PHYSICS.—*High centrifugal fields.*[1] J. W. BEAMS, University of Virginia. (Communicated by KARL F. HERZFELD.)

Gravitational and centrifugal forces no doubt were recognized by man very early in his development. The gravitational pull of the earth and the centrifugal force on his body whenever he moved about in a curved path certainly were ever present. It will be recalled from studies of mechanics that the gravitational force between any two bodies is proportional to the product of their gravitational masses divided by the square of their distance apart, while the centrifugal force is equal to the inertial mass of a moving body multiplied by the square of its velocity divided by the radius of curvature of its path. Also it will be recalled that the gravitational and inertial masses of any given body are proportional. Consequently, the action of a gravitational field and a centrifugal field on matter, for most purposes at least, may be considered to be essentially equivalent (1). In view of this equivalence we usually express centrifugal acceleration in terms of the standard acceleration of gravity at the surface of the earth since this is one of our most familiar concepts.

The intimate contact with centrifugal force in our every-day life undoubtedly accounts for its wide use in a multitude of our activities. One of its important uses, which we shall now attempt to discuss, is the production and application of high centrifugal fields to a few problems in physics, chemistry, biology, and medicine. Space does not permit a complete review of this rapidly growing use of centrifugal fields, so a large amount of important work necessarily will have to be passed over. Emphasis will be

placed upon certain problems not because they are thought to be more important than others but rather because my colleagues and I happen to have had more firsthand experience with them in our laboratory at the University of Virginia.

One important use of centrifugal fields is in the purification or separation of different substances and in the determination of particle or molecular weights, sizes, and shapes. It is, of course, common knowledge that larger particles settle out of a liquid or a gas faster than smaller ones. Just one hundred years ago Stokes (2) showed that the velocity of fall v of a spherical particle of density ρ_p and radius a in a liquid of density ρ_d and coefficient of viscosity η, in a gravitational or centrifugal field F, is given by the relation

$$v = \frac{2}{9\eta}\Big(\rho_p - \rho_d\Big) a^2 F. \tag{1}$$

It will be observed from this relation that the rate of settling in either a gravitational or centrifugal field increases with density and with size of particle and hence may be used for separating particles of different sizes and different densities. However, from the above equation of Stokes alone, all uncharged particles or molecules in a convection-free suspension or solution might be expected to settle out after a sufficient time has elapsed. This, of course, is contrary to observation since, for example, an unsaturated solution of sugar or salt may be left to stand or centrifuged in a low-speed centrifuge as long as desired without appreciable sedimentation. This apparent absence of sedimentation is due to the fact that diffusion always opposes sedimentation. Diffusion arises from the thermal agi-

tation or Brownian motion of the particles and molecules and tends to produce a transport of the particles or molecules from regions of high concentration to regions of low concentration. If the particles are large enough, their velocity of sedimentation in the gravitational field or low-speed centrifuge is much larger than their back diffusion, and so they are precipitated. However, as the size of the particle decreases the magnitude of the centrifugal force must correspondingly be increased in order to overcome the increased back diffusion. When the particle reaches molecular dimensions the centrifugal field required for appreciable sedimentation becomes quite large, but with the centrifuging technique now available appreciable sedimentation of almost any molecules or atoms can be obtained.

Unfortunately, the general theory of sedimentation in real liquids has never been worked out completely, but a good theory does exist for sedimentation in ideal incompressible liquids. Mason and Weaver (3) solved this problem for sedimentation in a uniform gravitational field in 1924, and Archibald solved it for sedimentation in a centrifuge in 1938 (4). According to Lamm (5) the differential equation for sedimentation of uncharged particles or molecules in an ideal, dilute, incompressible solution enclosed in a sector shaped centrifuge cell rotating with an angular velocity ω is given by

$$\frac{1}{r} \frac{\partial}{\partial r} \left\{ \left(D \frac{\partial c}{\partial r} - \omega^2 r S c \right) r \right\} = \frac{\partial c}{\partial t}, \quad (2)$$

where r is the distance of the material from the axis of rotation, c is the concentration, t the time, D the diffusion constant, and S the Svedberg sedimentation constant or the velocity of settling of the solute or particles in unit centrifugal field. Faxen (6) and Soyten (7) each have given approximate solutions to this equation, and Archibald has given the general solution. Recently Archibald has greatly simplified the calculations necessary in applying the theory to experimental results (8). Many years ago Svedberg (9) derived the equations for sedimentation in a sector-shaped centrifuge

cell (equation 2 above) for two most important special cases. In the first case he assumed that if the centrifuging process is continued long enough, diffusion will exactly balance the sedimentation and equilibrium will be established, i.e., $\partial c/\partial t = 0$, and the change in free energy throughout the centrifuge vanishes. This gives

$$M_e = \frac{2 RT \ln c_1/c_2}{(1 - V\rho)\omega^2(r_1{}^2 - r_2{}^2)} \quad (3)$$

and

$$V_e = \frac{2 RT \ln c_1/c_2}{N(\rho_p - \rho_d)\omega^2(r_1{}^2 - r_2{}^2)}, \quad (4)$$

where M_e is the particle or molecular weight, V_e is the volume of the particle or molecule, c_1 and c_2 are the concentrations at distances r_1 and r_2 from the axis of rotation, respectively, ρ, ρ_p, and ρ_d are the densities of the solution, particle and solvent respectively, V is the partial specific volume of the substance, R is the gas constant, N is the Avogadro number, and T is the absolute temperature. It will be observed that equation (3) is merely the statement of the Maxwell-Boltzmann distribution in the centrifugal field. It subsequently has been derived from thermodynamic reasoning by Svedberg and Tiselius. If the solution is an electrolyte which dissociates into m n-valent cations and n m-valent anions, where the numbers n and m contain no whole number factor,

$$M_e = \frac{2(m + n) RT \ln c_1 f_1{}^{\pm}/c_2 f_2{}^{\pm}}{(1 - V\rho)\omega^2 (r_1{}^2 - r_2{}^2)}, \quad (5)$$

where $f_1{}^{\pm}$ and $f_2{}^{\pm}$ are the activity coefficients (10). In most cases where the molecular weight is comparatively large and the solutions are not at the isoelectric point, or where the particles are charged, the electrostatic effects of the ions can be repressed by the addition of a low molecular weight electrolyte such as NaCl and the simpler equations (3) and (4) may be used instead of (5). Electrical charges on the sedimenting particles always reduce the velocity of sedimentation. In case the solute is ionized the sedimentation of the heavier ion is decreased and that of the lighter ion is in-

creased since the heavier ion drags the lighter ion along with it. When the centrifugal field is first applied to a solution containing ions an electrical potential appears between the axis and periphery. This electrical potential decreases as the sedimentation continues and vanishes when equilibrium occurs (11, 12).

In applying the above equations the concentrations and temperature are measured under equilibrium conditions, and hence to a first approximation the values of M_e are independent of particle or molecular shape. The partial specific volume V is usually measured outside the centrifuge and does not take account of hydration or solvation effects. However, Lamm (10), Kraemer (10), Pickels (13), and their collaborators have shown how to correct for these effects and have found that, in most practical cases, the correction is comparatively small, although there are exceptions. In cases where the centrifuge cell contains a solution with a number of different solutes equation (3) gives the concentration of each substance independently of the others as a function of the radius provided the solution is sufficiently dilute. Consequently by measuring the concentration in the centrifuge cell at a number of different radii, it is possible to determine the distribution of particle weights in the solution.

Although the above equilibrium method of equations (3), (4), and (5) gives most reliable values for M_e and V_e, the centrifuging time required for equilibrium to be established is very long when the molecular weight M_e is large. In fact, for many of the biologically important substances which have large molecular weights, this equilibrium time is of the order of several days or weeks.

In order to avoid this long centrifuging time Svedberg (9, 20) devised the rate of sedimentation method which is especially applicable to the measurement of the molecular weights and sizes of large molecular weight substances. In this rate of sedimentation method both the molecular weight and centrifugal field must be large enough to produce a sedimentation velocity that can be measured directly. If the particles or molecules are uncharged and the solution is ideal and dilute and if the experimental conditions are such that molecular or particle reflection from the ends of the sedimenting column can be neglected, then the sedimentation force per mole may be equated to the frictional force per mole

$$M_s(1 - V\rho)\omega^2 r = f \frac{dr}{dt}. \qquad (6)$$

The frictional force $f = RT/D$ where D is the diffusion constant. This gives for the molecular weight M_s

$$M_s = \frac{RT}{D(1 - V\rho)} \frac{dr/dt}{\omega^2 r}$$

$$= \frac{RT}{D(1 - \rho V)} S, \qquad (7)$$

where S is the velocity of sedimentation in a unit field, and is usually expressed in Svedberg units, i.e., in units of centimeters per second per unit field of force multiplied by 10^{13}. Actually most of the values given for S have been reduced to the equivalent velocity of sedimentation S_{20} in water at 20°C by the relation

$$\frac{S_t}{S_{20}} = \frac{\eta_{20}}{\eta_t} \frac{1 - V_t\rho_t}{1 - V_{20}\rho_{20}}.$$

In this method each molecular species gives rise to an easily distinguishable boundary between the solvent and the sedimenting column so that dr/dt and hence S can be quite accurately measured. As the time of centrifuging increases, this sedimenting boundary becomes progressively blurred because of diffusion. However, for a comparatively large molecular weight substance, this blurring is small, and furthermore diffusion theory shows that the position which the boundary would have occupied had there been no diffusion is the place where the concentration is one half that in the unaffected sedimenting column adjacent to the boundary. The concentration in the column of sedimenting material decreases slowly as the centrifuging progresses since the particles move out along the radius and hence diverge. Also the centrifugal field increases toward the periphery which

in turn increases the rate of sedimentation and hence reduces the concentration in the sedimenting column. If c_o is the original concentration and c_t is the concentration at any time, t, then Svedberg and Rinde (9, 10) have shown that

$$c_t = c_o \frac{r_o^2}{r_t^2} \tag{8}$$

where r_o is the radius of the meniscus and r_t is the radius of the boundary at the time t. When the solution contains several sedimenting substances, each forms its characteristic sedimenting boundary so that a molecular weight analysis of the solution is automatically obtained. Also if the solution contains a distribution of molecular weights and sizes rather than a homogeneous molecular species, the rate of sedimentation method can give this distribution.

It will be observed from equation (7) that in order to get the molecular weight M_s the diffusion coefficient D must be measured. This may be carried out either during the centrifuging process or in separate experiments. Both methods give essentially the same results so the latter method is often used. If the particles or molecules are known to be spherical and hydration effects can be neglected, the frictional constant f_o can be calculated from the following equation of Stokes:

$$f_o = 6\pi\eta N \left(\frac{3MV}{4\pi N} \right)^{1/3}, \tag{9}$$

which when substituted into equation (6) gives the molecular weight M. The ratio of the measured frictional constant f of equation (6) to the calculated value f_o [equation (9)] is always equal to or greater than one. If the amount of hydration is known the approximate shape of the molecules or particles can be estimated.

The equilibrium method [equation (3)], which has its greatest application in the smaller molecular weight range, and the rate of sedimentation method [equation (7)], which is adapted principally to the larger molecular weight range, recently have been supplemented by a method made possible by the solution of equation (2) by Archibald. Archibald (8) has put the results of his

mathematical solution in a convenient form for use, and it is hoped that it will provide increased reliability for the results especially in the large molecular weight range.

Although the above outline of the theory is very brief and incomplete, it does show that a sufficiently powerful centrifuge may be used as an analytical tool for the determination of particle or molecular weights, sizes, and shapes or that it may be utilized for the purification of materials or the separation of a mixture into its various pure components. The value of this tool in biological, and medical research is enhanced by the fortunate fact that most of the biological compounds in solution are not deactivated by high centrifugal fields so that the measurements obtained are for this material in its natural state.

For analytical work the centrifuge must produce a centrifugal field sufficiently intense to give an appreciable sedimentation and the solution being centrifuged must be convection free. It can be shown that in order to meet the latter requirement the centrifuge cell should be sector shaped so that the sedimenting particles can all move freely in the direction of the centrifugal field and that the centrifuge cell should be at as nearly uniform temperature as possible.

SVEDBERG ULTRACENTRIFUGES

Svedberg and his associates not only pioneered in the use of the convection free centrifuge for analytical work, but they have provided us with a major portion of the molecular weight data obtained so far by the centrifuge method (10). No attempt will be made here to discuss the Svedberg ultracentrifuges, since they are described in detail in a book by Svedberg and Pederson (10), except to mention that they consist of two principal types. The first type develops comparatively low centrifugal fields (500 to 15,000 g). It is driven by an electrical motor and supported in ball bearings. The rotor spins in hydrogen and carries a sector-shaped cell with quartz windows which contains the material to be centrifuged. The sedimentation is viewed optically through the quartz windows. This type of ultracentrifuge is used principally

for sedimentation equilibrium measurements. The second type of ultracentrifuge develops much higher centrifugal fields (15,000 to 750,000 g) and is driven by twin oil turbines. The material being centrifuged also is contained in a sector-shaped cell, which has quartz windows so that the sedimentation can be recorded by optical methods. The rotor is surrounded by hydrogen at about 20 mm pressure which conducts the heat generated by the friction in the bearings, by gaseous friction on the rotor, and by oil impinging on the turbines, to the casing. This type of ultracentrifuge has been used primarily for rate of sedimentation measurements.

AIR-DRIVEN VACUUM-TYPE ULTRACENTRIFUGE

The vacuum-type air-driven ultracentrifuge originally was developed to avoid the troublesome effects of air or gaseous friction on high-speed rotors or centrifuges (14, 15, 16) This gaseous friction at high rotor speeds not only consumes a large amount of power but also it heats the centrifuge rotor. This heating of the centrifuge rotor is greater on the periphery than on the axis, and thermal gradients in the rotor are set up which cause convection in the centrifuge cell. Since the forces that produce this convection increase with the centrifugal field thermal gradients necessarily should be as small as possible if the data are to be reliable. Essentially this machine consists of a large centrifuge rotor located inside a vacuum-tight chamber, a small air-driven air-supported turbine situated above or below the chamber, and a thin flexible shaft which fastens them together and which is coaxial with their common axis of rotation. Several variations in the design of these convection-free vacuum-type ultracentrifuges are in use by different workers (16), but Fig. 1 gives a diagram of a machine used at Virginia for analytical work which will serve to illustrate the principles of the apparatus (17). The rotating members consist of the flexible steel shafts A (0.1 inch) and A' (0.1 inch), the driving turbine T, the centrifuge rotor RA, and a magnet M. The shafts are vertical and coaxial and turn in the hard babbitt bearings G_1, G_2, G_3 and a

very loose (15-mil clearance) sliding guide SB. G_1, G_2, and G_3 are mounted in flexible round neoprene rings and are oil sealed. G_3 is lubricated and sealed with a low vapor pressure oil so that a good vacuum can be maintained in the steel vacuum chamber V. The guide bearing SB serves to prevent "swinging" of the rotor at low rotational speed. At running speed the shaft A' runs free without touching in the 15-mil clearance. The rotating members are supported by an air-thrust bearing formed between the Bakelite collar B and the turbine T. This collar is fastened to a flexible neoprene support D. The centrifuge rotor RA is a Duralumin analytical rotor with the cell C, which contains the solution to be centrifuged 65 mm from the axis of rotation. Fig. 2 shows a picture of this rotor and the cell. The rotor is made oval shaped to increase its strength, and the cell is sector shaped with crystal quartz windows cut perpendicular to the optic axis for observing the sedimentation optically. The permanent magnet M together with the field FC provides a speed control for the ultracentrifuge. M is a magnetized, hardened steel rectangular bar $1\frac{1}{2}$ inches long, $\frac{1}{2}$ inch wide, and 9/16 inch high. It has a hole in the center for the shaft A, to which it is rigidly attached by set screws or a steel clutch. The field coils FC consist of a large number of turns of well-insulated copper wire wound on a laminated field core. The laminations were (0.02 to 0.0075 inch thick) silicon steel laminations to give a total thickness of 9/16 inch. The copper wire was no. 21 enameled, and sufficient turns were used to give an inductance greater than 30 millihenrys for the coils connected in series.

To operate the centrifuge the vacuum chamber V and rotor RA are first thermostated to the desired running temperature. The material to be centrifuged is then placed in the vacuum-tight centrifuge cell which is tightly sealed and placed in RA. Electrical condensers are next connected in series with the field coils FC, which possess the proper capacity to make the circuit resonate at a frequency slightly above the desired running speed. Oil is next forced into G_1, G_2, and G_3 and the vacuum chamber V evacuated to less than 10^{-5} mm Hg through

P. Compressed air then is applied through *SI* to the air support at a pressure (10 to 15 lbs/in²), which allows the rotating members to turn freely. The driving air is next admitted through *DI* to the turbine *T*, and the centrifuge starts spinning. The centrifuge will continue to accelerate until electrical resonance starts in the circuit. The rotating magnet *M* induces an e.m.f. in the field coils with a frequency equal to its frequency of rotation, but the current in the circuit is negligible until the circuit starts resonating. When resonance starts the current in the circuit increases abruptly and the reaction is such as to brake the magnet. If now the air pressure to the turbine is set a few lbs/in² above that necessary to maintain this speed of the centrifuge, the resonant circuit will absorb the surplus energy, and the rotor speed will be maintained

constant. In practice, with an ordinary air-pressure regulator in the air line, the speed of the centrifuge automatically remains constant to less than 0.1 per cent as long as desired. Also, if no changes are made in the driving air pressure or circuit, the rotor speeds will check to about 0.1 per cent on successive runs. An idea of the critical braking action of this speed control can be obtained from the fact that, in our experiments, doubling the driving air pressure increased the rotor speed (at about 1,000 r.p.s.) by less than 1.5 per cent. Also our air turbines are not able to drive the centrifuge through resonance with six times the normal driving air pressure, which was all we had available on our pressure line. It will be observed that no over-heating occurs when energy is absorbed in the control due to the fanning action of the magnet *M*.
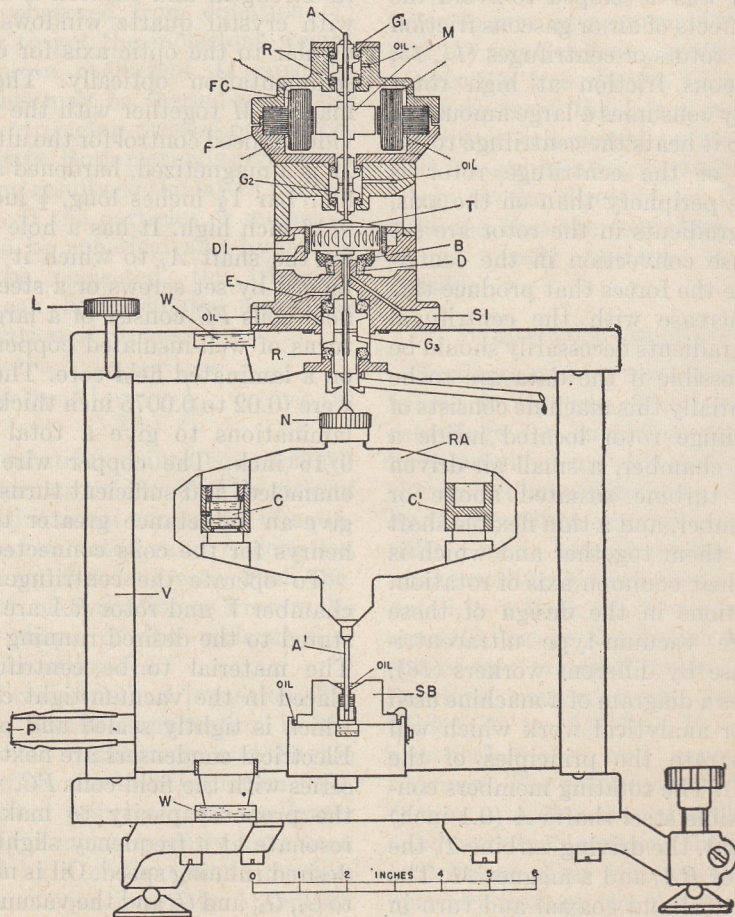

FIG. 1.—Air-driven air-supported vacuum-type ultracentrifuge with speed control.

Also any heat from the control is isolated from the vacuum chamber by the air passing through the air turbine below it. It should be pointed out that the only factor that limits the maximum centrifuge rotor speed is the mechanical strength of the rotor. Clearly the running speed can be set anywhere below this value. Since the centrifuge rotor spins in a good vacuum, no appreciable heat is developed and hence no temperature gradients are formed in the centrifuge cell. In some experiments hydrogen at a pressure of a few mm of Hg has been used to surround the rotor. In these experiments it was desirable to have a good thermal connection between the vacuum chamber walls V and the rotor. The low viscosity and hence low resultant rotor friction of hydrogen together with its comparatively large heat conductivity make it the best gas to use for this purpose. If the pressure of the hydrogen in V is not over a few mm Hg, convection-free sedimentation is obtained.

Several methods are available for measuring the concentration or sedimentation in the centrifuge cell optically. Most of these methods make use of the fact that the solute has a different absorption spectrum than the solvent or they employ the gradient of the refractive index in the cell. For many proteins, in order to get sufficient differential absorption, ultraviolet light must be used. This requires quartz optical parts throughout and hence is rather expensive. However, for the refractive index gradient methods, glass is used. Except in certain special cases the refractive index methods are preferable and a number of modifications of it are in use. Lamm (18, 10) developed a method in which a scale is photographed through the centrifuge cell. From the distortions in the images of this scale the refractive index gradient is determined. Philpot (19), Svensson (20), Anderson (21), Pickels (13), Williams (22), and others have used methods based upon the Toepler schlieren idea. Special tests carried out by Pickels showed that deviations of the light beam produced at any level of the centrifuge cell (at least up to 0.02 radian) can be determined photographically with a precision of less than 1 per cent and that the image positions of every radial distance in the cell can be produced with an accuracy of less than 1 per cent of the total centrifuge cell height. In our laboratory at Virginia, I. Foster is using a Jamin interferometer method for measuring the sedimentation in very dilute solutions. It is obvious, of course, that other than optical methods may be used for measuring sedimentation such as the radioactive radiations from atoms tagged to the sedimenting molecules, etc. It will be recalled that the present theory of sedimentation holds only for dilute solutions, therefore it is important in some cases at least to use a method of recording the sedimentation in as dilute solutions as possible. There is indeed a genuine need for better methods of recording sedimentation in very dilute solutions than are in use at present.

ELECTRICALLY DRIVEN MAGNETICALLY SUPPORTED VACUUM-TYPE ULTRACENTRIFUGE

The air-driven air-supported vacuum-type ultracentrifuge just described possesses practically all of the desirable features of an ideal centrifuge. However, it requires a supply of compressed air, which is not always readily available. Also a noisy air compressor is sometimes a nuisance to be avoided. Because of this we (23, 24, 16) developed at Virginia before the war the electrically driven magnetically
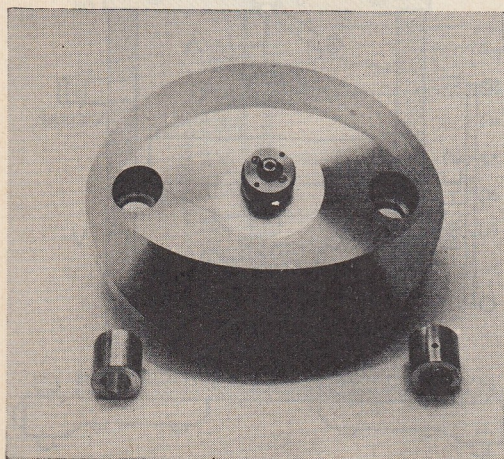

FIG. 2.—Analytical ultracentrifuge rotor.

supported vacuum-type ultracentrifuge shown in Fig. 3. The rotating components consist of the steel motor armature D, the steel magnetic support core R, the flexible shaft A, and the centrifuge rotor C. The shaft is hollow from G_3 to G_2, through which cooling water passes. The rotor C, flexible shaft A, vacuum chamber V, and oil glands G_1 and G_2 are the same as in the air-driven vacuum-type centrifuge described previously. The only differences are that an electrical motor is substituted for the air drive and a magnetic support is used instead of an air support. The small remaining weight is carried by a small thrust bearing consisting of the upper babbitt surface of G_3 and a small collar on the shaft. This small oil thrust bearing is designed to support the total weight of the rotating system in case the power supply of the lifting solenoid should fail. This type of magnetic support is almost friction free. The four field coils F of the motor are supplied with a high-frequency 2-phase alternating current in such a way that the field rotates with the same frequency as the alternating current. This rotating field induces currents in the steel armature D and causes it to rotate. This type of induction motor is well suited to this problem because a comparatively large starting and accelerating torque exists. Several ways may be used to produce the alternating power required. At Virginia an electronic generator is used with which the rotational speed of the centrifuge remains constant to 0.1 per cent as long as one desires. Fig. 4 shows a couple of rotor speed versus time curves taken on successive days. The entire apparatus operates from the 110-volt AC lines and is automatic. With the 6-inch Duralumin rotor of Fig. 3 weighing about 9 pounds, 1 kilowatt input to the motor at a frequency of 1,180 cycles per second accelerated the rotor to 1,000 r.p.s. in a little over 15 minutes, which compares favorably with the air drive. The power input to the motor required to maintain the speed constant at 1,000 r.p.s. was less than 500 watts.

The precision with which molecular weights can be measured with the air-drive (Fig. 1) and electrically driven (Fig. 3) centrifuges is about the same. In most

experiments the rotational speed can be measured to 0.1 per cent, the temperature to about 0.1°C, and the rate of sedimentation to at least 1 per cent. In the equilibrium method the ratio of the concentrations at the various radial distances of the cell can be measured to less than 1 per cent and the temperature and rotor speed held constant for the run to at least 0.1 per cent. The most uncertain element in the molecular weight determination of ultracentrifugation is in finding the true value for the partial specific volume V. As pointed out before this arises from the uncertain knowledge as to the amount of hydration or solvation present. There is urgent need for a reliable method of determining V in the actual solution. The three centrifuge methods, including the equilibrium, the rate of sedimentation, and the new method which makes use of Archibald's solution of equation 2, provide comparatively good molecular-weight data over a range in
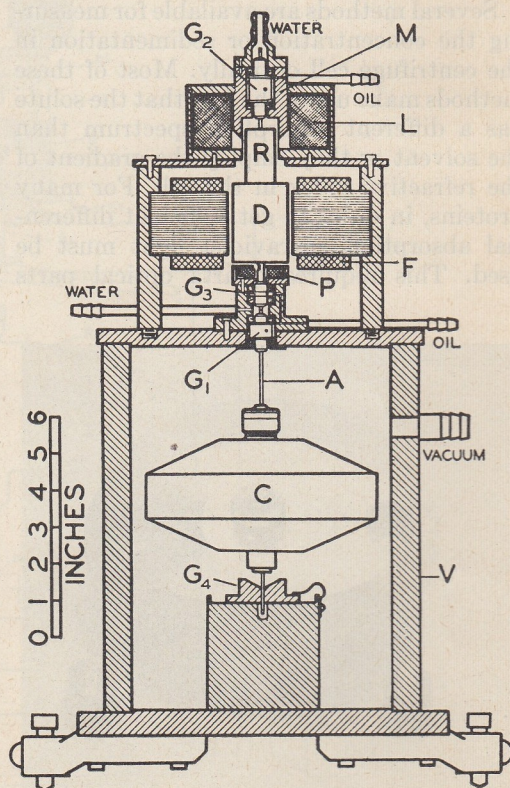

Fig. 3.—Electrically driven magnetically supported vacuum-type ultracentrifuge.

molecular weights from the lightest to the heaviest molecules known. The first equilibrium method (equations 3, 4, and 5) is used primarily for molecular weights below about 10,000, while the latter two methods are best suited for molecular weights above 10,000.

From the standpoint of biophysics, one of the most interesting facts that have come out of the molecular-weight data is the apparent existence in nature of very high molecular weight homogeneous compounds. Some of these compounds have molecular weights that exceed 10^7 molecular-weight units. These compounds are quite stable and, in some cases as far as can be determined, are composed of a single molecular species in which each molecule has exactly the same mass. Not all of these naturally occurring substances such as keratin, fibroin, and myosin are composed of homogeneous molecular species, but most of the easily soluble proteins have definite molecular weights. In some cases such as the respiratory proteins, which have been extensively studied in the analytical ultracentrifuge, the molecules dissociate into submultiples. For example, when a solution of haemoglobin is made very dilute, the molecules split into halves. In the case of a haemocyanin (*Helix pomatia*) solution at the isoelectric point, whole molecules exist alone with molecular weight of 8.9×10^6 provided the salt concentration is not too high. When the solution is made weakly acid, half molecules appear. If it is made alkaline, half molecules appear first followed by molecules one-eighth the original molecular weight, etc. Within the region of *p*H 4 to *p*H 9 the dissociation is reversible and the whole molecules will be reformed at the isoelectric point. In the case of some proteins Svedberg and, more recently, Pedersen (*10*) have interpreted their molecular-weight data as indicating that certain proteins they have studied have molecular weights that are integral multiples of 17,600. In other words, the building blocks of these proteins have a mass of 17,600 molecular-weight units. These conclusions have been questioned by a number of workers (*25, 26*) on the ground that the molecular-weight data are not good enough definitely to prove that the 17,600 unit exists. Whatever may be the answer to this most interesting and important suggestion of Svedberg, all workers in the field will agree that it would be highly desirable to obtain more precise data if it is possible. For example, an error of ± 1 per cent in a molecular weight of 10^7 is $\pm 10^5$ molecular units. Just before the war some experiments (*27*) were started at Virginia with the hope of examining more closely the homogeneity of some of these proteins, but these have been interrupted until the past few months.

Several alternate methods were considered including the electron microscope. This beautiful method, while giving the shape of the largest molecules, has its precision
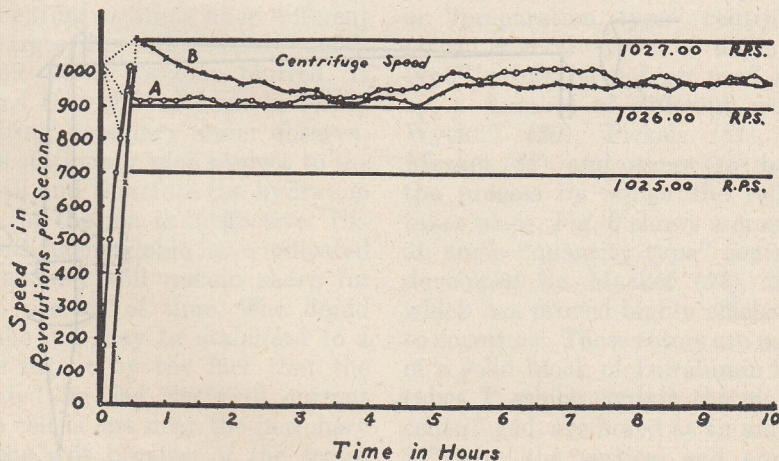

Fig. 4.—Rotor speed versus time curves on successive days for the electrically driven magnetically supported ultracentrifuge.

limited by a resolving power of from 10 to 20 Ångstroms (28). It also may deactivate the molecules under study. Therefore it seemed necessary to reexamine the centrifuge method, especially since the electron microscope technique was being vigorously pursued elsewhere.

In the rate of sedimentation method the resolving power or ability of the centrifuge to resolve two molecular species is approximately proportional to $\omega^2 rh$ where $\omega^2 r$ is the centrifugal field and h is the height of the cell. The strength of a centrifuge rotor is roughly proportional to $\omega^2 r^2$ where ω is the angular velocity and r is the radius of the rotor. In practice $\omega^2 rh$ is very roughly proportional to $\omega^2 r^2$, and so the resolving power is proportional to the bursting strength of the centrifuge rotor. Therefore, since, in the present centrifuges, the rotor speed is limited only by the strength of the rotor, there does not seem to be much promise of increasing the resolving power by increasing the rotor strength at least

until better rotor material is available. However, if we let T be the time that the sedimenting boundary remains in the field of view of the centrifuge cell, the resolving power becomes $(\omega^2 r)^2 ST$ or directly proportional to T. Now it is possible to increase the time T by flowing the solvent through the centrifuge cell at approximately the same speed with which the material settles out. This makes it possible to keep the sedimenting boundary in the field of view for long periods of time, thus greatly increasing the resolving power of the machine. Fig. 5 shows a schematic diagram of the arrangement in which the straight schlieren method is used for observing the sedimenting boundary. The solvent is injected at a controllable rate through the axis of the spinning centrifuge and flows through the centrifuge cell from the periphery toward the axis where it is collected in a large cavity in the rotor. If there is any nonhomogeneity in the substance being centrifuged, or if it consists of two or more
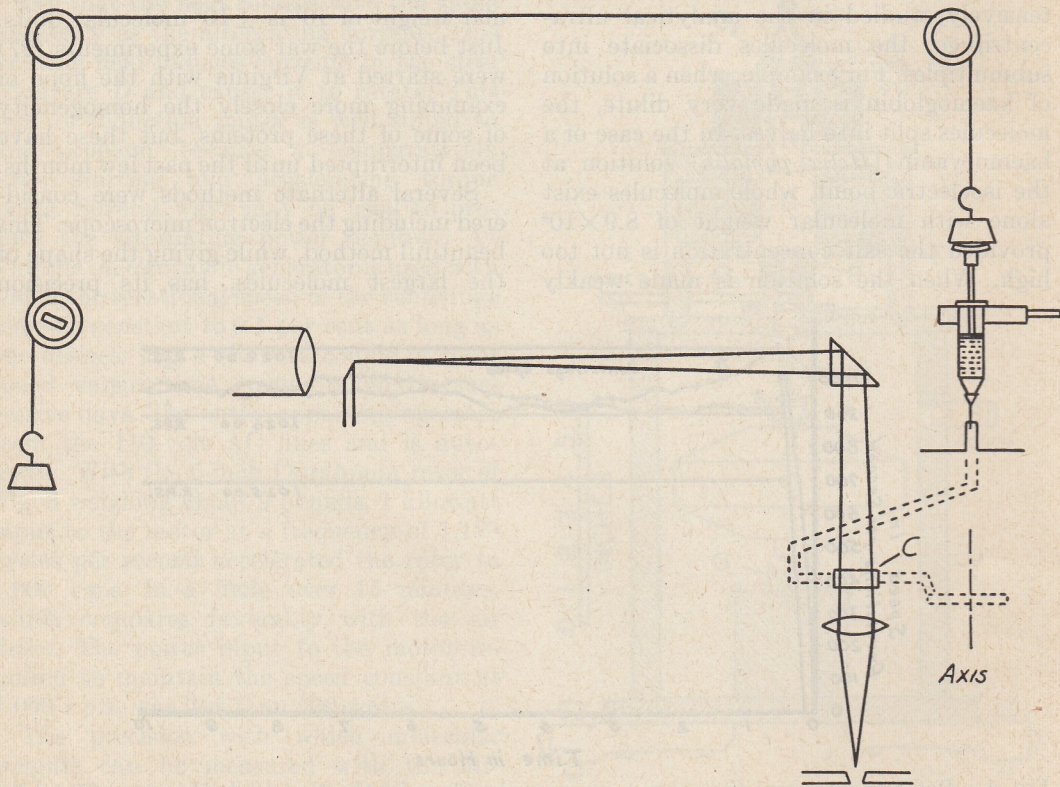

Fig. 5.—Schematic diagram of high-resolving-power ultracentrifuge.

molecular species, the sedimenting boundary will blur or split up into two or more sedimenting boundaries respectively. In some preliminary experiments haemoglobin was apparently homogeneous to at least 50 molecular-weight units. The ultimate limiting factor with the method seems to be a gradual dilution produced by the effect described in equation (8), but, with better optical methods of measuring sedimentation in dilute solutions, the method has promise of precision much greater than any yet obtained.

A second method of testing the homogeneity of large molecular weight substances also is being developed at Virginia. In this method the sedimentation in the cell is balanced by electrophoresis. Electrodes are placed in the centrifuge cell in such a way that a current can be passed through the cell along the radius. The solution containing the materials to be centrifuged is brought to a pH value not on the isoelectric point. The centrifuge is then spun to full speed and sedimentation allowed to take place until the sedimenting boundary moves to the middle of the cell. The electrical current is then passed through the cell in such a direction that this boundary remains stationary. Under these conditions, if the solute that forms the sedimenting boundary is either nonhomogeneous or composed of different molecular species, the sedimenting boundary will no longer remain sharp. Also, if the molecules or ions in the sedimenting columns have different electrical charges, the sedimenting boundary will split up or become blurred. It may be noted that the molecule or ion in the sedimenting boundary under observation remains stationary with respect to the centrifuge cell and therefore the hydration or solvation of the ion is ineffective. Experiments with haemoglobin have indicated that the boundary will remain sharp for considerable periods of time. The liquid column in the cell may be stabilized to a considerable extent by the fact that the heat generated by the electrical current through the cell is less near the periphery than near the axis because of the sector shape of the centrifuge cell.

The high-speed centrifuge not only may be employed as an analytical tool as discussed above but also it may be used as a means of purifying or separating substances that have different rates of sedimentation or different densities. As a matter of fact, this latter function is perhaps more important than the former. A great many substances of utmost importance in biology and medicine exist in nature in very dilute solution and in mixtures. In order properly to study these substances, it is usually necessary to purify and concentrate them by some means which does not destroy their biological activity. Often comparatively small changes in pH or temperature will destroy them so that the usual chemical means of concentration cannot be used. However, as mentioned previously, a high centrifugal field does not seem to deactivate them in any way; also the temperature and pH in the vacuum type ultracentrifuge can be accurately adjusted to any desired value within the working range. In most cases it is desirable to centrifuge comparatively large amounts of the natural solution in order to obtain a sufficient quantity of the purified substance to carry out the desired experiments.

The vacuum-type high-speed ultracentrifuges described in Figs. 1 and 3 may be used for the purification or concentration of substances instead of as an analytical centrifuge by simply removing the analytical centrifuge rotor from the shaft and placing a so-called angle "quantity type" or "preparation type" centrifuge in its place. Several types of angle "quantity type" rotors have been used successfully by a number of different workers, and Wyckoff (30), Pickels (31), Rosenfeld, Masket (32), and others (16) have studied the process by which the sedimentation takes place. Fig. 6 shows a cross section of an angle "quantity type" centrifuge rotor developed by Masket (32), at Virginia, which has proved highly efficient and easy to construct. These rotors are usually made of a solid block of Duralumin ST 14. The tubes T, which contain the material to be centrifuged, are bored at an angle of about 12° with the vertical and are drawn to scale in Fig. 6. A Lusteroid tube which fits snugly into T is completely filled with the
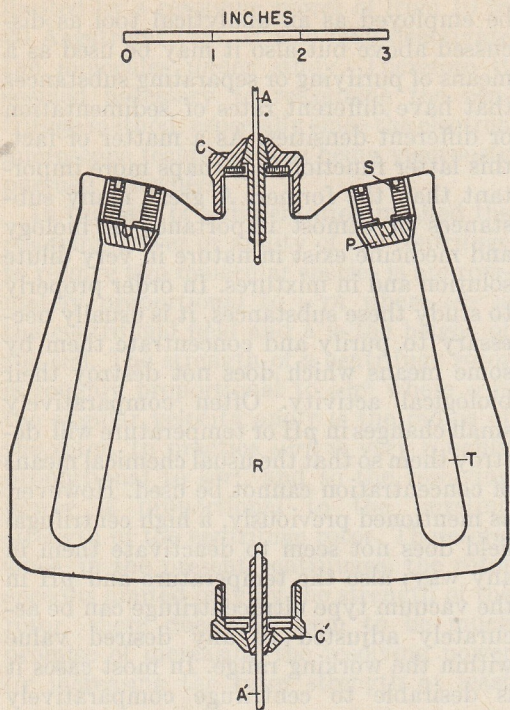
INCHES
0   1   2   3

FIG. 6.—Preparative or "quantity" type ultracentrifuge rotor.

weights, high strength steel no doubt could be used to a considerable advantage. The sedimentation that takes place in the tubes T is not completely convection free as in the case of the analytical rotors, but what convection exists aids the separation instead of hindering it. The material settles out almost to the outer wall and thus increases the density in this outer region. The force on this concentrated solution is then such as to cause is to move down to the bottom of the tube where it settles out. Several of the viruses actually crystallize out in the bottom of the tube. After the sedimentation is completed, the centrifuge rotor is allowed to decelerate to zero. The rotor may be decelerated rather rapidly down to about 300 r.p.s. and then move slowly on down

solution or suspension to be centrifuged. A leak-tight plug P is forced into T and the screw S tightened until a leak-tight seal is made. The tubes T are accurately spaced so that the rotor is in good dynamical balance when either full or empty. The rotor shown in Fig. 6 holds about 100 cc when all the tubes are filled, and the maximum centrifugal field in the cell is about 300,000 gravity. With the apparatus of Figs. 1 and 3, the strength of the rotor Fig. 6 is the only factor that limits the rotor speed and centrifugal field. The strength of a homogeneous centrifuge rotor depends not on its absolute strength directly, but upon this divided by the density of the rotor material. This is not quite true for the rotor of Fig. 6, which carried 100 cc of solution, but it is roughly correct. Duralumin ST 14, therefore, is almost as good as steel and possesses the added advantage of being machinable after heat treatment. Also it is considerably lighter, which allows it to be accelerated rapidly and lessens the damage done if an accidental explosion occurs. On the other hand, for the substances of lower molecular
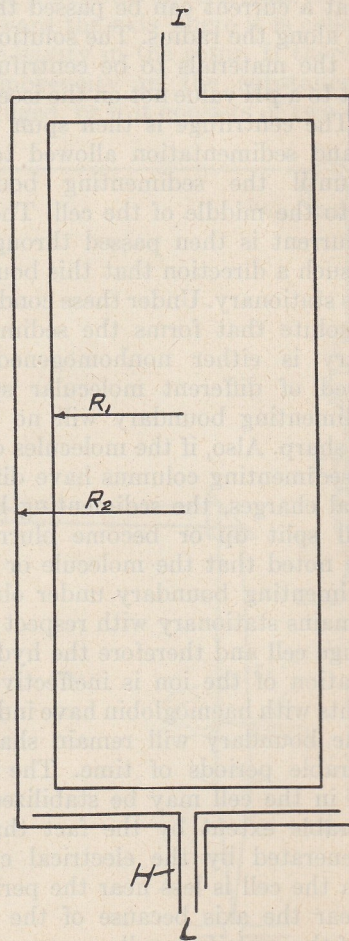

FIG. 7.—Schematic diagram of tubular centrifuge rotor.

to rest. The deceleration must be smooth in order to avoid remixing. The Lusteroid tubes which contain the material to be centrifuged are carefully removed from the rotor and placed in a vertical holder. The lighter fractions are then drawn off through a hypodermic needle or other arrangement which does not stir or remix the contents of the tube. Sometimes, when no material is actually deposited on the bottom of the tube, a method of displacement with a heavier nonmixable liquid may be used to separate the lighter from the heavier fractions. Pickels (31), Masket (32), and others have shown that a fairly precise analysis of the molecular weights of the substances being centrifuged can be obtained if this procedure is carried out with care. This analytical technique is most useful in many cases where the substance being concentrated is a very dilute active agent whose absorption spectrum or other optical properties are unknown.

In many cases it is desirable to process a very large volume of material, and then the above "quantity type" of high-speed centrifuge becomes impracticable. About 10 years ago at Virginia we attacked this problem by means of a continuous-flow tubular high-speed centrifuge (16). This work was dropped in 1940 because of the war and has not as yet been taken up again, although the results showed that the method was strikingly successful.

Fig. 7 shows a schematic diagram of the tubular centrifuge rotor used. This rotor consists of two concentric steel cylinders with radii R_1 and R_2 and length L. The inner cylinder is solid steel. The material to be centrifuged enters at I and then flows vertically down the annular space between the two cylinders where sedimentation takes place. The light fraction emerges through L and the heavy fraction through H. If the inner wall of the outer cylinder is accurately cylindrical some of the heavy material settles out on the wall, but if it is slightly cone shaped with the apex of the cone toward the top, the concentrated material will move down along the wall and if the exits are properly made will emerge through H, provided of course the concentration is not allowed to become so great

that it clogs the outlets. However, in most practical cases, the sedimented material is in dilute solution and it is desirable to have the concentrated material deposited on the wall. In practice, H is plugged and this material is deposited in a smooth film or coating on the inside of the outer cylinder and does not interfere with the sedimentation until it becomes too thick. At this stage the centrifuge rotor is stopped and the film may be ripped or scraped off of the wall and the rotor again put into operation. If the solution contains two materials which are sedimented out, the deposit on the top of the cylinder contains a larger percentage of the heaviest of these materials while the deposit on the bottom part of the cylindrical surface contains mostly the lighter component. Consequently by proper adjustments, the various components can be purified. Also from an analysis of this deposit as a function of the distance from the top of the cylinder an estimate of the sedimentation constants S_1 and S_2 and hence the molecular weights M_1 and M_2 of the two substances respectively can be obtained. If the solution contains an uncharged homogeneous molecular species as a solute with a sedimentation constant S, and if all this solute is deposited on the inner cylindrical wall of radius R_2, then the amount of solution F, in which the centrifuge can completely remove all of the solute per second, is approximately (16):

$$F = \frac{\pi L \omega^2 S}{2} \left(R_2 + R_1 \right)^2. \quad (10)$$

From this it can be seen that with the tubular centrifuges we described about 10 years ago, when we were working on this problem, one tubular centrifuge was equivalent to about 40 machines of the "quantitative type" of Fig. 6. Therefore this type of tubular centrifuge should be used when large quantities of material are to be processed.

If only very small quantities of solution are available the tubes T in Fig. 6 should be reduced in size so that they are always filled. Whenever possible, the vacuum-type centrifuge should be used because of the absence of thermal gradients. However, if

the material has a sedimentation constant above about 4 to 5 Svedberg units and only a very small amount of solution is available a rotor similar to Fig. 6 may be mounted on a small air-driven turbine shown in Fig. 8. Such apparatus is easy to make and costs only a few dollars (*16*). However, extreme care must be taken in stopping the rotor to prevent remixing. Also we have found that an artificial radial temperature gradient (decreasing from axis to periphery) introduced by focusing a hot-pointed heater on the region near the axis of the rotor tends to stabilize the sedimentation. Such thermal gradients also may be introduced by electrical means into the vacuum-type centrifuge rotors if desired, but if the pressure is below 10^{-5} mm Hg or if hydrogen at a pressure of less than a millimeter of Hg surrounds the rotor it is unnecessary. Also the vacuum-type ultracentrifuge can easily be operated between 4°C and 0°C, over which range in aqueous solution an increase of temperature increases the density, so the periphery may be made warmer than the axis of the rotor.
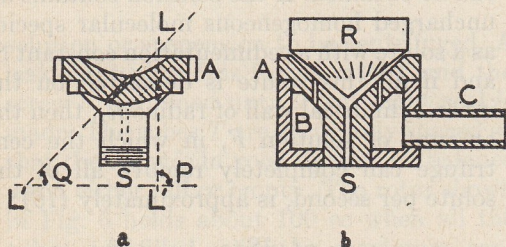

FIG. 8.—Small air-driven air-supported centrifuge.

In equation (6) it will be observed that if $V\rho = 1$ then $dr/dt = 0$ and the sedimentation stops. In other words, no sedimentation takes place if the density of the solvent ρ_d and the density ρ_p of the solute are exactly the same. Consequently, if a radial density gradient exists in a centrifuge cell over a range of densities which would include the density ρ_p of the solute, then the solute will seek a radial level in the centrifuge where the density of the solution ρ coincides with ρ_p. Clearly the sharpness of this radial level gives a measure of the homogeneity of the material being cen-

trifuged. Also the density $\rho = 1/V$ can be determined directly. The value of the partial specific volume V obtained in this way clearly may not be the same as the value of V in dilute solution due to possible changes in hydration brought about by the material added to the solution to increase its density. In the case of proteins, the value of ρ_p in the unsolvated state is about 1.3 gm/cc although values from 1.1 to 1.5 gm/cc have been reported. Hence in order to carry out experiments with proteins, a comparatively low molecular weight inactive material must be added to the water solvent such as pure sugar.

The radial stratification of substances of different densities can be carried out very conveniently with the vacuum-type centrifuges of Fig. 1 where the process can be observed. The smallest density gradient is set by the compressibilities of the solvent and solute where a pure liquid solvent is used, but where other low molecular weight substances are added, the major portion of the density gradient can be calculated by equation (3), since equilibrium must exist before a stable density gradient is established. With the vacuum type of centrifuge rotor of Fig. 1, the radial density gradient in the centrifuge cell which contains pure water is approximately 1 per cent per cm with a rotor speed of 1,000 r.p.s. E. N. and E. B. Harvey, H. W. Beams, and others have discussed the possibility that this density gradient centrifuge method might be used for the separation of the chromosomes and other cell components. Recently E. N. Harvey (*33*) has calculated the density gradients necessary to separate certain X and Y chromosomes in man and in some animals and has concluded that they might be separated in the vacuum-type ultracentrifuge similar to that in Fig. 1. The high resolving power ultracentrifuge of Fig. 6 also would seem to be well suited to this problem of the separation and collection of the chromosomes or other cell components.

The relative displacement of the various components of the biological cell in centrifugal fields has been studied by a number of different workers with most interesting and important results. No attempt can be
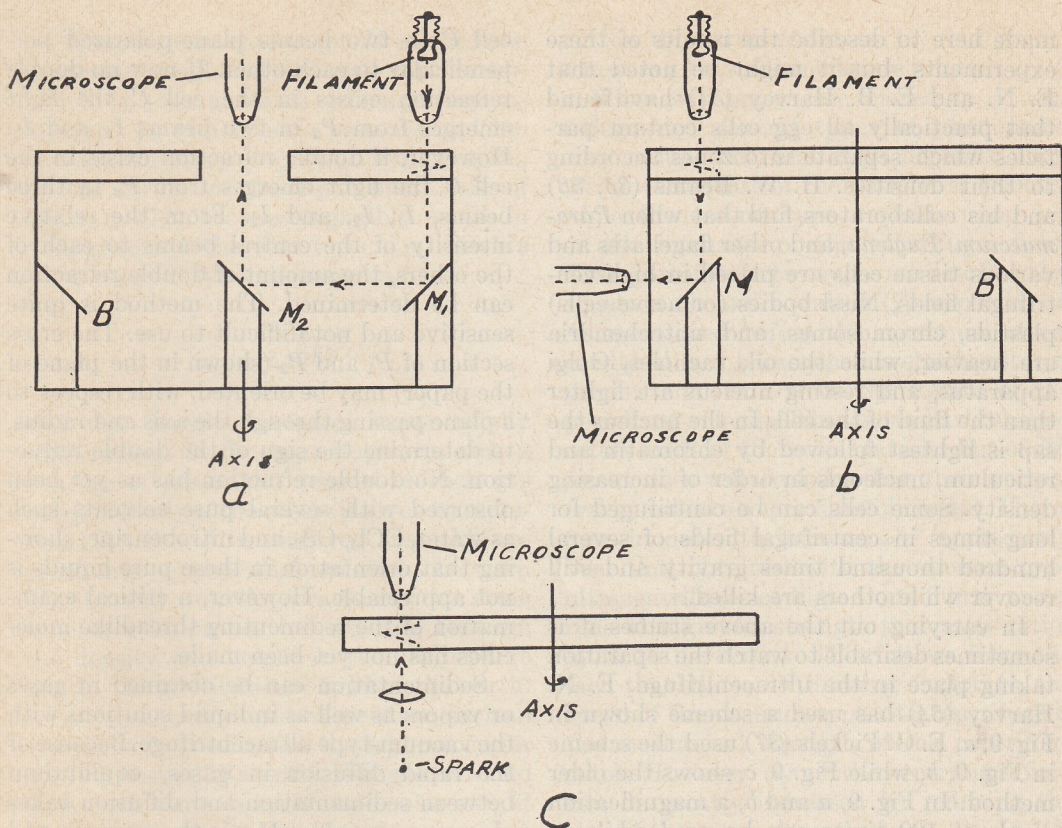
Fig. 9.—Schematic diagram of methods of viewing or photographing objects in a centrifuge by means of a microscope.
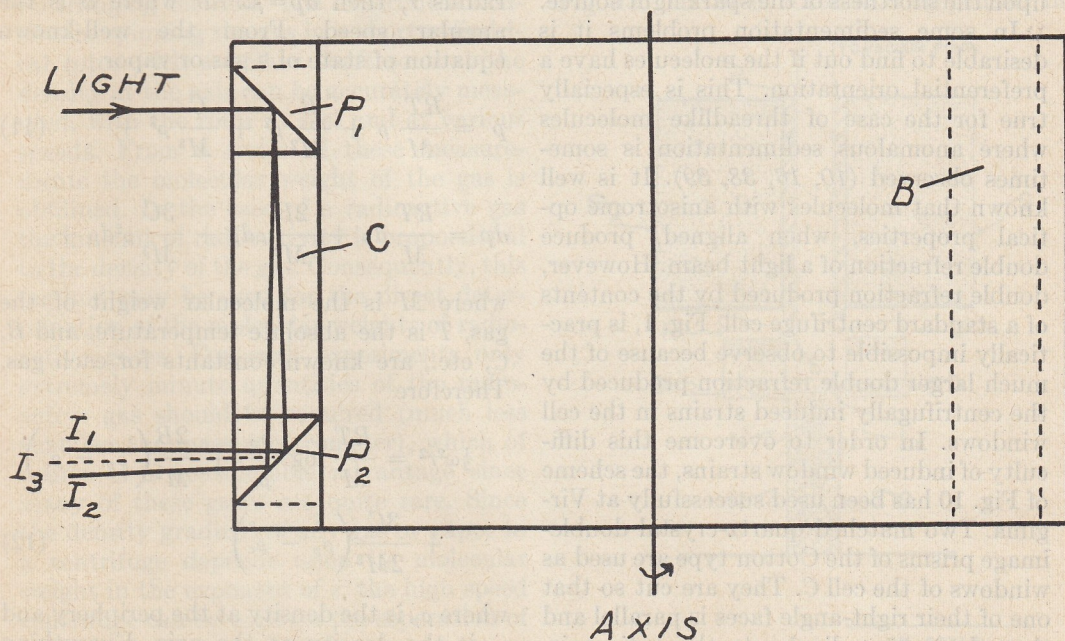
Fig. 10.—Schematic diagram of an optical method for viewing double refraction in an ultracentrifuge.

made here to describe the results of these experiments, but it might be noted that E. N. and E. B. Harvey (*34*) have found that practically all egg cells contain particles which separate into zones according to their densities. H. W. Beams (*35, 36*) and his collaborators find that when *Paramaecium, Euglena*, and other flagellates and various tissue cells are placed in high centrifugal fields, Nissl bodies (or nerve cells) plastids, chromosomes, and mitochondria are heavier, while the oil, vacuoles, Golgi apparatus, and resting nucleus are lighter than the fluid of the cell. In the nucleus the sap is lightest followed by chromatin and reticulum, nucleolus in order of increasing density. Some cells can be centrifuged for long times in centrifugal fields of several hundred thousand times gravity and still recover while others are killed.

In carrying out the above studies it is sometimes desirable to watch the separation taking place in the ultracentrifuge. E. N. Harvey (*34*) has used a scheme shown in Fig. 9, *a*, E. G. Pickels (*37*) used the scheme in Fig. 9, *b*, while Fig. 9, *c*, shows the older method. In Fig. 9, *a* and *b*, a magnification of about 100 times can be used while in 9, *c*, the possible magnification depends upon the shortness of the spark light source.

In some sedimentation problems it is desirable to find out if the molecules have a preferential orientation. This is especially true for the case of threadlike molecules where anomalous sedimentation is sometimes observed (*10, 16, 38, 39*). It is well known that molecules with anisotropic optical properties, when aligned, produce double refraction of a light beam. However, double refraction produced by the contents of a standard centrifuge cell, Fig. 1, is practically impossible to observe because of the much larger double refraction produced by the centrifugally induced strains in the cell windows. In order to overcome this difficulty of induced window strains, the scheme of Fig. 10 has been used successfully at Virginia. Two matched quartz-crystal double-image prisms of the Cotton type are used as windows of the cell *C*. They are cut so that one of their right-angle faces is parallel and the other perpendicular to the optic axis. If light falls upon P_1 it emerges into the cell *C* in two beams plane-polarized perpendicular to each other. If now no double refraction exists in the cell *C* the light emerges from P_2 in two beams I_1 and I_2. However, if double refraction exists in the cell *C* the light emerges from P_2 in three beams, I_1, I_2, and I_3. From the relative intensity of the central beams to each of the others, the amount of double refraction can be determined. The method is quite sensitive and not difficult to use. The cross section of P_1 and P_2 (shown in the plane of the paper) may be oriented, with respect to a plane passing through the axis and radius, to determine the sign of the double refraction. No double refraction has as yet been observed with several pure solvents such as water, CCl_4, CS_2, and nitrobenzine, showing that orientation in these pure liquids is not appreciable. However, a critical examination of the sedimenting threadlike molecules has not yet been made.

Sedimentation can be obtained in gases or vapors as well as in liquid solutions with the vacuum-type ultracentrifuge. Because of the rapid diffusion in gases, equilibrium between sedimentation and diffusion takes place very quickly. If p is the pressure and ρ the density of the gas or vapor at any radius r, then $dp = \rho \omega^2 r dr$ where ω is the angular speed. From the well-known equation of state of a gas or vapor

$$p = \frac{RT}{M}\rho + \frac{B}{M^2}\rho^2 + \frac{C}{M^3}\rho^3 + \qquad (11)$$

$$dp = \frac{RT}{M}d\rho + \frac{2B}{M^2}\rho d\rho + \frac{3C}{M^3}\rho^2 d\rho +$$

where M is the molecular weight of the gas, T is the absolute temperature, and B, C, etc., are known constants for each gas. Therefore

$$\tfrac{1}{2}\omega^2 r^2 = \frac{RT}{M}\log\frac{\rho_p}{\rho_c} + \frac{2B}{M^2}\left(\rho_p - \rho_c\right)$$

$$+ \frac{3C}{2M^3}\left(\rho_p^2 - \rho_c^2\right) \qquad (12)$$

where ρ_p is the density at the periphery and ρ_c is the density at the axis. Everything in equation (12) can be measured in a cen-

trifuging experiment except M so that the molecular weight of the gas can be obtained. In some gases, at ordinary temperature and at atmospheric pressure and below, and in many vapors at low pressure, all the terms on the right hand side of equation (12) become negligibly small in comparison to the first term; i.e., Boyle's law is obeyed. When this occurs,

$$\rho_p = \rho_c e^{\frac{M\omega^2 r^2}{2RT}},$$

and the density increases exponentially with radius. If the centrifuge contains a mixture of gases, the density of each gas independently of the others is given by equation (12). It will be observed that this approximate exponential relation between density and centrifuge radius is the same type of relation as that existing between the density and height in the atmosphere due to the gravitational field of the earth. As a consequence we can produce such an atmospheric gradient in the laboratory for study if desired. Recently, it has been found that, with some care, the molecular weight of a gas can be measured with a precision of the order of 0.1 per cent by the above centrifuging method. The experiment consists in spinning the gas in a short cylindrical rotor with a hollow shaft so that the density at the axis can be accurately measured, with the rotor at rest and at various speeds. From a series of these measurements the molecular weight of the gas is obtained. In the case of a radioactive gas the amount of radioactivity is proportional to the density of the gas. Consequently, this method may be used for the direct determination of the molecular weights of radioactive gases. For such experiments only extremely minute quantities of the radioactive gas should be required (much less than for the mass spectrometer), which of course is a considerable advantage since many of these gases are quite rare. Since the density gradient of any gas or vapor in a centrifuge depends upon its molecular weight in the exponent of e, the high speed centrifuge can be used for the separation of gases or the isotopes in gas or vapor. A considerable amount of work has been carried out on this latter problem (16), but no attempt can be made to discuss it here.

When substances are placed in a centrifuge two general types of phenomena may occur. The first type depends upon $\omega^2 r^2$ or the square of the peripheral velocity while the second type depends upon $\omega^2 r$ or the intensity of the centrifugal field. Examples of these two types are described by equation (3) and equation (7) respectively. As mentioned before the maximum stress produced in a homogeneous elastic rotor is proportional to $\omega^2 r^2$ or the square of the peripheral velocity. That is, similarly shaped rotors made of the same material but of different sizes will explode when their peripheral speeds are approximately equal. Therefore, in order to increase $\omega^2 r^2$ appreciably over that obtained with the vacuum type ultracentrifuges of Figs. 1 and 3 new materials with greater strength to density ratios must be developed. This follows from the fact that the only limiting factor to the speed of the vacuum-type ultracentrifuge is the mechanical strength of the centrifuge rotor. However, $\omega^2 r$ or the centrifugal field can be greatly increased by reducing the size of the rotor. A method (40, 41, 42, 43) for doing this is shown schematically in Fig. 11. The steel rotor R is suspended in
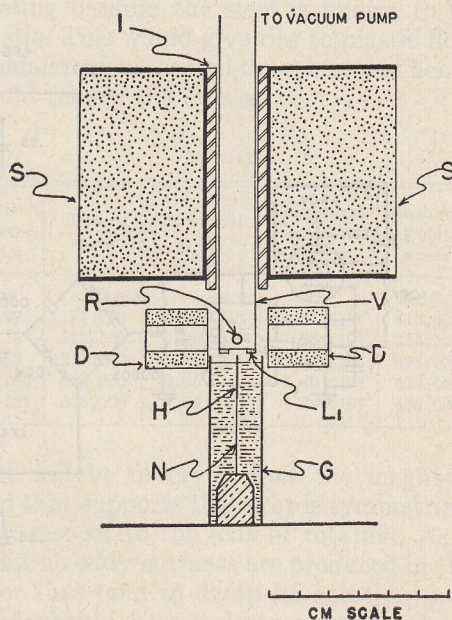

FIG. 11. Diagram of small-rotor apparatus.

the axial magnetic field of the solenoid S and is spun by a 2-phase rotating magnetic field in the two pairs of coils D. The ver-

tical position of the steel (or other ferromagnetic) rotor is maintained by an automatic regulation of the current through the

FIG. 12.—Suspension circuit.

FIG. 13.—Driving circuit.

solenoid S while its horizontal position is maintained by the symmetrically diverging magnetic field of the solenoid. The small "pick up" coil L_1 is in the grid circuit of a tuned-grid tuned-plate radio-frequency oscillator, which regulates the current through S (Fig. 12). This circuit is so arranged that when the rotor R rises the current through S decreases. These current changes are such as to position accurately the rotor without observable hunting. It will be observed from Fig. 12 that this is accomplished by introducing a derivative or "anti-hunt" signal along with the signal from the pickup coil L_1. An idea of the stability of the rotor can be obtained from the fact that no vertical motion could be observed with a 30-power microscope focused on the markings of the rotor. A small iron wire H mounted in a glass tube filled with a liquid assists in damping any horizontal motion of the rotor. The glass chamber V surrounding the rotor R was evacuated by the usual cold trap, diffusion pump, and fore pump combination. The pressures surrounding the rotor were measured by an ionization gauge. Fig. 13 shows a diagram of the driving circuit which produces the rotating magnetic field in the coils D. The output of the transition oscillator is passed through a volume control and then a phase-splitting bridge. Each of the phases is amplified and applied to the coils so that the current in the coils D are approximately 90° out of phase. The frequency was varied up to 750,000 cycles/sec, which was used for accelerating a 0.521-mm rotor. The rotor speed is measured by the arrangement schematically shown in Fig. 14. One-half of the rotor is polished and the other half darkened by dipping in dilute H_2SO_4, which had been in contact with metallic antimony. The dark layer is very thin and adheres to the rotor until the centrifugal field reaches the order of 4×10^8 gravity. Light from an incandescent lamp A is focused on the rotor R and the resulting scattered light focused on the electron multiplier photocell P. The variable output from the photocell, owing to the spin of the rotor, is amplified and fed to one pair of plates of a cathode ray oscilloscope. The frequency of the rotor is then compared with the sweep frequency applied to the other pair of plates of the oscilloscope. This sweep frequency is calibrated by a standard frequency oscillator (accurate from 0.1 to 0.01 per cent) up to 50,000 cycles/sec. Above this frequency multiples of the calibrated frequencies are used (45).

It is well known that, for rotors made of a given elastic material, the highest peripheral speed should be obtainable when the shape is such that the radial and tangential stresses are of constant value throughout the rotor. In practice small hardened steel rotors are difficult to grind to the shape required by the above conditions so hard-spherical steel rotors (from ball bearings) were used. Each spherical rotor had a small flat (about 1 mil) ground off of it in order to determine its axis of rotation. Table 1 gives some of the results obtained just before the rotors explode. It will be observed that the maximum peripheral speed for all of the rotors is roughly 10^5 cm/sec, which is in agreement with the theory. In the fifth column are tabulated the maximum stresses in the rotors calculated by the method of Chree (44), assuming the rotors are perfectly elastic. These stresses reach a maximum at the center of the rotor and fall off toward the surface. The calculated values may be somewhat larger than those actually existing because the steel is known to be plastic. This would give rise to plastic flow in small regions around the center and hence would relieve the stresses.

TABLE 1

Diameter rotor	Rotor speed	Peripheral speed	Centrifugal acceleration ×gravity	Maximum calculated stress
mm	*r.p.s.*	*cm/sec*		*lb./in.²*
3.97	77,000	9.60×10^4	4.71×10^7	410,000
2.38	123,500	9.25×10^4	7.20×10^7	385,000
1.59	211,000	1.05×10^5	1.43×10^8	498,000
.795	386,000	9.65×10^4	2.40×10^8	420,000
.521	633,000	1.04×10^5	4.28×10^8	488,000

It will be observed that the magnetic field that supports the rotor is symmetrical with respect to the axis of rotation. As a result no eddy currents are produced in the rotor that tend to damp its rotation; i.e., there should be no electromagnetic drag. With a 1/16-inch or 1.59-mm rotor spinning

at 100,000 r.p.s., the driving current in the coils D was turned off and the rotor allowed to coast while the deceleration was measured. It was found that it required about 1 hour to lose 0.1 per cent of its speed. The air pressure surrounding the rotor during these experiments was approximately 2×10^{-6} mm of Hg. The drag on the rotor due to air friction can be calculated from the relation (42)

$$\log_e \frac{N}{N_o} = \frac{-5p}{rd} \left(\frac{M}{2\pi RT} \right)^{1/2} \left(t - t_o \right)$$

where N_o is the number of r.p.s. at the time t_o, N is the number of r.p.s. at time t, p is the pressure in bars, d is the density of the steel (7.8 gms/cm⁵), T is the absolute temperature, M is the molecular weight of the gas, and r is the radius of the spherical rotor. Substituting into this equation, it is found that practically all the observed drag can be accounted for by air friction alone, although there may be a very small residual drag due to inhomogeneities. Clearly this spinning rotor arrangement should serve as an excellent absolute pressure manometer for measuring low gas pressures.

The very small observed drag on the rotor suggested that the rotor would continue to accelerate until it reached the driving frequency. That is, during the acceleration period the rotor would operate in a way similar to that of an armature of an induction motor, but as soon as it approached closely enough to the frequency of the rotating field in the driving coils D (Fig. 11) it would shift over and run as a synchronous motor. This would be expected from the fact that the rotor is steel and should become magnetized when the rotor speed and driving frequency become nearly equal. Experiment showed that the rotor actually reaches the speed of the driving frequency and then "locks into" this frequency. It is well known that the frequency of a piezoelectric crystal will remain extremely constant if it is cut properly and connected into the proper circuit and if its temperature is held constant. The driving oscillator in the circuit of Fig. 13 was replaced by such a piezoelectric crystal oscillator with a frequency of 100,000 cycles/sec.

A 1.59 mm rotor surrounded by a gas pressure of 2×10^{-6} mm Hg or less was allowed to accelerate up to the frequency of the piezoelectric crystal and "lock in" with it. In order to determine when this "locking in" takes place a signal from the piezoelectric crystal driving circuit was applied to one pair of plates of a cathode-ray oscilloscope while the signal from the electron multiplier photoelectric cell (Fig. 14) was applied to the other pair of plates of the same oscilloscope. The two signals were made to give approximately the same deflection so that when the rotor speed reached exactly the driving frequency the Lissajous figure became a stationary circle on the screen. It is interesting to note that when the rotor speed first reaches the driving frequency, a slight hunting occurs. However, this hunting damps out and as far as can be determined, completely vanishes in a few minutes. This damping of the hunting is apparently electromagnetic. Therefore, the speed of these rotors can be made as constant as the frequency of a piezoelectric crystal circuit.

The temperature of the spinning rotor has not been measured directly but an upper limit for it can be obtained by measuring the temperature of the rotor at rest in the vacuum chamber with the full power applied to the driving coils D. This was ac-
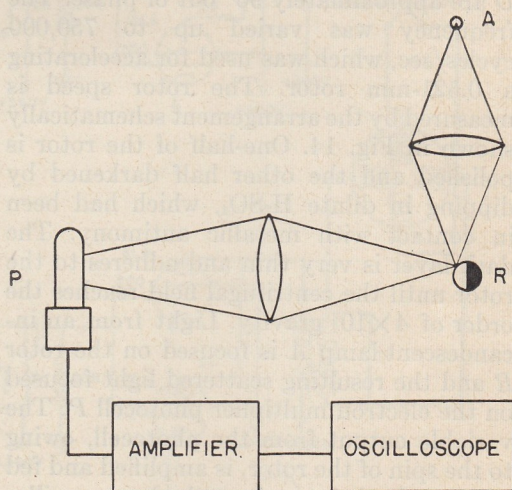

Fig. 14.—Schematic arrangement for measuring rotor speed.

complished by fastening a thermocouple made of fine wires to the rotor, then evacuating the vacuum chamber surrounding the rotor and applying the power to the coils D. The maximum increase in temperature was between 5° and 6°C. Therefore the rise in temperature of the spinning rotor is less than 6°C. Small mirrors have been ground on these magnetically suspended rotors without introducing appreciable added friction. Consequently rotating mirrors are available whose rotor speed not only is very high but extremely constant, and it should be possible to obtain greater precision for example in such determinations as the velocity of light. Obviously this technique of producing both very high centrifugal fields and very high and constant rotor speeds opens many new avenues for interesting investigations.

LITERATURE CITED

(1) Eddington. *Mathematical theory of relativity.* Cambridge, 1924.
(2) Stokes. Cambridge Phil. Trans. **8**: 287. 1847.
(3) Mason and Weaver. Phys. Rev. **23**: 412. 1924. Weaver. Phys. Rev. **27**: 499. 1926.
(4) Archibald. Phys. Rev. **53**: 746. 1938; **54**: 371. 1938.
(5) Lamm. Ark. Mat. Astr. Fys. **21B** (2). 1929; Zeitschr. Phys. Chem. **143A**: 177. 1929.
(6) Faxen. Ark. Mat. Astr. Fys. **21B** (2). 1929.
(7) Soyten. Proc. Phys.-Math. Soc. Japan (3) **18**: 9. 1936. Oka, Proc. Phys.-Math. Soc. Japan (3) **19**: 1094. 1937.
(8) Archibald. [Private communication, 1946.]
(9) Svedberg. *Colloid chemistry.* Chemical Catalogue Co. 1928.
(10) Svedberg and Pedersen. *The ultra centrifuge.* Oxford, 1940. (See for other references.)
(11) Tolman. Journ. Amer. Chem. Soc. **33**: 121. 1911.
(12) MacInnes. Ann. New York Acad. Sci. **43**: 243. 1942.
(13) Pickels. Chem. Rev. **30**: 341. 1942.
(14) Pickels and Beams, J. W. Science **81**: 342. 1935.
(15) Beams, J. W., and Pickels. Rev. Sci. Inst. **6**: 299. 1935.
(16) Beams, J. W. Journ. Appl. Phys. **8**: 795. 1937; Rev. Mod. Phys. **10**: 245. 1938; Rep. Prog. Phys. (Physical Soc.) **8**: 31. 1941. (See for other references.)
(17) Beams, J. W., Linke, and Sommer. Rev. Sci. Inst. **18**: 57. 1947.
(18) Lamm. Nova. Acta. Reg. Soc. Sci. Upsaliensis (4) **10** (6). 1937.
(19) Philpot. Nature **141**: 283. 1938.
(20) Svensson. Kolloid—Z, **87**: 181. 1939.
(21) Andersson. Nature **143**: 720. 1939.
(22) Bridgman and Williams. Ann. New York Acad. Sci. **43**: 195. 1942.
(23) Skarstron and Beams, J. W. Rev. Sci. Inst. **11**: 398. 1940.
(24) Beams, J. W. Ann. New York Acad. Sci. **43**: 177. 1942.
(25) Rothen. Ann. New York Acad. Sci. **43**: 299. 1942.
(26) Bull. *Physical biochemistry.* Wiley, 1943.
(27) Beams, J. W. Science in Progress (2) **9**: 232. Yale, 1940.
(28) Zworkin, Morton, Ramberg, Hillier, and Vance. *Electron optics and the electron microscope.* Wiley, 1945.
(29) Beams, J. W. Phys. Rev. **69**: 546A. 1946.
(30) Wyckoff. Proc. Amer. Phil. Soc. **77**: 455. 1937; Journ. Biol. Chem. **121**: 219. 1937; **122**: 239. 1937; **124**: 585. 1938.
(31) Pickels. Journ. Gen. Physiol. **26**: 341. 1943.
(32) Masket. Rev. Sci. Instr. **12**: 277. 1941.
(33) Harvey, E. N. Journ. Hered. **37**: 71. 1946.
(34) Harvey. Journ. Franklin Inst. **214**: 1. 1932; Biol. Bull. **66**: 48. 1934; Journ. Appl. Phys. **9**: 68. 1938. (See for other references.)
(35) Beams, H. W., and King. Anat. Rec. **59**: 363, 395. 1934.
(36) Beams, H. W. Biol. Symposia **10**: 71 1943. (See for other references.)
(37) Pickels. Science **83**: 471. 1936.
(38) Stanley and Lauffer. Science **89**: 345. 1939. Lauffer. Journ. Phys. Chem. **44**: 1137. 1940.
(39) Jullander. Ark. Kemi. Min. Geol. **21A** (2). 1945.
(40) Holmes and Beams, J. W. Nature **140**: 30. 1937.
(41) MacHattie. Rev. Sci. Inst. **12**: 429. 1941.
(42) Beams, J. W., Young, and Moore. Journ. Appl. Phys. **17**: 886. 1947.
(43) Beams, J. W., and Young. Phys. Rev. **71**: 131. 1947.
(44) Chree. Proc. Roy. Soc. **58**: 39. 1895.
(45) Shapiro and Butt. Rev. Sci. Inst. **8**: 34. 1937.