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CONTENTS

IX

THE ULTRACENTRIFUGE

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Since the dawn of civilization man probably has been more or less conscious of the fact that larger particles settle through a liquid faster than smaller ones. In fact, he undoubtedly used this phenomenon in his crude mining and manufacturing operations. However, it remained for Galileo about 1590 to state clearly the fundamental laws underlying the phenomenon and to establish them by logical experimental procedure. From the equation established by Stokes [1] in 1847 it is clear why the process of settling in liquids is so effective for the separation of particles of different sizes, since, if the particle diameter is doubled, its speed of fall in the liquid is increased fourfold. If this equation of Stokes described the only phenomenon taking place in the process of sedimentation, one should expect, after sufficient time had elapsed, to find all uncharged particles or molecules completely settled out of suspension or solution. However, when the particles are very small, this is not the case because of another phenomenon, known as diffusion, which opposes complete sedimentation. This phenomenon of diffusion arises from the fact that all particles have thermal agitation or Brownian motion and are moving in random directions in the liquid.[2] Diffusion always

1. Stokes showed the theoretical relation between the rate of fall, v, under the force of gravity of a spherical particle of density ρ_p and its radius r in a liquid having a density ρ_d and coefficient of viscosity η to be

$$v = \frac{2}{9\eta} (\rho_p - \rho_d) g r^2.$$

2. Each particle has an amount of energy equal to $3/2\ RT/N$, where T is the absolute temperature, R the gas constant per mole, and N is the Avogadro number.

operates in such a manner that there is a net transport of particles from a region where the particles are closer together to where they are further apart. Consequently, in the process of sedimentation the settling out of small particles, which concentrates the particles at the bottom, will proceed only to the point where the sedimentation is balanced by diffusion. When the size of the particles approaches that of molecular dimensions, the diffusion is so great in comparison to the velocity of sedimentation that practically no particles will settle out under the force of gravity, unless, of course, the solution is completely saturated. The quantitative mathematical theory for the settling of particles in liquids under the force of gravity has been worked out by Mason and Weaver. The variation in the density of the air with height above the surface of the earth is an example of the process of sedimentation of a gas.

If, instead of allowing the small particles to settle out of a liquid or gas under their own weight in the gravitational field of the earth, the liquid or gas containing the particles is placed in a centrifuge and subjected to a centrifugal force many times that of gravity, the sedimentation becomes more complete. In addition to producing a greater amount of sedimentation, the larger centrifugal field makes possible the separation of particles with smaller size or mass differences. Because of these advantages of the centrifuge, it has been widely used, both in industry and in research. In this chapter an attempt will be made to describe some of the recent important developments in high-speed centrifuging, as well as some of their applications to research.

ULTRACENTRIFUGES

Recent progress in the technique of high-speed centrifuging has been made in two directions: first, the rotational speed of the centrifuges, and hence the centrifugal force, has been increased so that the rate of sedimentation is increased; and, second, the centrifuge has been made convection free so that no remixing of the material being separated can occur. Although it might seem

obvious that the latter development would always accompany the former if genuine progress is to be made, it was not until about 1924 that Svedberg first succeeded in obtaining convection-free sedimentation in centrifugal fields as high as 5,000 times gravity. In this pioneering work of Svedberg and his students it was shown that the centrifuge could be used effectively for the determination of particle or molecular size and shape. The results obtained with this early machine, which they named the ultracentrifuge, especially in the determination of the molecular weights and sizes of proteins, were of such great interest and fundamental importance that Professor Svedberg set about to improve the centrifuging technique still further. The results of this systematic investigation are the two modern types of "Svedberg ultracentrifuges," adapted especially to Professor Svedberg's epoch-making studies of particle or molecular weights and sizes.

The first type of Svedberg ultracentrifuge gives centrifugal fields from approximately 500 to 15,000 gravity. It is provided with ball bearings and is driven by an ordinary electrical motor. The rotor spins in hydrogen at atmospheric pressure and is surrounded by a water-cooled casing. The material to be centrifuged is contained in a sector-shaped cell with quartz or glass windows so that the concentration of the material at various radial distances in the cell can be determined by optical means while the rotor is spinning. This low-speed machine is usually used for the determination of particle or molecular weights by the so-called equilibrium method, which will be described later. The second type, or "oil turbine" Svedberg ultracentrifuge, is his famous high-speed machine which is used to give centrifugal forces in the range from 15,000 to 750,000 g. The rotor of this machine is made of a nickel steel alloy and shaped for maximum strength. It carries a sector-shaped cell with crystal quartz windows for observing optically the concentration of the material being centrifuged. The rotor is supported in horizontal bearings and is spun by twin oil turbines, one on each end of the rigid shaft. The oil

under pressure which drives the turbines is supplied by a special oil compressor and is filtered and thermostated at a suitable temperature before striking the turbines. The rotor is surrounded by a thermostated, heavy steel case. Hydrogen is continuously admitted at the periphery and pumped off at the center at such a rate as to maintain a pressure of about 20 mm. surrounding the rotor. The purpose of this hydrogen is to conduct the heat generated in the bearings by the oil impinging on the turbines and by the gas friction on the rotor to the casing, thus preventing the temperature of the cell from changing or becoming nonuniform. The rotor containing the cell and its contents is carefully balanced, both statically and dynamically, before running the machine. This machine is used principally for the determination of molecular weights and sizes by the rate of sedimentation method, which will be described later. Although the leading role which the Svedberg ultracentrifuges have played in the fundamental experiments of Professor Svedberg and his collaborators assuredly entitles them to a very detailed description in any analysis of high-speed centrifuging, my personal knowledge of them is secondhand, so I shall confine my discussion to the type of centrifuges and their uses which are more familiar to me.

Air-Driven Centrifuges

In general there are two different types of air-driven ultracentrifuges in use at the present time. The first type spins in air or other gases at approximately atmospheric pressure, while the second, or vacuum type, spins in an air-tight chamber which may or may not be highly evacuated. For most problems the second, or vacuum type, ultracentrifuge is definitely much superior, yet the first type still is often used because of its extreme simplicity of construction.

It has long been known that a ball may be supported and spun on a jet of air. However, it was not until 1925 that Henriot and Huguenard succeeded in constructing small rotors that could be

supported and spun to very high rotational speeds by properly directed jets of air. The original design of Henriot and Huguenard has been modified, improved, and stabilized by a number of different workers until at the present time the rotors are very stable. Figure 100 shows a diagram and Figure 101 a photograph of one of these simple air-driven, air-supported centrifuges which

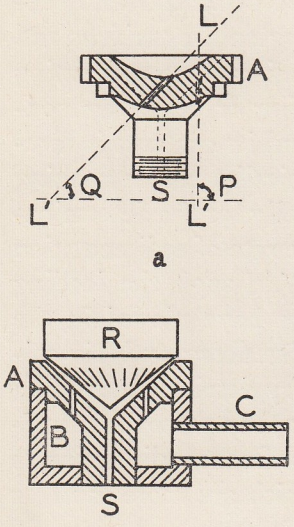

FIG. 100. Diagram illustrating air-driven, air-supported centrifuge as described on page 236. (a) Section through the strator cone (noncentral); (b) Section through complete machine.

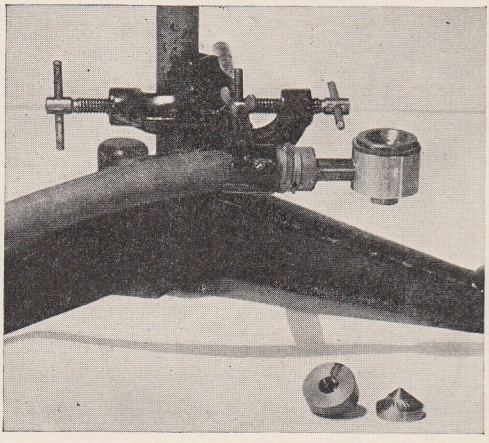

FIG. 101. Photograph of air-driven centrifuge shown in Fig. 100, mounted in clamp stand.

we designed and which has operated very satisfactorily in our laboratory for about ten years. The machine consists of three parts: a so-called stator cone A, an air box B, and a cone-shaped rotor R. Figure 100, b, shows the first two screwed together and the rotor in place. Parts A and B may be made of any machinable

material such as brass or duralumin, but the rotor R should be constructed of alloy steel or duralumin ST14 and shaped to give it a maximum bursting strength.

The construction of this machine is so simple that often we have given it as a problem in machine-shop practice to first-term

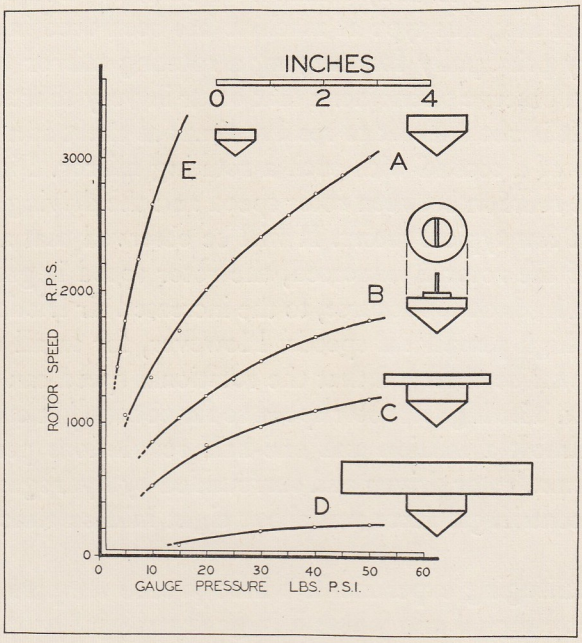

FIG. 102. Curves showing the relation of air pressure to rotor speed for rotors spinning in air at atmospheric pressure. The size of rotor for each curve is shown at right of the curve.

graduate students. To operate the machine, compressed air, that is forced into the air box B, emerges through the tubes LL^1 in jets which impinge upon the flutings of the rotor R. These air jets lift the rotor and start it spinning. The cone-shaped surfaces of A and R are so constructed that the Bernouilli forces prevent the rotor from flying out of the stator and compel it to spin on a thin cushion of air between them. The channel S allows air to

flow into the stator from the atmosphere and stabilize the supporting air cushion. This stable air cushion permits the rotor to seek its own axis of rotation within limits and therefore avoids the necessity of dynamically balancing the rotor with high precision. Also the rotor may be loaded or unloaded while at full speed or be made to carry a mirror or other superstructure. As a matter of fact, this type of machine has been used in our own laboratory principally for carrying a rotating mirror to photograph and observe phenomena that occur in very short intervals of time. It is not difficult to resolve times as short as a hundred millionth of a second with such a rotating mirror. Figure 102 shows the relation between rotor speed and the driving air pressure for a few typical rotors. It will be observed that as the diameter of the rotor is increased, the rotor speed is greatly decreased. This is due, of course, to the increased air friction on the rotors at high peripheral speeds. However, for small diameter rotors it will be observed that the rotational speed can be made very great. The highest rotor speed so far obtained in our laboratory is almost a million and one-half revolutions per minute with a 9 mm. rotor driven and surrounded by hydrogen. In this case the centrifugal force was about eight million times that of gravity.

In centrifuging experiments where precise temperature control is not essential and where a large centrifugal field is necessary only over a small radial distance, this simple machine has been used very effectively. For example, H. W. Beams, R. L. King, Harvey, Guyer, McIntosh, Selbie, Gatenby, and others have used this type of machine in very interesting studies of the relative displacement of the different components of plant and animal cells produced by centrifuging. Harvey and Pickels have devised optical methods of viewing material with a microscope while it is being centrifuged. In both methods the material is illuminated by a very narrow filament of light and the optical parts are so arranged that the plane of the image of the material observed moves or rotates only a very small amount during the

time of viewing. It might be noted that the focal length of the microscope objective can be decreased when the rotor diameter is decreased. Hence for the highest resolving power the rotor should be small. Although these methods of Harvey and Pickels have not been used very extensively, it is probable that the adaptation of very small rotors would render them more useful.

This type of simple centrifuge has also been used by a number of different workers for the sedimentation of small particles and molecules out of suspension or solution. However, in these experiments great care must be taken to maintain temperature equilibrium throughout the centrifuge "bowl." The necessity for this can be seen when one considers that in a vertical vessel of water an increasing temperature from top to bottom of a degree or so will cause convection currents because of the smaller density at the bottom. One of the principal factors in producing this mixing or convection is the product of the difference in densities and the acceleration of gravity. Consequently in a centrifuge filled with liquid, where the field may be many hundred thousand times gravity, the temperature must be held extremely uniform to prevent convection. Of course there are other forces which oppose convection, such as the increase in density toward the periphery due to pressure, but these are small enough to require an extremely uniform temperature in an ultracentrifuge. In the centrifuge of Figures 100 and 101 it will be observed that temperature gradients exist in the rotor due to the cooling of the expanding air jets on its undersurface and the heating resulting from air friction on its periphery. Several devices have been used to make these rotors convection free. Dr. E. G. Pickels and I have used a thin rotor cell with a transparent window for observing optically the rate of sedimentation of hemoglobin. Uniform temperature of the cell was maintained by a second thicker cell directly below it, containing the same liquid, which broke up the temperature gradients by convection. We also obtained convection-free sedimentation by using disk-shaped baffles coaxial with the rotor, spaced one just above the other in the centrifuge

cell. McBain and his co-workers also have used various devices to prevent convection in these centrifuges and have reported the successful determination of sedimentation constants with their "transparent ultracentrifuge," as well as in ones in which the concentrations in the centrifuge cell are determined after the centrifuge is stopped. McIntosh, Selbie, and others have placed their material in small glass tubes in the rotor in a manner somewhat similar to the technique of Elford, with good results. However, for the measurement of rates of sedimentation, and thus particle or molecular size and weight, a little consideration will show that the precision is increased as the centrifuge rotor diameter is increased. Mason and Weaver, as well as Svedberg and his collaborators, have shown that the ability of a centrifuge to resolve two molecular species with almost the same sedimentation constants is proportional to w^2rh, where w is the angular velocity, r the radius, and h the height of the cell. In a general way h can be made larger as r is increased, so this resolving power is roughly determined by the square of the peripheral velocity of the rotor.

Now it will be observed from Figure 102 that a very large amount of energy is required to spin a rotor of more than about an inch in diameter to high peripheral speeds because of the increased air friction on rapidly moving surfaces. Consequently, in addition to troublesome thermal gradients set up in the rotor, the diameters of the rotors are too small to give high precision in this problem. High peripheral velocities are also essential for the concentration of isotopes, while in other experiments it is undesirable for the field to vary too rapidly. Because of these limitations we undertook the development of a high-speed centrifuge about seven years ago that would spin in vacuo.

The Vacuum-Type, Air-Driven Ultracentrifuges

The first vacuum-type, air-driven ultracentrifuge constructed in our laboratories consisted of a large rotor "centrifuge" situated inside a vacuum-tight chamber. This large rotor was suspended from and driven by an air turbine similar to that shown in Figure

100, located outside and vertically above the chamber. The turbine and centrifuge were connected by a piano wire which was coaxial with their vertical axis of rotation and passed through a vacuum-tight oil gland which sealed the vacuum chamber. Since the piano wire shaft was flexible the large rotor could seek its own axis of rotation and could be spun through any critical speeds without difficulty. Also the small diameter shaft permitted low friction losses in the oil gland or bearing even at high rotational speeds. The vapor pressure of the oil in the oil gland which limits the vacuum attainable was very low (10^{-4} to 10^{-6} mm. for vacuum pump oils) so that the air or gaseous friction on the large rotor was negligible. In actual practice it was found that the efficiency of the air turbine was not changed appreciably by attaching the large rotor and shaft to it provided the residual pressure in the vacuum chamber was below 5×10^{-4} mm. As it is possible to explode the small turbine by its own centrifugal force, the only limiting factor on the rotational speed of this machine is the strength of the large rotor. Since in a vacuum there is no heat generated by the rotor, the temperature of the rotor not only remained extremely uniform but could be accurately controlled by thermostating the vacuum chamber. Consequently, convection-free sedimentation was easily obtained. This vacuum-type machine has been improved and adapted to many different special problems by a number of workers and has been found to be a very efficient machine.

Figure 103 is a sectional diagram and Figure 104 a photograph of a vacuum-type centrifuge which we have used in an attempt to concentrate the isotopes by the method of evaporative centrifuging. The rotating parts consist of the centrifuge C inside the vacuum chamber, the flexible hollow shaft A (hypodermic needle gauge 15), and the air turbine T. The air turbine T is supported by air entering at I and forming a cushion between the Bakelite cup and the underside of T. The turbine is driven by air entering the air box S through D and impinging upon the turbine flutings. The vacuum-tight oil glands G_1 and G_2 are supported in Neoprene rings and have good thermal contact with their supports. They are arranged so that

oil may be circulated through them, but this is very seldom necessary. Vacuum-pump oil is forced into them under a pressure slightly above the air pressure in I. In experiments on the separation of chlorine isotopes, about 14 cc. of liquid carbon tetrachloride is injected into the hollow rotor C. It is then spun to 1,560 r.p.s. and evacuated through the hollow shaft A. The carbon tetrachloride evaporates at the periphery, diffuses in the

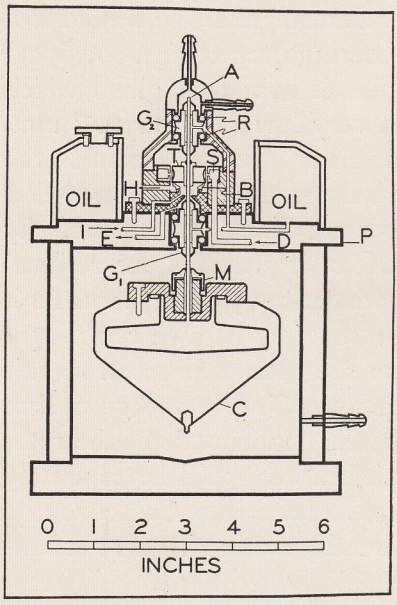

FIG. 103. Section of air-driven, vacuum-type centrifuge as described on page 241.

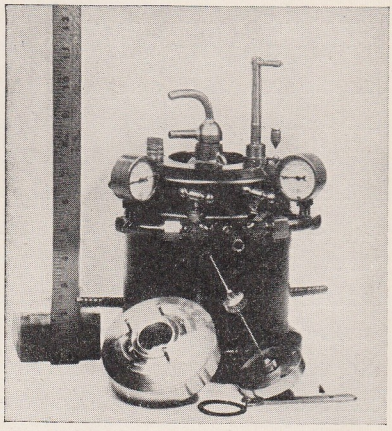

FIG. 104. Photograph of centrifuge shown in Fig. 103.

vapor state through the centrifugal field to the axis of rotation, out through the hollow shaft, and is collected in a succession of 2 cc. (liquid) fractions in dry-ice traps placed in the pumping line. It was found that the first fraction collected had the light isotope 35 concentrated, while the last samples had the heavy isotope 37 concentrated. When the rate of withdrawal was not large enough to destroy the equilibrium conditions in the rotor, the separation obtained was in accord with the equilibrium theory. It has been shown by Aston and Lindemann, Mulliken, Chapman, and others that for the case of a substance with two isotopes which behave as ideal gases, the

ratio K_0 of the concentrations of light to heavy isotopes at the axis divided by the same ratio K at the periphery

$$S = \frac{K_0}{K} = exp\,\frac{(M_2 - M_1)v^2}{2\,RT} \qquad (1)$$

where S is the so-called separation factor, v is the peripheral velocity in cm./sec., $R = 8.3 \times 10^7$, and T is the absolute temperature. Mulliken has further shown that this same equation holds when the element is centrifuged in any of its compounds. It will be noted from the above theory that the separation factor S depends only upon the differences in masses of the isotopes and not their absolute value, so that the method should be practically as successful for heavy as for light elements. It will also be observed that the separation factor is greatly increased with increasing peripheral velocity v and decreasing temperature T. Table IV gives some values of S for peripheral velocities normally used in the apparatus in Figure 104 at room, dry-ice, and liquid-air temperatures:

TABLE IV

$M_2 - M_1$	v cm./sec.	S		
		300° A	200° A	90° A
1	4.5×10^4	1.04	1.06	1.13
2	4.5×10^4	1.08	1.12	1.30
3	4.5×10^4	1.12	1.19	1.47
4	4.5×10^4	1.17	1.27	1.68

Mulliken has also shown that in his evaporative centrifuge method used above, the change in atomic weight of the element ΔA is given by

$$\Delta A = \frac{(M_2 - M_1)^2 X_1 X_2 v^2}{2RT} \log_e C = B \log_e C \qquad (2)$$

where X_1 and X_2 are the mole fractions of the light and heavy isotopes and C is the so-called cut or the ratio of the total amount of material initially to the amount of residue remaining in the centrifuge. Figure 105 shows a graph of ΔA versus the cut.

In order to take advantage of the increased separation factor at reduced temperature, the apparatus was modified to permit

the centrifuging of materials at other than room temperature. Essentially it is the same machine, but with the large rotor insulated thermally from the driving mechanism. The shaft is a long stainless steel tube, and the guides to keep it from developing standing waves at certain frequencies are mounted on nonconducting Bakelite. The large rotor is surrounded by a metal

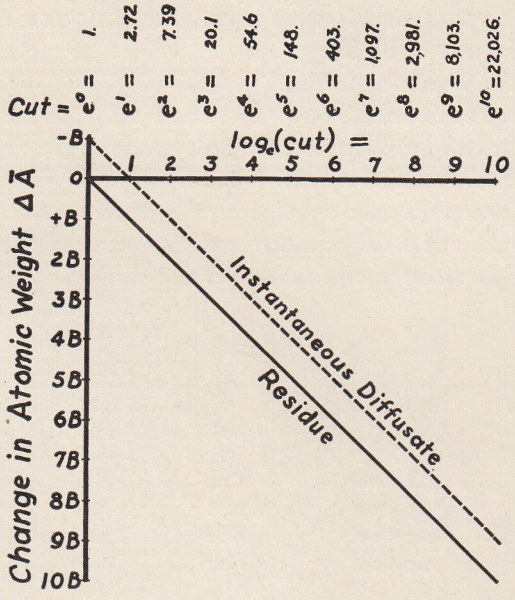

FIG. 105. Relation of atomic weight change to the natural logarithm of the cut for the evaporative centrifuge method of concentrating isotopes.

"thermos flask." With this apparatus the centrifuge has been spun at liquid air temperature, and the separation of the isotopes of chlorine in ethyl chloride at dry-ice temperatures has been shown to be in accord with the theory just given.

The practicability of any centrifuging process for the separation of isotopes depends not only on the separation factor S, but upon the amount of material that can be centrifuged per unit of time. The amount of material that can be centrifuged per unit of time depends upon the time required for rough equilib-

rium to be established between sedimentation and diffusion in the centrifuge (page 242). When the material is passed through the centrifuge too rapidly, the separation factor is decreased. This question has been treated theoretically by Humphreys and by Witmer and Wilson. In order to increase the amount of material centrifuged per unit of time, it was obviously necessary to increase the size of the centrifuge. A little consideration will show that it was more practical to increase the length than the diameter of the rotor. We therefore undertook the development of the technique for spinning long rotors (Fig. 106).

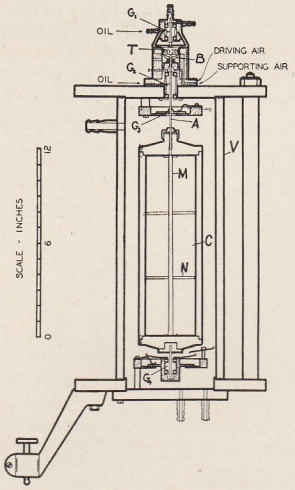

FIG. 106. Section of air-driven, vacuum-type tubular ultracentrifuge used in the evaporative centrifuge method of separating isotopes.

The only necessary change in the apparatus shown in Figure 103 is the flexible sliding bearing at G_3 and G_4. The long rotor is a chrome moly-steel tube approximately 1 foot long and 3 inches in internal diameter, with ½ inch wall thickness. The tube contains a large number of very small holes for drawing off the vapor uniformly along the axis of the tube.

Figure 107 shows a graph of the ratio of the separation expected from the theory to that observed versus the rate of removal of carbon tetrachloride per minute. The circles show the results ob-

tained with a hollow tube. It will be observed that the efficiency starts to fall off at rates of removal of about .2 cc. liquid CCl_4 per minute. This falling off was eventually traced to the remixing in the rotor at the high rates of withdrawal of the vapor. As the vapor moves from the periphery toward the center, its angular momentum is conserved and hence a sort of whirlwind is formed, spinning in the same direction as the rotor which gives

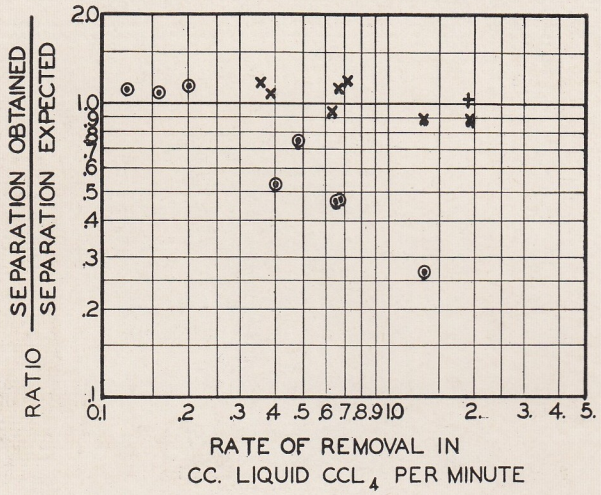

FIG. 107. Ratio of isotopic separation obtained to that expected on equilibrium theory versus rate of removal of CCl_4. The circles are for a hollow tube, and the crosses for the same tube with baffles.

rise to remixing. By putting spider baffles in the rotor to absorb the angular momentum of the gas and thus prevent stirring, the experimental values shown by the crosses in Figure 107 were obtained. The magnitude of the angular momentum given up by the gas in diffusing to the axis was demonstrated by the fact that after the baffles were installed the driving air to the turbine could be considerably reduced, yet the rotational speed of the centrifuge was maintained constant by the loss of the angular momentum of the CCl_4 vapor. This method of concentrating isotopes is very effective for changing the isotopic ratio by about

20 per cent or less in comparatively large quantities of material, but to get large changes in the isotopic ratio the process becomes very laborious.

In addition to the evaporative centrifuge method, we have also experimented with a continuous-flow method. Figure 108A shows a vertical section and Figure 108B a picture of the centrifuge used. The air turbine is placed below rather than above the

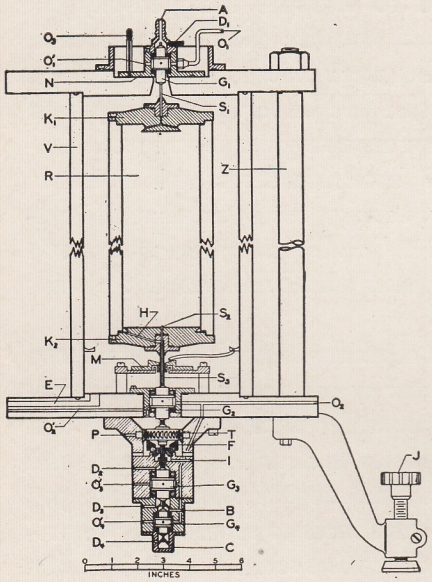

FIG. 108A. Section of continuous flow, air-driven, vacuum-type centrifuge used in the separation of gases and vapors as described on pages 247–248.

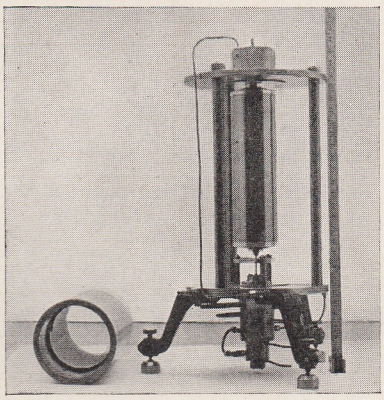

FIG. 108B. Photograph of centrifuge shown in Fig. 108A.

vacuum chamber; otherwise the driving and supporting mechanism is identical to that shown in Figure 103.

The tubular rotor R (4″ O.D., 15″ long, with ½″ wall thickness) is made of alloy steel and capped by duralumin end plates K_1 and K_2. The gas or vapor to be centrifuged flows at a continuous rate into A, through the stainless steel tube S into R, and as it flows down R separation takes

place. Upon reaching K_2 the heavy fraction flows out through the loosely fitting circular flange into the tubes H, then through the region between the coaxial tubular shafts S_2 and S_3 and is collected at B. The lighter fraction flows down S_2 and is collected at C. The ratio of the quantity of material collected at B and at C can be regulated by adjustable valves at B and C. In other words, the gases or vapors pass through the machine at a continuous rate and are collected in a heavy and a light fraction.

The machine has been used to separate nitrogen-oxygen, nitrogen-carbon dioxide mixtures, and the chlorine isotopes in ethyl chloride vapor. When baffles were placed in the centrifuge to prevent stirring, the ethyl chloride vapor could be passed through the machine at the rate of about 2 gms. per minute without appreciable reduction in the separation factor, which was in approximate accord with theory. It might be noted that this cylinder with its baffles weighed 36 lbs. and when spinning at 1,100 r.p.s. had stored in it something over a half million foot-pounds of energy.

The air-driven vacuum-type centrifuges, which I have been describing, were developed primarily for use in the concentration of isotopes. Since the isotopes differ usually by 1 or 2, and in rare cases, 10 atomic weight units, it is clear that they are, with the exception of compounds with almost equal molecular weight, among the most difficult of all substances to separate by centrifuging. Hence a centrifuge which gives even a small separation of the isotopes should be a very effective means of purifying many substances. This has been found to be especially true in the case of the very large molecular weight substances such as hormones, viruses, enzymes, etc., which are known to play an important role in our well-being. Many of these biological materials occur naturally in great dilution, and it is of first importance to concentrate and purify them. Often this is difficult, especially by chemical means, because small changes in pH value or temperature will deactivate them. Fortunately, however, their properties apparently are not affected by strong centrifugal fields, and the temperature of the types of centrifuges which I have

described can be controlled accurately. Hence these centrifuges are well suited for their purification.

Quantity-Type Ultracentrifuges

Although many different workers have used various modifications of the air-driven, vacuum-type ultracentrifuges in the purification of their materials, Figures 109 and 110 contain the general principles usually employed. The driving and supporting mechanism is essentially the same as that in Figure 103 except that additional provision is made for a set of reverse jets which may be used for decelerating the machine in a comparatively short time. The rotor C is a large metal block (usually duralumin ST14) machined to the approximate shape shown. The holes bored at an angle contain Lusteroid test tubes which, in turn, contain the material to be centrifuged. The vacuum-tight top is clamped to the rotor and the surrounding chamber evacuated to less than 5×10^{-4} mm. of mercury pressure. The rotor is then spun for the desired time and, upon stopping, the heavy materials are found to collect near the bottom of the tubes, whereas the lighter fractions are found near the top. Wyckoff has shown that, within limits, the smaller the angle which the axes of the tubes make with the vertical, the better the separation. An 8-inch rotor may hold 150 cc., while a 6-inch will hold 80 or 90 cc. In their first experiments Bauer and Pickels obtained a concentration of the yellow-fever virus of ten thousand times, whereas Wyckoff and Corey found that tobacco mosaic virus was crystallized on the bottom of the tube in one centrifuging.

This type of machine is well suited to the concentration of comparatively large molecular weight substances. However, since the time required to complete one centrifuging is usually several hours, the amount of material that can be centrifuged with one machine is very limited.

Recently the question has arisen whether or not the tubular vacuum-type centrifuge (Figures 107 and 108) used for gases and vapors might be used for the concentration of materials in

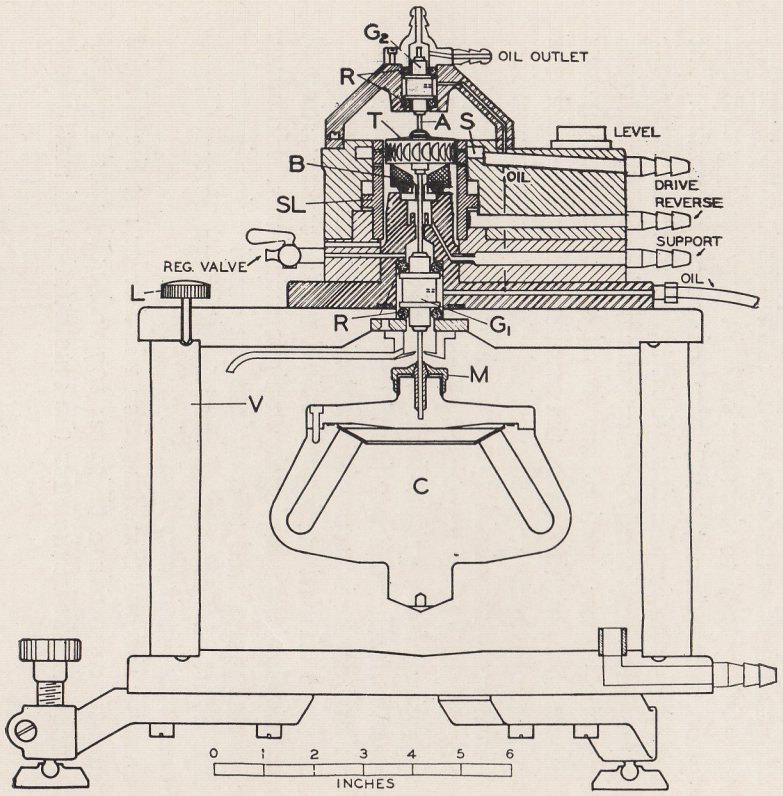

FIG. 109. Section of air-driven, vacuum-type centrifuge used for purification of large molecular weight substances as described on page 249.

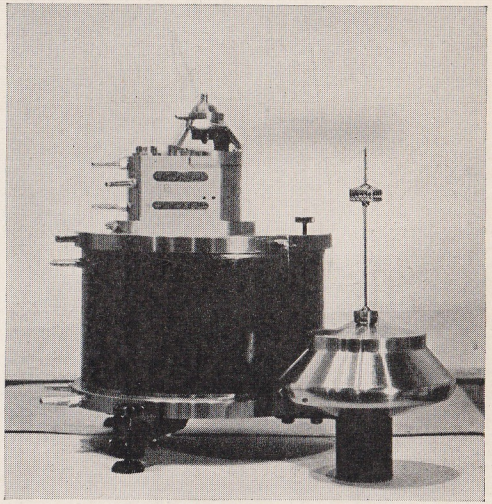

FIG. 110. Photograph of centrifuge shown in Fig. 109. The rotor-turbine and shaft are in the foreground.

solution. In collaboration with Drs. Chanutin and Masket, of the University of Virginia Medical School, we have been making some preliminary tests with a small tubular machine on a few biological substances such as egg albumen and hemoglobin. Several modifications in the machine of Figures 107 and 108 were, of course, necessary. A solid rod or core was placed inside the tubular rotor and coaxial with it, in order to force the materials being centrifuged into the strongest part of the centrifugal field, i. e., in the annular space between the core and inner wall of the tube where separation takes place. It was also necessary to make the rotor and core of stainless steel or else to plate them with gold to prevent corrosion. The inlet and exit chambers were made of Lucite. Guards were also placed between the inlet and exit chambers to prevent the material being centrifuged from coming in contact with the oil leaking from the vacuum-tight oil glands. With this arrangement the material flows into the centrifuge at the top at a continuous rate, and a heavy fraction and a light fraction are separately collected at the bottom in a manner similar to that of the cream separator. This machine is not yet fully developed, but our preliminary results show that the separation attained is in approximate accord with the predictions of the theory. This shows that no stirring or remixing is taking place. A little consideration will show that the maximum separation through this ultracentrifuge is proportional to the number of revolutions of the centrifuge per second squared, to the sum of the outer radius of the core and the inner radius of the tube squared, and to the length of the tubes. Since tubes several feet long can be spun up to their bursting point, this tubular ultracentrifuge for liquids holds considerable promise as a method of separating materials rapidly.

Analytical Ultracentrifuges

As previously mentioned, Svedberg and his collaborators have shown that the particle or molecular weights or sizes can be obtained from centrifuging data. There are two general cen-

trifuging methods, known as the equilibrium and the rate-of-sedimentation methods, used for molecular or particle weight and size determination at the present time. In the equilibrium method the centrifuging is continued a sufficient time for equilibrium between sedimentation and diffusion to be established at every point in the centrifuge.

When this equilibrium state is reached in a dilute solution contained in a sector-shaped cell,

$$M = \frac{2RT \log_e C_1/C_2}{(1 - \rho V)w^2(r_1^2 - r_2^2)} \qquad (3)$$

$$v = \frac{2RT \log_e C_1/C_2}{N(\rho_p - \rho_d)w^2(r_1^2 - r_2^2)} \qquad (4)$$

where M is the molecular weight, R the gas constant per mole, T the absolute temperature, C_1 and C_2 the concentrations at the points r_1 and r_2 distance from the axis of rotation, respectively, ρ the density of the solution, V the partial specific volume of the molecule, N the Avogadro number, ρ_p and ρ_d the densities of the particle and solvent respectively, and v the volume of the molecule.

The equilibrium method is the simplest from a theoretical standpoint and requires only the measurement of two concentrations at their respective distances from the axis of rotation in addition to the constants of the centrifuge and of the solution given in eq. (3) and (4) for a determination of the molecular weight or molecular volume. On the other hand, the time required for equilibrium to be established in a centrifuge is usually very long. According to Archibald, who has worked out the general theory for the sedimentation in centrifuges, as well as from experiment, this time required for equilibrium to be established may be several days in the case of comparatively large molecular weight compounds. Besides requiring a long centrifuging time, throughout which the temperature, rotational speed, etc., of the centrifuge must remain constant, the method cannot be used in the case of many of the most interesting (biological) substances be-

cause they will decompose before equilibrium can be established.

Fortunately, the rate-of-sedimentation method supplements the equilibrium method because it operates best where the equilibrium time is longest.

If the solution is ideal and dilute and if the particles or molecules are not electrically charged, and, also, if no particles are reflected from the ends of the sector-shaped sedimenting column, then the centrifugal force per mole can be set equal to the frictional force per mole—that is,

$$M(1 - V\rho)w^2r = f\,dr/dt \qquad (5)$$

where the frictional constant $f = RT/D$ from Einstein's diffusion equation (D being the diffusion constant). Hence,

$$M = \frac{RT(dr/dt)}{D(1 - V\rho)w^2r} = \frac{RT}{D(1 - \rho V)}S \qquad (6)$$

where S is the sedimentation constant which is the velocity of sedimentation in a unit field.

It will be observed that with this velocity-of-sedimentation method, it is necessary to measure both the sedimentation constant S and the diffusion constant D in order to determine the molecular weight. However, the measurements can be made in a short time if the centrifugal field and the molecular weight are large. The methods of measuring S (eq. 6) as well as C_1 and C_2 (eq. 3 and 4) usually are optical or analytical. The optical methods which have been developed by Svedberg and his students consist in determining the concentration of the material in a sector-shaped transparent centrifuge cell as a function of the distance from the axis of rotation while the centrifuge is spinning by the amount of light absorbed or by the refractive index. The index-of-refraction method has taken several forms such as the Lamm scale method, the schlieren method, etc., and is usually preferable except in cases where the material being centrifuged has convenient absorption bands.

A photograph of an ultracentrifuge rotor is shown in Figure 111, which contains a sector-shaped cell with crystal quartz windows which may be used for either equilibrium or velocity-of-sedimentation measurements. It is made elliptical in shape to increase its strength. The driving mechanism for this rotor is

the same as that shown in Figures 109 and 110. Figure 112 shows a series of photographs using the absorption method for determining the rate of sedimentation in diluted fresh human blood. It will be observed that, in addition to the downward motion of the boundary between the hemoglobin and the clear solvent, there is a slight blurring due to diffusion. With the aid of microphotometer measurements across these boundaries, it is not only possible to measure the sedimentation constant S but the diffusion constant D as well. However, D is usually measured in a separate experiment. If the solution contains more than one molecular species, then each will form a separate sedimentation boundary which moves out with its characteristic velocity. Also, if, instead of homogeneous molecular species, the solution contains a distribution of particle sizes or weights, this distribution can be determined by the "blurring" of the sedimenting boundary. For precise measurements the molecules or particles must be uncharged; otherwise the settling out of the heavier ions will set up electrical potentials which may oppose the sedimentation. This trouble is usually eliminated by centrifuging at the proper pH value or by adding a small amount of a low molecular weight electrolyte to the solution. Since the specific volumes used are based upon dry weight measurements, both the equilibrium and the velocity-of-sedimentation methods take no account of hydration effects but Svedberg and Kraemer and Lansing have shown that as long as the density of the hydration or absorption shell around the molecule does not differ appreciably from the density of the solution, the results are not affected. In fact, in cases of consid-

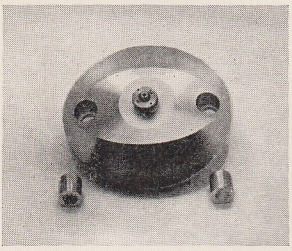

FIG. 111. Rotor used in equilibrium and rate of sedimentation methods for determining particle or molecular weight and size. Right foreground, cell with transparent crystal quartz windows. Left foreground, dummy cell necessary for balancing.

erable differences they show that the errors introduced are quite small provided the concentration of the protein and salt is small.

It might also be noted that the above methods can be used for determining particle or molecular sizes and shapes as well as volumes and masses.

In some cases it is not practical to measure the concentration as a function of the radius in the centrifuge by optical methods. Consequently it is necessary to make analyses of samples of the material taken from different distances from the axis of rotation of the centrifuge by the ordinary methods of analytical chemistry. These samples can be taken from the centrifuge while it is spinning or after it is stopped, provided no remixing takes place. Tiselius, Pedersen, Svedberg, and others have divided the sector-shaped centrifuge cell into approximately equal compartments by means of a partition which is perpendicular to the direction of sedimentation. The partition is usually made of thin Bakelite perforated with a large number of fine holes, which is covered with a piece of hard filter paper. This arrangement does not appreciably disturb the sedimentation but prevents remixing upon stopping.

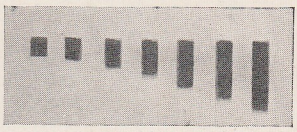

FIG. 112. Series of successive photographs illustrating rate of sedimentation of dilute human blood by the absorption method.

They have shown that using the velocity-of-sedimentation method, the sedimentation constant S is given by

$$S = -\frac{1}{2w^2t} \log_e \left(1 - \frac{2\Delta}{q \times c_0}\right) \qquad (7)$$

where Δ is the total change in amount of the material above or below the partition, x the distance from the axis of rotation, q the area of the partition, and c_0 the concentration of the substance at the beginning. However, this method is not very precise because for large molecular weight substances the time required to accelerate the centrifuge to full

speed and to stop it is an appreciable part of the running time t. Since the product $t\,w^2$ enters into eq. (7), a considerable error is introduced.

In order to increase the precision of this method, Mr. Fox and I have constructed the machine shown in Figures 113 and 114. The centrifuge is first accelerated to full speed and then the material to be centrifuged is introduced through the hollow

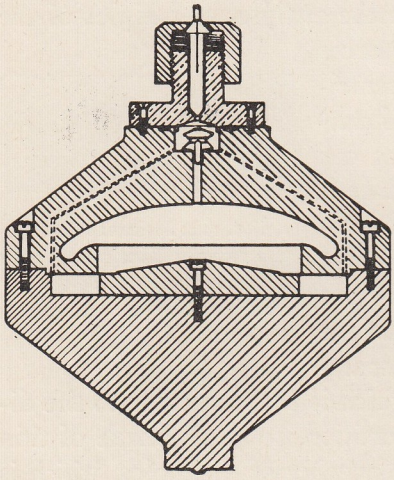

FIG. 113. Section of rotor which permits division of cell contents while machine is spinning, as described on page 256. The rotor is made in two parts and fastened together with screws.

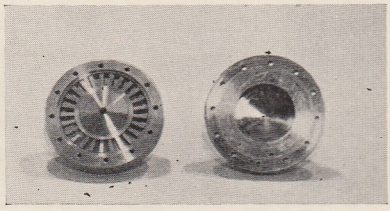

FIG. 114. Photograph of rotor illustrated in Fig. 113 with the two parts separated. Upper part, right; lower part, left.

shaft until the sector-shaped cells are all just filled. The centrifuging is then continued at a constant angular velocity w for the desired time t. A liquid heavier than the solution is then introduced through the hollow shaft. This liquid flows into the sector-shaped cells near their periphery and about half of the solution is forced into a chamber above the cells. After the rotor is stopped, the sedimentation constant is determined from analysis of the original solution and of the material collected in the upper chamber of the centrifuge. The rotor is spun by the same

driving mechanism as shown in Figure 104. The method has been used to measure S for egg albumen and for hemoglobin. It seems to be capable of high precision. Obviously the same apparatus can be used with the equilibrium method as well as the velocity-of-sedimentation method.

As mentioned before, it has been shown by Mason and Weaver for a uniform field and by Svedberg and his collaborators for the centrifugal field that the resolving power is equal to the product of the strength of the field and the height of the sedimenting column. That is, the ability of a centrifuge to separate the sedimenting boundaries of two homogeneous molecular species is proportional to the w^2rh, where h is the height of the cell, r is the radius, and w is the angular velocity. The quantity is roughly proportional to the strength of the rotor, which in turn is the limiting factor in all of the experiments described above. In the case of substances with very large molecular weights, such as tobacco mosaic virus, the whole resolving power of the centrifuge cannot be used because the material settles almost out of the cell before the machine can reach full speed. Recently we have been experimenting with a scheme by which the solvent is made to flow through the cell from the periphery toward the axis of rotation at about 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. For example, tests with this machine show human hemoglobin to be very homogeneous. It might also be noted that in addition to increasing resolving power, this method increases the flexibility by making possible changes or variations in the solvent during the centrifuging. In addition to maintaining the sedimenting boundary stationary in the cell, by the above flow method it can be done electrically in some cases. By making two electrical connections to the two ends of the insulated sedimenting columns with insulated lead wires which pass out through the shafts and connect with mercury cups outside, it has been found possible to maintain

an electrical field in the cell in a radial direction. If the solution being centrifuged is properly buffered, and the substance is not at its isoelectric point, it is possible to hold the sedimenting boundary in the field of view by balancing the centrifugal force on the molecules by the force of the electrical field upon their charges. Some preliminary experiments show that this can easily be done. However, the flow method is far superior in both theory and practice.

The Electrically Driven, Magnetically Supported Ultracentrifuge

The air-driven vacuum-type machines described above are practically ideal centrifuges because they are convection free and will produce centrifugal fields as high as it is possible to construct rotors to withstand. Also rotors of almost any size can be spun successfully. However, they require a supply of compressed air, which is not always available. Also, if constant rotational speed is required, an automatic speed control which actuates the driving pressure is necessary. In order to obviate these inconveniences, the magnetically supported, electrically driven, vacuum-type ultracentrifuge has been developed. It employs the same mechanical principles as the air-driven vacuum-type machine except that the air support has been replaced by a magnetic support and the air turbine drive has been replaced by a small electrical motor. Figures 115 and 116 illustrate the machine which Dr. Skarstrom and I have found very successful.

The rotating parts consist of the armature of an electrical motor D, an iron rod R, the centrifuge C, and the flexible shaft A. The flexible shaft passes through the vacuum tight oil glands G_1, G_2, and G_3. The sliding guide at G_4 serves to prevent the rotor C from wobbling when it is first started. The rotating parts are almost but not quite supported by the upward pull of the electromagnet L on the iron rod R. The remaining small thrust is taken by the top bearing of G_3. Oil leaking from G_3 which is under pressure must pass out through this thrust bearing, so that the surfaces of the thrust bearing are separated by a film of oil. This bearing

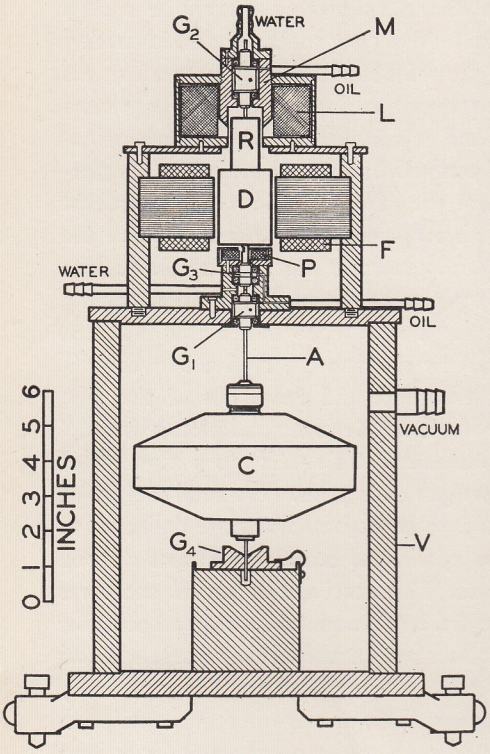

FIG. 115. Section of magnetically supported, electrically driven, vacuum-type centrifuge as described on page 258.

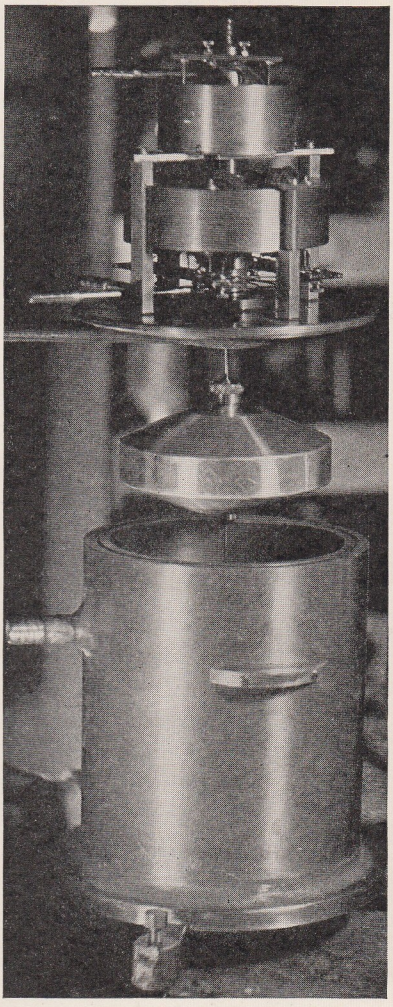

FIG. 116. Photograph of centrifuge shown in Fig. 115.

is able to support the whole weight of the rotating members for several hours without harmful wear in case the current in the solenoid L is interrupted. It, however, has considerably more friction than the magnetic support, which is almost frictionless because the symmetry of the magnetic field practically eliminates electromagnetic drag. The field coils of the motor are actuated by a high frequency alternating current in such a way that the field rotates with the same frequency as that of the alternating current. This induces currents in the solid steel armature D and causes the centrifuge to spin. The high frequency alternating current is generated by amplifying the power from a fixed or variable audiofrequency oscillator, feeding this into a network composed of two parallel condenser-inductance circuits, one tuned above the other below resonance, so that two-phase power is generated. This two-phase power is applied to the opposite pairs of the field coils F and produces the rotating field. The induced currents in D produce considerable heat during the starting period when the machine is accelerated rapidly, so that water is forced in at the top down through the hollow shaft to the region between G_1 and G_3, where it emerges from the shaft and flows out of the tube marked "water." The rotor C shown in the drawing and picture is 6¼" in diameter and is made of solid duralumin ST14. It has a moment of inertia of 80,000 gm./cm.^{2} and weighs over 7 lbs. (the total weight of the rotating members is over 8 lbs.). When 1,000 watts are delivered to the motor at a frequency of 1,200 cycles, the centrifuge attains a speed of 1,060 r.p.s. in about 18 minutes. This acceleration period compares very favorably with the air turbine drive. To maintain the speed constant at any desired value (say, at 1,060 r.p.s. in the above case), a small magnetic pick-up at P delivers a signal voltage whose frequency critically controls the power input to the motor. This arrangement will maintain the rotational speed constant at 1,060 r.p.s. to $\pm$ .03 per cent for at least a day, i. e., to $\pm$ .3 r.p.s. The power input to the motor required to maintain this speed is about 500 watts with a vacuum in V below 5×10^{-4} mm. of mercury. There is no reason why the above type of machine cannot be applied to any centrifuging problem, and it is our opinion that it is much superior to the air drive for the majority of cases.

Finally, attention should be called to another type of centrifuge which spins in a very high vacuum and is suspended by an axial magnetic suspension. This suspension was invented by F. T.

Holmes and has been developed at Virginia by Holmes, Smith, and MacHattie. The centrifuge is attached to a vertical iron rod which is suspended by the coaxial field of one or more solenoids. A steady direct current in one of the solenoids is not quite sufficient to support the rotating members, while the current through the other solenoid is controlled by a photoelectric cell arrangement. The amount of light striking the cell is increased as the centrifuge moves downward and decreased as it moves upward, making it possible to so adjust the current in the solenoid that the rod is automatically maintained at a predetermined height. Since the magnetic field is symmetrical around the vertical axis of the rod, there is no appreciable electromagnetic drag when the rod rotates. Consequently, when the rotor is mounted in vacuo there is almost a vanishing frictional torque against axial rotation. The "centrifuge" has been spun by rotating magnetic as well as electrostatic fields. These machines are especially suited to problems requiring extremely constant high rotational speed or to centrifuging at a reduced temperature or in a high vacuum.[3]

APPLICATIONS

The applications of high-speed centrifuges are so wide and varied that it will be impossible to discuss them here in detail. However, it might be of interest to mention some of the researches in which they have been used. Perhaps their widest and most important use up to the present time has been in the fields of medicine and biology. A great many biological and medical substances have been purified and their molecular weights and sizes studied. Svedberg and his collaborators, as well as Bauer, Pickels, Wyckoff, McFarland, Williams, Stern, Dietz, Severinghaus, Chiles, and others, have shown that most of the easily soluble native proteins are composed of homogeneous molecular species. Also they have found a regularity in the molecular weights of these proteins, which Svedberg interprets as indicating that their molecules are made up of definite fundamental

3. Recently MacHattie spun a .1 in. steel ball 6½ million r.p.m.

units which have masses of about 17,600. He assumes that only a few of these aggregates are stable, and the greater the molecular weight the fewer are the possibilities of stable aggregation. Some of the proteins have their structure altered by such things as dilution, change in pH value, or small amounts of foreign matter. For example, if the pH value of haemocyanin [4] of molecular weight 674×10^4 is changed by a small amount, it dissociates by steps into halves, eighths, and sixteenths, each dissociation product being a homogeneous molecular species. When the pH is changed back to the original value, the components recombine to form the original compound of molecular weight, 674×10^4. The homogeneity of the molecular species is determined by the sharpness of the sedimenting boundary in the ultracentrifuge, and within the limits of the resolving power of the ultracentrifuge most of the easily soluble proteins give sharp sedimenting boundaries. Substances such as tobacco mosaic virus and vaccinia virus, studied by Wyckoff and his collaborators, which have molecular weights well in excess of fifty millions, present important theoretical problems in molecular stability. Interesting differences in the number of sedimenting boundaries, as well as the relative concentration in each, between normal and pathological sera have been found. It is possible that the centrifuge may be very useful as a means of determining the correct diagnosis of disease.

In addition to proteins, many other substances have been studied by the centrifuge. For example, Lamm has shown that the masses of the molecules of starch depend upon its previous treatment and history and are, in general, polydisperse.[5] Signer has investigated Polystyrenes in different organic solvents and found results which indicate that in these solutions neighboring molecules greatly affect each other's free movement, except in very dilute solution. Nichols and Kraemer and others have

4. Respiratory pigment of the snail *Helix promatia* and other mollusks.

5. Recently Beckmann and his students have made a more detailed study of starch and arrived at a somewhat different conclusion.

studied a number of materials used industrially, such as rubber, Neoprene, cellulose, and cellulose derivatives, and have found relationships between the shape and weight of molecules and their macroscopic properties such as viscosity, etc. They have found that a great many of the synthetic large molecular weight substances are polydisperse. The particle sizes of both organic and inorganic colloids have been determined by centrifuging. Also it has been shown that the ultracentrifuge can distinguish between a simple mixture of molecules and chemical equilibrium. Furthermore, it gives the relationship between the equilibrium constant, pressure, temperature, and centrifugal field.

The electrical potentials developed in electrolytes when subjected to gravity or centrifugal fields were studied many years ago by Colley, Des Coudres, Burton, and Tolman. In the ultracentrifuge these potentials may reach values of the order of .1 volt when some electrolytes are first subjected to the centrifugal field. However, as the centrifuging is continued, the potential between the center and periphery decreases and theoretically, at least, should disappear completely when equilibrium is established. Comparing the molecular weight of the electrolyte obtained by the equilibrium method of centrifuging with the known value obtained from chemical data, it is possible to determine the activity coefficient or amount of dissociation of the electrolyte. Pedersen and Drucker have determined a number of activity coefficients in this way. In our own laboratory we have recently been studying both the electrical potentials and the concentration gradients set up in electrolytes in the ultracentrifuge. It might be noted in passing that with this type of technique it may be possible to identify the masses of artificially radioactive isotopes of an element.

As mentioned earlier, the high-speed ultracentrifuge has been used for the study of the relative densities of the materials in the living cell. Such things as "viscosity" and "surface tension" of the living substance, protoplasm, as well as the elasticity of the cell membranes, have been estimated. Also, it is helpful in dis-

tinguishing between real structures and so-called artifaxes. In some cases, after the cell has been centrifuged, abnormal development occurs, whereas in others this is not the case. The magnitude of the centrifugal fields required to kill some cells is rather surprising. H. W. Beams and R. L. King centrifuged fertilized eggs of the parasitic worm *Ascaris suum* at 400,000 gravity for an hour. The eggs were then removed from the centrifuge and observed under a microscope. At first the cell contents were stratified into definite layers. Twelve hours later the eggs had lost their stratification and most of them lived. However, the above treatment will kill a great many cells.

The centrifuge is of use in studies of the adhesion of films to surfaces. The experiments consist in determining the centrifugal force necessary to throw the film off the periphery of the centrifuge. Since the peripheral velocity of some of the air-driven, vacuum-type centrifuges may exceed twice the velocity of sound in air, interesting studies of the friction of different gases at reduced pressures on rapidly moving surfaces are possible. Several other applications could be discussed if space permitted, but perhaps the material presented will illustrate the extensive and important fields for the application of high-speed centrifuging technic.

CHAPTER IX

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R_1 = inner radius of tubular centrifuge

R_2 = radius of solid rod

a = clearance between flange and inner wall of the tube

C_0 = concentration of material to be centrifuged

C = concentration of the heavy fraction

then for most efficient operation

$$\frac{C_0}{C} = \frac{a(2R_1 - a)}{R_1^2 - R_2^2}$$

If T = time material remains in Centrifuge and L = length of centrifuge tube

$$T = \pi(R_1^2 - R_2^2)L / (\text{volumetric rate of flow})$$

$$S = \frac{1}{2\omega^2 T} \log_e \left\{ \frac{R_1^2}{R_2^2}\left(1 - \frac{C_0}{C}\right) + \frac{C_0}{C} \right\}$$

volumetric rate of flow $= \dfrac{\pi L \omega^2 S (R_1^2 - R_2^2)(R_1 + R_2 - a)}{2(R_1 - R_2 - a)}$

$= \dfrac{\pi L \omega^2 S (R_1 + R_2)^2}{2}$ where a is small

$300 \times 3 \times 10^5 \cdot 100 \times 10^{-11}$

10^{-1} cc.