The expenses of this investigation were defrayed through a grant in aid received from the Schering Corporation, of Bloomfield, N.J. The author is especially indebted to Drs. G. Stragnell and E. Schwenk of the above Corporation for the synthetic hormones used in these experiments, and to Miss L. Bassett and Messrs. K. Nielsen, H. Torunski and C. Rasmussen of this Department for technical assistance.

REFERENCES

1. KORENCHEVSKY, V., HALL, K. AND BURBANK, R.: The manifold effects of prolonged administration of sex hormones to female rats, *Biochem. J.*, 1939, 33: 372.
2. KORENCHEVSKY, V., HALL, K. AND ROSS, M. A.: Prolonged administration of sex hormones to castrated rats, *Biochem. J.*, 1939, 33: 213.
3. SELYE, H.: Studies on adaptation, *Endocrinol.*, 1937, 21: 169.
4. *Idem*: The effect of adaptation to various damaging agents on the female sex organs in the rat, *Endocrinol.*, 1939, 25: 615.
5. SELYE, H. AND STEVENSON, J.: The toxic effect of œstrogens as influenced by progesterone, Canad. Physiol. Soc. Meeting, Kingston, 1939, this issue of the *Canad. M. Ass. J.*, p. 190.
6. SELYE, H.: On the protective action of testosterone against the kidney-damaging effect of sublimate, Montreal Physiol. Soc., December, 1939. (See also this issue of the *Canad. M. Ass. J.*, p. 189.)
7. MCEUEN, C. S., SELYE, H. AND COLLIP, J. B.: Effect of testosterone on somatic growth, *Proc. Soc. Exper. Biol. & Med.*, 1937, 36: 390.
8. SELYE, H.: Thymus and adrenals in the response of the organism to injuries and intoxications, *Brit. J. Exper. Pathol.*, 1936, 17: 234.

THE CONTRIBUTION OF THE ELECTRON MICROSCOPE TO MEDICINE

(I. THE ELECTRON MICROSCOPE DESCRIBED)

BY E. F. BURTON, J. HILLIER AND A. PREBUS

Department of Physics, University of Toronto, Toronto

THE microscope is an instrument peculiarly serviceable to the biologist and the medical scientist. Its early developments, in England at any rate, were in the hands of the natural scientists, and even today, if one wants the best technique in the use of a microscope, one is inclined to turn to the biologist rather than to the physicist. The biologist is a specialist in using the instrument and is not concerned overmuch with the theory; the physicist very often can reel off pages of mathematical theory regarding the working of the instrument but would often find great difficulty in setting one up for actual use. With the electron microscope both the technique and the theory are of a new type and the trained physicist is indispensable.

1. LIMITATIONS OF THE ORDINARY MICROSCOPE

When one examines the geometrical drawing showing the method of formation of the image in the ordinary compound microscope (Fig. 1b), one can see no apparent reason why the magnification produced should not be made greater and greater merely by enlarging the dimensions of the instrument. This appears possible because we represent the light by *rays, i.e.,* we treat the light as though it were propagated exactly in straight lines. However, such is not the case. Light has the characteristics of a wave motion and can consequently bend around obstacles, a phenomenon which becomes very important when the obstacle becomes extremely small.

Ordinary light is just an electromagnetic wave similar in many particulars to the waves employed in wireless telegraphy. The difference is in the wave-length. Whereas wireless waves have wave-lengths of the order of 100 metres and we receive the signals in our radios, light has a wave-length of from four to ten-millionths of a metre, and we receive such signals on the retina of our eyes, or on a photographic plate. However even such short wave-lengths are considerably larger than the linear dimensions of many bodies in which the microscopist is interested. It is this fact that sets a minimum to the size of particles made visible to us by the microscope.

The essence of microscopic vision is to reproduce in an image on the retina, or on a photographic plate, a replica of an object with the essential fine structures in the object separated in the image; that is, we wish to see greater and greater detail in the object. Satisfactory performance of the instrument is judged by its ability to depict for us very fine points or fine particles present in the object. On account of the fact that light is a wave motion it is impossible for any instrument to separate in an image two fine particles of an object if those particles are really closer together than the wave-length of the light used. This means that no microscope can be constructed to be used with ordinary light that will enable us to see *in detail* a particle less than about two one-hundred-thousandths, $\frac{2}{100,000}$ths of a cm. (0.2 μ or 0.2 microns), in diameter, or a fibre or thread less than this amount in cross-sectional diameter.

This lower limit can be extended to smaller

magnitudes if ultra-violet light, the wave-length of which may be less than one-half the average wave-length of visible light, is used. The microscope devised by Barnard and Gye (about 1925) made use of ultra-violet light, and they were able to improve microscopic images to a certain extent. However, the magnification they attained was only double the old.

2. X-RAYS AND ELECTRONS

Scientists have always fostered the hope that a form of radiation would some day be discovered which would have a much shorter wave-length and consequently might lend itself to photographing in detail still smaller objects. In the last decade of the nineteenth century both electrons and x-rays were discovered and the question arose as to whether these might not lead to a solution of the problem.

On account of their effect on a photographic plate and their power to penetrate matter, x-rays were from the first thought to be a form of light, but all attempts to make x-rays amenable to refraction by any kind of lens failed. They have proved to be of the greatest use in depicting the intimate structure of crystals but this is due to phenomena not directly related to microscopic vision.

One reads so much about electron tubes and the miracles that can be performed with them through engineering developments, that electrons seem to be very familiar to us. They can be produced in so many ways that we have come to look upon them as one of the primary constituents of all matter. From the time of their first discovery over forty years ago they have been looked upon as very minute, negatively-charged particles moving in clouds or streams, often with a great velocity, which may even approach the velocity of light.

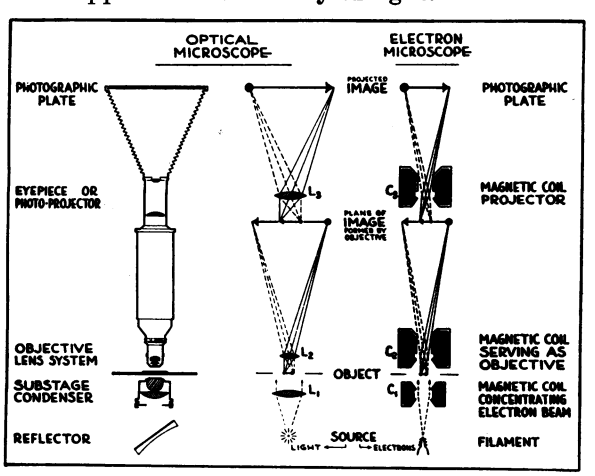

Fig. 1a Fig. 1b Fig. 1c

3. RELATIONSHIP BETWEEN LIGHT AND ELECTRONS

During the present century our views regarding the true nature of light have changed greatly. In some experiments, as for example with the microscope, light acts as though it were merely a wave-motion. In other experiments, *e.g.*, the photoelectric cell, we must regard light as corpuscular in nature—a beam of light, according to this view, being a stream of discrete bundles of energy to which the name photons has been given.

When this duality of light became evident to the physicist, he began to enquire whether

TABLE I.

CONTRAST BETWEEN LIGHT AND ELECTRON MICROSCOPES. (SEE FIG. 1)

Elements	*Light microscope*	*Electron microscope*
1. Source of illumination.	Sunlight or electric light.	Electron source: cold cathode or hot filament.
2. Control of illumination.	Sub-stage condenser, L_1.	Converging action of the magnetic field of the first coil, C_1.
3. Specimen support.	Microscope slide of glass about 1 mm. thick.	Film of collodion about 10 millimicrons thick.
4. First image forming system.	The objective, L_2.	Converging action of the magnetic field of the second coil, C_2, forming the first image.
5. Accessory magnification.	The eyepiece or photographic projector, L_3.	Converging action of the magnetic field of the third coil, C_3, forming a second enlarged image of a portion of the first image.
6. Medium.	Air and glass.	A very high vacuum.
7. Viewing image.	The eye directly.	The eye (image projected on fluorescent screen).
8. Method of focussing.	Movement of lenses as a result of judging the sharpness of the image with the eye.	Alteration of the intensity of the magnetic fields in the coils as a result of judging the sharpness of the image on a fluorescent screen.
9. Recording of image.	Photographic plate.	Photographic plate.
10. Smallest particle photographed in detail.	$\frac{2}{100{,}000}$ cm.	Even less than $\frac{1}{1{,}000{,}000}$ cm.

similar duality should not be ascribed to the electron beam. Our first conception of an electron beam was that it consisted of a stream of small negatively charged corpuscles, each particle having a mass about 1/2,000th of that of a hydrogen atom. In 1925 it was shown experimentally that an *electron beam displayed the same dual nature displayed by light.* Consequently, an electron beam must sometimes be looked upon as a wave-motion. Moreover its wave-length is only a very small fraction of that of ordinary light. The question arose: "Can we have an electron microscope?" and "Will it enable us to see particles or structures much finer than the ordinary microscope can?" The answer to both questions is "Yes". It is now possible to photograph particles smaller than ten millimicrons (10 mμ or one-millionth of a cm.) and likewise to see in microscopic objects details which are many times finer than those made visible by the most powerful light microscope.

4. The Use of an Electron Beam

The essential principle underlying the operation of an ordinary microscope is the bending of the rays of light emanating from an illuminated object by a system of glass lenses so that the rays produce an image on the photographic plate (or the retina of the eye). In the electron microscope this essential feature of the action of a glass lens on light is performed by the action of a particular type of magnetic field on the beam of electrons. That is, *a type of magnetic field has been discovered which has the same effect on a beam of electrons, as a glass lens has on a beam of light* (Figs. 1b and 1c).

5. Comparing Light and Electron Microscopes

The accompanying table sets out the contrasts and similarities between the light microscope and the electron microscope (see Fig. 1).

6. Precise Designation of Magnifying Power

In both the electron microscope and the light microscope the detail which is visible in the final image is determined by the objective lens. In each case the objective produces an image which contains all the detail that will be visible in the final picture. However, the image produced by the objective is seldom at a magnification of more than x100 in either instrument and hence most of the detail present still cannot be seen by the eye. The subsequent magnification by the eye-piece in the case of the light microscope, and the projector coil in the case of the electron microscope, merely serves to increase the dimensions of these details until they are visible to the eye. In order to be visible, the finest detail of the picture must be larger than 0.02 cm. A simple calculation shows that in the case of the light microscope an eye-piece magnification of x10 is necessary, thus bringing the total magnification up to x1,000; in the case of the electron microscope the second magnification must be considerably over x200 in order to produce a final

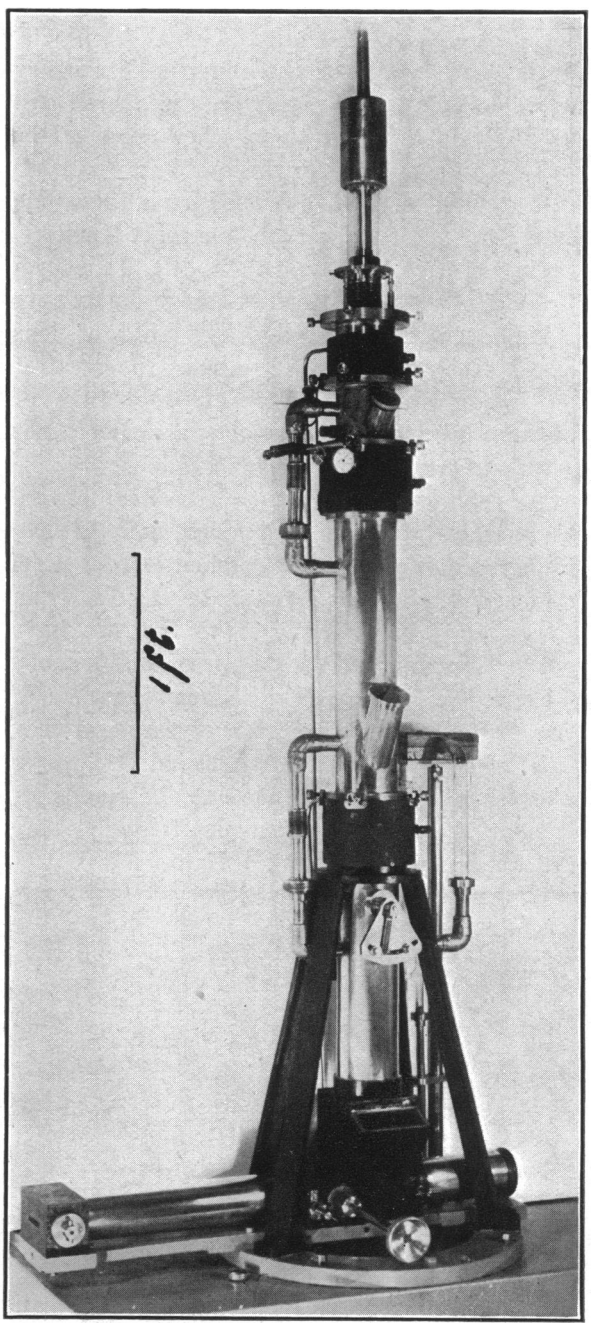

Fig. 2.—Photograph of electron microscope.

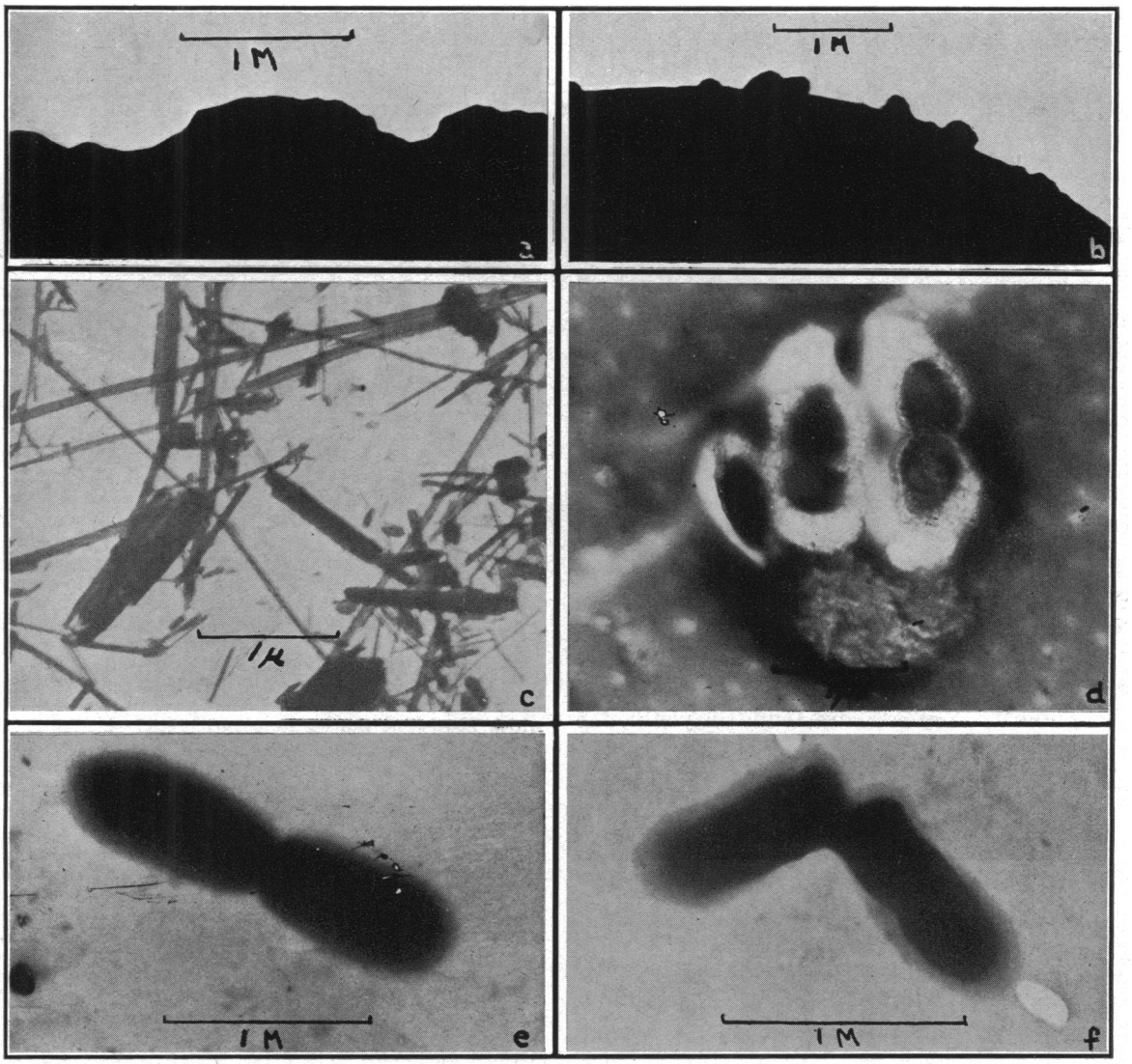

Fig. 3a.—Portion of edge of new razor blade about 1/8,000 of an inch. x24,000. **Fig. 3b.**—Edge of a pollen particle from *Cupressus sempervirens.* x16,000. **Fig. 3c.**—Small fibres of asbestos making up asbestos dust. x19,000. **Fig. 3d.**—Group of pneumococci from peritoneal fluid of a dead mouse. x18,000. **Fig. 3e.**—Method of division of a *B. prodigiosus.* x28,000. **Fig. 3f.**—Method of division of diphtheroid bacillus. x33,000. In each cut the straight line represents $1\mu = 0.0001$ cm.

magnification of x20,000 or over. We may call these final numbers x1,000 and x20,000 the respective magnifying powers of the instruments, since further magnification, in either case, would reveal no more detail to the eye. For practical reasons, a large number of photographs taken with the electron microscope are taken at magnifications near x12,000. Hence, there is considerable detail on the plates that is still invisible to the eye. In order to make this detail visible, it is necessary to enlarge the photographs optically until the total magnification is over x20,000.

Often, to overcome difficulties of reproduction or demonstration, it is necessary to enlarge the photograph to a total magnification considerably above this value. This subsequent magnification has no physical significance.

The photographs given in this and succeeding papers will all be marked by a number representing the final magnification. Each reproduction will be accompanied by a line indicating the scale; thus |————| 1μ, the symbol μ being one ten-thousandth of a centimetre.

7. Samples of Results Obtained with the Electron Microscope

In Fig. 3 (a to e) are shown some typical results obtained so far with the electron microscope. The legend attached explains these sample results.