A STUDY OF THE ELECTRON MICROSCOPE
TYPE EMC-2 AND PROPERTIES OF SPECIMEN MOUNTINGS

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M. B. M.

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PREFACE

The natural evolution of instruments for revealing the nature of minute objects has led to the electron microscope. The limit of resolution for objects viewed with light has been reached; and in order to go beyond the limit set by the inherent nature of light, a system of "viewing" without light has been developed. High-speed electrons have been found to possess properties similar to those of light and, in addition, to afford greater resolving power. The electron microscope has been the natural result.

The electron microscope is now in widespread laboratory use with the development of commercial models. Among these models the type EMC-2 microscope manufactured by RCA is one of the most compact and easy-to-operate instruments. It is the purpose of this paper to describe briefly the type EMC-2 electron microscope, the theoretical basis for it, its applications, and its limitations.

Chapter 1

HISTORY OF THE ELECTRON MICROSCOPE

The development of the electron microscope followed the discovery that electrons have the dual properties of particles and waves. Electrons had long been considered as particles, but their wave nature was demonstrated in 1927 when C. J. Davisson and L. H. Germer showed that electron beams could be diffracted. The dual nature of the electron had been mathematically demonstrated back in 1923 by de Broglie, and his value for the wave length of the electrons was confirmed by experimental results from the diffraction of electron beams at the surface of a crystal.[1] The relationship for the wave length as de Broglie had calculated was $\lambda = h/mv$. Planck's constant is h, m is the mass of the electron, and v is the velocity of the electron.

The interaction of an electric current and a magnetic field which was first noted by Oersted was utilized by H. Busch in 1926 in showing that a beam of electrons can be focused by a solenoid.[2] Busch also studied the focusing properties of axially symmetrical electric fields as well as magnetic. Experimental work on the lens properties of circular apertures was

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[1] E. F. Burton and W. H. Kohl, The Electron Microscope, p. 148.

[2] R. B. Barnes and C. J. Burton, "The Electron Microscope," The American Dyestuffs Reporter, XXXI (May, 1942), 254.

carried on by C. J. Davisson and C. J. Calbick in 1931, and they obtained a value for the focal length of the slit and circular aperture. Their work became the basis for the electrostatic electron lens.[3]

In Germany the study of geometrical electron optics was pursued most actively, and many articles were written both there and in other countries on the theory of geometrical optics. As a result of these studies both magnetic and electrostatic electron microscopes were developed. In 1932 E. Bruche and H. Johannson obtained images of the filament in an electrostatic lens microscope, and M. Knoll and E. Ruska produced images in the first magnetic electron microscope ever built. The electrostatic instrument used three-hundred volt electron beams, whereas the magnetic instrument had an accelerating potential of sixty-thousand volts.[4]

After the first electron microscopes were built, research on further improvements went on rapidly, but the microscope remained a research instrument which was too complicated and too laborious to use effectively in industrial research until the commercial model was designed in 1940 at the R.C.A. laboratories.[5] The need for an electron microscope which could be operated rapidly and without complicated adjustments led to the development of a small console model microscope for use in industrial laboratories as well as the more complicated and

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3 Burton and Kohl, Loc. cit.

4 Burton and Kohl, Op. cit., p. 149.

5 Ibid., p. 6.

more versatile universal type. The console model could be made very compact by making the magnification fixed, and it still was capable of performing most of the research which arose in industrial laboratories. The universal type of electron microscope was designed to operate at several different magnifications and to use a considerably higher accelerating voltage than the console model.

At the present time many electron microscopes are in use throughout the country, and although the instrument itself has reached a stage of development such that there is not much possibility of any major improvement unless it is a complete departure from the present design, a great deal is being learned about the application of the microscope to various fields of study. The number of articles on electron microscope research being published has increased greatly in the past five years.

Experimental research on the electron microscope itself is largely directed towards the use of voltages higher than the one hundred-thousand volts used for the accelerating potential in the largest type of commercial microscope. A two hundred-thousand volt microscope has been designed, but the main advantage of the higher voltage lies in the penetration of thicker specimens. The extra shielding necessary to stop the x-rays produced by the high voltage and the complicated apparatus necessary for handling the large voltage safely make the two hundred-thousand volt instrument too bulky and expensive for it to replace the present models. Some work is also being done toward further simplification of the small models with the possibility of a portable model being devised.

Chapter 2

THEORETICAL BASIS OF THE ELECTRON MICROSCOPE

As improvements were made on the light microscope, the magnification which could be obtained was steadily increased; but the resolving power did not keep pace with the improvements in magnification. The limit of the useful magnification of a microscope, i.e., the magnification which reveals further detail, was attributed to mechanical imperfections in the lenses until Ernst Abbe was able to show that the limiting factor was the wave length of the viewing medium.[1]

According to Abbe's Law the resolving power of a microscope, that is, the minimum distance apart at which two objects can be distinguished as being separated, may be expressed in the formula; $R_{OPT} = K \frac{\lambda}{\mu \sin\theta} = K \frac{\lambda}{\text{NUMERICAL APERTURE}}$. μ is the refractive index, $\sin\theta$ is the sine of the angle between the central ray and the outermost ray from the object, and K is a constant equal to 1.22. The minimum resolving power for a light microscope is greater than .1 micron, and this resolution can only be accomplished by means of an oil-immersion lens, ultraviolet light (wave length = .25 microns), and oblique illumination which increases $\sin\theta$. The resolving power of a light microscope can

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[1] Zworykin, Morton, Ramberg, Hillier and Vance, <u>Electron Optics and the Electron Microscope</u>, p. 81.

be shown to be approximately one-half the wave length of the light used.[2]

High magnification light microscopes have a very short depth of focus. This depth of focus is a function of the reciprocal of the numerical aperture, and for the best light microscopes is equal to .04 microns. Thus some of the light microscopes have depths of focus shorter than the limits of their resolving powers.

The magnification may be determined from the equation $R \cdot M = \frac{0.61 \lambda \cdot M}{\sin\theta}$. Since the product R.M must equal the limit of resolution for the average human eye, .02 cm. when visible light is being used in the microscope, the magnification $M = \frac{0.033 \sin\theta}{\lambda}$ which has a maximum value of 1100 diameters for visible light and 1800 for ultraviolet light.[3]

In order to produce higher magnification and increased resolving power, some radiation of shorter wave length than that of light was needed. That radiation had also to be capable of being focused. There was no known radiation which satisfied the requirements for use in a higher resolution microscope until Louis de Broglie demonstrated that electrons had a wave length associated with them.

Electrons with extremely short wave lengths can be obtained since the wave length varies inversely as the square root of the potential difference through which the electron

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[2] R. B. Barnes and C. J. Burton, American Dyestuffs Reporter XXXI (May, 1942) p. 254.

[3] Zworykin, Morton, Ramberg, Hillier, and Vance op. cit., p. 82.

has fallen. The relation of the wave length λ and the electric potential φ can be derived from de Broglie's equation, $\lambda = h/mv$, and the expression for the total energy of a body, $E = mc^2$. The kinetic energy of the accelerated electron, which is expressed as the product of the electron charge e and the potential φ , is equal to the total energy E less the rest mass energy $m_0 c^2$. The equation is $e\varphi = mc^2 - m_0 c^2$. Since the mass of a particle in motion is not the same as the rest mass but is the rest mass multiplied by a corrective factor which takes into account the variation of m with the velocity v, the expression for m is given by $m = \frac{m_0}{(1 - \frac{v^2}{c^2})^{\frac{1}{2}}}$. Substituting for m gives the equation

$$e\varphi = m_0 c^2 \left[\frac{1}{(1 - \frac{v^2}{c^2})^{\frac{1}{2}}} - 1\right].$$

Solving the equation for v/c gives

$$\frac{v}{c} = \frac{m_0 c^2}{e\varphi + m_0 c^2}\left[\left(\frac{e\varphi}{m_0 c^2}\right)^2 + \frac{2e\varphi}{m_0 c^2}\right]^{\frac{1}{2}}.$$

Multiplying both sides by m and c gives the expression

$$mv = m_0 c \left[\left(\frac{e\varphi}{m_0 c^2}\right)^2 + \frac{2e\varphi}{m_0 c^2}\right]^{\frac{1}{2}}$$

and substituting this value in the original equation for the wave length results in the equation

$$\lambda = \frac{h}{m_0 c\left[\frac{2e\varphi}{m_0 c^2} + \left(\frac{e\varphi}{m_0 c^2}\right)^2\right]^{\frac{1}{2}}}.$$

Substituting in the numerical values for Planck's constant h, the rest mass m, the charge on an electron e, and the velocity of light c gives the following value for the wave length expressed in angstrom units,

$$\lambda = \frac{12.26}{[\varphi + 0.978 \cdot 10^{-6}\varphi]^{\frac{1}{2}}}.$$

From this relation the wave length of any electron can be determined when the potential difference through which it has been accelerated is known. If the potential difference is not very large, the wave length is closely approximated by

$\lambda = 12.26/\varphi^{\frac{1}{2}}$. When the voltage does not exceed twenty-thousand, this approximation is not in error by more than one percent.[4]

Focusing of electron beams is possible because they are bent by magnetic fields, and their paths may be calculated by geometrical optics in much the same way as those of light rays. A stream of electrons when moving through a magnetic field is acted upon by a force perpendicular to both the electron path and the direction of the field. The force on each electron is $F = Bev$. B is the flux density of the magnetic field, e is the charge on the electron, and v is the velocity of the electron. When the force F is equal to the centripetal force necessary to keep the electron moving along a circle of radius r, the electron follows the circular path until acted upon by some other force. Equating the two forces gives the equation $Bev = \frac{mv^2}{r}$ when m is the mass of the electron. If electrons moving in a circular path in a uniform magnetic field are given a velocity parallel to the field, they will describe a helix. Electrons leaving a certain point with the same velocity components in the direction of the field come together again after one revolution, and so an image of the original point is formed once every revolution of the helix. The images formed in this way are not enlarged, and to obtain enlargement a short magnetic field lens is used as shown in Figure 1.

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4 Zworykin, Morton, Ramberg, Hillier, and Vance, Op. cit., pp. 347-351.

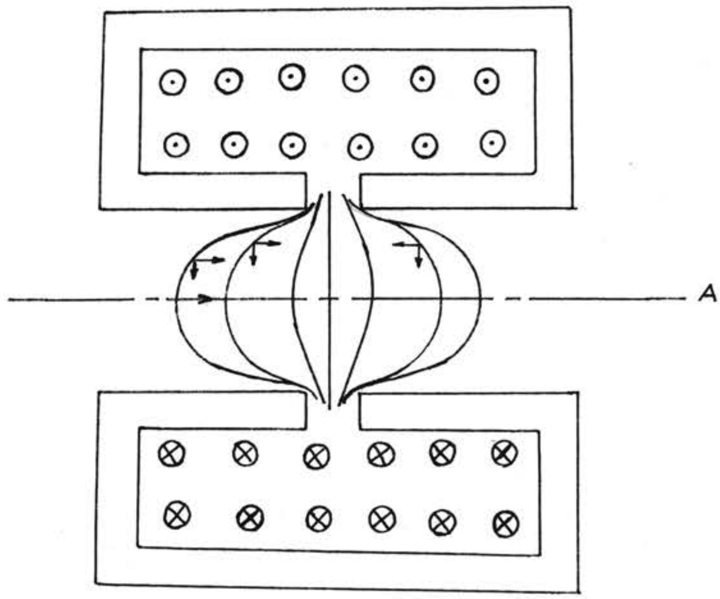

Fig. 1

Electrons entering the field parallel to the axis A are given an acceleration toward the axis. The components of the magnetic field parallel to A cancel their effect as the electron traverses the total field. The normal components, however, accelerate the electrons toward the axis throughout the total field. There are many types of magnetic lenses, but they all have the common characteristic of decreasing rather than increasing the divergence of the beams passing through them. After the electrons emerge from the magnetic field, they will travel in a straight line unless acted upon by another magnetic or electric field or until they collide with some substance thick enough to absorb or deflect them. The electron lenses have focal planes which can be determined.

One of the two main advantages of the electron microscope is its increased resolving power. The practical limit of resolution of the electron microscope is ten angstrom units. This resolving power is a gain of approximately one hundred times that of the light microscope.[5] The actual resolution obtained with an instrument depends upon the precision of its construction, but the fundamental limiting factor is the diffraction effect which limits the resolving power of any electron microscope to slightly more than one-half the wave length of the electrons when they strike the object.[6] The resolving power can be experimentally determined by measurement of the distance between two very small objects which are close enough together that they can just be distinguished as two separate objects. This experimental determination is necessarily rather rough and depends upon the contrast present. Since the resolution may vary for the different areas in a single micrograph, the value given is usually the optimum resolving power of the instrument.

The other major advantage of the electron microscope over the light microscope is its greater depth of focus. The electron microscope uses much smaller apertures than does the light microscope, and the depth of focus is a function of the reciprocal of the numerical aperture.

When electrons strike a substance they are either reflected, scattered, or transmitted depending upon the energy of the electrons and the product of the thickness and density of the substance.[7] Since, in general, objects are viewed by means of the

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[5] Zworykin, Morton, Ramberg, Hillier, and Vance, Op. cit., p. 84.

[6] Ibid., p. 123.

[7] R. B. Barnes and C. J. Burton, "The Electron Microscope," <u>American Dyestuffs Reporter</u>, XXXI (May 1942), p. 254.

transmitted electrons, the object must be thin enough to permit a sufficient number of them to pass through with little change in direction to form an image. Collisions with air molecules would scatter a beam of electrons so that they would diffuse instead of forming an image; therefore any instrument utilizing an electron beam as the medium for producing an enlarged image must be kept in a very high vacuum. The disadvantages of the electron microscope lie in the necessity for a vacuum in all parts of the instrument which the electrons traverse, in the fact that only very thin objects can be penetrated, and in the limitations of the high temperatures which a specimen must withstand. The electron bombardment heats the object to a very high temperature, and so any substance which is altered in appearance by the heat cannot be viewed satisfactorily in an electron microscope.

Chapter 3

CHARACTERISTICS OF THE MAGNETIC ELECTRON MICROSCOPE IN GENERAL

At the present stage of development the magnetic lens microscope is superior to the electrostatic lens instrument. The resolution obtained from the magnetic type is equal or superior to that obtained from any other type of electron microscope. Most of the research up to the present time has been done on the magnetic lenses. The basic parts that make up a magnetic electron microscope are the electron source, the lens system, the voltage supply, and the vacuum system.

The electron source for the microscope may be either the cold-cathode of a gas-discharge tube or the hot filament of a thermionic vacuum tube. In the gas-discharge tube the electrons are emitted when positive ions, formed in the gas, strike the cathode. These secondary electrons are accelerated by the difference in potential between the cathode and the anode and then pass through a very small aperture in the anode.

In the thermionic vacuum tube the filament is heated and emits electrons. The filament is surrounded by a cathode which serves as a guard cylinder when it is maintained at the filament potential or as a control when the potential of the cap can be varied with respect to that of the filament. The electrons pass through a fairly large opening in the cathode cap and are accelerated until they pass through the anode aperture.

The hot filament source is preferable to the cold-cathode since the former can be operated in a vacuum, whereas the latter requires a pressure of 10^{-2} mm. of Hg for its operation.[1] The small anode aperture makes it possible to maintain a better vacuum in the remainder of the system than in the gas-discharge tube itself, but even so the gas pressure may cause a fluctuation in the voltage and render the tube operation uneven. The hot filament tube has enough advantages over the gas-discharge tube that nearly all magnetic electron microscopes employ thermionic emission.

After emerging from the anode aperture, the electron beam passes through two or more magnetic lenses which serve to focus it on a fluorescent screen which gives off visible light rays when struck by electrons. Many of the instruments employ three lenses -- condenser, objective and projector. The condenser lens serves to control the convergence and intensity of the beam between the source and the object. The image of the filament formed by the condenser lens has a magnification of unity and is formed at the object. The condenser lens is omitted in some types, and in its place a smaller aperture is used; however for high resolution a condenser must be used.

The magnetomotive force for the condenser lens is supplied by a small iron-enclosed coil. The magnetomotive force for the objective and projector lenses is supplied by separate iron coils for microscopes which have variable magnifications. For

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[1] Zworykin, Morton, Ramberg, Hillier, and Vance, Electron Optics and the Electron Microscope, p. 126.

fixed-magnification instruments the two lenses can operate with just one coil. The objective and projector lenses both have pole pieces of some highly permeable material surrounding a small air gap. The objective lens produces an enlarged image of the object (the amount of magnification depending upon the magnetizing current), and the projector lens magnifies the image again just as the eyepiece lens does in a light microscope. The magnetic system, or at least the path between the objective and projector lenses, is shielded by some metal such as Permalloy so that stray magnetic or electric fields cannot cause distortion of the image.[2]

The magnetic electron microscope requires an extremely stable high voltage supply for the electron accelerating potential and an equally stable current supply for the lens coils. For some of the types of magnetic electron microscopes the high voltage is supplied by means of high voltage transformers used with a rectifier and a resistance-capacity filter to smooth out the output voltage. In this type of supply the output voltage varies with the input voltage, so a line-regulator of the saturated-core type is used to decrease the fluctuations in the output due to changes in the line voltage. This system of voltage supply gives poor regulation, and so much energy is stored up in the condensers of the filter that breaking the vacuum while the high voltage is on seriously damages the instrument.[3] A system which gives better results and is more widely used has an

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[2] Ibid., p. 160.

[3] Ibid., p. 224.

electronically-controlled rectifier with a high degree of feedback; this system gives an output voltage which is insensitive to changes in either the input voltage or the output current.

The current for heating the filament was first supplied by batteries, but it was found that alternating current could be used when precautions are taken to minimize the effect produced by the alternating magnetic field connected with the alternating current. The use of very small diameter tungsten wire for the filament reduces this effect sufficiently so that the alternating current can be used. Some microscopes can now take their entire power supply from 110 volt, 60 cycle lines. The lens current can be supplied from alternating source by using an inverse feedback loop. Since the magnetizing current must be constant, the resistances provided for varying the focus are made of wire with a temperature coefficient of zero.

In the vacuum system of the electron microscope, a rotary oil pump is used together with some type of diffusion pump. If the pump is a mercury diffusion type, a freezing-out trap is required since mercury vapor does not have a sufficiently low vapor tension at room temperatures. Some kind of oil with a low vapor tension is used in most of the microscopes so that no freezing-out trap is necessary. The vacuum systems are provided with a by-passing arrangement so that all the air which is evacuated will not have to be pumped through the small apertures in the column of the microscope. In large instruments a valving system makes it possible to shut the column off from the remainder of the vacuum system when it is necessary to admit air during the loading of specimens or photographic slides. Some small instruments can be completely evacuated in such a short time that it is not necessary to provide valving arrangements. The pumping-out time for most types of microscopes during operation should not exceed a few minutes when the vacuum system is functioning properly. A pressure of 10^{-4} mm Hg is low enough to give the electrons a mean free path of nearly three meters.[4] This pressure is small enough to prevent any gas discharges from taking place between the cathode and anode and to prevent deterioration of the filament by bombardment of positive ions. The number of electrons which collide with gas molecules at this pressure is practically negligible, so a pressure of 10^{-4} mm Hg is sufficient evacuation for electron microscopes.

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4 Ibid., p. 195.

Chapter 4

CHARACTERISTICS OF THE TYPE EMC-2 ELECTRON MICROSCOPE

The model EMC-2 microscope is a compact desk style with the electron gun and lens column assembly nearly horizontal. This arrangement is possible because the low fixed magnifications allow use of a comparatively short lens assembly. The main parts of the microscope are the electron gun and high voltage supply, the magnetic lens system with its low voltage supply, and the vacuum system.

In Figure 2 the electron gun is shown in position on the microscope, and in Figure 3 it is shown removed from the instrument with the cathode cap off so that the filament can be seen. The filament is a very fine V-shaped tungsten wire. The electrons are emitted from the vertex of the filament. The high voltage unit supplies the current for the filament through a step-down transformer. The leads to the filament extend through the back of the gun and are electrically insulated from the rest of the gun. The filament current is regulated by the intensity control knob which varies the resistance in the primary winding circuit. The cathode cap acts as a control for the electron emission from the filament. The cathode cap is at a negative thirty-thousand volt potential with respect to ground. There also exists a difference in potential of thirty-thousand between the cathode cap and the anode since the anode and anode aperture, as well as the case of the microscope, are grounded.

Fig. 2--Electron Gun on Column

Fig. 3--Gun with Cathode Cap Removed to Show Filament

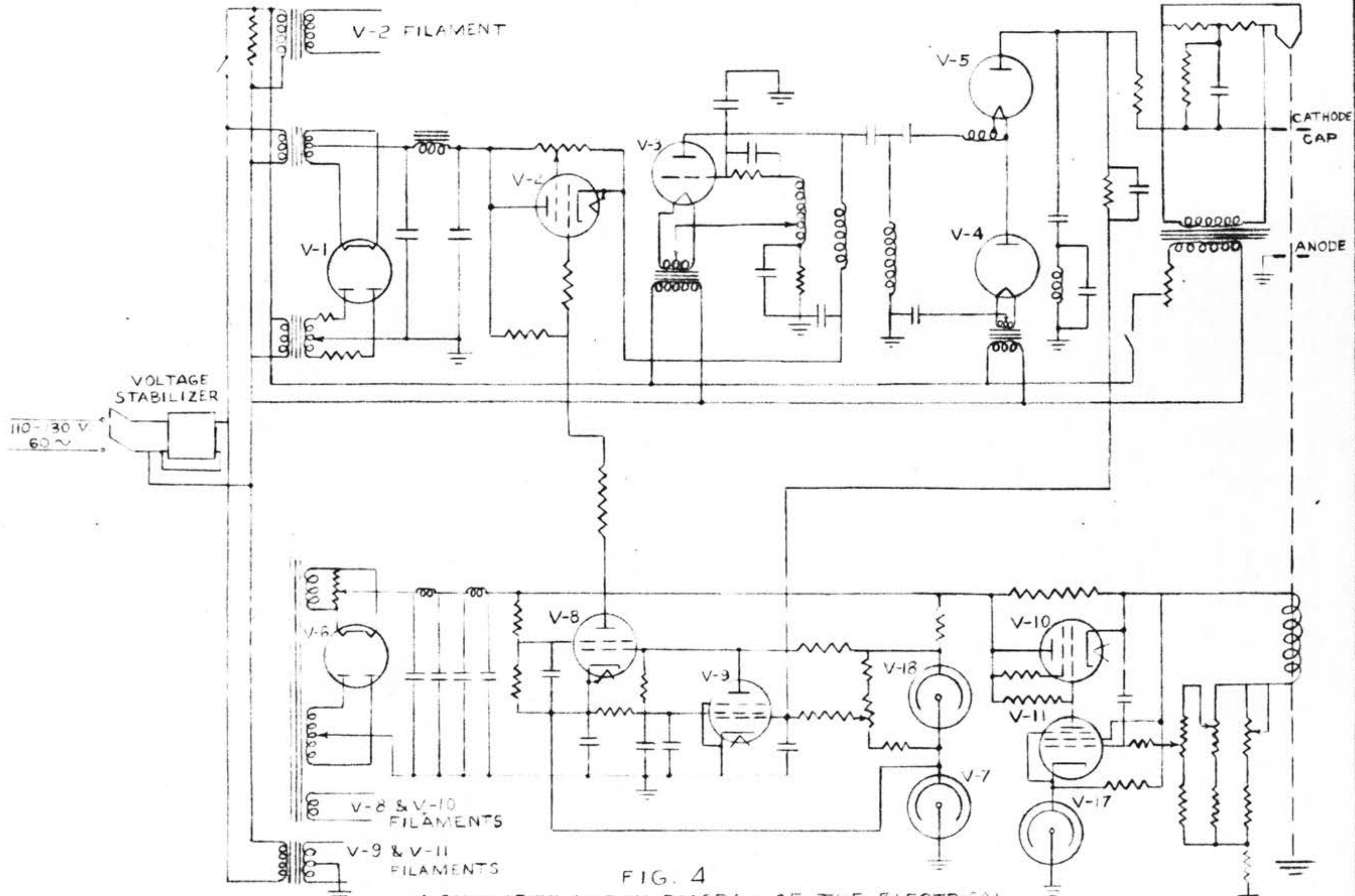

FIG. 4

A SIMPLIFIED WIRING DIAGRAM OF THE ELECTRICAL SYSTEM FOR THE ELECTRON GUN AND LENS COIL OF THE TYPE EMC-2 ELECTRON MICROSCOPE

As shown in the wiring diagram of Figure 4, the anode is positive with respect to the filament since the filament and cathode cap are connected to the negative high potential. The electrons are accelerated in passing from the cathode to the anode, and most of them pass through the anode aperture since the original pencil of electrons is narrow even with respect to the small aperture. The anode is in contact with the specimen chamber and helps conduct away the heat produced from the electron bombardment. The anode is made of fairly thick copper to facilitate cooling. The specimen itself is mounted just in front of the anode by means of the specimen holder.

After passing through the specimen, the electrons must be focused so that they produce an enlarged image on the fluorescent screen at the front of the column. The focusing is accomplished by the magnetic lens system which consists of objective and projector lenses. The gaps for the two lenses are connected in series in the magnetic system of the single electromagnet. The lenses fit into the pole piece assembly which also contains the objective aperture, the projector aperture, and the adjusting nuts. The objective aperture can be omitted from the assembly without seriously effecting the image produced. In fact, the omission of this aperture makes the alignment of the column easier, and the only loss is a slight reduction in the contrast obtained. The adjusting nuts make it possible to vary the distance of the lenses from the viewing screen, but in ordinary operation of the instrument the nuts remain fixed after being adjusted to give an approximate focus, and the focusing is done by varying the magnetizing current for the lenses. The single magnetizing coil is supplied with a very steady magnetizing current to prevent shifts in the focus after it has been set.

All parts of the microscope which the electron beam traverses must be evacuated, and for this a mechanical pump and an oil diffusion pump are provided. A valving arrangement shuts the diffusion pump off from the column until the mechanical forepump has evacuated it to a pressure around 150 microns of Hg. The diffusion pump is then connected to the chamber, and the evacuation continued to one micron of Hg. The graph in Figure 5 shows the pressure in microns of Hg for the different readings of the thermocouple gage on the microscope. In Figure 6 the curve shows the rate of decay of the vacuum when the pumps are turned off but are left connected to the column. The diffusion pump is never connected to the other system when the pressure is high and the oil in the diffusion pump is hot because air molecules cause the hot oil to deteriorate. When air must be admitted to the column, the pumps are shut off from it by another setting of the valve system; and the air which is admitted passes through a filter and drier. Keeping moisture and dust out of the instrument is important both in cutting down the amount of cleaning which the parts need and in minimizing the time to evacuate the chamber.

In order for the microscope to produce a clear, useful image of an object, the elements of the microscope column must be properly aligned with respect to each other. The proper spacing from the cathode cap to the cathode filament is very critical in the operation of the instrument. A good approximate

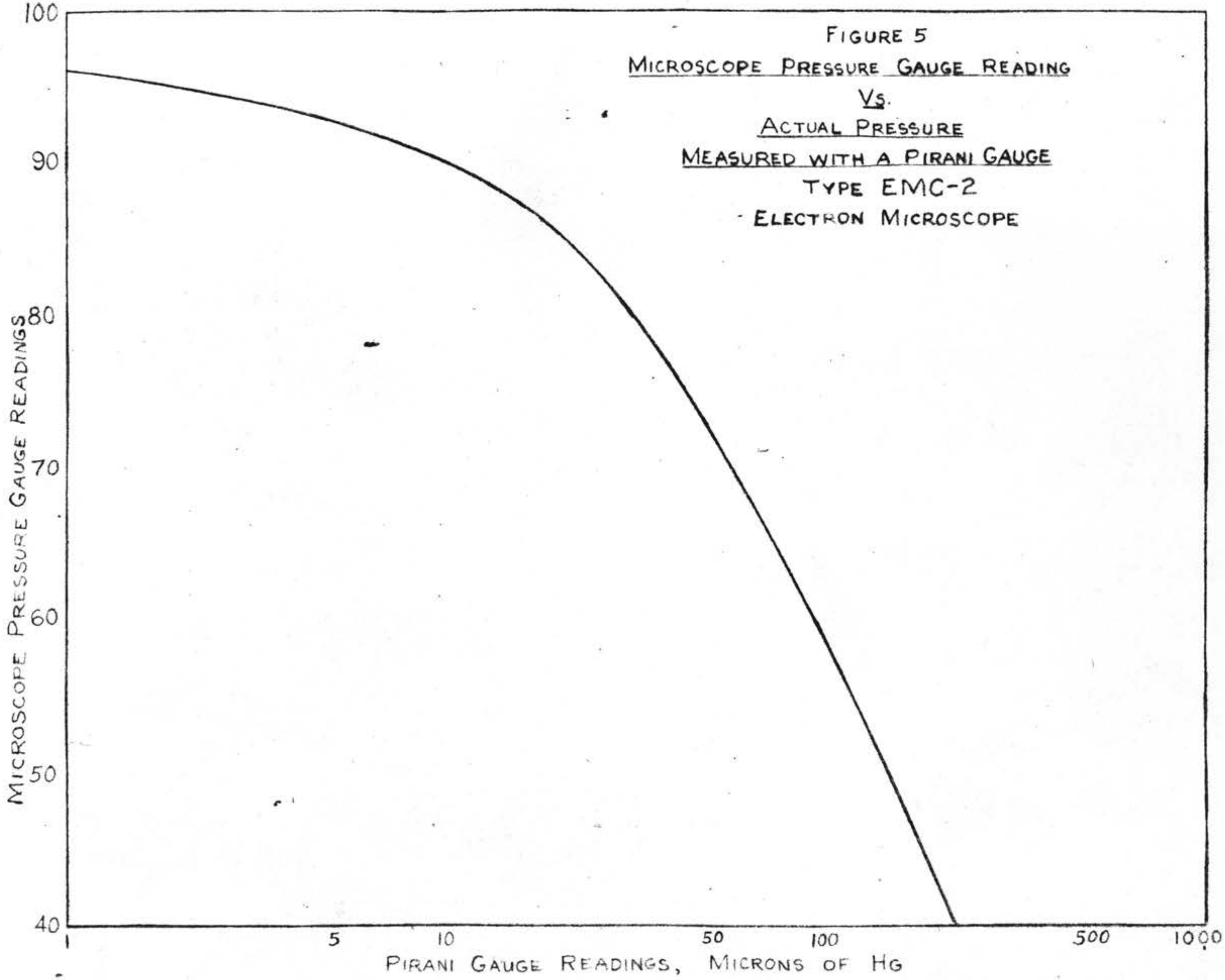

Figure 5
Microscope Pressure Gauge Reading
Vs.
Actual Pressure
Measured with a Pirani Gauge
Type EMC-2
Electron Microscope

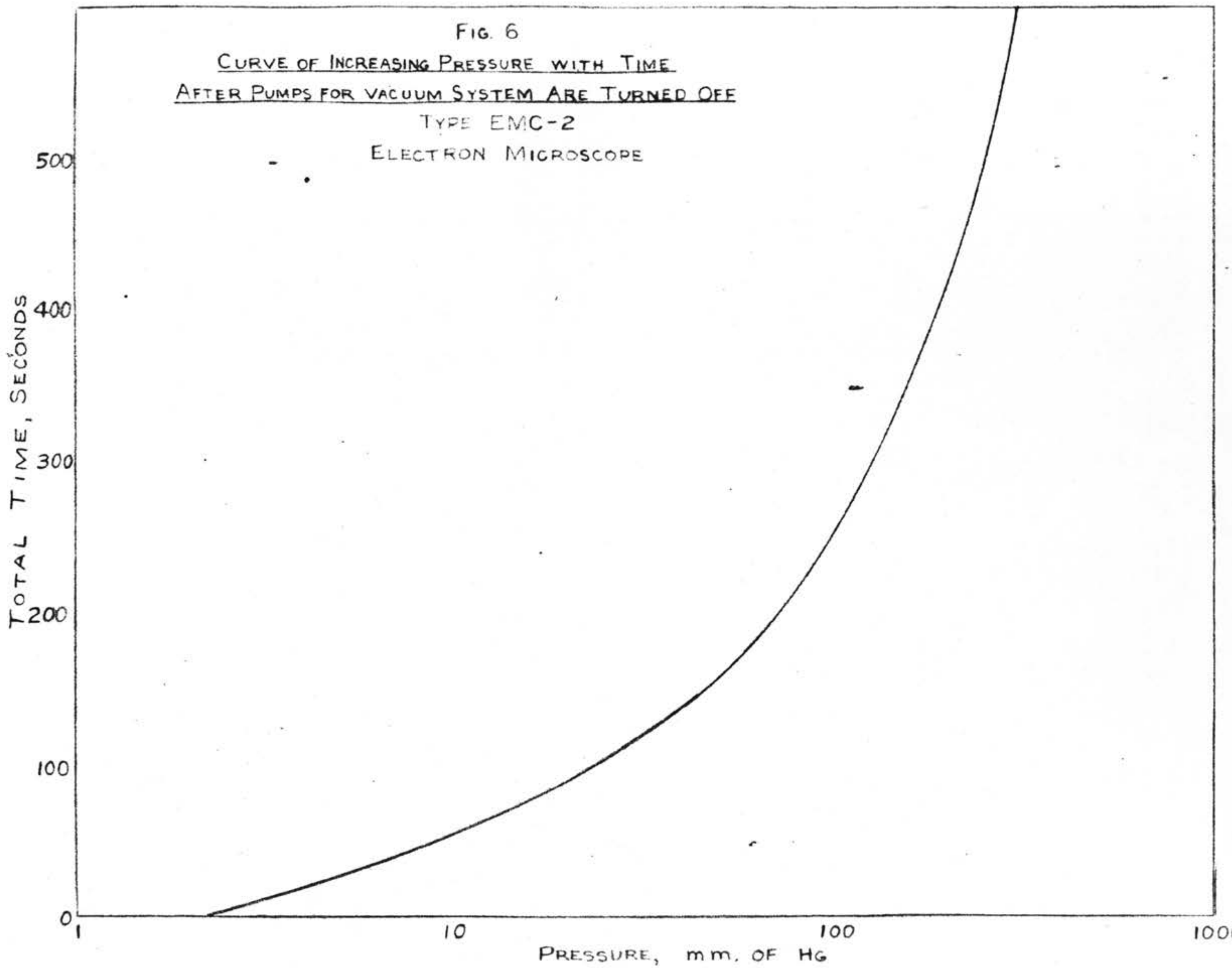

Fig. 6

Curve of Increasing Pressure with Time After Pumps for Vacuum System Are Turned Off

Type EMC-2 Electron Microscope

Fig. 7

Electron Gun in Height Adjusting Tool

Fig. 8

Rear View of the Electron Microscope with Hoods Removed adjustment is given by means of the height adjusting tool provided for the purpose. The electron gun is shown in Figure 7 placed in the height adjusting tool. A metal depth gage is inserted in the cap aperture, and as the cap distance from the filament is varied, the gage makes contact with the filament; consequently an electrical circuit is completed, and the light at the base of the tool flashes on. The electron gun is then replaced on the column and a check made to see if the image of the filament is visible at low beam intensities when the lenses and apertures are removed from the pole piece. If an image of the filament with bright striations cannot be seen at low intensities, the cathode cap should be adjusted slightly until it is possible to see the image. As the intensity is increased, the image should diffuse into bright general illumination. When the anode aperture is returned to the assembly, it is aligned by centering its image in the viewing screen by means of the two anode aperture adjustment screws. The remainder of the adjustments necessary to permit a beam of electrons to traverse the column and strike the viewing window are made by changing the position or tilt of the gun. Since the lenses are fixed with respect to each other and to their apertures, no adjustment of them is required. In Figure 8 the lateral adjustment screws by which the gun can be shifted laterally and the gun tilt screws by which the distance of the gun from the fixed part of the column can be varied are shown. Throughout all the adjustments, care must be taken to keep dust from the parts, and the parts should all be cleaned periodically with acetone. Dirt in the instrument gives unsteady images which shift or tend to fly apart.

The type EMC-2 instrument has a high enough resolving power that more details of the image are available than just those which are visible on the fluorescent screen. In order to make these details visible, the image is photographed. With fine grain development, a useful magnification of approximately ten diameters can be made. The amount of enlargement which is necessary to reveal all the detail depends upon the exactness of the focus and upon the contrast available in the specimen. This microscope uses a two inch square glass photographic plate, only one of which can be loaded into the instrument at a time. After each picture is taken, the vacuum must be broken so that the exposed film can be removed and another slide inserted. Before the picture is taken, the plate in its holder is rotated into position in front of the viewing screen. The photograph itself is made when the shield in front of the slide is rotated down during the desired time of exposure.

Chapter 5

SPECIMEN MOUNTING

The preparation of specimens to be viewed in the electron microscope is entirely different from that for light microscopes since the electron beam cannot pass through a substance much thicker than one micron. In place of the usual glass slide for supporting the microscope specimen, a small circular fine-mesh screen is used. The screen is usually either steel with approximately two hundred meshes per inch or copper with even smaller openings etched in the metal. Several of the openings can be viewed while the screen is in the microscope by use of the specimen stage controls which move the stage in any direction in a plane normal to the electron beam. Thus there is a better chance of finding the desired configuration on any given specimen screen. The screen is fitted into a small cylindrical cap which is then mounted on the specimen holder. The screen serves not only as mechanical support for the specimen but also conducts away some of the heat produced and protects portions of the object from electron bombardment. For that reason it is desirable to place the specimen on the side of the screen away from the electron source, whenever possible.

The specimen must be mounted in the openings of the screen firmly enough that the bombardment of electrons will not dislodge it. Only a few objects will serve as their own support. These fall into two classes--objects such as smoke which form chains of particles extending far enough across the openings to be clearly visible and substances such as plastics which form thin films across the screen apertures when the screens are dipped into the solutions and then dried. The magnesium oxide in the picture of Figure 9 shows how chains of smoke particles can extend beyond the support.

Self-supporting specimens are rare, and in general a separate means of supporting them must be found. The nature of the electron microscope makes it necessary for the supporting medium to be structure-free within the range of the microscope, mechanically very strong to withstand the bombardment, and not much more than one hundred angstrom units in thickness. The actual thickness which can be used depends on the density of the substance as well as the accelerating potential for the electrons. Some organic materials which have the necessary characteristics to make a satisfactory supporting film are collodion and polyvinyl formal. Films made of aluminum oxide are used when they must withstand a great deal of heat, but this substance has a higher mass density and is not as free of structure as the other types of films. Silica films can also be made, but the preparation is more difficult.[1]

The cellulose nitrates, such as collodion, are dissolved in amyl acetate in order to form a film of the desired thinness. For collodion, the solution is usually 2% by weight of collodion in amyl acetate. The amyl acetate has a very low

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[1] C. H. Gerould, "Preparation and Uses of Silica Replicas in Electron Microscopy", _Journal of Applied Physics_ XVIII (April, 1947), pp. 333-343.

surface tension in comparison to that of water so when a drop of the solution comes into contact with a water surface, the acetate spreads evenly over a large area. When the solvent has evaporated (amyl acetate is very volatile), an extremely thin film of collodion remains on the surface of the water. Only the rippled edge of this film can be seen. The specimen screens are placed on the film surface and pressed down to make sure that the film adheres to the screen. The screens can be picked up with a coating of film by pushing a clean glass slide vertically downward near the screens. The film folds up on either side of the slide, and the screens are lifted up between the glass and the film. Distilled water is used so that the film will be free of impurities which might prove confusing when a specimen mounted on the film is being viewed and also because impurities cause the film to rupture both in the mounting process and under bombardment by the electrons.

Collodion films are so easily ruptured that their use is not practical except in cases where the lack of contrast or some other quality of the specimen makes it essential to have the fine structure and extreme thinness of the collodion film. Even increasing the concentration of the collodion solution adds little to the strength of the film produced. The ruptured film in Figure 10 is a 2.5% solution of collodion which ruptured at ordinary operating intensities in the microscope. Collodion solutions deteriorate in a few months as can be seen from Figure 11 which is a photograph of a collodion film formed from a solution prepared six months before the film was made.

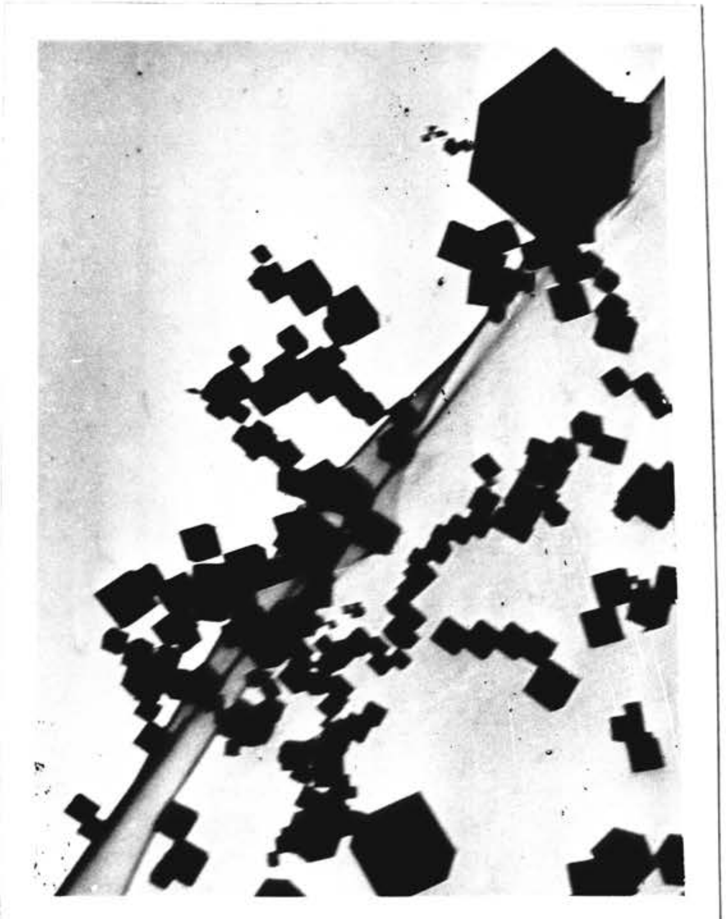

Fig. 9--Magnesium Oxide Smoke

Fig. 10--Ruptured 2.5% Collodion Film

Fig. 11--Film from Six Month Old Collodion Solution

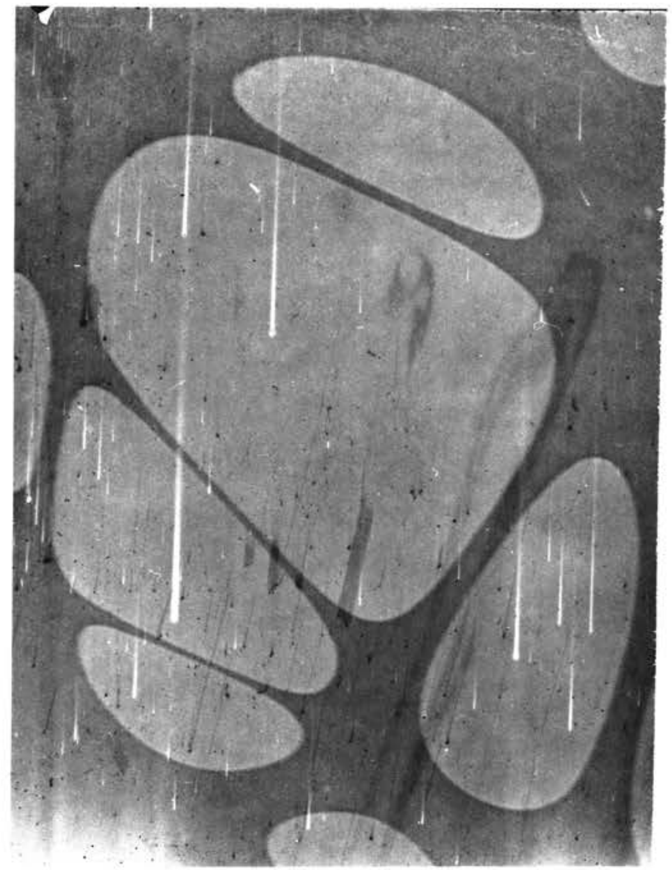

Fig. 12--Film Bubbles in Formvar

For films which can be prepared rapidly and stand up well under the electron bombardment, polyvinyl formal is preferable.

Polyvinyl formal (the trade name is formvar) is commonly dissolved in ethylene dichloride for forming a film. When the solution is to be formed on water, as in the case of collodion, a 1% by weight solution of formvar in ethylene dichloride gives good results. Since the ethylene dichloride has a higher surface tension than the amyl acetate, the solution in this case does not cover nearly as large an area as a collodion film does. For this reason the solution needs to be less concentrated for the formvar.

Another method of preparing formvar films, which has some special applications, consists of dropping the solution on a very smooth surface and then stripping the film after it has dried. For this method of preparation, a .5% solution of formvar in ethylene dichloride is used. If the surface of the object is sufficiently irregular, i.e., variations of 200-300 angstrom units in elevation, the film shows the impression of the surface when viewed in the microscope. Replicas of various surfaces are made in this way when the object is too thick to be viewed directly in the electron microscope. For making replicas of metal surfaces dioxane is the solvent to use since ethylene dichloride reacts with the metal. Several different methods can be used for stripping the film from the surface. When one edge of the film has been loosened with a razor blade or rolled slightly by the use of tweezers, the film can be floated off on the surface of water by slowly thrusting the object down into the water with the film surface vertical. The specimen screen is then pressed down onto the floating film. The screen and film are removed together on a glass slide. This method is easier to use when dioxane is the solvent. The film has more tendency to wrinkle when it is being removed from the water than film which is formed originally on the water surface since the area of the former is so much smaller. The film cannot be pulled directly from the surface and mounted on a screen because it would become distorted and wrinkled in the process. Films formed on solid surfaces instead of on water have the advantage of being relatively free of air bubbles. These bubbles lead to breaks in the film as shown in Figure 12. Another method of dry-stripping films consists of placing the screen on the film and pressing scotch tape down over both. The screen and film can then be pulled off with the tape. Sometimes the tape does not adhere very well; in which case, the application of moist air to the film surface makes the tape hold better. The tape is removed from the screen with a razor blade without damaging the film since it is on the opposite side. Any particles which remain on the screen from the tape are on the side away from the film and do not show in the microscope image since they are only on the wire.

Replicas produced directly from the surface to be studied give a negative film, i.e., the depressions in the surface are the thickest part of the film and so appear the darkest when viewed in the microscope. In order to produce a positive film, two impressions must be made. First, a metal such as silver is evaporated onto the surface, and then mechanically stripped from it. A film of formvar is then formed on the surface of the first replica. The metal can be dissolved by a suitable solvent, leaving a positive film.[2]

Some types of specimen can be dissolved in the film solution itself, and no additional means of mounting them on the film is necessary. However, a great many substances are changed in form when dissolved in the solution, and a different means of mounting the specimen on the film must be used for them.

Finely divided particles suspended in the air can be made to adhere to the film simply by passing the film through the air. Smokes, in particular, are mounted in this manner. There is sufficient adhesion to keep most of the particles on the film in spite of the electron bombardment as can be seen by the picture of magnesium smoke in Figure 13.

Many specimens can be suspended in some liquid, dropped on a film-covered screen, and allowed to dry. The liquid used must not be a solvent of the film material and preferably should not have a high surface tension since that causes the specimen to form groups of particles instead of spreading out evenly over the film. However, water is used in spite of its high surface tension since it dissolves so many substances without changing their structure, and it does not effect the film.

It is extremely important that extraneous material is not included with the object to be studied. The method of preventing this varies with the material; some preparations are

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[2] V. K. Zworykin and E. G. Ramberg, "Surface Studies with the Electron Microscope," Journal of Applied Physics, XII (September 1941), p. 692.

filtered, and some are centrifuged to eliminate the unwanted material. Since only extremely small particles can be observed in the electron microscope, it is frequently necessary to break down the material into smaller particles by means of grinding and homogenizing. Distilled water is used to clean all the objects which come into contact with the specimen, and the specimen is shielded as much as possible from the impurities in the air.

A three-dimensional effect is obtained by a process of metallic shadow-casting. After the specimen has been mounted in the usual way, it is placed in a vacuum system where a metal such as gold can be sputtered or evaporated onto the surface. When the specimen screen is placed at an angle other than ninety degrees, there are portions of the object which are not coated by the metal since the gold molecules travel in straight lines; thus shadows are formed behind all elevations on the specimen. These shadows increase the contrast and make small details easier to distinguish as well as giving a means of measuring the thickness of various parts of the specimen. The thickness can be calculated from the angle at which the metallic coating was deposited and the length of the shadow. The shadows produced by this process are reversed--instead of being dark, they are light. Pictures can be obtained with the shadows showing dark again by making a second negative, but the rest of the object would also be reversed, except in the case of film replicas which gave a negative picture originally. The pictures of film replicas which have been shadow-cast are easier to interpret if the second negative is made.

Fig. 13--Magnesium Oxide Smoke

Fig. 14--Material Soaked from Cotton

Fig. 15--Starchy Pollen Grain

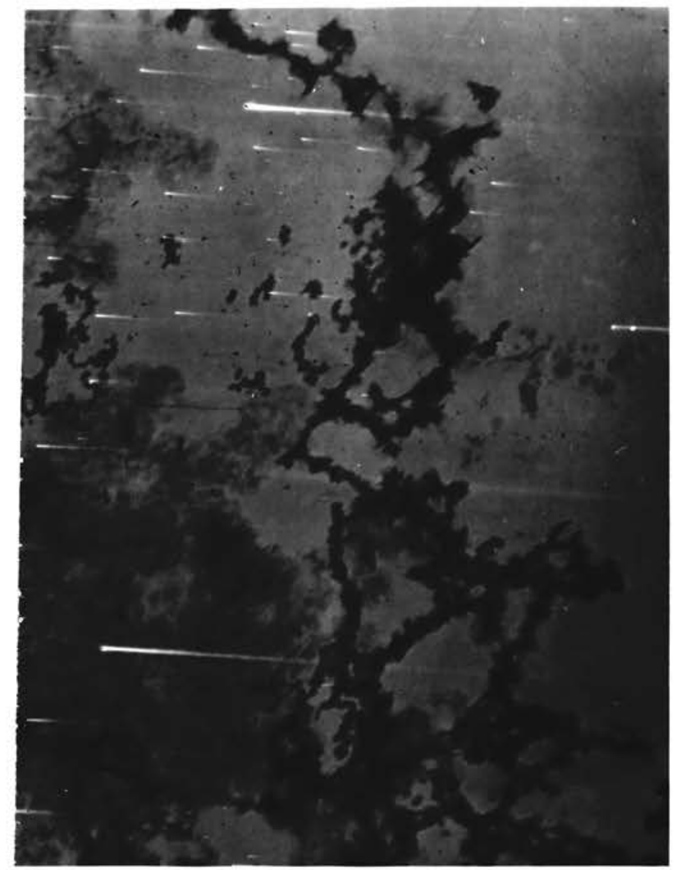

Fig. 16--Photographic Emulsion Exposed to Light

Chapter 6

APPLICATIONS OF THE TYPE EMC-2 ELECTRON MICROSCOPE

The electron microscope produces what is essentially a shadow picture of the object viewed. For sufficiently thin objects the shadow reveals internal structure as well as the outline of the object. In interpreting the micrographs, one must make allowance for the fact that the relative shading of the areas is due to a variation in mass density of the material as well as in the thickness.

There are several methods of obtaining at least an approximate value of the thickness of an object viewed in the microscope. A direct method which can be used for small uniform particles consists of using a collodion film and finding a break in the film where it has curled back, and a particle can be viewed on edge. A three-dimensional effect, which gives a measure of the thickness of an object, can be obtained by making stereomicrographs. In order to obtain stereoscopic views, the specimen holder in this type of microscope can be rotated to either five or ten degrees on either side of its normal position which gives a maximum angle of twenty degrees difference in the direction of the incident rays with respect to the specimen. Since the vacuum must be destroyed in order to rotate the specimen holder and also to change the photographic slide, a great deal of care must be exercised to see that the two photographed fields match.

The thickness determination of thinner objects can be made by comparison of the density of different areas of the photographic plate. The thickness x is given by $X = \frac{1}{SN} \text{Log} \frac{t_o}{t}$; t_o is the transmission of the negative for a part of the plate showing only the supporting film, t is the transmission for that part of the plate where the object supported by the film was photographed, S is the total cross section for scattering outside the angle limited by the objective aperture, and N is the number of scattering atoms per cubic centimeter. N can be found from the equation $N = N_o \rho / M$ where N_o is Avogadro's number, M is the molecular weight of the object, and ρ is the density.[1] The relative transmission of light through the negative, t_o/t, may be measured with a photometer. Measurement of particle thickness by means of shadow-casting has already been discussed.

Some specimens suffer a change under the bombardment by electrons in the microscope. While this change in the nature of the materials limits the use of the electron microscope, it also provides a means of studying the effect of the bombardment on various substances such as photographic emulsions. The effect of electron bombardment on the silver halide crystals of the emulsion can be compared with that of light.[2] The electron bombardment produces changes in many substances,

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[1] L. Marton and L. I. Schiff, "Object Thickness Determination," Journal of Applied Physics, XII (October, 1941),

[2] C. E. Hall and A. L. Schoen, "Application of the Electron Microscope to the Study of Photographic Phenomena," Journal of the Optical Society of America, XXXI (March, 1941) p. 281.

especially if the particle size is relatively large, but most of these changes are due to the heating effect or the collection of charge on the particles. The heating effect causes many substances such as cotton fibers to expand and char. The expansion makes the density of the specimen less as shown in Figure 14 of particles soaked from cotton fibers. The effect of a collection of charge on particles can be noted by the fuzzy outline of one particle when the remainder of the field is in focus. The charge on the one particle repels the electrons which pass close, and make that part of the specimen appear to be out of focus. Particles appear to charge and discharge periodically by coming into contact with other parts of the specimen which are not insulated from the metal support. The negative charges on the insulated particle attract the positive charges in the grounded particle, and the attractive force is strong enough to draw them together until the charge is neutralized.

Actual particle size can be determined by calibrating the instrument. The value of five thousand for the magnification obtained from the type EMC-2 microscope is approximate; for accurate determination of the size of objects viewed in the microscope, the magnification should be redetermined after any adjustments of parts of the pole piece or the electron gun. A quick and relatively easy calibration can be made by photographing a dry-stripped formvar replica of a diffraction grating used as the object in the microscope. Whether the replica has been distorted or not can be checked by determining its number of lines per unit length in a spectrometer. By mounting the specimen and replica together, it is possible to obtain a scale on the picture of the specimen; thus any distortion of the specimen would show a distortion of the scale also.

The applications of the type EMC-2 microscope are limited by the fixed magnification of approximately five thousand diameters which can be obtained directly and the maximum accelerating potential of thirty-thousand volts. Even though the photographs obtained can be magnified to some fifty-thousand diameters or more, the usefulness of the microscope is limited to objects which can be distinguished sufficiently well at five-thousand diameters to allow selection of the desired configuration. The accelerating voltage is the limiting factor in determining how thick a specimen can be penetrated by the electron beam.

In the field of biology the type EMC-2 electron microscope is less applicable to viewing sections of tissues than is the higher-voltage microscope. New high-speed microtomes which are capable of producing extremely thin sections are being developed. New sectioning methods may eventually make this low voltage microscope useful in this field of biology as it is in studies of bacteria, bacteriophage, and diatoms. Bacteria and bacteriophage have been photographed in various stages and states, and a great deal of inner structure, which could not be seen without the use of the electron microscope, is shown by micrographs.[3] The larger types of virus particles

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[3] Zworykin, Morton, Ramberg, Hillier, and Vance, <u>Electron Optics and the Electron Microscope</u>, pp. 285-300.

are within the range of this type of microscope, but they can be distinguished only after being separated from plant material by ultracentrifuging and filtering.

The type EMC-2 microscope is particularly well adapted to chemical and metallurgical studies. Many particles such as clays, smokes, crystals, colloidal suspensions, and pigments are the right size for viewing with this type electron microscope. Smokes, such as the magnesium oxide shown in Figure 13, give high contrast micrographs which show clearly the particle arrangement in chains and the particle shape. Aside from viewing directly many substances of small particle size, the microscope is also useful in viewing surface structures by means of film replicas for metallurgical and chemical studies.

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