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## THE NEW MICROSCOPES.

A DISCUSSION BY

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It is, to speak conservatively, of extreme interest to review the recent progress made by the scientist in his endeavor to penetrate the unseen world of the minute and disease-causing organisms, in particular a world of viruses—suspected, yet lying just beyond the scope of human vision and the power of the microscope to reveal; for the laboratory research worker, the doctor, the technician long have been familiar with the effects of these unseen enemies they have been called upon to treat and to cope with in man, animal, and plant, and while their knowledge of the infinitesimal has been growing steadily, they were, until very recently, unable to make the slight step “beyond” which would enable them to “see.” But today, Science is exploring—looking for the first time upon totally new worlds through the eyes of totally new types of microscopes, microscopes new in principle of construction and in principle of illumination.

### THE ELECTRON MICROSCOPE.

One of these new instruments, the Electron Microscope, has received considerable attention and is now being used extensively in both industrial and medical research. Based on the principles of geometric electron optics, this microscope utilizes electrons as a source of illumination instead of the light source of the ordinary light microscope.

Electrons, practically speaking, are the smallest, lightest particles of matter and electricity. Like light, they behave like corpuscles guided by waves. Unlike light, however, they travel in a straight line in a vacuum where, subject to the action of electric and magnetic fields, their behavior coincides with the laws and principles set down by Sir William Hamilton who, more than a century ago, demonstrated the existence of a close analogy between the path of a light ray through refracting media and that of a particle through conservative fields of force.

We know that these negatively-charged particles, the electrons, revolving about in their various orbits in the atom, serve to maintain the balance of the atom while the nucleus exerts the “positive” force which holds it together; and we also know that when this balance is upset, due to gain or loss of electrons, we think of the atom as “charged,” since it is this circumstance which causes the tiny particle to attract or

repel other electrons according to the state of its unbalance. And Science has succeeded in unbalancing the atoms to such an appreciable extent that the negative electricity may be withdrawn and harnessed for use in such instruments as the Electron Microscopes.

The fact has long been established that atoms are in a constant state of vibration in a heated body and that the greater the heat of the body, the greater the agitation of the atoms. According to the electron theory of metals, electrons circulate about a three-dimensional network, or lattice, of positive ions, some of the electrons being comparatively free, that is to say, the attractions of the ions are practically cancelled by the repulsions of the other electrons. It does not necessarily follow, however, that the same electrons consistently remain free. They may be controlled by the ions eventually, but regardless of this, there is always a fixed number of them that are free. Moreover, there is a critical value of speed above which the electrons are able to rise in metals and thus escape from their restraining positive charges, though at ordinary temperatures the proportion of them moving rapidly enough to do this is relatively small. However, as the heat applied to the metal is increased, not only is the thermal agitation of the electrons increased also, but the proportion among them possessing sufficiently high speeds to enable them to leave the metal.

Thus is heat applied to the electron source of the Electron Microscope which, in the case of most instruments of this kind, is a tungsten filament surrounded by a guard cylinder. After leaving the filament, or cathode, the electrons enter an electric field wherein are large accumulations of charge which serve to steadily speed up the motion of these freely-moving particles. Since the electrons travel in vacua, none of the kinetic energy gained in crossing the field is lost, the total kinetic energy, or energy of motion, gained in passing through this region being proportional to the voltage applied. We may deduce, therefore, that since increase of charge in an electric field means a proportional increase of kinetic energy of these electrons, the higher the voltage applied, the greater the speed of the electrons—all of which has been calculated mathematically and confirmed experimentally.

After traversing the electric field and passing through the anode, the electrons are concentrated on the specimen under examination by the first of three magnetic fields which are created by currents flowing through coils enclosed in soft iron shields, molded so as to concentrate the magnetic fields on a short section of the microscope's axis. Whereas in the ordinary light microscope glass lenses serve as the refractive media through which light rays are deflected, in the Electron Microscope it is these magnetic fields of rotational symmetry which are the refractive media and serve as the "lenses" which deflect the beams of electrons. The first of these, the condenser lens coil, corresponding to the substage condenser of the ordinary light microscope, concentrates

the beam of electrons upon the specimen. The convergence of the beam falling on the specimen is controlled by varying the current through this condenser lens. Now, having passed through the specimen, the objective coil, similar in effect to the objective lens, focuses the electrons, and an intermediate image enlarged about one hundred diameters is formed. Finally, the projection coil, corresponding to the projection lens or ocular, produces a further magnified image on a large fluorescent screen. In some of the Electron Microscopes, there is a periscope-like attachment by means of which it is possible to locate and adjust for study the most interesting portion of the specimen, or that which it is desired should be examined, before the projection lens coil forms the final magnified image upon the screen, since it is sometimes difficult to accomplish this at high magnification. Also, if it is desired that a photographic record be made, the screen can be removed and a photographic plate substituted.

The specimen itself is supported on a thin nitrocellulose membrane less than one-millionth of an inch thick, and clamped in the tip of a cartridge which is inserted between the pole pieces of the objective coil. The membrane is suspended across the opening of a fine mesh screen, and a plate, serving as the movable stage, supports the cartridge. The image is projected onto the screen according to the density and atomic weight of the specimen. In other words, whereas in the ordinary light microscope the image is seen due to refraction of the specimen or differences in absorption, in the Electron Microscope the image is seen due to scattering of the electrons, and since electrons travel in a straight line in a vacuum, it stands to reason that even a fairly thin specimen will prove sufficient to deflect such particles. Electrons which strike a thick or solid portion of the specimen will, of course, not continue on in a straight line to the screen but will be either completely absorbed by the specimen or scattered too far out of the beam, thus failing to enter the narrow aperture of the objective, so that that portion of the screen corresponding to the thick portion of the specimen will remain dark. However, those electrons which are able to escape complete absorption or too great deflection because they do not happen to come in contact with too solid a portion of the specimen and either pass along on all sides of it or penetrate the thinner portions where it is possible they may encounter only a single heavy nucleus for considerable scattering (the angle of deflection being proportional to the square root of the thickness), continue on to the screen where they impinge and cause the chemically-treated screen to fluoresce, thus providing a study in light and shadow. If the atoms of a particular substance are heavy, they will also deflect more electrons than if they were light. It may be readily seen, therefore, that the thinner the specimen and its mounting, or the greater the variations in density of the specimen, the more internal structure and detail which may be seen, since too great density

tends to absorb or interrupt the straight-line progress of too many of the electrons.

Focusing of the image is accomplished by varying the strength of the fields and thereby altering the focal length of the "lens" coils at will, so that the need of changing the specimen's position in relation to a fixed optical system as would be the case with an ordinary light microscope, is avoided. Thus, magnification in an Electron Microscope can be continuously varied.

Some specimens may be mounted directly on the fine mesh screen while others may be embedded in collodion, sealed between films of collodion, or suspended in a gelatin film, itself supported on collodion film. The supporting films beside being very thin must be homogeneous lest an artefact be created. For the most part, no staining of bacteriological specimens is done since usually they exhibit sufficiently high contrast in density to readily reveal flagella and other detail without any preparation except that of suspending the specimen in distilled water or other liquid and allowing a drop of the suspension to dry on the film surface which method is also utilized for specimens of colloidal particles, pigments, and other chemical preparations. At times, however, as Dr. L. Marton of Stanford University has mentioned in his article on the Electron Microscope (written for *The Journal of Bacteriology*, March 1941, when he was associated with the R. C. A. Research Laboratories), virus particles may show decided low contrast. One method which Dr. Marton mentioned for overcoming this is to secure a number of electron micrographs at various focuses and simply select the best one for study. Or the virus may be permitted to absorb colloidal gold which would result in an image of high contrast. Dr. Marton points out that there may be future need for a staining in density and that already osmic acid has been tried and used for this purpose.

In this microscope, voltages of between 30,000 and 60,000 are used. It has been previously stated that the higher the voltage, the greater the speed of the electrons. This might now be augmented to read, the higher the voltage, the greater the speed of electrons; hence, the shorter the wavelength. An explanation of this may be approached through a brief discussion of short-wave diffraction as considered by Dr. Karl K. Darrow of Bell Laboratories in his book, "The Renaissance of Physics." In order to obtain convenient angles of refraction with the ordinary diffraction grating, it is necessary that the wavelengths of light be smaller, but not many times smaller, than the spacing between the wires or grooves. Naturally, a limit of measurement is reached in the region of ultra-violet light since it is impossible to further lessen the spacing of these gratings. However, this limitation was overcome when von Laue conceived the idea of substituting a crystal for an artificial grating since the atoms in a crystal are a thousand

times more closely set together than are the wires or grooves of a grating and are arranged in precise regular order or "lattices," and, like gratings, are unable to diffract waves which are longer than the spacings between their atoms. Von Laue suggested that if a beam of light were directed across a crystal and made to strike a photographic plate, there would appear a spray of narrow rays each composed of a single wave train instead of the broad fan-like arrangement of the grating, and a pattern of star-like spots where the rays come in contact with the plate instead of the dark irregular blot when a grating is used. Of course, the rays are disposed according to the spacings of the atoms in the lattice and according to the character of the lattice. Von Laue confirmed this idea for waves short enough to be so diffracted and then advanced the theory that this principle might hold true for x-rays as well, which theory was almost immediately confirmed by Friedrich and Knipping. Shortly after Schroedinger began to develop De Broglie's wave theory of electrons, Elsasser conceived the idea that possibly these tiny particles might also be diffracted by crystals, and Doctors Davisson and Germer of the Bell Telephone Research Laboratories, using as part of their apparatus an electron gun, set out to test and to prove this theory. Due to their experiments and those of G. P. Thomson, it was established beyond a doubt that electron beams are diffracted just as are x-ray beams. However, it was also demonstrated in the course of these experiments that electrons of slow speeds and feeble kinetic energies are unable to penetrate the crystals. It was Thomson who utilized faster electrons and demonstrated that not only are electrons diffracted like x-rays, but like x-rays also they make an imprint upon a photographic plate at increased speeds. These three men, together with others, then measured the wavelengths which they compared with the momenta of these electrons by their diffraction. To these experiments and measurements were then applied the following Rules of Correlation: "Energy ( $E$ ) is proportional to frequency ( $\nu$ ), and momentum ( $p$ ) is inversely proportional to wavelength ( $\lambda$ ), the same constant ( $h$ ) appearing in both relations. (Frequency is interpreted as the velocity ( $V$ ) of the waves divided by their wavelength.)" These Rules can be applied mathematically to the Electron Microscope to better illustrate the principles of its operation. In making use of the first Rule, however, it is necessary to substitute "voltage" for "frequency," and in so doing, therefore, the Rules of Correlation explain the increase of energy in relation to the increase of voltage as well as the increase of speed of electrons in relation to the decrease or shortening of wavelength when we say—the higher the voltage, the greater the speed; hence, the shorter the wavelength of electrons. It is interesting to note in passing that a 150-volt electron has a wavelength of one angstrom unit, this being more than  $10^{-3}$  times smaller than the wavelength of visible or ultra-violet light.

Because the wavelengths utilized in an Electron Microscope are so much shorter than those employed in an ordinary light microscope, it is possible to obtain greatly increased resolution and magnification. As a matter of fact, resolution up to 20,000 or 25,000 diameters may be realized, and increased magnifications beyond this point up to 100,000, even 200,000 diameters, can be obtained, such magnifications, however, constituting enlargement of the image. (Definitions of "Resolution" and "Magnification" discussed under "The Ordinary Microscope.") This high magnification is greatly desirable since otherwise the eye would be unable to distinguish the fine detail of internal structure at a resolution of the order of 25,000. As a result of this increase in resolution and magnification over that of the ordinary light microscope which is between 1,600 and 2,500 diameters and in the ultra microscope between 2,500 and 5,000 diameters, many surface cells and much intricate internal structure hitherto unsuspected, or at least undetected by ordinary microscopes, have been revealed. To cite a few examples:

The streptococcal cells appear, not as individual cells, that is, separate and apart from one another, but as chain-like groups, the cells in each chain being bound together apparently by the strong rigid membrane or outer cellular wall which extends over a number of these cells and which is so plainly evident under the Electron Microscope. Subjected to sonic vibration, these cells suffer a loss of protoplasmic material from their interior, causing them to become mere "ghost" cells, which makes them more transparent to electron beams. That there exists considerable difference between the surface structure and internal composition of these cells has also been determined and demonstrated.

Using the Electron Microscope, Dr. Harry E. Morton of the Department of Bacteriology of the University of Pennsylvania Medical School and Dr. Thomas F. Anderson of R. C. A. Research Laboratories were able to demonstrate that in at least one instance where chemical reaction is induced by bacteria this reaction takes place "inside" the cells. The fact that diphtheria bacilli reduce potassium tellurite to metallic tellurium has been known for some time, but whether this reaction occurred inside the cell or on the cell surface or both had never been definitely shown until the Electron Microscope was made available. Then, securing unstained preparations of *Corynebacterium diphtheriae* grown on blood infusion agar, Drs. Morton and Anderson demonstrated that the typical polar granules appear as dense spherical masses, or possibly plates, of a very black color and that in unstained preparations of this same *Corynebacterium diphtheriae* grown on potassium tellurite chocolate agar, not only the polar granules are in evidence but also the tiny needle-like crystals inside the cell which disappear along with the black color of the cell masses when a drop of bromine water is added to 1 cc. of a suspension of the cells on potassium tellurite chocolate agar.

From this the experimenters were able to deduce that tellurium metal occurs in the form of needles and is the cause of the black color, and that this reaction occurs within the cells since the crystals have never been observed to lie totally outside the cell wall, although at times there is some distortion of the wall.

The Electron Microscope also affords such study and observation as that carried out by Dr. W. M. Stanley of the Rockefeller Institute for Medical Research and Dr. Thomas F. Anderson in their recent investigation of plant viruses. By means of electronmicrographs, they were able to judge the exact manner and extent of attack made on the tobacco mosaic virus by the protein antibodies in the blood stream of rabbits in which an artificial immunity to the virus had been produced.

Structures like that of the spirochete of Weil's disease, typhoid flagella, unusual internal structure of pertussis organisms, tubercle bacilli, the isolation and recognition of the influenza virus, the spores of trychophyton mentagrophytes, spirochaeta pallida with its accompanying flagellar appendages, and colloidal particles are but a few of the interesting revelations of the Electron Microscope for medical science. Industrial science, too, has found this new research tool of great value in the study of metals, alloys, and plastics, as well as in the study of size, shape, and distribution of particles in chemical compounds and elements.

The Electron Microscope herein described is that manufactured by the Radio Corporation of America. There are, of course, variations in construction of the different instruments of this kind but all types are built along similar lines and upon the same general principles. In the Electron Microscope there is some aberration plus the additional disadvantages of having the specimen in a vacuum, not to mention the probable protoplasmic changes induced by the terrific bombardment of electrons, and finally, what is perhaps the greatest disadvantage insofar as medical science is concerned—that of being unable to view living organisms. Nevertheless, the disadvantages of the microscope are far overshadowed by its increased resolving and magnification powers which have combined to make it an invaluable research tool.

#### RESOLUTION AND MAGNIFICATION OF ORDINARY MICROSCOPE.

We have stated that the resolving power of the ordinary light microscope is restricted to between 1,600 and 2,500 diameters and that of the ordinary ultra microscope to between 2,500 and 5,000 diameters, resolution in any microscope being the ability of the instrument to reveal the most minute of component parts of a specimen so that each may be seen as a distinct and separate image. For instance, let us suppose an object is examined through which run two very fine parallel lines closely set together. If the two lines are visible under the microscope and are revealed as two separate images, then, appar-



ently, no limit of resolution has been reached; but if the two lines are merged or revealed as only one, and upon further magnification the image merely becomes enlarged without separation of the lines, then a limit of resolution apparently has been reached and additional magnification would constitute only enlargement. Assuming now that the object is a point object in which case the images of the points would be diffraction disks, the disks should likewise be sufficiently resolved so that each may be distinguished as a single image. If, when these disks are seen to overlap, additional magnification fails to extend the distance between them, their size simply increasing in proportion to the increase of magnification, or, if they are all but completely merged and the image becomes just a spurious disk of light, it is evident that a definite limit of resolution has been attained and that further magnification would be useless. Resolution, in a broad sense, then, is the ability of the microscope to bring out or reveal internal structure and detail of a specimen, the shortest distance it is possible to separate two component parts, according to Abbe, being not less than the wavelength of light by which the specimen is illuminated divided by the numerical aperture of the objective lens plus the numerical aperture of the condenser lens, or, about one-third the wavelength of light utilized.

The several factors which are generally acknowledged to be responsible for the limitation of resolving power are inter-related. Now when light passes from one medium into another of different density, in the instance which we are considering that of light refracted by the specimen and passing from air into glass, the light rays are deviated from their straight-line course; that is to say, that when they come to within a very short distance of this denser medium, they are acted upon by a very powerful force in such a manner that they execute a short rapidly curving motion, or an angle, and are pulled into the medium of greater density. When the rays of light undergo such a force, the momentum of the corpuscles is increased and the speed of the waves decreased, resulting, of course, in a shortening of the wavelengths. Here, again, we may make use of the second of the Rules of Correlation—"Momentum (of corpuscles) varies inversely as wavelength (of waves)." Once well inside the new medium, however, the light rays straighten themselves out again (unless the medium is so constructed that it possesses gradation of density in which case they follow a curved path). They do this in spite of the fact that the same forces are still acting upon them, although now these forces issue from all sides of them and so cancel each other out, the momentum of the photons or light corpuscles continuing to increase while the speed of the waves is proportionately retarded. If the light is refracted normally to the surface, however, it does not bend, but tends to cause a shortening of the optical path although the wavelength is shortened regardless. It is only when it is refracted obliquely to the surface that the light is bent, the greater

being the obliquity of the incident ray and the denser the medium, the greater the bending of the angle of the cone of light and the shorter the wavelength. It might therefore seem desirable to obtain as great an angle of refraction as possible. However, shortening of the wavelength is not in exact proportion to the amount of bending except in the case of the diffraction grating. And regardless of how great a change there is in its angle, the numerical aperture of the light, or angular aperture as it is more properly called, remains constant.

In order, then, that the cone of light be large enough to supply the aperture of the objective with sufficient light to produce an accurate, bright, and enlarged image of the specimen, it is first necessary that the specimen be refracting or emitting light of an adequate quantity, since both magnification and resolution are largely dependent upon the amount of light which the objective utilizes and receives into the tube of the microscope and since such light as the objective does receive should be only that emitted by the specimen. It is obvious, therefore, that it is of primary importance for the specimen itself to be amply illuminated. This would seem to depend entirely on the actual light source, yet no matter how powerful a light source is employed, it is of little avail unless the condenser is of sufficient quality and aperture dimensions to accommodate the light which it receives from the source. If, for instance, the numerical aperture of the objective is 1.25, the width of the cone of light emanating from the specimen should completely fill this aperture in order for the fullest powers of the microscope to be realized. Now, since the condenser supplies the light to the specimen, it stands to reason that it, also, should have a numerical aperture of at least 1.25. However, if the condenser and specimen slide are separated by air, the condenser can provide light of only 1.00 N. A. to the specimen since, according to a law of optics, no aperture greater than 1.00 N. A., (this being the refractive index of air), can pass from a denser medium into air. To remedy this situation, an immersion fluid is placed between the top of the condenser and the lower side of the specimen slide as well as between the specimen and the objective lens.

Since no optical medium has an index of refraction greater than three and no immersion fluid an index of refraction greater than 1.7, to further increase resolving power, then, might it not be feasible to widen the apertures of the objective and condenser lenses, thus affording additional illumination for utilization by both specimen and objective? This idea would be entirely practical except for the fact that such enlargement of the lenses would increase aberration, both spherical and chromatic, and apparently present-day lenses are now as highly corrected as it is possible for human ingenuity and skillful workmanship to make them. Spherical aberration, caused by the paraxial rays coming to a focus at the center of the lens before those rays near the

principal axis, is corrected by using concave and convex lenses of different material and, consequently, of different refractive index. In this manner spherical aberration of a convex lens, for instance, can be overcome, without its converging action being altered, by adding to the optical system a concave lens in which there is an equal and opposite aberration. Chromatic aberration, occurring when more than one wavelength of light is used to illuminate the specimen, is due to the fact that the shortest waves of the spectrum are refracted most and the longest waves least, thus causing the blue-violet waves to come to a focus ahead of the red waves and resulting in a series of colored foci all along the axis. Now since, as we have said, the shortening of the different groups of wavelengths is not in exact proportion to their bending and since this circumstance varies according to the substance the light rays pass through, it is possible to combine lenses or lens systems in such a way that white light may be obtained. For instance, a small concave flint-glass prism produces the same amount of dispersion as a large convex crown-glass prism. Thus, if these two prisms are placed with their edges opposite, the crown glass will bring together the spectrum produced by the flint glass and white light will be the result. However, the rays of white light will not extend parallel with the original direction but will bend toward the base of the crown glass since the mean refraction of the crown glass is greater than that of the flint glass. Achromatic objectives, corrected spherically for one color, chromatically for two; semi-apochromatic objectives, possessing moderate refractive indices and very small dispersion, in which a lens of fluorite is substituted for one of the glass lenses; apochromatic objectives, corrected spherically for two colors, chromatically for three; and also certain monochromatic lenses for use with light of one wavelength only are available for overcoming, at least in part, one of the conditions which tends to interfere with better resolution. Condensers, also, can be corrected for both spherical and chromatic aberration and must be achromatic-aplanatic if the light which enters the objective is to come only from the specimen, for condensers with spherical and chromatic aberration are unable to direct their entire cone of light upon the specimen.

In addition to being as highly corrected as possible and possessing a large numerical aperture, an objective should also be capable of adequately magnifying the image, being aided in this by the ocular which also serves at times to compensate for the defects in chromatic magnification which cannot be managed conveniently by high-power objectives, the magnification of the final image being the product of the magnification of the objective multiplied by the magnification of the ocular. An amplifier is sometimes inserted between the objective and ocular which causes the rays of light from the objective to diverge to a greater extent, thus doubling the size of the image. Magnification may also

be improved by increasing the tube length, by increasing the distance from which the image is projected, and by altering the positions of the various lenses in an adjustable objective. In general, the greater the magnification, the smaller will be the specimen field, but, as has been stressed, high powers of magnification should always be accompanied by equally high powers of resolution.

As we have seen, resolution in the ordinary light microscope is definitely restricted by a number of inter-related elements. Even when monochromatic light is employed, there is always present some spherical aberration with which to contend. True, better visibility of specimens is provided by dark-field microscopy in which the specimen is viewed by the high contrast of its own scattered or reflected light against a dark field, although in this type of illumination objects in the field must be well separated. Much fine detail and brilliant color of specimens can be observed by means of the polarization of light. Further, it is possible to illuminate the specimen with shorter and shorter wavelengths of light, the shorter the wavelength of light used, the more of the fine detail of the specimen which can be seen, but a limit is reached here, also, for ordinary glass lenses are not transparent to ultra-violet rays. However, in the ultra-violet microscope, having a resolution twice that of the instruments using "visible light," the condenser, objective, and ocular are all made of quartz and, by substituting the photographic plate for direct observation, many excellent micrographs of numerous varieties of organisms and cellular structures can be made. But when viewed directly, nothing of the nature or structure of the specimen can be ascertained; only the light scattered by the specimen is distinguishable, the size of the specimen being roughly estimated by the amount of light refracted.

These seemingly unsurmountable obstacles of the ordinary microscopes would appear to indicate that Abbe's law and the contention of physicists that "any object which is smaller than one-half the wavelength of light by which it is illuminated cannot be seen in its true form or detail" are destined to remain undefined.

#### REDUCTION IN THEORETICAL LIMIT OF RESOLUTION DEMONSTRATED.

But Dr. Francis F. Lucas of the Bell Telephone Research Laboratories and Doctors Louis Caryl Graton and E. C. Dane, Jr., of the Department of Geology, Harvard University, have very convincingly demonstrated a reduction in these theoretical limits of resolution and visibility with their instruments, designed for use in the visible light region of the spectrum.

The Graton-Dane microscope is mounted on a 360 kg. steel foundation bed which, in turn, is supported by six rubber-in-sheer marine-engine mountings—this for the purpose of eliminating all vibration and insuring stability of parts, two factors upon which both men have laid

great stress. Any type source, such as the carbon arc, metallic arc, incandescent filament, Point-O-Lite, Mercury Vapor, or any of the special forms of monochromators, can be used for illuminating the specimen with direct and dark-field transmitted, vertical and oblique reflected, or polarized light. The image beam itself follows a straight-line path in passing from the objective, the objective ranging anywhere from the shortest to the greatest in working distance, through the tube to the ocular, as few lenses as possible being placed in its way. The spiral-cut rack and pinion which moves the stage and sub-stage assembly in longitudinal tracks or guides can be operated by hand or by an electric motor and is independent of the fine adjustment, also motor-driven, which moves only the objective and the carriage carrying the objective. Whereas manual operation of the fine adjustment which is one hundred times more sensitive than that of the ordinary instruments necessitates five hundred turns of the knob to move the objective a distance of but one millimeter, (an adjustment calculated to require a time period of twenty-five minutes), by means of the motor it is possible to move the objective at the rate of 0.01 mm. per second or 0.004 mm. per second, depending upon which of the two speeds is desired, rapid motion being used when the image appears considerably out of focus and decreased speed being used when the image seems to be reaching a point of perfection.

Resolution up to 6,000 diameters and magnification up to 50,000 diameters have been achieved with this high precision microscope which photographs or enables observation of both opaque and transparent preparations; in fact, polishing scratches measuring, in width, but one-tenth the wavelength of light used have been clearly distinguished. It is the opinion of both Dr. Graton and Dr. Dane that some present-day lenses are really capable of better resolution than claimed for them by their manufacturers, it having been their experience to use objectives exhibiting superior qualities of resolution over those of identical medium and numerical aperture, proving that not only have already available lenses surpassed their theoretical limits of resolution, indicating that it might be possible to design objectives with still greater numerical apertures, but that the accepted theory regarding this resolution is sadly in need of revision. Dr. Lucas's microscope utilizing an objective with a numerical aperture of 1.60, for instance, in combination with monochromnaphalene immersion fluid, also yields resolution up to 6,000 diameters being, like the Graton-Dane scope, a high precision instrument constructed with the idea of maintaining absolute stability of parts. Dr. Lucas also has expressed doubt as to the complete validity of the generally accepted theory of resolution.

In working with a high precision ultra-violet micro-camera, into which a tri-color filter system has been incorporated, which he has just recently perfected, Dr. Lucas is able to obtain a minimum magnification

of 30,000 diameters and a maximum magnification of 60,000 diameters. With this instrument it is possible to view living cells and organisms, no staining or killing of organisms being necessary, and Dr. Lucas has succeeded in obtaining excellent photomicrographs (both still and motion pictures). Of special significance to industry, for instance, is the ability of this scope to demonstrate the size, shape, and reactions in motion and affinity of the tiny particles of which rubber is composed under varying conditions of temperature, etc., while its ability to reveal living rat and mouse sarcoma and carcinoma cells and to demonstrate the development and behavior of the syphilitic organism is of far more than average interest to medical science.

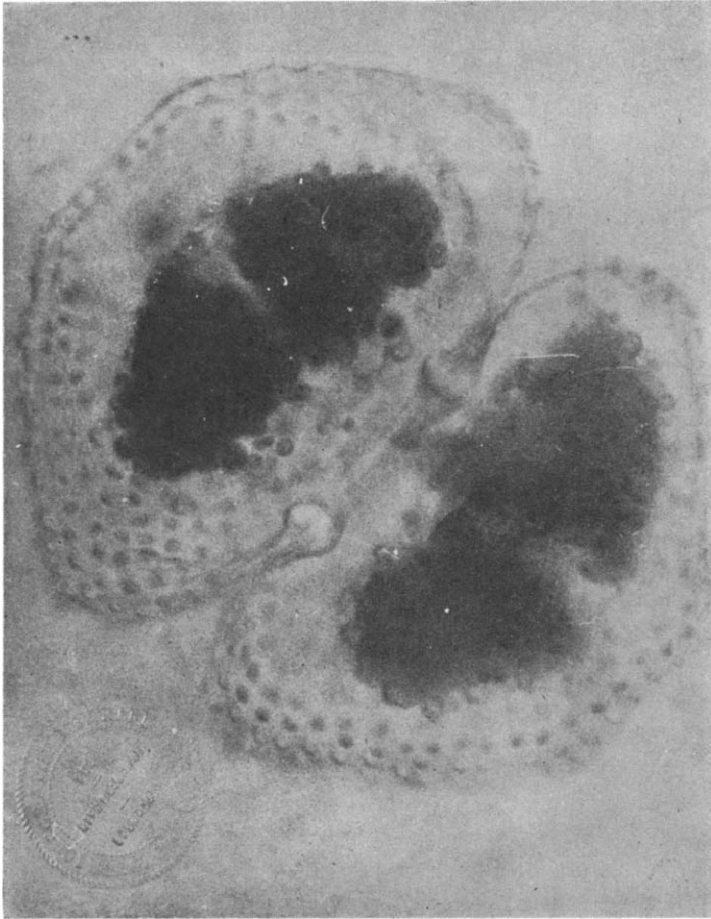
England's Dr. J. E. Barnard has succeeded in obtaining resolution up to 7,500 diameters with his ultra-dark-field scope in which he uses a combined illuminator. In this, an outer system of glass acts as the immersion dark-field illuminator while the inner immersion system of quartz makes possible the passage of a transmitted beam of light through the specimen. Both condensers have the same focus, one for visible light, the other for ultra-violet radiation, and both can be stopped out at will. When, for instance, bacteria are being observed, immersion contact is made between the condenser and quartz slide, the dark-field illuminator being used, thus revealing the bacteria with visible light. When the dark-field illuminator is closed, however, a beam of ultra-violet light may be directed up through the quartz condenser and focused on the bacteria. The object-glass, of course, has to be adjusted since it does not possess the same focus for ultra-violet that it does for visible light. Staining of specimens is thus unnecessary, making it possible to secure photomicrographs of living minute organisms.

In addition to these four microscopes, a fourth, belonging to the Canadian Department of Mines and located at Ottawa, and almost identical in principle and construction to that of Doctors Dane and Graton, has demonstrated ability to attain equally high resolution. This, like the scopes of Doctors Dane, Graton, and Lucas, is fitted with a tube for visual observation although intended mainly for micro-photographical work in the field of metallurgy. It is Dr. Graton's belief, however, that his instrument and that of Dr. Dane might also be adaptable to the purposes of biological research. Referring, in the description of their "Precision, All Purpose Microcamera" (*Journal of the Optical Society of America*), to the necessity or "desirability" of "reëxamining the classical conception of the limit of useful magnification," Doctors Dane and Graton have this to say:

"So long as the makers accepted the conventional limit as valid and had already attained it, there was little incentive toward progress. But with that limit apparently surpassed, there is no present knowledge as to how far ahead the true limit may lie. If present-day objectives do substantially better than the 'limit' for which they were designed,

is it not reasonable to suppose that effort to do better still may conceivably be rewarded?"

To such an inquiry there can be but one logical answer—an agreement which, while perhaps not concurred in by all, must, for those stimulated to more intense interest and effort by the possibilities of uncovering new facts, pose further questions; for, if the improvement of one part results in the improved performance of the whole, is it not



Chlorophyll Cell (algae) (The Universal Microscope). 17,000 $\times$  on 35 mm. film.

also reasonable to suppose that additional changes of additional parts, yes, even changes with respect to principle and method might likewise bear fruit?

#### THE UNIVERSAL MICROSCOPE.

It is not only a reasonable supposition, but already, in one instance, a very successful and highly commendable achievement on the part of Dr. Royal Raymond Rife of San Diego, California, who, for many

years, has built and worked with light microscopes which far surpass the theoretical limitations of the ordinary variety of instrument, all the Rife scopes possessing superior ability to attain high magnification with accompanying high resolution. The largest and most powerful of these, the Universal Microscope, developed in 1933, consists of 5,682 parts and is so-called because of its adaptability in all fields of microscopical work, being fully equipped with separate substage condenser units for transmitted and monochromatic beam, dark-field, polarized, and slit-ultra illumination, including also a special device for crystallography. The entire optical system of lenses and prisms as well as the illuminating units are made of block-crystal quartz, quartz being especially transparent to ultra-violet radiations.

The illuminating unit used for examining the filterable forms of disease organisms contains fourteen lenses and prisms, three of which are in the high-intensity incandescent lamp, four in the Risley prism, and seven in the achromatic condenser which, incidentally, has a numerical aperture of 1.40. Between the source of light and the specimen are subtended two circular, wedge-shaped, block-crystal quartz prisms for the purpose of polarizing the light passing through the specimen, polarization being the practical application of the theory that light waves vibrate in all planes perpendicular to the direction in which they are propagated. Therefore, when light comes into contact with a polarizing prism, it is divided or split into two beams, one of which is refracted to such an extent that it is reflected to the side of the prism without, of course, passing through the prism while the second ray, bent considerably less, is thus enabled to pass through the prism to illuminate the specimen. When the quartz prisms on the Universal Microscope, which may be rotated with vernier control through 360 degrees, are rotated in opposite directions, they serve to bend the transmitted beams of light at variable angles of incidence while, at the same time, a spectrum is projected up into the axis of the microscope, or rather a small portion of a spectrum since only a part of a band of color is visible at any one time. However, it is possible to proceed in this way from one end of the spectrum to the other, going all the way from the infra-red to the ultra-violet. Now, when that portion of the spectrum is reached in which both the organism and the color band vibrate in exact accord, one with the other, a definite characteristic spectrum is emitted by the organism. In the case of the filter-passing form of the *Bacillus Typhosus*, for instance, a blue spectrum is emitted and the plane of polarization deviated plus 4.8 degrees. The predominating chemical constituents of the organism are next ascertained after which the quartz prisms are adjusted or set, by means of vernier control, to minus 4.8 degrees (again in the case of the filter-passing form of the *Bacillus Typhosus*) so that the opposite angle of refraction may be obtained. A monochromatic beam of light, corresponding exactly



to the frequency of the organism (for Dr. Rife has found that each disease organism responds to and has a definite and distinct wavelength, a fact confirmed by British medical research workers), is then sent up through the specimen and the direct transmitted light, thus enabling the observer to view the organism stained in its true chemical color and revealing its own individual structure in a field which is brilliant with light.

The objectives used on the Universal Microscope are a 1.12 dry lens, a 1.16 water immersion, a 1.18 oil immersion, and a 1.25 oil immersion. The rays of light refracted by the specimen enter the objective and are then carried up the tube in parallel rays through twenty-one light bends to the ocular, a tolerance of less than one wavelength of visible light only being permitted in the core beam, or chief ray, of illumination. Now, instead of the light rays starting up the tube in a parallel fashion, tending to converge as they rise higher and finally crossing each other, arriving at the ocular separated by considerable distance as would be the case with an ordinary microscope, in the Universal tube the rays also start their rise parallel to each other but, just as they are about to cross, a specially-designed quartz prism is inserted which serves to pull them out parallel again, another prism being inserted each time the rays are about ready to cross. These prisms, inserted in the tube, which are adjusted and held in alignment by micrometer screws of one hundred threads to the inch in special tracks made of magnesium (magnesium having the closest coefficient of expansion of any metal to quartz), are separated by a distance of only thirty millimeters. Thus, the greatest distance that the image in the Universal is projected through any one media, either quartz or air, is thirty millimeters instead of the 160, 180, or 190 millimeters as in the empty or air-filled tube of an ordinary microscope, the total distance which the light rays travel zig-zag fashion through the Universal tube being 449 millimeters, although the physical length of the tube itself is 229 millimeters. It will be recalled, that if one pierces a black strip of paper or cardboard with the point of a needle and then brings the card up close to the eye so that the hole is in the optic axis, a small brilliantly-lighted object will appear larger and clearer, revealing more fine detail, than if it were viewed from the same distance without the assistance of the card. This is explained by the fact that the beam of light passing through the card is very narrow, the rays entering the eye, therefore, being practically parallel, whereas without the card the beam of light is much wider and the diffusion circles much larger. It is this principle of parallel rays in the Universal Microscope and the resultant shortening of projection distance between any two blocks or prisms plus the fact that objectives can thus be substituted for oculars, these "oculars" being three matched pairs of ten-millimeter, seven-millimeter, and four-millimeter objectives in short mounts, which make possible not only the unusually high magnification

and resolution but which serve to eliminate all distortion as well as all chromatic and spherical aberration.

Quartz slides with especially thin quartz cover glasses are used when a tissue section or culture slant is examined, the tissue section itself also being very thin. An additional observational tube and ocular which yield a magnification of 1,800 diameters are provided so that that portion of the specimen which it is desired should be examined may be located and so that the observer can adjust himself more readily when viewing a section at a high magnification.

The Universal stage is a double rotating stage graduated through 360 degrees in quarter minute arc divisions, the upper segment carrying the mechanical stage having a movement of 40 degrees, the body assembly which can be moved horizontally over the condenser also having an angular tilt of 40 degrees plus or minus. Heavily-constructed joints and screw adjustments maintain rigidity of the microscope which weighs two hundred pounds and stands twenty-four inches high, the bases of the scope being nickel cast-steel plates, accurately surfaced, and equipped with three leveling screws and two spirit levels set at angles of 90 degrees. The coarse adjustment, a block thread screw with forty threads to the inch, slides in a one and one-half dovetail which gibs directly onto the pillar post. The weight of the quadruple nosepiece and the objective system is taken care of by the intermediate adjustment at the top of the body tube. The stage, in conjunction with a hydraulic lift, acts as a lever in operating the fine adjustment. A six-gauge screw having a hundred threads to the inch is worked through a gland into a hollow, glycerine-filled post, the glycerine being displaced and replaced at will as the screw is turned clockwise or anti-clockwise, allowing a five-to-one ratio on the lead screw. This, accordingly, assures complete absence of drag and inertia. The fine adjustment being seven hundred times more sensitive than that of ordinary microscopes, the length of time required to focus the Universal ranges up to one hour and a half which, while on first consideration, may seem a disadvantage, is after all but a slight inconvenience when compared with the many years of research and the hundreds of thousands of dollars spent and being spent in an effort to isolate and to look upon disease-causing organisms in their true form.

Working together back in 1931 and using one of the smaller Rife Microscopes having a magnification and resolution of 17,000 diameters, Dr. Rife and Dr. Arthur Isaac Kendall of the Department of Bacteriology of Northwestern University Medical School were able to observe and demonstrate the presence of the filter-passing forms of *Bacillus Typhosus*. An agar slant culture of the Rawlings strain of *Bacillus Typhosus* was first prepared by Dr. Kendall and inoculated into six cubic centimeters of "Kendall" K Medium, a medium rich in protein but poor in peptone and consisting of one hundred mg. of

dried hog intestine and 6 cc. of tyrode solution (containing neither glucose nor glycerine) which mixture is shaken well so as to moisten the dried intestine powder and then sterilized in the autoclave, fifteen pounds for fifteen minutes, alterations of the medium being frequently necessary depending upon the requirements for different organisms. Now, after a period of eighteen hours in this K Medium, the culture was passed through a Berkefeld "N" filter, a drop of the filtrate being added to another six cubic centimeters of K Medium and incubated at 37 degrees centigrade. Forty-eight hours later this same process was repeated, the "N" filter again being used, after which it was noted that the culture no longer responded to peptone medium, growing now only in the protein medium. When again, within twenty-four hours, the culture was passed through a filter—the finest Berkefeld "W" filter, a drop of the filtrate was once more added to six cubic centimeters of K Medium and incubated at 37 degrees centigrade, a period of three days elapsing before the culture was transferred to K Medium and yet another three days before a new culture was prepared. Then, viewed under an ordinary microscope, these cultures were observed to be turbid and to reveal no bacilli whatsoever. When viewed by means of dark-field illumination and oil immersion lens, however, the presence of small, actively-motile granules was established, although nothing at all of their individual structure could be ascertained. Another period of four days was allowed to elapse before these cultures were transferred to K Medium and incubated at 37 degrees centigrade for twenty-four hours when they were then examined under the Rife Microscope where, as was mentioned earlier, the filterable typhoid bacilli, emitting a blue spectrum, caused the plane of polarization to be deviated plus 4.8 degrees. Then when the opposite angle of refraction was obtained by means of adjusting the polarizing prisms to minus 4.8 degrees and the cultures illuminated by a monochromatic beam coordinated in frequency with the chemical constituents of the typhoid bacillus, small, oval, actively-motile, bright turquoise-blue bodies were observed at a magnification of 5,000 diameters, in high contrast to the colorless and motionless debris of the medium. These observations were repeated eight times, the complete absence of these bodies in uninoculated control K Media also being noted.

To further confirm their findings, Doctors Rife and Kendall next examined eighteen-hour old cultures which had been inoculated into K Medium and incubated at 37 degrees centigrade, since it is just at this stage of growth in this medium and at this temperature that the cultures become filterable. And, just as had been anticipated, ordinary dark-field examination revealed unchanged, long, actively-motile bacilli; bacilli having granules within their substance; and free-swimming, actively-motile granules; while under the Rife Microscope were demonstrated the same long, unchanged, almost colorless bacilli; bacilli, prac-

tically colorless, inside and at one end of which was a turquoise-blue granule resembling the filterable forms of the typhoid bacillus; and free-swimming, small, oval, actively-motile, turquoise-blue granules. By transplanting the cultures of the filter-passing organisms or virus into a broth, they were seen to change over again into their original rod-like forms.

At the same time these findings of Doctors Rife and Kendall were confirmed by Dr. Edward C. Rosenow of the Mayo Foundation, the magnification with accompanying resolution of 8,000 diameters of the Rife Microscope, operated by Dr. Rife, was checked against a dark-field oil immersion scope operated by Dr. Kendall and an ordinary 2 mm. oil immersion objective,  $\times 10$  ocular, Zeiss scope operated by Dr. Rosenow at a magnification of 900 diameters. Examinations of gram and safranin stained films of cultures of *Bacillus Typhosus*, gram and safranin stained films of cultures of the streptococcus from poliomyelitis, and stained films of blood and of the sediment of the spinal fluid from a case of acute poliomyelitis were made with the result that bacilli, streptococci, erythrocytes, polymorphonuclear leukocytes, and lymphocytes measuring nine times the diameter of the same specimens observed under the Zeiss scope at a magnification and resolution of 900 diameters, were revealed with unusual clarity. Seen under the dark-field microscope were moving bodies presumed to be the filterable turquoise-blue bodies of the typhoid bacillus which, as Dr. Rosenow has declared in his report ("Observations on Filter-Passing Forms of *Eberthella Typhi*—*Bacillus Typhosus*—and of the *Streptococcus* from Poliomyelitis," Proceedings of the Staff Meetings of the Mayo Clinic, July 13, 1932), were so "unmistakably demonstrated" with the Rife Microscope, while under the Zeiss scope stained and hanging drop preparations of clouded filtrate cultures were found to be uniformly negative. With the Rife Microscope also were demonstrated brownish-gray cocci and diplococci in hanging drop preparations of the filtrates of streptococcus from poliomyelitis. These cocci and diplococci, similar in size and shape to those seen in the cultures although of more uniform intensity, and characteristic of the medium in which they had been cultivated, were surrounded by a clear halo about twice the width of that at the margins of the debris and of the *Bacillus Typhosus*. Stained films of filtrates and filtrate sediments examined under the Zeiss microscope, and hanging drop, dark-field preparations revealed no organisms, however. Brownish-gray cocci and diplococci of the exact same size and density as those observed in the filtrates of the streptococcus cultures were also revealed in hanging drop preparations of the virus of poliomyelitis under the Rife Microscope, while no organisms at all could be seen in either the stained films of filtrates and filtrate sediments examined with the Zeiss scope nor in hanging drop preparations examined by means of the dark-field. Again using the Rife Microscope

at a magnification of 8,000 diameters, numerous nonmotile cocci and diplococci of a bright-to-pale pink in color were seen in hanging drop preparations of filtrates of Herpes encephalitic virus. Although these were observed to be comparatively smaller than the cocci and diplococci of the streptococcus and poliomyelitic viruses, they were shown to be of fairly even density, size, and form and surrounded by a halo. Again,



Tetanus Spores (The Universal Microscope). 25,000 $\times$   
on 35 mm. film, enlarged 227,000 $\times$ .

both the dark-field and Zeiss scopes failed to reveal any organisms, and none of the three microscopes disclosed the presence of such diplococci in hanging drop preparations of the filtrate of a normal rabbit brain. Dr. Rosenow has since revealed these organisms with the ordinary microscope at a magnification of 1,000 diameters by means of his special staining method and with the Electron Microscope at a magnification of 12,000 diameters. Dr. Rosenow has expressed the opinion that the

inability to see these and other similarly revealed organisms is due, not necessarily to the minuteness of the organisms, but rather to the fact that they are of a non-staining, hyaline structure. Results with the Rife Microscopes, he thinks, are due to the "ingenious methods employed rather than to excessively high magnification." He has declared also, in the report mentioned previously, that "Examination under the Rife Microscope of specimens containing objects visible with the ordinary microscope, leaves no doubt of the accurate visualization of objects or particulate matter by direct observation at the extremely high magnification obtained with this instrument."

Exceedingly high powers of magnification with accompanying high powers of resolution may be realized with all of the Rife Microscopes one of which, having magnification and resolution up to 18,000 diameters, is now being used at the British School of Tropical Medicine in England. In a recent demonstration of another of the smaller Rife scopes (May 16th, 1942) before a group of doctors including Dr. J. H. Renner of Santa Barbara, California; Dr. Roger A. Schmidt of San Francisco, California; Dr. Lois Bronson Slade of Alameda, California; Dr. Lucile B. Larkin of Bellingham, Washington; Dr. E. F. Larkin of Bellingham, Washington; and Dr. W. J. Gier of San Diego, California, a Zeiss ruled grating was examined, first under an ordinary commercial microscope equipped with a 1.8 high dry lens and  $\times 10$  ocular, and then under the Rife Microscope. Whereas fifty lines were revealed with the commercial instrument and considerable aberration, both chromatic and spherical noted, only five lines were seen with the Rife scope, these five lines being so highly magnified that they occupied the entire field, without any aberration whatsoever being apparent. Dr. Renner, in a discussion of his observations, stated that "The entire field to its very edges and across the center had a uniform clearness that was *not* true in the conventional instrument." Following the examination of the grating, an ordinary unstained blood film was observed under the same two microscopes. In this instance, one hundred cells were seen to spread throughout the field of the commercial instrument while but ten cells filled the field of the Rife scope.

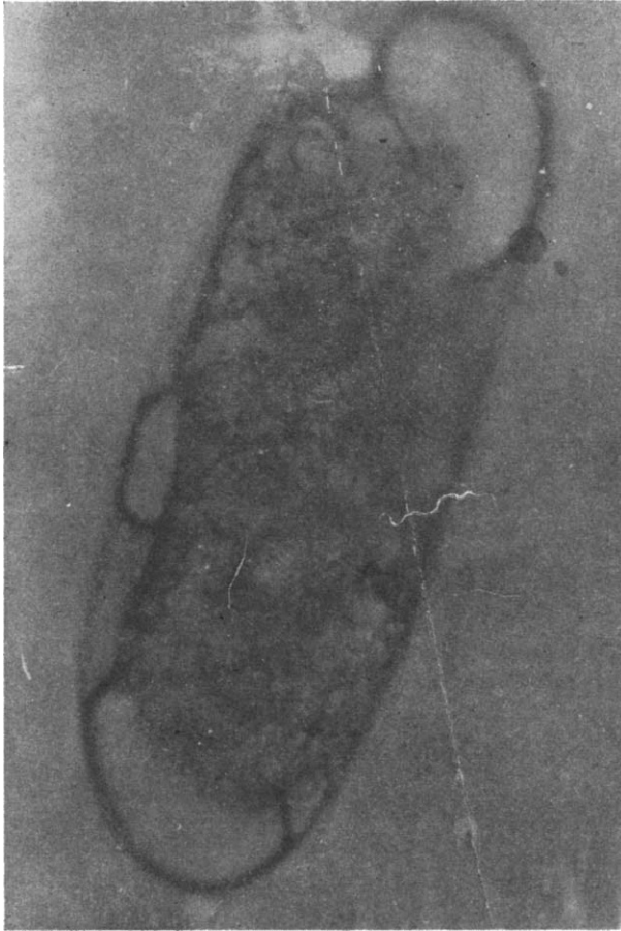
The Universal Microscope, of course, is the most powerful Rife scope, possessing a resolution of 31,000 diameters and magnification of 60,000 diameters. With this it is possible to view the interior of the "pin point" cells, those cells situated between the normal tissue cells and just visible under the ordinary microscope, and to observe the smaller cells which compose the interior of these pin point cells. When one of these smaller cells is magnified, still smaller cells are seen within its structure. And when one of the still smaller cells, in its turn, is magnified, it, too, is seen to be composed of smaller cells. Each of the sixteen times this process of magnification and resolution can be repeated, it is demonstrated that there are smaller cells within the

smaller cells, a fact which amply testifies as to the magnification and resolving power obtainable with the Universal Microscope.

More than 20,000 laboratory cultures of carcinoma, were grown and studied over a period of seven years by Dr. Rife and his assistants in what, at the time, appeared to be a fruitless effort to isolate the filter-passing form, or virus, which Dr. Rife believed to be present in this condition. Then, in 1932, the reactions in growth of bacterial cultures to light from the rare gasses was observed, indicating a new approach to the problem. Accordingly, blocks of tissue one-half centimeter square, taken from an un ulcerated breast carcinoma, were placed in triple-sterilized K Medium and these cultures incubated at 37 degrees centigrade. When no results were forthcoming, the culture tubes were placed in a circular glass loop filled with argon gas to a pressure of fourteen millimeters, and a current of 5,000 volts applied for twenty-four hours, after which the tubes were placed in a two-inch water vacuum and incubated at 37 degrees centigrade for twenty-four hours. Using a specially designed 1.12 dry lens, equal in amplitude of magnification to the 2 mm. apochromatic oil immersion lens, the cultures were then examined under the Universal Microscope, at a magnification of 10,000 diameters, where very-much animated, purplish-red, filterable forms, measuring less than one-twentieth of a micron in dimension, were observed. Carried through fourteen transplants from K Medium to K Medium, this B. X. virus remained constant; inoculated into four hundred and twenty-six Albino rats, tumors "with all the true pathology of neoplastic tissue" were developed. Experiments conducted in the Rife Laboratories have established the fact that these characteristic diplococci are found in the blood monocytes in 92 per cent. of all cases of neoplastic diseases. It has also been demonstrated that the virus of cancer, like the viruses of other diseases, can be easily changed from one form to another by means of altering the media upon which it is grown. With the first change in media, the B. X. virus becomes considerably enlarged although its purplish-red color remains unchanged. Observation of the organism with an ordinary microscope is made possible by a second alteration of the media. A third change is undergone upon asparagus base media where the B. X. virus is transformed from its filterable state into cryptomyces pleomorphia fungi, these fungi being identical morphologically both macroscopically and microscopically to that of the orchid and of the mushroom. And yet a fourth change may be said to take place when this cryptomyces pleomorphia, permitted to stand as a stock culture for the period of metastasis, becomes the well-known mahogany-colored *Bacillus Coli*.

It is Dr. Rife's belief that all microorganisms fall into one of not more than ten individual groups (Dr. Rosenow has stated that some of the viruses belong to the group of the streptococcus) and that any alteration of artificial media or slight metabolic variation in tissues will

induce an organism of one group to change over into any other organism included in that same group, it being possible, incidentally, to carry such changes in media or tissues to the point where the organisms fail to respond to standard laboratory methods of diagnosis. These changes can be made to take place in as short a period of time as forty-eight



Typhoid Bacillus (The Universal Microscope).  $23,000\times$   
on 35 mm. film, enlarged  $300,000\times$ .

hours. For instance, by altering the media—four parts per million per volume—the pure culture of mahogany-colored *Bacillus Coli* becomes the turquoise-blue *Bacillus Typhosus*. Viruses or primordial cells of organisms which would ordinarily require an eight-week incubation period to attain their filterable state, have been shown to produce disease within three days' time, proving Dr. Rife's contention that the incubation period of a microorganism is really only a cycle of reversion. He states:



“In reality, it is not the bacteria themselves that produce the disease, but we believe it is the chemical constituents of these micro-organisms enacting upon the unbalanced cell metabolism of the human body that in actuality produce the disease. We also believe if the metabolism of the human body is perfectly balanced or poised, it is susceptible to no disease.”

In other words, the human body itself is chemical in nature, being comprised of many chemical elements which provide the media upon which the wealth of bacteria normally present in the human system feed. These bacteria are able to reproduce. They, too, are composed of chemicals. Therefore, if the media upon which they feed, in this instance the chemicals or some portion of the chemicals of the human body, becomes changed from the normal, it stands to reason that these same bacteria, or at least certain numbers of them, will also undergo a change chemically since they are now feeding upon a media which is not normal to them, perhaps being supplied with too much or too little of what they need to maintain a normal existence. They change, passing usually through several stages of growth, emerging finally as some entirely new entity—as different morphologically as are the caterpillar and the butterfly (to use an illustration given us). The majority of the viruses have been definitely revealed as living organisms, foreign organisms it is true, but which once were normal inhabitants of the human body—living entities of a chemical nature or composition.

Under the Universal Microscope disease organisms such as those of tuberculosis, cancer, sarcoma, streptococcus, typhoid, staphylococcus, leprosy, hoof and mouth disease, and others may be observed to succumb when exposed to certain lethal frequencies, coördinated with the particular frequencies peculiar to each individual organism, and directed upon them by rays covering a wide range of waves. By means of a camera attachment and a motion picture camera not built into the instrument, many “still” micrographs as well as hundreds of feet of motion picture film bear witness to the complete life cycles of numerous organisms. It should be emphasized, perhaps, that invariably the same organisms refract the same colors when stained by means of the monochromatic beam of illumination on the Universal Microscope, regardless of the media upon which they are grown. The virus of the *Bacillus Typhosus* is always a turquoise-blue, the *Bacillus Coli* always mahogany-colored, the *Mycobacterium li prae* always a ruby shade, the filter-passing form or virus of tuberculosis always an emerald green, the virus of cancer always a purplish-red, and so on. Thus, with the aid of this microscope, it is possible to reveal the typhoid organism, for instance, in the blood of a suspected typhoid patient four and five days before a Widal is positive. When it is desired to observe the flagella of the typhoid organism, Hg salts are used as the media to see at a magnification of 10,000 diameters.

In the light of the amazing results obtainable with this Universal Microscope and its smaller brother-scopes, there can be no doubt of the ability of these instruments to actually reveal any and all micro-organisms according to their individual structure and chemical constituents.

With the aid of its new eyes—the new microscopes, all of which are continually being improved—Science has at last penetrated beyond the boundary of accepted theory and into the world of the viruses with the result that we can look forward to discovering new treatments and methods of combating the deadly organisms—for Science does not rest.

To Dr. Karl K. Darrow, Dr. John A. Kolmer, Dr. William P. Lang, Dr. L. Marton, Dr. J. H. Renner, Dr. Royal R. Rife, Dr. Edward C. Rosenow, Dr. Arthur W. Yale, and Dr. V. K. Zworykin, we wish to express our appreciation for the help and information so kindly given us and to express our gratitude, also, for the interest shown in this effort of bringing to the attention of more of the medical profession the possibilities offered by the new microscopes.

#### REFERENCES.

- ANDERSON, T. F., AND STANLEY, W. M.: "A Study by Means of the Electron Microscope of the Reaction Between Tobacco Mosaic Virus and its Antiserum," *Jour. Biol. Chem.*, **139**: No. 1, 339-344, 1941.
- ANDRADE, E. N. DA C.: "The Mechanism of Nature," G. Bell & Sons, Ltd., London, 1932.
- APPERLY, F. L.: "Relation of Solar Radiation in North America," *Cancer Research*, **1**: 191-195, March 1941.
- BATEMAN, J. B., CALKINS, H. E., AND CHAMBERS, L. A.: "Optical Study of the Reaction Between Transferred Monolayers of Lancefield's 'M' Substance and Various Antisera," *Jour. Immun.*, **41**: 321-341, 1941.
- BURTON, E. F.: "The Electron Microscope," *Proc. Roy. Can. Inst.*, **5**: Series 3A, Feb. 10, 1940.
- CORRINGTON, JULIAN D.: "Under the Microscope," *Nat. Mag.*, **34**: 117-120, Feb. 1941.
- DARROW, KARL, K.: "The Renaissance of Physics," Macmillan Co., 1939.
- DAVIS, WATSON: "Exploring A New World," *Science News Letter*, **38**: 227-230, Oct. 12, 1940.
- Same: Abr. with title, "30,000 Times Life-Size," *Readers Digest*, **37**: 13-16, Nov. 1940.
- DUNN, H. H.: "Movie New Eye of Microscope in War on Germs," *Pop. Science*, **118**: 27, June 1931.
- ELDRIDGE, JOHN A.: "The Physical Basis of Things," 1934.
- "Electron Microscope Magnifies Invisible World 25,000 X," *Life*, **8**: 54, April 29, 1940.
- "Electron Microscope Shows 'Strep' Has Outer Membrane," *Science News Letter*, **38**: 301, Nov. 9, 1940.
- "Filterable Germ Forms Seen with New Super-Microscope," *Science News Letter*, Dec. 12, 1931.
- "Filterable Germs Need not be Extremely Small," *Science News Letter*, Nov. 12, 1932.
- FRIEDMAN, JOSEPH S.: "New Electron Microscope," *Amer. Photography*, **35**: 368-369, May 1941.
- GAGE, SIMON HENRY: "The Microscope," 16th. and 17th. Ed., Comstock Pub. Co., Inc., 1936-1941.
- GRATON, L. C., AND DANE, E. C.: "Precision, All Purpose Microcamera," *Jour. Opt. Soc. Amer.*, **27**: 355-376, Nov. 1937.

- HARDY, ARTHUR C., AND PERRIN, FRED H.: "The Principles of Optics," 1st. Ed., McGraw-Hill Book Co., Inc., 1932.
- HARRINGTON, JEAN: "Electron Microscope is now Ready for Industrial Use," *Scient. Amer.*, **164**: 155, March 1941.
- HEKTOEN, LUDVIG, MATHERS, GEORGE, AND JACKSON, LEILA: "Microscopic Demonstration of Cocci in the Central Nervous System in Epidemic Poliomyelitis," *Jour. Infect. Dis.*, **22**: 89-94, 1918.
- HILLIER, JAMES: "Personalities in Science," *Scient. Amer.*, **164**: 133, March 1941.
- HILLIER, J., AND VANCE, A. W.: "Recent Developments in the R. C. A. Electron Microscope," *Proc. Inst. Radio Engin.*, **29**: 167-176, No. 4, April 1941.
- HOUSTON, W. V.: "Telescopes and Microscopes," *Amer. Scholar*, **9**: No. 1, 63-73, Jan. 1940. Same: Abr. with title, "Electron Microscope," *Science Digest*, **7**: 43-48, April 1940.
- INGALLS, A. G.: "Who Ever Dares Say Never," *Scient. Amer.*, **163**: 9, July 1940.
- KENDALL, ARTHUR ISAAC: "Filtration of Bacteria; Studies in Bacterial Metabolism," *Science*, **75**: 295-301, March 18, 1932.
- KILLEFFER, D. H.: "Supermicroscopes," *Rotarian*, **57**: 47, Aug. 1940.
- LINDLEY, D.: "Smallest Ever," *Colliers*, **105**: 20, May 4, 1940.
- LUCAS, F. F.: "Late Developments in Microscopy," *Jour. Fr. Inst.*, **217**: 661-707, June 1934.
- LUCAS, F. F.: "A Precision High Power Metallographic Apparatus," *Trans. Amer. Soc. Steel Treating*, **21**: 1112-1134, 1933.
- LUCAS, FRANCIS F., AND STARK, MARY B.: "A Study of Living Germ Cells by Means of the Ultra-Violet Microscope," *Jour. Morph.*, **52**: 91-115, 1931.
- MALOFF, I. G., AND EPSTEIN, D. W.: "Electron Optics," 1938.
- MARTIN, L. C.: "The Electron Microscope," *Nat.*, **142**: 1062, Dec. 17, 1938.
- MARTON, L.: "A New Electron Microscope," *Physical Rev.*, **58**: 57-60, 1940.
- MARTON, L., BANCA, M. C., AND BENDER, J. F.: "A New Electron Microscope," *R. C. A. Review*, **5**: No. 2: 232-242, Oct. 1940.
- MARTON, L.: "Electron Microscopy of Biological Objects," *Nat.*, **133**: No. 3372: 911, 1934.
- MORTON, G. A., AND RAMBERG, E. G.: "Point Projector Electron Microscope," *Phys. Rev.*, **56**: 705, 1939.
- MARTON, L.: "The Electron Microscope: A New Tool for Bacteriological Research," *Jour. Bacter.*, **41**: 397-413, March 1941.
- McMILLEN, J. HOWARD, AND SCOTT, GORDON H.: "A Magnetic Electron Microscope of Simple Design," *Rev. Sci. Instru.*, **8**: No. 8: 288-290, Aug. 1937.
- MILLMAN, JACOB, AND SEELY, SAMUEL: "Electronics," McGraw-Hill Book Co., Inc., 1941, 1st. Ed.
- MORTON, G. A.: "A Survey of Research Accomplishments with the R. C. A. Electron Microscope," *R. C. A. Rev.*, **6**: No. 2: 131-166, Oct. 1941.
- MORTON, HARRY E., AND ANDERSON, THOMAS F.: "Electron Microscopic Studies of Biological Reactions. I. Reduction of Potassium Tellurite by *Corynebacterium Diphtheriae*," *Proc. Soc. Exper. Biol. and Med.*, **46**: 272-276, 1941.
- "Most Powerful Microscope," *Science Dig.*, **7**: 94-95, Feb. 1940. Same: *Science News Letter*, **36**: 411, Dec. 23, 1939.
- MOULLIN, C. M.: "What is Cancer? A Biologist's Answer," *Med. Press* **203**: 512-514, June 19, 1940.
- MOULTON, F. R.: "The Electron Microscope," *Science Monthly*, **49**: 189-192, Aug. 1939.
- MUDD, STUART, AND LACKMAN, DAVID B.: "Bacterial Morphology as Shown by the Electron Microscope," *Jour. Bact.*, **41**: No. 3: 414-420, March 1941.
- MUDD, STUART, POLEVITZKY, K., ANDERSON, T. F., AND CHAMBERS, L. A.: "Bacterial Morphology as Shown by the Electron Microscope. II. The Bacterial Cell Membrane in the Genus *Bacillus*," *Jour. Bact.*, **42**: 251-264, Aug. 1941.
- MUDD, STUART, AND ANDERSON, THOMAS F.: "Demonstration by the Electron Microscope of the Combination of Antibodies with Flagellar and Somatic Antigens," *Jour. Immun.*, **42**: No. 3: 251-266, Nov. 1941.

- "Never Seen Before; Electron Microscope Reveals Viruses for First Time," *Scient. Amer.*, **164**: 358, June 1941.
- "New Microscope," *Science, n.s.*, **87**: Sup. 8, Jan. 14, 1938.
- "New Electron Microscope May Some Day See Atoms," *Science*, **91**: Sup. 11, April 26, 1940.  
Same: *Science News Letter*, **37**: 275, May 4, 1940.
- Northwestern University Bulletin (Medical School) **32**: No. 8: Oct. 19, 1931.
- PATTEN, JAMES A.: Lecture, Northwestern University Bulletin **32**: No. 5; Sept. 28, 1931.
- PREBUS, A., AND HILLIER, J.: "The Construction of a Magnetic Electron Microscope of High Resolving Power," *Can. Jour. Research*, **17**: 49-63, 1939.
- "Probe Secrets of Germs with Electron Microscope," *Science News Letter*, **39**: 23, Jan. 11, 1941.
- RAMBERG, E. G., AND MORTON, G. A.: "Electron Optics," *Jour. Applied Phys.*, **10**: 465-478, 1939.
- RHEA, H. E.: "Scientific Apparatus and Laboratory Methods," *Science, n.s.*, **93**: 357-358, April 11, 1941.
- RHEA, H. E.: "The New Electron Microscope," *Science, n.s.*, **93**: Sup. 12, Jan. 3, 1941.
- RIFE, ROYAL R.: "Bacteria," Presented April 28, 1939.
- RIFE, ROYAL RAYMOND: "The Filterable Virus of Carcinoma," Presented April 26, 1939.
- RIFE, ROYAL RAYMOND: "The Rife Microscopes," Presented April 28, 1939.
- RIFE, ROYAL RAYMOND, AND KENDALL, ARTHUR ISAAC: "Observations on Bacillus Typhosus in its Filterable State," *California and Western Med.*, **35**: 409-411, Dec. 1931.
- ROSENOW, E. C.: "Microdiplococci in Filtrates of Natural and Experimental Poliomyelitic Virus Compared Under the Electron and Light Microscopes," *Proc. Staff. Meet. Mayo Clinic* **17**: No. 7: 99-106, Feb. 18, 1942.
- ROSENOW, E. C.: "Observations on Filter-Passing Forms of Eberthella Typhi (Bacillus Typhosus) and of the Streptococcus from Poliomyelitis," *Proc. Staff. Meet. Mayo Clinic* **7**: 408-413, July 13, 1932.
- ROSENOW, E. C.: "Observations with the Rife Microscope of Filter-Passing Forms of Microorganisms," *Science, n.s.*, **76**: 192-193, Aug. 26, 1932.
- Science, n.s.*, **74**: Sup. 10: "Filterable Bodies Seen with the Rife Microscope," Dec. 11, 1931.
- Science News Letter*, **33**: 55: "Powerful Microscope Makes Very Tiny Objects Visible," Jan. 22, 1938.
- "Smaller and Smaller," *Time*, **36**: 50, Oct. 28, 1940.
- SMITH, THEODORE A.: "The Electron Microscope," *Scient. Month.*, **52**: 337-341, April 1941.
- SOUTHALL, JAMES P. C.: "Mirrors, Prisms, and Lenses," 3rd. Ed., MacMillan Co., 1933.
- STANLEY, W. M., AND ANDERSON, T. F.: "A Study of Purified Viruses with the Electron Microscope," *Jour. Biol. Chem.*, **139**: No. 1: 325-338, 1941.
- STOKLEY, JAMES: "The Electron Microscope and Television," *Science*, **92**: Sup. 8, Nov. 1, 1940. Same: *Science News Letter*, **38**: 300, Nov. 9, 1940.
- Time* **30**: 37: "Precision and Stability," Dec. 20, 1937.
- "The Electron Microscope," Pamphlet issued by Engin. Prod. Divis., R. C. A. Manufacturing Co., Inc.
- "The Photographic Image," *Science, n.s.*, **92**: Sup. 10, Dec. 6, 1940. Same: *Science News Letter*, **38**: 355, Dec. 7, 1940.
- TODD AND SANFORD: "Clinical Diagnosis by Lab. Meth.," 1939.
- VANCE, A. W.: "Stable Power Supplies for Electron Microscopes," *R. C. A. Rev.*, **5**: No. 3: 293-300, Jan. 1941.
- WILE, UDO J., PICARD, ROBERT G., AND HEARNEY, EDNA B.: "The Morphology of Spirochaeta Pallida in the Electron Microscope," *Jour. Amer. Med. Assoc.*, **119**: 880-881, July 11, 1942.
- ZWORYKIN, V. K.: "An Electron Microscope for the Research Laboratory," *Science, n.s.*, **92**: 51-53, July 19, 1940.
- ZWORYKIN, V. K.: "Electrons Extend the Range," *Elect. Engin.*, **59**: 441-443, Nov. 1940.

- ZWORYKIN, V. K., AND RAMBERG, E. G.: "Surface Studies with the Electron Microscope," *Jour. Applied Phys.*, **12**: No. 9: 692-695, Sept. 1941.
- ZWORYKIN, V. K., HILLIER, J., AND VANCE, A. W.: "An Electron Microscope for Practical Laboratory Service," *Elect. Engin.*, **60**: 157-162, 1941.
- ZWORYKIN, V. K., HILLIER, J., AND VANCE, A. W.: "Preliminary Report on 300,000-volt Electron Microscope," *Jour. Applied Phys.*, Oct. 1941.
- HARRINGTON, JEAN: "Seeing With Electricity," *Scient. Amer.*, **163**: 20-22, July 1940.