The patient's present health, his medical history, and his family history are significant. His occupation, recreations, and amount of exercise, and the physical conditions of heat and ventilation in his home and place of work all require consideration. The climatic conditions of temperature and humidity can be countered only by protective measures. Warm clothing on the body and the extremities is important. Many soldiers on active service found two pairs of socks effectively prevented chilblains. In cases of severe chilblains of the legs in women, elastic understockings may be desirable in the winter for warmth and to prevent the stagnation of fluid in the tissues. Tight and restricting garments are unwise, and the application of warmth to cold extremities by fire or hot bottles is to be avoided.

In the home and at work central heating and the avoidance of draughts are desirable, though a current of air is preferable to stagnation. The maintenance of one posture over long periods is to be avoided, and exercise should be encouraged.

Treatment

Stimulation by electrical and radiation therapy, massage, and passive and active movements are useful. Ultra-violet light therapy to the whole body or fractional doses of x rays (100 r) to the affected areas may rapidly cure an attack, but the measures should only be employed by practitioners experienced in their use.

Where the affection is intractable and disabling the question arises of the patient's removal to a different location or climate, or of his reference to a surgeon with a view to sympathectomy.

Local stimulants are not indicated, except that the massage involved in their application may be beneficial. The risk of provoking dermatitis or blistering is considerable, since most of these preparations are irritants. Iodine (as ung. iodi denigrescens), menthol, and camphor are favourite measures.

Calcium by mouth or by injection, with or without vitamin D, has long been regarded as curative and is often demanded by the patient. There is no scientific evidence to support the claims for its efficacy, but this does not necessarily mean that it is of no value. Often, no doubt, the effect is psychological, the result of relentless advertisement upon profession and patient, but it would be rash to deny the possibility of some biological effect. Massive doses of calciferol, vitamin D₂, are said to produce remarkable results in many cases. Although Anning (1947) has shown that a high content of calciferol in the blood—as in patients with lupus vulgaris under treatment with the drug—does not protect against the development of chilblains, it may be that the metabolic shock occasioned by this therapy is effective in temporarily clearing the condition. It is not a measure that should be used for more than a short period, because of the dangers involved.

Equally dramatic results may follow the administration of thyroid extract in small doses ($\frac{1}{4}$ — $\frac{1}{2}$ gr.—16–32 mg.). This effect is seen more often in thin patients than in the heavy, exudative type of person, as would be expected from the slight stimulus to the circulation and the relaxation of the contracted arterioles caused by such therapy. Patients receiving thyroid do not feel the cold as readily as others. Nicotinic acid, 50 mg. t.d.s., acts as a peripheral dilator and may effect recovery in a proportion of patients. It is interesting to note that a purgative will often cut short an attack, probably by disturbing the fluid balance in the tissues.

It would not be possible to enumerate all the measures which have been advocated for the relief of chilblains. While each has a place in treatment if used with care, they should not be prescribed without regard to the physical factors concerned. It is important that these factors should be explained to the patient, who is best able to control them.

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AN APPROACH TO ELECTRON MICROSCOPY

BY

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The optical microscope is a supremely efficient instrument, but in the fineness of detail which can be distinguished it has already reached the limit of possible development. At the same time there has been progress recently in other directions. The phase-contrast microscope enables small transparent objects to be viewed intact and without staining. The reflecting microscope offers the special advantages that a longer working distance can be made available, and that focusing is independent of wavelength. But, with some qualification in the case of the reflecting microscope, they leave untouched the main problem of the fineness of detail, or resolution, attainable. The electron microscope, on the other hand, is a relatively crude instrument, judged by optical standards, which has completed only the first stage of its probable development. It is in the position of the optical microscope before the correction of lens faults became possible. Even so, it offers a gain in resolution of a thousand-fold. For an understanding of electron microscopy it is necessary to consider (1) requirements in microscopy; (2) theoretical limitations as they apply to particular forms of microscopy; and (3) the electron microscope in comparison with the optical microscope.

Requirements in Microscopy

For any section of material to be examined microscopically there must be formed a corresponding image. This image must be recognized by the human eye or recorded on a photographic plate, and each point in the object should be uniquely represented by a corresponding point in the image. The distances between image-points should be greater than those between object-points, and in their mutual separation and directions the pattern of image-points should provide a true rendering, apart from scale magnification, of the pattern of object-points. Since the object is three-dimensional and the image two-dimensional, it is further necessary that the thickness of the object should be limited. Finally, it is desirable that differences in the structure of the object, whether visible or not in the ordinary sense, should be represented by corresponding differences in the apparent structure of the image.

For an image to be formed, "something," whether waves or particles, must travel from the object to the image. This "something" can originate either in the object itself or in some outside "source of illumination." An example of the first type is the photomicrography of luminous material in its own light. But for general use the "something" must come from outside. Theoretically, whether neighbouring points in the object can, or cannot, be regarded as independently self-luminous makes a difference. But the difference introduced is numerically small, and for our purpose it is enough to state that the object must be induced to behave as if it were its own source of whatever radiation is to be used for its examination.

In the optical microscope these external requirements are met by the lamp and the condenser lens. These are independent of the main optical system in the sense that, if the object

were luminous in itself, they would be unnecessary. Internally, there must be variations within the object in the extent to which individual points can act as their own sources by scattering or otherwise modifying the "radiation." Otherwise, structure could not be shown. Any kind of irradiation to which the response of the object may show local variations offers a possible means for the formation of a useful image. On the other hand, the extent and character of the structural differences reproduced may depend on the means by which they are shown. This last should perhaps be emphasized, for there is a natural tendency to assume that a picture, however obtained, is complete within the limits of detail which can be distinguished; and, in particular, to give a mental priority to those formed directly by light which we can see. As an index of structure, any accurately formed picture has as much validity as any other, and the only important criteria are the fineness of detail and the extent of the local variations reproduced.

Further requirements follow from the original specification that a set of object-points is to be translated into a set of image-points. The first is that the "something" should travel in a predictable way—in practice, effectively in a straight line unless otherwise disturbed. The second arises from the fact that "radiation" diverging from a point is to be made to converge on another point. This means that the "something" must be capable of deflection in a way which can be controlled. Finally, there is the negative requirement that the object shall not itself be significantly affected; in other words, that the picture obtained shall show the "normal" state of the object, and not some modified state produced by irradiation. As a point of theory, there must always be some effect on the object; what matters practically is that this effect should not be excessive. There is fortunately an obvious test: that the structure shown should not be affected by minor variations in the duration of exposure to the "radiation."

Nothing in these general considerations points particularly to visible light as the means for examination, except that it is the "something" to which our own eyes are sensitive, and so also is the photographic plate. An advantage, not decisive, is that the picture can be directly viewed both by eyes and by the camera without translation or adjustment. As against this, we have become accustomed under different conditions to pictures formed in the first instance by ultra-violet (microscopy), infrared (wartime night observation), x rays (radiography), gamma radiation (industrial radiography), and electrons (television and radar display), although not all these would be suitable for microscopy.

The possibilities, therefore, still in general terms, are (1) any form of electromagnetic radiation, and (2) particles of small mass. Limitations in the case of the first will appear in the following section. Limitations in the case of the second can be immediately stated. The particles must be light enough to secure reasonable penetration and yet avoid disruption of the object; and they must be electrically charged in order that they may be controllably deflected.

Theoretical Limitations on Resolution

The limit of resolution in optical microscopy arises from the fact that light is a form of wave motion. It is a general rule, applicable to any kind of waves, that the smallest separation at which two wave sources can be distinguished is about one-half the wavelength. Crudely expressed, it may be said that if the waves get mixed up at the source, then nothing which can be done afterwards can disentangle them. The average wavelength of visible light is about 0.5 microns. This is usually expressed as 0.5μ , and a micron, or micromillimetre, is $1/1,000$ mm. The shortest wavelength to which the eye is sensitive is about 0.4 microns. The limit of resolution is accordingly about 0.25μ for white light, and about 0.2μ for violet. These figures determine the extent of magnification which can usefully be employed. Since the eye can just distinguish a separation of 0.1 mm. (or 100μ) at the limit of distinct vision, it follows that, even with the most perfect optical microscope, magnification $\times 500$ will suffice to show up all the detail which light waves, regardless of instruments, can inherently resolve. Any higher magnification is a matter of convenience;

and, although it may be worth while to go to $\times 1,000$ or even $\times 2,000$, no further detail will be obtained.

It is true that these limits can be extended by a factor of two or a little more by reducing the wavelength; either, with visible radiation, by immersing the object in oil (which has this effect) or by using ultra-violet radiation, of which the waves are inherently shorter. Finally, there may be some further improvement by working with a reflecting microscope and using shorter wavelengths of ultra-violet light than will penetrate a lens system even of quartz. But there is the limitation that, as the wavelength is reduced, the loss of energy on reflection is progressively increased. There may be an advantage, for comparison, in this limited extension. But for any major gain in resolution it would be necessary to proceed to x-ray wavelengths; and here there is no method of deflection and focusing comparable to optical methods.

There remains the second possibility of using particles of small mass. With the specification, already given, that the particles used must be both light and electrically charged, this can be taken for the present to mean the electron. There are, in fact, proposals for the use of at least one other kind of particle. It is the electron which offers the finest limit in theoretical resolution, as well as that almost startling gain of a thousand-fold in practical resolution mentioned at the beginning of this article.

Until 1926 it would have been said that there was no theoretical limit which need be considered. In that year it was shown by Davisson and Germer in the United States, and confirmed by Professor, now Sir George, Thomson in Great Britain, that in sufficiently small-scale effects the electron shows wave-like properties. The details can be omitted, except to state that the evidence is as direct and straightforward as in the case of light and x rays. It leads to the conclusion that, for the purpose of resolution, electrons act as waves of which the wavelength depends on the speed of the electrons, the fastest electrons having the shortest waves.

Numerically, electrons accelerated by passing across an electric potential of only 15,000 volts have a wavelength of one hundred-millionth of a millimetre—and voltages of 100,000, corresponding to still shorter wavelengths, are in current use. Even at 15,000 volts the electron wavelength is 50,000 times smaller than the average wavelength of visible radiation. It follows that the resolution of the electron microscope, compared with the optical, could be increased theoretically in the same proportion. This wave-like behaviour is a fundamental property of electrons, as we now know, and is experimentally established. But since this is somewhat difficult as a conception, it may be a help to recall the more general analysis already given. The possibility of electron microscopy depends on the simpler properties of straight-line travel, variable scattering by matter, and controllable deflection. It is the limit of resolution, and not the possibility, which is determined by this wave-like behaviour.

It is convenient at this point to define a different unit of length, the Ångström Unit (abbreviated as Å), which is $1/10,000 \mu$. In terms of this unit the theoretical limit of resolution for an electron microscope, operated at 15,000 volts, is about 0.05Å . For comparison, a representative protein molecule has been determined by x-ray methods as a cylinder of 57Å diameter and 34Å height, with internal spacings of about 5Å and 10Å , respectively.

If the electron microscope could be made as efficient as the optical microscope, then even at the relatively low voltage quoted there would be an ample margin for the direct viewing of molecular structure. In practice, voltages of up to 100,000 are used in standard instruments, but the resolution attained—conservatively 20Å —falls short of the theoretical 0.05Å already noted by a factor of 400. It is in this sense that the electron microscope is a relatively crude instrument by optical standards.

It is not that it is inaccurate, or should be mistrusted on the ground of inaccuracy; but rather that it is at the beginning of its powers, and that the lenses which it uses are more analogous to the thin and uncorrected lenses of early optics than to the thick, complex, and corrected lenses of modern microscopy.

The gain is due to the use of electrons, with their short equivalent wavelength, instead of light. It has taken us already to the beginning of that region at which molecular resolution will become possible. There are various proposals, still tentative or at the stage of exploration, by which that region may be entered. To appreciate the importance of this fact it is necessary to remember that it is the largest molecules, and particularly proteins, which are of most interest to medicine; and that x-ray analysis is a method of detail in which an assumed structure is compared with the actual structure. It is therefore at its best with small molecules, and progressively more difficult and less successful with those that are larger.

Electron Microscopes Compared with Optical Microscopes

There are both formal resemblances and practical differences between these two types of microscope. It is convenient to take the resemblances first, since they illustrate the character of the operations and the relationship between components.

The need for controllable deflection as a means of focusing has already been emphasized. This can be done either by lenses or by mirrors in the case of light. For the deflection of electrons it is necessary to use either an electrical field or a magnetic field. Either or both of these methods are in current use in television, where the picture is painted by a moving beam of electrons which by variations in its intensity conveys light and shade but is required also to "scan" the screen in a regularly repeated manner which is controlled. Both methods have also been used in electron microscopy, and in fact the first "electric lens" (Brüche and Johannson) and the first "two-

lens" electron microscope of magnetic type (Knoll and Ruska) were built in the same year, 1931.

Knoll and Ruska showed that the required form of magnetic field is produced by a compact coil of current-carrying wire around the axis of the instrument. The effect is analogous but not identical with that of a convergent optical lens. It is the same to the extent that electrons diverging from a point on the axis on one side of the lens are brought into a focus at a second point on the axis on the other side of the lens. It is different to the extent that the individual electron follows a spiral course, whereas the deflection of a light ray by a lens is within a single plane. It follows that the theory of a magnetic lens is more complicated than that of an optical lens; so the analogy, while useful, is not complete.

The simplest optical microscope would consist of three uncorrected lenses: a substage condenser (L_1) to illuminate the object; an objective lens (L_2) to form a magnified image of the object; and an eye-piece or photo-projector (L_3) through which this image is viewed or projected on to a photographic plate. As will be seen from the diagram (Fig. 1), there are the same elements in an electron microscope. In place of a lamp, a filament is shown from which electrons are projected. In place of three optical lenses, there are three or more magnetic lenses. But in their main outline the systems are identical.

The first difference is one of physical arrangement. Because of its greater size the electron microscope is commonly built upside down, compared with the optical microscope: that is, with the image end at the bottom instead of the top. This is the case with the instrument shown in Fig. 2. A second difference is that between the filament and the condenser the electrons

Fig. 1.—Comparison of light microscope and magnetic electron microscope.

are accelerated to a high speed or energy in what is generally described as an electron gun. This is done electrically and is part of an electron microscope for which high voltages are needed. Diagrammatically, the filament and gun are together equivalent to the lamp of the optical microscope.

The third and most important difference is that the electron microscope is a high-vacuum instrument. It is necessary for its working that the collisions of the electrons should be confined to the specimen to be examined, and that there should be no further collisions with the molecules of the air. Otherwise the electron beam would be scattered and dissipated, and no useful picture would be obtained. This is important in relation to the form in which the specimens are examined, and results also in a certain remoteness in the handling of the instrument. Either the specimen must be allowed to become dehydrated *in vacuo*, as when it is directly examined, or else the examination must be of a replica which will show its surface.

This is a convenient point to mention the question of specimen-size, which might be called a fourth difference. Whereas a section of 1–10 μ is "thin" for the purpose of

vided that the plate can record the available detail, that is all that matters in the first instance. For example, with electron magnification up to $\times 60,000$, photographic enlargement may take this to $\times 150,000$. A final magnification of even $\times 250,000$ may be justified for convenience.

On this scale the smallest separation which the naked eye can distinguish would be expanded to 25 m., or more than 80 ft. Such extreme magnifications are exceptional and are used only for such special purposes as the study of the cell wall of a bacterium. Other studies at high magnification have shown the fine structure of muscle fibrils and the lysis of bacteria by phage and the subsequent multiplication of the phage, as on the outpoured debris of the bacterium.

It is generally agreed that for the best work knowledge of the particular instrument is needed as well as experience. But it would be a mistake even for research use to place the whole emphasis on the largest magnifications. There is a field for the use of the electron microscope over the whole range from the limit of optical microscopy upwards; and in research, as for credibility, there is a value in the continuity linking this new technique and older methods. A number of more or less familiar objects at comparatively modest magnifications are shown on the special plate accompanying Dr. A. S. McFarlane's article in this issue of the *Journal*.

Finally, there are some points of convenience and method which it may be useful to mention. Provision is necessary in the electron microscope both for the fine adjustment of the position of specimens without breaking the vacuum and for the rapid interchange of specimens. Then there is the increase of contrast—equivalent to staining in optical microscopy—by impregnation of the specimens with compounds of heavy elements—for example, osmium salts and phototungstic acid. Surface structure may be shown up by the method of shadow-casting, in which the specimen is "illuminated" *in vacuo* before examination by a beam of metal atoms directed on to its surface at a known angle. (See Fig. 8b, Special Plate.) It must be emphasized, too, that, as in optical microscopy, results depend as much on the preparation of the specimen as on the microscopy.

We are indebted to Sir George Thomson and the Editor of *Endeavour* for permission to reproduce Fig. 1.

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SOCIETY OF MEDICAL OFFICERS OF HEALTH DINNER

Tributes to Sir Wilson Jameson

At the annual dinner of the Society of Medical Officers of Health, held at the Piccadilly Hotel on November 24, a number of speakers took the opportunity of paying a tribute to Sir Wilson Jameson, who relinquishes the post of Chief Medical Officer to the Ministry of Health next year. Mr. Aneurin Bevan, proposing the toast of the Society, described Sir Wilson as one of the very great public servants of Britain, a man who reinforced his profound academic knowledge by an equally profound sagacity. It was a testimony to medical officers of health, Mr. Bevan continued, that the present Chief Medical Officer and his successor had both been M.O.s.H. In the last century Britain had built up a very remarkable health service, and, while the prima donnas of medicine occupied the limelight, the more prosaic work of the M.O.H. and sanitary inspector made the largest contribution. It was universally agreed that the last four and a half years had been the most fertile in the history of public health, "but whether you approve of the offspring is a different matter. . . . What we must do now,

Metrovick Type EM3 electron microscope with d.c. cubicle.

optical microscopy, specimens 10 to 100 times thinner are required for electron microscopy. The usual procedure, therefore, is to deposit the material as a finely divided suspension on a cellulose supporting film, which in turn is mounted on metal gauze or a metal washer, the latter being outside the picture. This difficulty in preparation arises from the small thickness of material which the electrons will penetrate. A single intact cell is too thick an object.

The electron image is focused on a fluorescent screen, which is then replaced by a photographic plate. Incidentally, part of the final magnification is usually photographic. This is made possible by the fact that the grain of a photographic plate is finer than the resolving power of the human eye; and, pro-