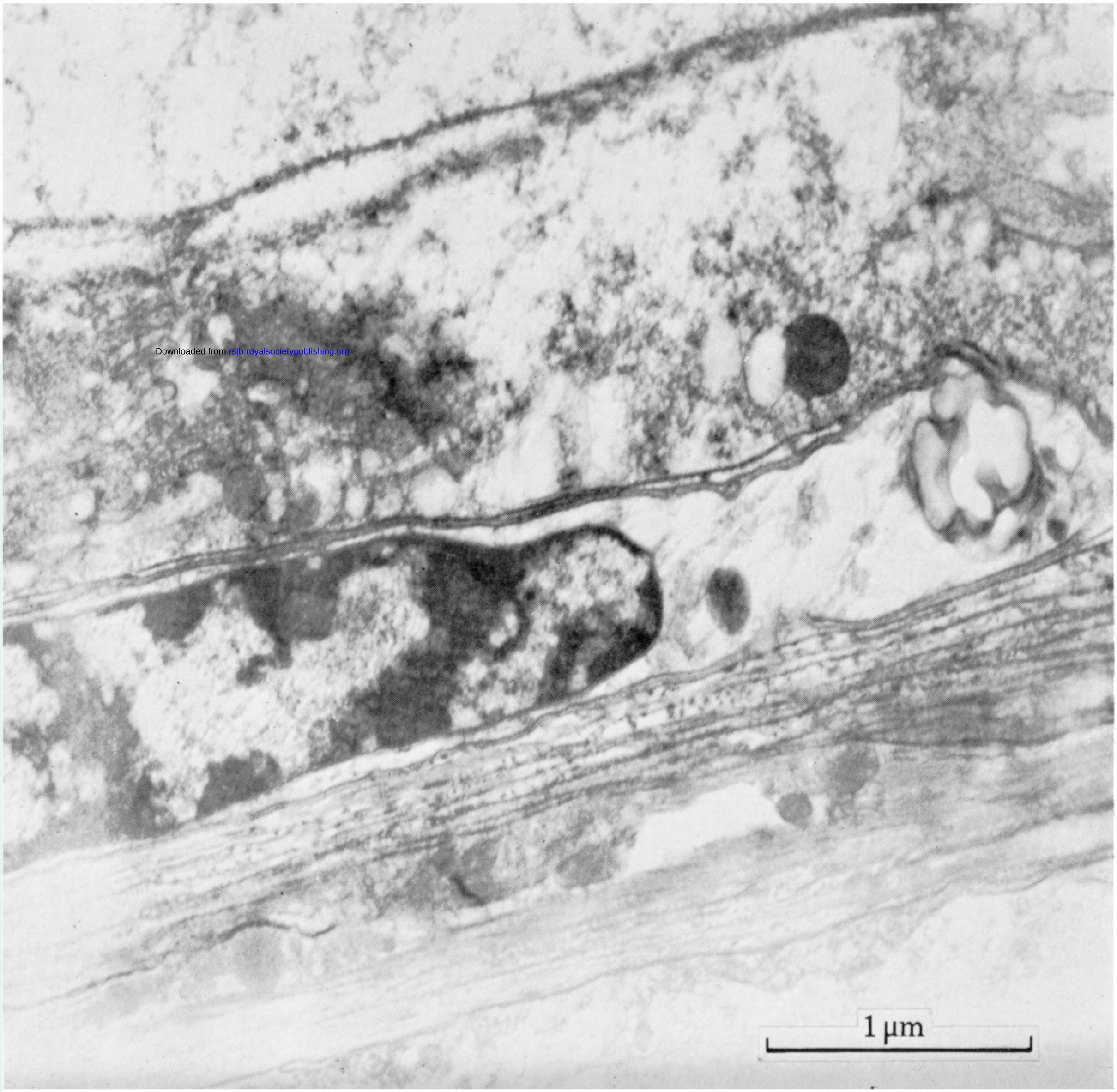

FIGURE 8. Boundary tissue of testis follicle of *Schistocerca gregaria* (after treatment with cyclophosphamide for 3 days). Section about 0.2 μm thick. Fixed in glutaraldehyde and post-fixed in OsO_4, stained with uranyl acetate, embedded in Epon. Section stained in uranyl acetate and lead citrate (30 min). Taken at 500 kV in the Cavendish 750 kV microscope. (Magn. $\times$ 30 000; F. M. O. Hawkes, unpublished.)

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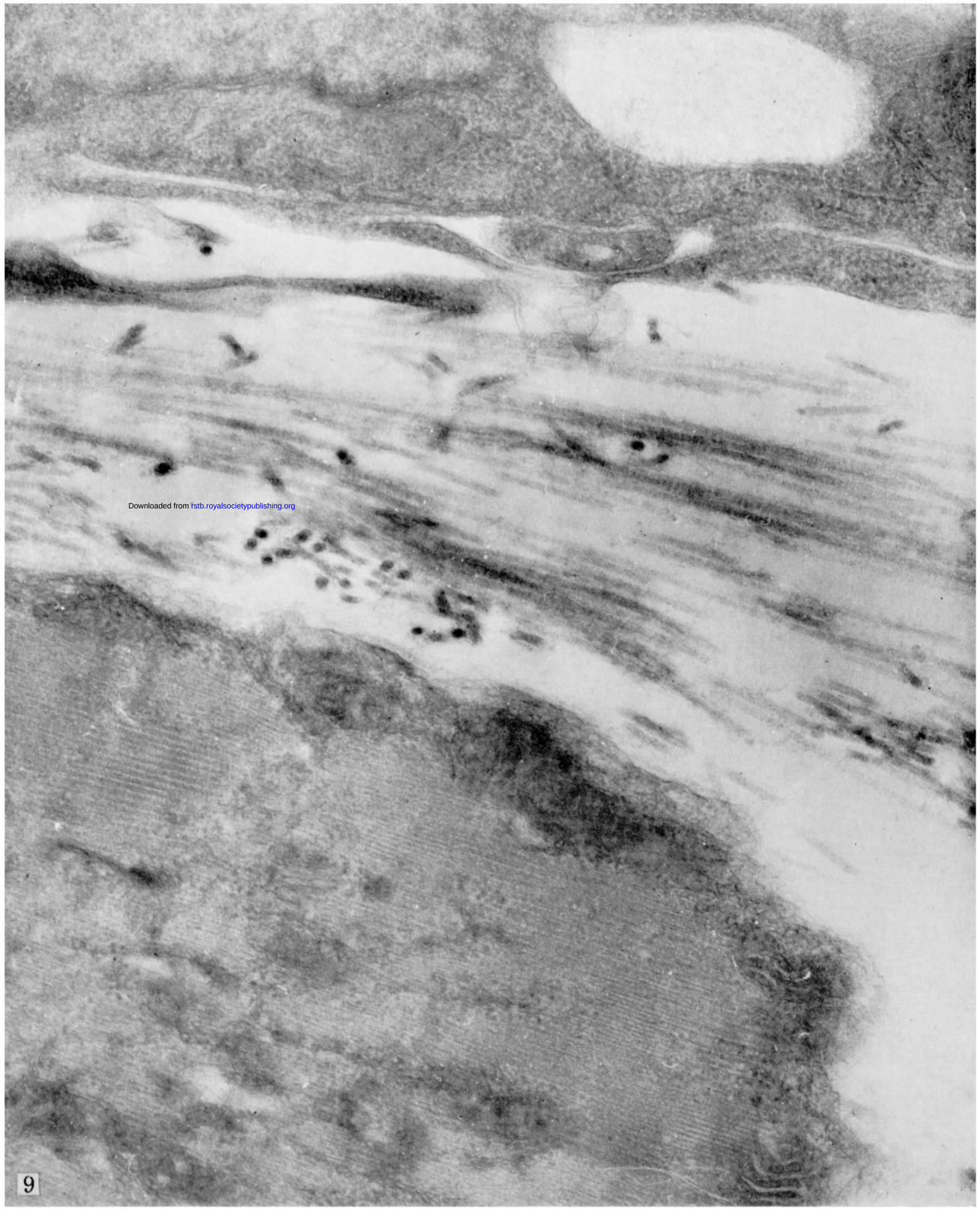

FIGURES 9 and 10. For legends see facing page.

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