The Design and Operation of the B-IV Zonal Centrifuge System [1]

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SUMMARY

A zonal centrifuge for either rate-zonal or isopycnic-zonal centrifugation with a total capacity of 1.7 liters useful with sucrose gradients at speeds up to 40,000 rpm (90,000 × *g* at R_{max}) is described. The rotor is loaded and unloaded during rotation through a coaxial face seal. The core divides the rotor chamber into four sector-shaped compartments and also serves to channel the sample layer into the rotor during loading, and the recovered fractions out of the rotor during unloading. Ancillary instrumentation is described for recording the absorbance of the recovered gradient and for integrating the square of the angular velocity during the entire interval between sample introduction and gradient recovery. The centrifuge system has been successfully used to isolate mitochondria, polysomes, ribosomes, ribosomal subunits, macroglobulins, and to fractionate glycogen.—Nat Cancer Inst Monogr 21: 137–164, 1966.

SERIES A ZONAL rotors have been designed for the separation of particles ranging in size from whole cells to chloroplasts and mitochondria (*1*, *2*). For the isolation of particles ranging down to very large molecules such as serum macroglobulin, B-series intermediate-speed rotors have been developed (*3–5*). The present paper describes the B-IV rotor system, which is applicable to the separation of subcellular organelles, including nuclei, mitochondria, microsomes, polysomes, ribosomes, ribosomal subunits, viruses, and macroglobulins. The mass of viral material which may be purified and concentrated with the B-IV and with subsequent rotors in the series designed more specifically for viral isolation (B-V, VIII, IX, and XII) is large; therefore, it has been considered advisable to develop suitable virus-tight enclosures for the upper end of the rotor, the seals, and the ancillary systems that will come in contact with the recovered gradient (*6*).

The B-II prototype zonal centrifuge previously described (*3*) has been useful in both the extension of zonal centrifuge theory and in the experi-

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[2] Operated for the U.S. Atomic Energy Commission by the Nuclear Division of Union Carbide Corporation.

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mental studies on particle separation. Instabilities observed with this rotor stimulated an extended study and redesign program (*4*, *7*, *8*) that led to the development of the B-IV system. The B-IV rotor has been used routinely at its maximum design speed of 40,000 rpm (*5*).

DESIGN OF THE B-IV CENTRIFUGE SYSTEM

A number of problems must be simultaneously solved in the design of an intermediate-speed, dynamically loaded centrifuge rotor (*4*, *8*). The rotor configuration must allow ideal sedimentation to occur in sector-shaped compartments. The compartmentation must also prevent mixing caused by Coriolis forces as fluid moves radially during loading and unloading. Interior surfaces must be arranged to allow the sample layer to spread uniformly over the surface of the density gradient as it flows into the rotor, and for the separated zones to be recovered without appreciable loss of resolution at the conclusion of a centrifuge run. The fluid lines and seals must be arranged to allow fluid to flow into and out of the rotor at low exterior pressure without heating, excessive shearing, spraying, foaming, or cross-leaking. The rotor configuration should be a stable one at all speeds, of as simple design as possible, and easily disassembled for cleaning and inspection. Methods for meeting some of these requirements have been listed previously (*4*). In long cylindrical rotors, however, more attention must be paid to the problem of funneling the gradient out of the rotor without loss of resolution than in short, flat A-type rotors (*2*).

The difference in hydrostatic pressure between the centrifugal and centripetal edges of the rotor fluid compartments during high-speed operation is considerable. It is imperative that the fluid pressure in the lines leading to the center and to the edge of the rotor compensate each other. This is done by leading both lines back as close as possible to the axis of rotation and by using a small-radius seal system for the coaxial fluid lines. A schematic presentation of the operating sequence of the B-IV rotor has been included in a previous paper (*4*).

The Seal

The coaxial seal developed for the B-II zonal centrifuge (*3*) worked in a satisfactory manner only at low speeds, since the mass of the stationary portion of the seal was too large to allow it to follow the rotating seal if the plane of the latter was not absolutely normal to the axis of rotation. In addition, fluid leaking out of the seal could form aerosols in the surrounding atmosphere. The seal system has therefore been completely redesigned to reduce the mass and to contain solutions or suspensions which may leak out during operation at high speed.

The seal system developed for the B-IV centrifuge consists of a housing that completely encloses the seal; a manifold to channel the edge, center,

and coolant streams; a two-path, high-speed face seal; and a secondary seal to retain the coolant solution that may also contain a disinfectant (text-fig. 1). The sealing surfaces are preloaded by a coil spring located inside the seal housing. O ring seals at all connections permit the seal to be readily disassembled for cleaning and inspection without the use of special tools or fixtures. The life expectancy of this seal has not been established. One seal has been used for over 100 hours at 40,000 rpm with only minor maintenance. Satisfactory operation at 50,000 rpm has been attained in an experimental test stand constructed to determine whether this seal could be used at higher speeds.

The amount of cross-leakage occurring in the seal during use depends on the condition of the seal, back pressure in the fluid lines, and, possibly, on rotor speed. In continuous flow studies, negligible cross contamination has been observed with viruses. With high flow rates and viscous fluids, cross flow as high as 1 percent may be encountered. In the experiments performed to date, leakage across the seal has not presented a problem.

Fluid leaking out of the primary face seal mixes with the coolant stream, which is contained in a closed loop. For leakage to the outside to occur, the contaminated coolant must also leak past the lower or secondary face seal. Centrifugal force tends to prevent this leakage from occurring.

The seal surfaces are composed of a filled fluorocarbon [3] which presses against stainless steel. The components of the seal are shown in figure 1. It should be noted that the fluid lines do not cross in the seal or manifold in the most recent modification of this centrifuge, the rotor center line flows through the center line of the seal, and the rotor edge line flows through the outer seal line. The sample and the recovered zones are therefore subject to minimal shear during passage through the seal.

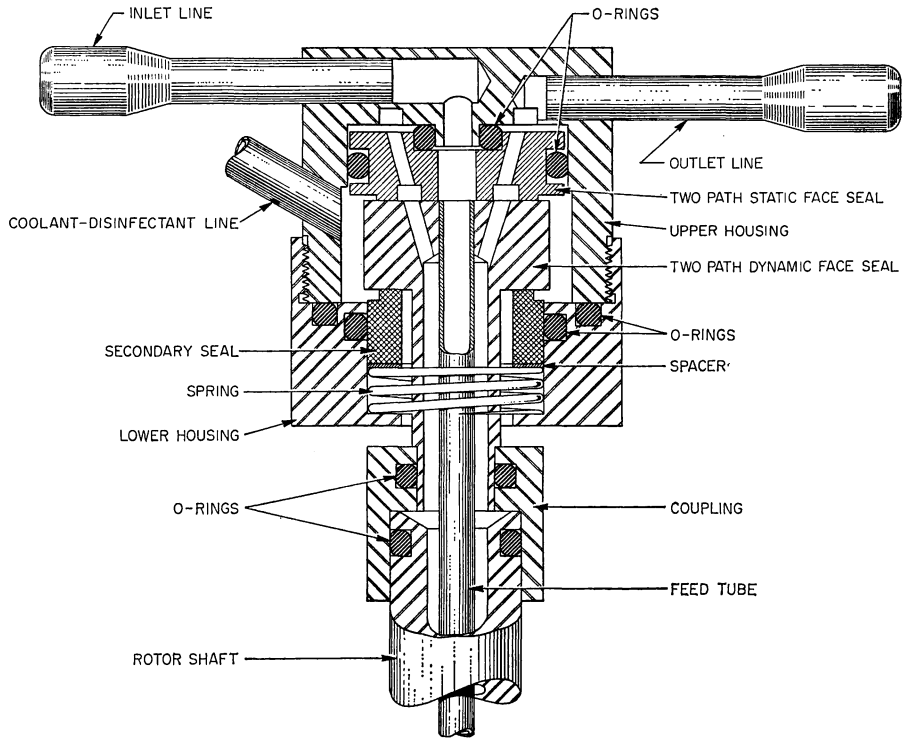
Coolant flow through the seal during high-speed operation is essential; if the flow is interrupted the seal will heat up rapidly. It is desirable to have a large squeeze bottle filled with water available to cool the seal externally should this occur.

The Upper Damping Bearing

The B-II zonal centrifuge rotor previously described (*3*) was destructively unstable in the region of 31,000 rpm. In the original tests, low-speed instability was observed in the region around 5000 rpm. This instability diminished when the number of septa was increased. Initially it was thought that fluid movements in the rotor contributed to this instability, and a large number of septa, 36 in all, were used to achieve a high degree of compartmentalization. The concept that compartmenting would solve the instability problem and that the number of compartments should equal the rotor speed in thousands of revolutions per minute (*9*) has not been supported by recent work.

In an effort to detect sources of vibration in the system the natural fre-

[3] Rulon, available from the Dixon Corporation, Bristol, Rhode Island



TEXT-FIGURE 1.—Enclosed seal for Series B rotors.

quencies were determined for the Spinco Model L drive system, rotor, upper bearing, and the supporting framework of the centrifuge.

For definitive studies, proximity probes[4] have been deployed in four positions to monitor vibrations occurring in the upper bearing, in the top and bottom ends of the rotor, and in the transmission of the centrifuge drive. The outputs are simultaneously displayed on a four-channel oscilloscope or recorded on tape, and photographs taken of oscilloscope traces. The phase relation of the motions at various points may be observed along with the frequencies of vibrations and the amplitudes. As shown by the oscillograms in figure 2, the B-II rotor exhibited a high amplitude precessional motion associated with a natural forward precessional mode of vibration. Efforts to eliminate this vibration involved three areas of the system: (*a*) drive quill and lower damper bearings, (*b*) transmission box mounting system, and (*c*) top centrifuge damper bearing. The first two were investigated in detail and found not to be the primary cause of the instabilities observed at the higher speeds.

The original design of the B-II top bearing was radically revised to incorporate more damping and a lower spring rate between the bearing and the vacuum chamber. Immediate improvements in stability were noted. The maximum amplitude of the instability was reduced from

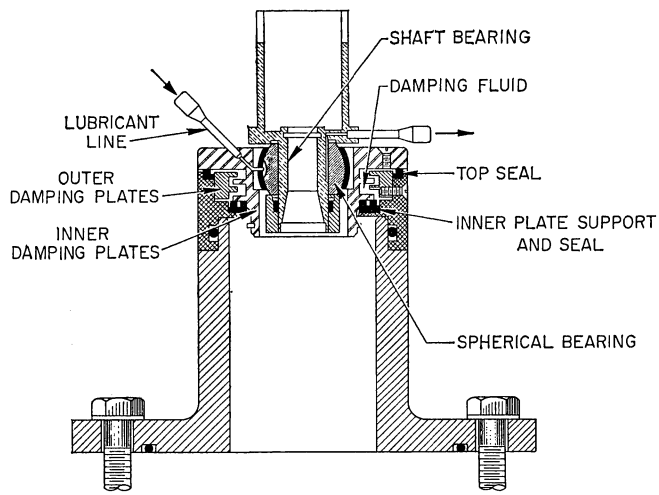
[4] Bentley Nevada Corp., Minden, Nevada, H-3020 and H-1-074-3.

greater than 0.120 inch total indicator reading (TIR) to the maximum amplitude of 0.003 inch TIR, as shown by the oscillogram in figure 3. This amplitude produced no detectable noise, and at about 33,000 rpm the amplitude markedly diminished, permitting the rotor to be driven successfully to 40,000 rpm. The actual value of the runout (variation in distance between the rotor and the probe during rotation) of the original B-II system during unstable operation at 30,000 rpm is not known since the probes positioned 0.060 inch from the rotor were completely destroyed.

This work led to the development of an enclosed journal bearing with the proper damping constants (text-fig. 2 and fig. 4). The bearing is self-aligning and is cooled by a stream of water. Damping is achieved by shearing a viscous oil between parallel plates. One set of plates is attached to the bearing and mounted to allow a small translational movement and by the so-called "squeeze film effect" of the cylindrical surfaces of the damper bearing. The other set of plates is mounted rigidly to the centrifuge top closure. With this bearing, the B-II centrifuge rotor could be rotated at 40,000 rpm filled with water *and without septa.*

The B-IV Core

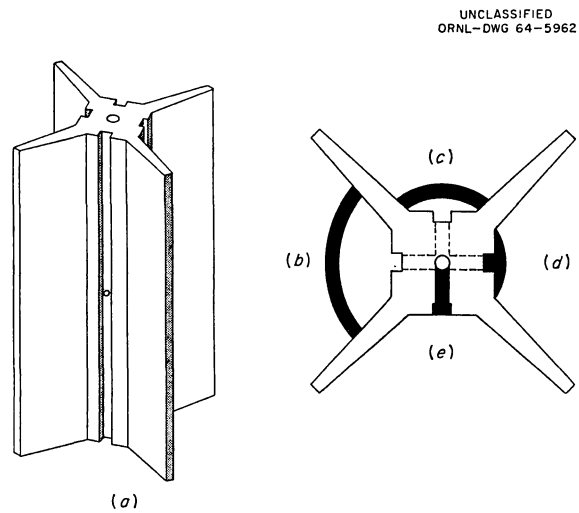
The core of a dynamically loaded zonal rotor serves to divide the rotor volume into sector-shaped compartments, to connect the seal lines to the center and edge of these compartments, and to funnel the gradient into and out of the rotor with minimal mixing and consequent loss of resolution. When it was shown that multiple compartmentation was not necessary to achieve rotor stability, the possibility of using a simple four-vaned core was reconsidered.



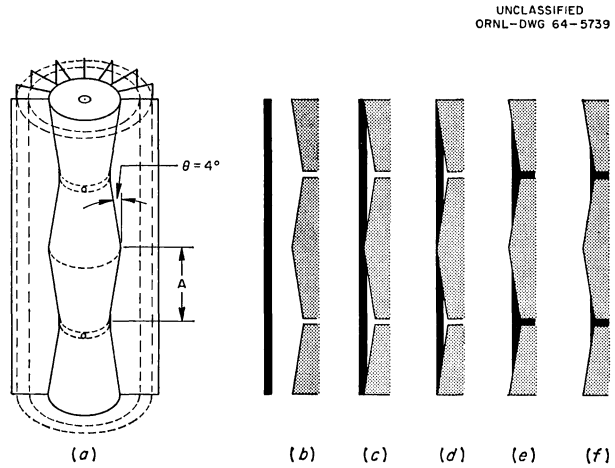
TEXT-FIGURE 2.—Top damper bearing for B-IV rotors.

The B-IV core, shown schematically in text-figure 3, is machined from two pieces of aluminum, which are then permanently joined. Structural considerations require that the vanes taper toward the edges, resulting in a small departure from an ideal sector shape. As a zone approaches the center of the rotor during unloading, it makes contact with the square center section of the core next to the root of the vane. The angle between a given density zone and the flat face of the core is initially 48.9°, resulting in flow of the zone toward the center of the core face. This effectively sweeps each density zone toward one of the four grooves in the center of each core face. Centripetal flow of particle-containing fluids in a centrifugal field has certain characteristics that are used to advantage. Thus, in rate-zonal centrifugation the particle concentration in the film of fluid in contact with the core during unloading is being continually decreased by sedimentation. As a result, laminar flow does not decrease resolution as much as one might expect. The maximum horizontal distance a particle flows in contact with the core face is 1.5 cm. The B-II core is also shown in text-figure 4 for comparison.

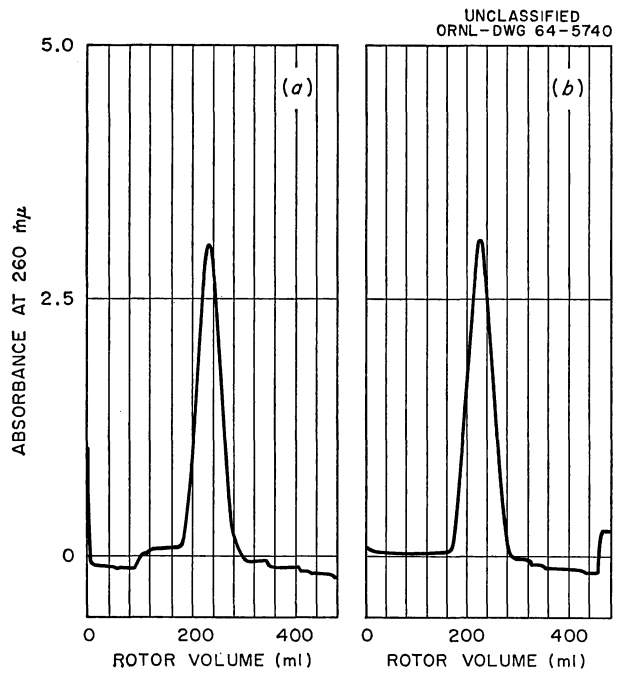
To compare the resolution obtainable with the B-II and B-IV cores, a number of experiments, including the rate separation of T2 and T3 phage under identical conditions, have been performed. No differences were noted. To determine whether the starting zone thickness was comparable in the two rotors, bovine serum albumin samples were run into the two rotors under comparable conditions and unloaded through the ultraviolet (UV) absorbance monitor, as indicated in the legend of text-figure 5. Essentially identical patterns were obtained, a result showing that the volume occupied by the sample was the same in both rotors.



TEXT-FIGURE 3.—Zone recovery in B-IV core. (*a*) B-IV core; (*b–e*) zone recovery; (*b*) zone approaching core; (*e*) part of zone recovered, part within core.



TEXT-FIGURE 4.—Zone recovery in B-II core. (*a*) Schematic of B-II core, with some septa removed. (*b–f*) Details of zone recovery in B-II core; (*b*) zone approaching core, (*f*) part of zone recovered, part within core.



TEXT-FIGURE 5.—Comparison of widths of sample zones in B-II (*a*) and B-IV (*b*) rotors. Sample, 25 ml of 2.5 percent bovine serum albumin in 4.2 percent sucrose; overlay, 216 ml of water; gradient, 17 w/w percent sucrose grading into 55 w/w percent sucrose; rotor loaded at 5000 rpm, accelerated to 25,000 rpm, maintained at that speed for 15 minutes, and unloaded at 5000 rpm.

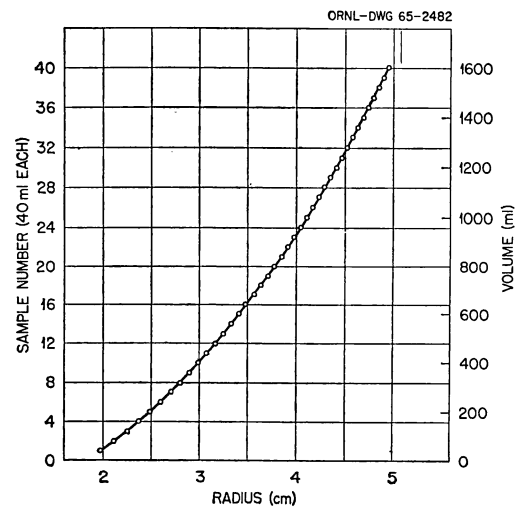
For convenience a plot of rotor volume versus radius is shown in text-figure 6. The sample used in text-figure 5 has a calculated width of 0.07 cm in the rotor. The observed width at half-peak height is equivalent to 0.13 cm, indicating relatively small boundary widening. Equations relating rotor volume to radius are given by Bishop (*10*).

Rotor Length

The length of a zonal rotor is limited by rotor stability considerations and by the fact that two forces act on particle or density zones during rotation. These are centrifugal and gravitational forces. The net result is that all zones of equal density form paraboloids of revolution about the axis of rotation. The equation for the intersection of an axial plane with the paraboloid of revolution (*3*) is given by

$$L = \frac{r^2\omega^2}{2g}$$

where L (cm) is the height from the apex of the paraboloid of a given point on the isodensity surface, r (cm) is the radius at that point, ω is the angular velocity in radians per second, and g is the acceleration due to gravity. It is evident that at various heights, L, isodense zones in a long rotor will be subjected to different centrifugal forces. Indeed, if the rotor is long enough to allow the apex of a zone to be in the rotor, the centrifugal field in a given zone will vary down to zero. For this reason, low-speed rotors generally have a very short height and are of large diameter, while high-speed rotors may be long and narrow. Fortunately, considerations of rotational stability and strength of materials generally favor the same configurations (*8*).



TEXT-FIGURE 6.—Rotor radius versus rotor volume for the B-IV zonal ultracentrifuge. Volume includes the 11 ml volume of the line leading from the point of fraction collection to the rotor.

Ancillary Instrumentation

The technique of zonal centrifugation in the B-IV rotor can yield analytical results of considerable precision. However, to realize its full potential it was necessary to develop several ancillary systems. Among these were systems for rotor temperature control, for determining the quantity of sample material recovered in the gradient, and for measuring the total centrifugal force exerted on a particle from the time of its introduction into the rotor until it is recovered in a fraction collector.

All controls (speed, time, temperature, vacuum) are mounted on a rack (fig. 5, right), which also holds the speed indicating and integrating system. The latter presents continuously a digital indication of the integral of $\omega^2 dt$ or G_c. A Bentley proximity probe [4] with associated power supply and amplifier,[5] used to detect rotor speed, is sensed by a frequency meter,[6] which provides a *dc* output voltage proportional to rotor speed, ω. This voltage drives a slide wire potentiometer,[7] which was modified by the addition of a squaring circuit to give ω^2. The squaring circuit output is electronically integrated by an integrator,[8] which provides a digital indication of the time integral of the square of the rotor's angular velocity,

$$\int_{t=t_o}^{t=t_1} \omega^2 dt$$

where ω is the angular velocity in radians per second, t_o is the time at which the sample is moved into the rotor, and t_1 is the time when collection is begun. A calibrating frequency source and locked timer are used for calibrating the entire integrating system. The digital readout is multiplied by 10^6 to yield the actual value in units of ω^2 seconds.

An absorbance monitoring system is arranged so that fluid flows from the rotor seal directly up through a 0.2 cm light path quartz cell (*11*) mounted in a Beckman DU monochromator. The monochromator has a photomultiplier and amplifier system[9] so that absorbance is indicated linearly on a recording strip-chart potentiometer. The hold-up volume from the point at which fluid leaves the core to collection is 11 ml. The spectrophotometer is mounted on a movable stand so that it may be positioned directly over the upper rotor bearing or may be retracted to allow the bearing and rotor to be moved away from the centrifuge. Forty ml volume fractions are collected by hand using 40 ml calibrated screw-top centrifuge tubes.[10] As the effluent line is moved from one tube to the next, a foot switch is depressed which moves an event-marking pen on the absorbance recorder. The position of any fraction in the

[5] Bentley Nevada Corp., Minden, Nevada, Distance Detector Energizer, Model B-15, and Bentley Distance Detector, Model D-152.

[6] Hewlett Packard, Palo Alto, Calif., Model 500BR.

[7] Leeds & Northrup Co., Philadelphia, Penna., Speedomax Model "H."

[8] Royson Engineering Co., Hatboro, Penna., Lectro Count.

[9] Gilford Instrument Laboratories, Inc., Oberlin, Ohio, Absorbance Indicator Model 220, Light-Source Stabilizer, Model 220, Optical Density Converter Model 220.

[10] Available from Bellco Glass Inc., Vineland, N.J.

absorbance monitor diagram can therefore be determined. For most work the monochromator is set at 260 mμ, and the amplifier set to record 0 to 2.5 absorbancy full scale. With correction for the short light path, the actual recorded scale is 0 to 12.5 absorbance units. It should be emphasized that when mitochondria and other microscopic particles are separated, a large fraction of the observed absorbance is the result of turbidity.

Containment

The B-IV system is suitable for work with nonpathogenic materials. For work with virus-containing suspensions, it is desirable to provide additional containment to prevent (*a*) the inadvertent introduction of contaminants into the fluids being fractionated and (*b*) the possible leakage of highly concentrated materials into the environment. Experimental containment systems for the B-IV centrifuge have been described by Cho *et al.* (*6*).

OPERATION OF THE B-IV CENTRIFUGE SYSTEM

Assembly of the Rotor System

The components of the B-IV rotor are shown in figure 6. The two end caps of the rotor are sealed with O rings. The four-vaned core is placed in the rotor after the bottom end cap has been screwed on. The O ring for the upper end cap is placed in position in the groove in the body of the rotor, and the manifold plug is screwed into position on the under side of the upper end cap (after the guide for the stem of the seal has been inserted). The top end cap is then screwed down, and the end caps are tightened until small bench marks are aligned. The stem guide is essential to avoid displacing or tearing the O ring, located within the manifold plug, when inserting the seal.

After ethylene oxide sterilization, the assembled rotor is placed in the centrifuge (fig. 7), and the upper chamber closure is screwed into position. The damper and bearing are positioned next. The oil lines for the bearing and the cooling water lines for bearing and seal are visible in figures 8 and 9.

Seals should be handled with care at all times. When the seal is positioned on the assembled rotor it should be pushed gently into running position. Coolant and feed lines should be attached without exerting undue force on the seal housing. *The seal is never operated without a continuous flow of water through both the seal and the upper bearing.*

After the rotor has been accelerated to approximately 3000 rpm, a check is made for (*a*) excess water in the oil drain line in addition to the few drops normally observed when the rotor is first started, (*b*) water leaking out the lines leading to the rotor center and the rotor edge, and (*c*) droplets of liquid in the rotor center line while the gradient

is loaded into the empty rotor. Appearance of droplets indicates leakage across the inner sealing dam if the droplets contain gradient solutes, or leakage across the outer sealing dam if the droplets are coolant fluid. It is important to detect signs of seal failure early, especially if a valuable sample is to be used.

After use, the seal is thoroughly cleaned by passing hot tap water through the seal after it has been removed from the rotor. The seal is then completely disassembled (the seal housing has left-hand threads), and the rubbing surfaces inspected. The lower seal and spring are cleaned carefully and all parts dried in a stream of dry air. Since cesium chloride corrodes stainless steel, the seal is cleaned as soon as possible after an experiment.

Before the seal is reassembled, the rubbing surfaces are inspected for scratches or indentations. Sharp instruments are not used and minimal force is exerted during seal assembly. The long center tube is aligned before insertion into the rotor.

The assembled seal is positioned by gently inserting the stem through the rotor upper shaft extension and, with gentle pressure, moving it through the O ring located inside the manifold plug. The upper portion of the seal is supported partially by the metal ring around the damper and bearing. In practice, an O ring is often used to hold the seal in a given position.

The cooling lines and the oil lines are next connected to the proper outlets provided on the seal and bearing assemblies. The completed assembly at this point is shown in the centrifuge and glove box in figure 8, and outside the centrifuge in figure 9. A schematic diagram of the rotor connections is shown in text-figure 7.

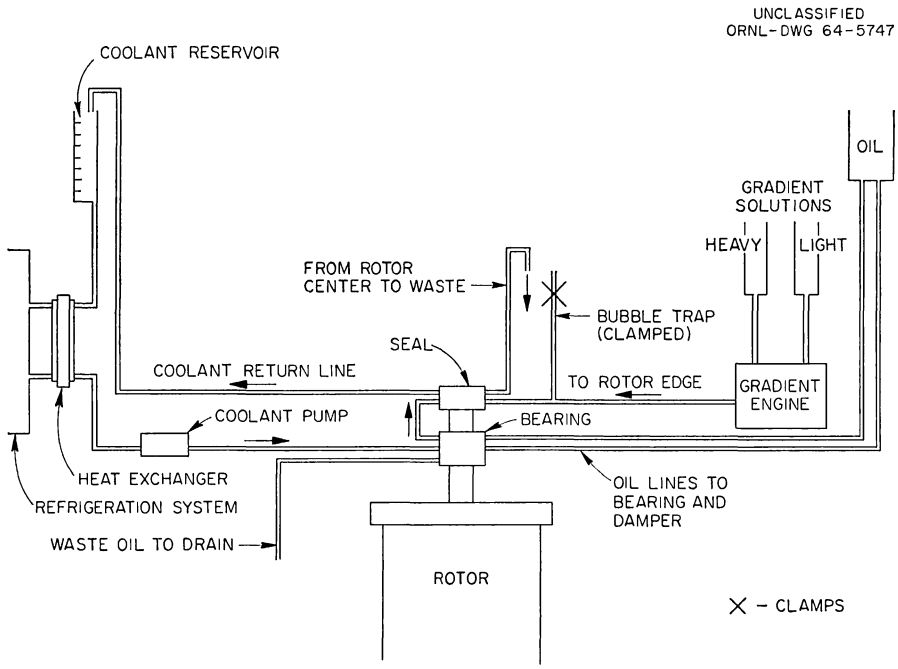
Cooling water flows, respectively, from the pump [11] through the upper rotor bearing, seal, graduated reservoir (not visible in photographs), refrigerated heat exchanger, and back to the pump. The cooling water circulates entirely inside the glove box enclosure, while the refrigerant circulates outside. The graduated reservoir indicates the amount of water in the system and provides visual indication of flow rate.

Oil lines from the reservoir within the glove box connect to two inlets on the bearing. The oil not only lubricates the bearing but aids in sealing the vacuum chamber. The overflow oil line from the bearing drains inside the glove box into a small plastic bottle.

Introduction of Gradient and Sample

After the rotor and associated tubing are positioned, the gradient engine is attached to the gradient solution bottles. The line from the gradient pump to the rotor seal edge line is connected to a glass "T" joint, which serves as a bubble trap and drain line when the sample is being introduced into the rotor.

[11] Sigma Pump Model AL 4-300.

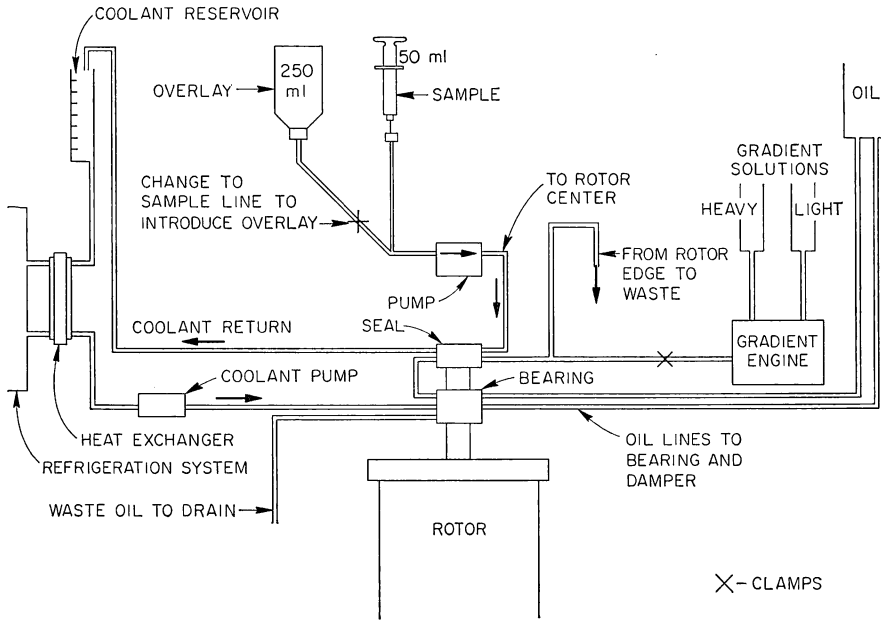


TEXT-FIGURE 7.—Schematic diagram of fluid lines when gradient is loaded into rotor.

At this time the rotor chamber is evacuated, the refrigeration system turned on, and the rotor accelerated to 3000 to 5000 rpm. The gradient, light end first, enters the rotor, is distributed uniformly over the rotor wall and is forced, by denser fluid pumped in subsequently, toward the center of the rotor. The gradient, normally 1000 to 1200 ml, does not fill the rotor completely. An underlay (or "cushion") of fluid denser than that used to form the gradient is pumped in until the first part of the gradient emerges through the center line. At this time, the gradient pump is stopped, and the gradient line is clamped off. The gradient is now ready to receive the sample. This is introduced through the center line (text-fig. 8), so that the sample is placed on top (centripetal end) of the gradient. The sample line is part of an intravenous set.[12]

A small pump[13] on the sample line close to the seal ensures constant flow during sample and overlay introduction. The drip-chamber probe is inserted into the overlay bottle, and the line is clamped. The needle of the sample syringe is inserted into the medicinal entry; this line is clamped also. The clamp on the bubble-trap line between the T and the gradient pump prevents the solution from emerging out of the rotor into the exterior of the glove box. The clamp from the sample syringe line is removed, and the sample is introduced into the rotor with the syringe, displacing part of the underlay from the edge of the rotor through the bubble-trap line to

[12] Intravenous set—Abbott No. 4540; Cutter Code No. 860-08.
[13] New Brunswick PA-56 pump.



TEXT-FIGURE 8.—Schematic diagram of fluid lines during sample introduction into B–IV rotor.

a waste container inside the glove box. To rinse the sample syringe, the clamps are arranged to allow a small amount of overlay solution to be drawn into it. The clamps are then rearranged to allow this rinse to flow into the rotor. When the fluid lines are clear of visible sample, the integrator counter is switched on, the sample syringe line clamped, the overlay line opened, and the small pump turned on and adjusted to pump from the overlay solution bottle to the rotor center.

Approximately 200 ml of overlay is pumped to the rotor (displacing part of the underlay from the edge) to move the sample zone out from the core. An additional 40 ml of overlay is introduced, after which the clamp from the gradient engine is removed and the pump started. The flow from the edge line of the rotor and from the gradient engine both pass through the bubble-trap line to waste. When all overlay solution has entered the rotor, the line from the bottle is clamped, and the sample pump is turned off and removed from the line, the sample syringe line opened, and the bubble-trap line clamped. Flow extends from the gradient engine to the edge of the rotor, and overlay is displaced from the center of the rotor to the syringe. By raising the sample line and tapping it gently, air bubbles are removed from the rotor.

When the 40 ml of overlay is recovered in the syringe, the gradient pump is stopped and the pump line clamped near the rotor. The only line open to the rotor leads from its center to the syringe, which is mounted vertically in a clamp. The rotor is accelerated to achieve the separation desired.

During a run, the level can be observed in the syringe reservoir and leakage can be detected. The volume in the syringe will decrease below 25 ml due to rotor expansion at higher speeds, but after deceleration to unloading speed the volume is restored.

Gradient Recovery

The rotor is decelerated to a speed of 5000 rpm and emptied by displacement. The rotor center line, which had been connected to the sample introduction syringe during the run, is removed and replaced by a line to the quartz flow cell (0.2 cm light path) and through it to the sample collection tubes.

The Gilford absorbance indicator and optical density converter unit used on the Beckman spectrophotometer and instrument panel, respectively, permit readings of 0.25 to 2.5 optical density units full chart range on the Honeywell recorder. With a 0.2 cm flow cell, the actual range becomes 1.25 to 12.5 absorbance units. Normally, a wavelength of 260 $m\mu$ is used.

The gradient pump is adjusted to pump only the most dense material used (55% sucrose in most virus-isolation studies). The only open lines lead to the rotor edge from the gradient pump and from the core of the rotor to the flow cell (text-fig. 9). As the contents of the rotor are displaced by 55 percent sucrose, the fluid running through the DU monochromator cell is collected in 40 ml centrifuge tubes. As each 40 ml tube is collected, a foot switch is depressed to activate an event-marking pen on the strip-chart recorder. This provides a visual record along the base of the chart which may be used to correlate the presence of a given absorbance peak with its location in the recovered fractions.

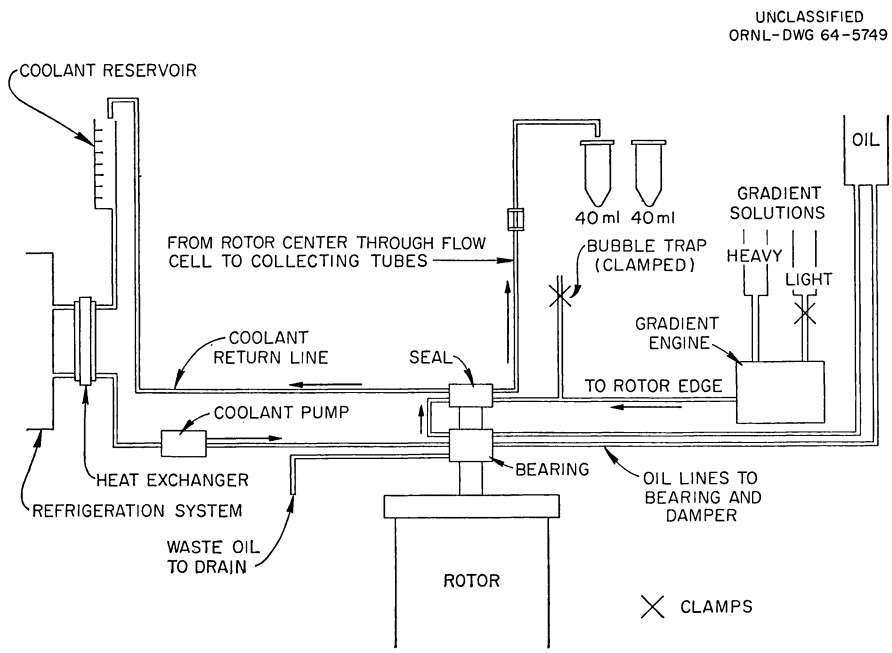
Density and Refractive Index Determinations

Until recently, the density of the recovered fractions was determined by using a manually operated Abbe-type refractometer. It is extremely difficult to do this aseptically and without contaminating the operator. To provide a semiautomatic method for determining the density of the recovered fractions, a modified torsion balance mounted in the glove box is adjusted to indicate density directly. The 40 ml fractions are shaken with the screw tops in place, opened, and individually placed under the glass bob. (The torsion balance has been modified to electronically read and display the results of the Archimedes measurement on tape outside the glove box. The printout mechanism is activated by a foot pedal.)

Gradient Pump Assembly

The gradient pump [14] is shown in figure 10. The pump is sterilized with the pistons partially disassembled to permit ethylene oxide exposure.

[14] Available from Beckman Instruments, Spinco Division, Palo Alto, Calif.



TEXT-FIGURE 9.—Arrangement of fluid lines during sample collection.

A program cam produces a 1200 ml gradient that is linear with respect to rotor volume. The pump is located outside the glove box. A single Tygon tube carries the gradient to the rotor from the pump and into the glove box through a metal seal. Gradient solutions are kept in sterile bottles and connected to the pump by disposable transfusion tubing connectors.

Cleaning and Sterilizing

All parts of the system in contact with samples or contaminated must be sterilized. At present, reusable components are treated with formaldehyde where appropriate, washed, and rinsed with distilled water, and either steam- or gas-sterilized. The rotor and its components [including seal, damper bearing, gradient engine and associated tubing, disposable Oak Ridge No. 30 rotor tubes (*6*), and the glass 40 ml screw-top centrifuge tubes used for gradient recovery] are gas-sterilized in a regular steam sterilizer (American Sterilizer Co.) fitted with an ethylene oxide system. Gas sterilization, which requires approximately 8 hours, is usually done overnight. The components are placed in paper bags, which are opened in the glove box, or in the case of the gradient engine, when the tubes leading to the sterile solutions and to the rotor are ready to be attached.

The glove box in which the rate-zonal centrifuge is housed is sterilized by overnight exposure to ethylene oxide (two 20 oz cans). The outlet valves from the box are closed during this process; filtered air is pumped through the box, beginning early the next morning, to remove residual gas.

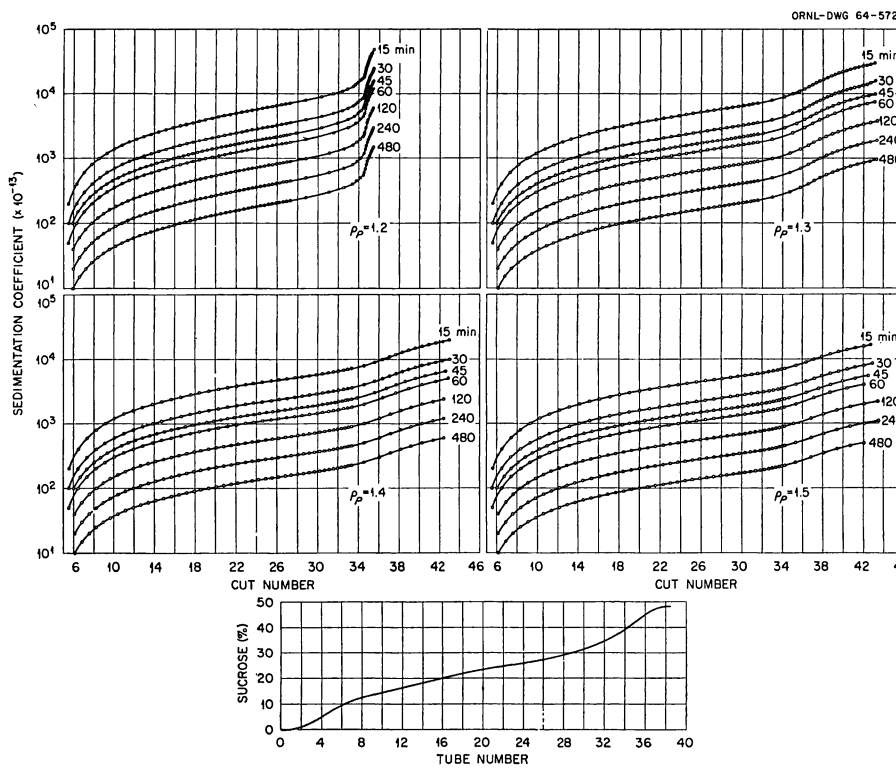
All solutions used are sterilized either by filtration or, when feasible, by steam.

DETERMINATION OF SEDIMENTATION RATES

A computer program (*10*) is used to calculate the equivalent sedimentation rates (S^*) in water at 20° C of spherical particles that do not behave osmotically. The basic equation is a modification of that used by Martin and Ames (*12*).

In experiments designed for searching tissue breis for virus particles, it is desirable to centrifuge long enough to spread known virus particles between the soluble protein zone and the mitochondrial zone. Rat liver mitochondria band isopycnically in sucrose gradients at a density level of 1.203 g per cm^3 [44.5% (w/w) sucrose]. In practice, virus particles are not allowed to reach this density during rate-zonal centrifugation.

The positions that particles having a range of densities and sedimentation coefficients would have after various centrifugation times at 20,000 rpm were calculated. These are plotted in text-figure 10. With the gradient used for these calculations, a suitable spread is obtained in 1 hour



TEXT-FIGURE 10.—Calculated positions in a sucrose density gradient in the B-IV rotor for nonosmotic particles having indicated densities and sedimentation coefficients as a function of centrifugation time at 20,000 rpm.

at 20,000 rpm. This speed and time have been used for most of the studies on viruses, glycogen, and microsomes.

EXPERIMENTAL SEPARATIONS

During the development of the B-IV zonal ultracentrifuge a variety of biological materials have been isolated. Among the viruses studied are polio (*3*, *13*), respiratory syncytial virus (*14*), Rauscher and Moloney mouse leukemia viruses, tobacco mosaic virus, bromgrass mosaic virus, and T2 and T3 coliphages (*14*, *15*). Nuclei, a membrane fraction, mitochondria, microsomes, polysomes, and ribosomes have been separated from rat liver homogenates (*16*, *17*). Cytochrome oxidase was found to be associated exclusively with the mitochondrial peak (*18*). Polysomes, ribosomes, and ribosomal subunits have been isolated from *Escherichia coli* (*5*, *19*, *20*).

By use of the two dimensional s-ρ technique, viruses added to tissue homogenates have been recovered in a high state of purity (*21*). The glycogen spectrum has been separated into a series of homogeneous subfractions from liver (*22*) and from *Tetrahymena pyriformis* (*23*). Lipid peroxidation has been shown to occur in all membrane fractions in rat liver, brain, kidney, and testis separated in sucrose gradients in the B-IV rotor (*24*). The relaxing activity in muscle was found to be associated with a particle separable from mitochondria (*25*). Good separations of serum macroglobulins have been obtained in prolonged runs (*26*, *27*).

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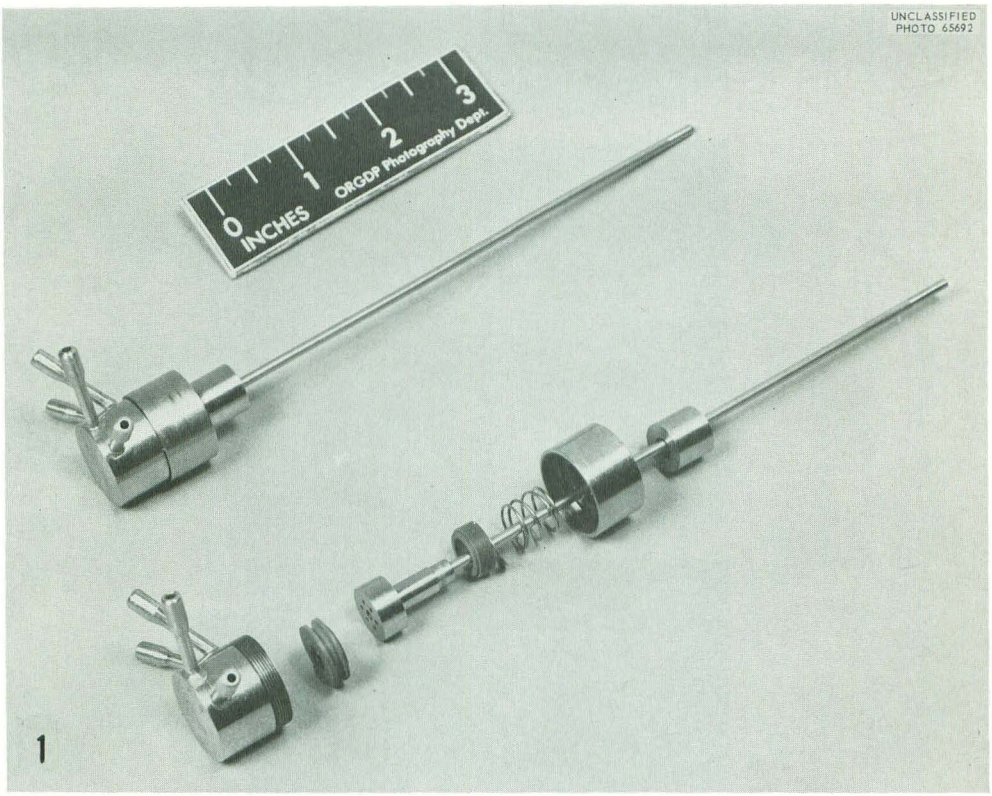

FIGURE 1.—Assembled and disassembled seal for B-series rotors.

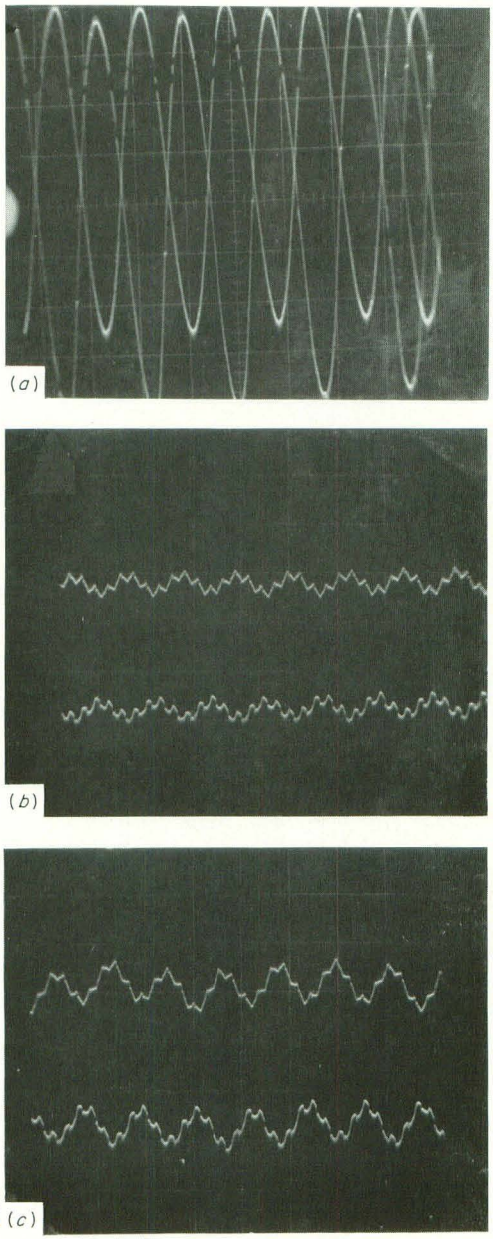

FIGURE 2.—Oscillograms of B-II rotor taken at three speeds. The *upper traces* are monitoring the top end cap, the *lower traces* the bottom end cap. (*a*) Taken at 28,000 rpm; vertical sensitivity 0.005 in/cm; horizontal sweep rate 0.005 sec/cm; high-amplitude frequency, 114 cps; low-amplitude frequency, not detectable but approximately 28,000 rpm. (*b*) Taken at 23,500 rpm; vertical sensitivity, 0.005 in/cm; horizontal sweep rate, 0.010 sec/cm; high-amplitude frequency, 86.5 cps; low-amplitude frequency, approximately 23,500 rpm. (*c*) Taken at 23,000 rp vertical sensitivity, 0.005 in/cm; horizontal sweep rate, 0.010 sec/cm; high-amplitude frequency, 87 cps; low-amplitude frequency, approximately 23,000 rpm.

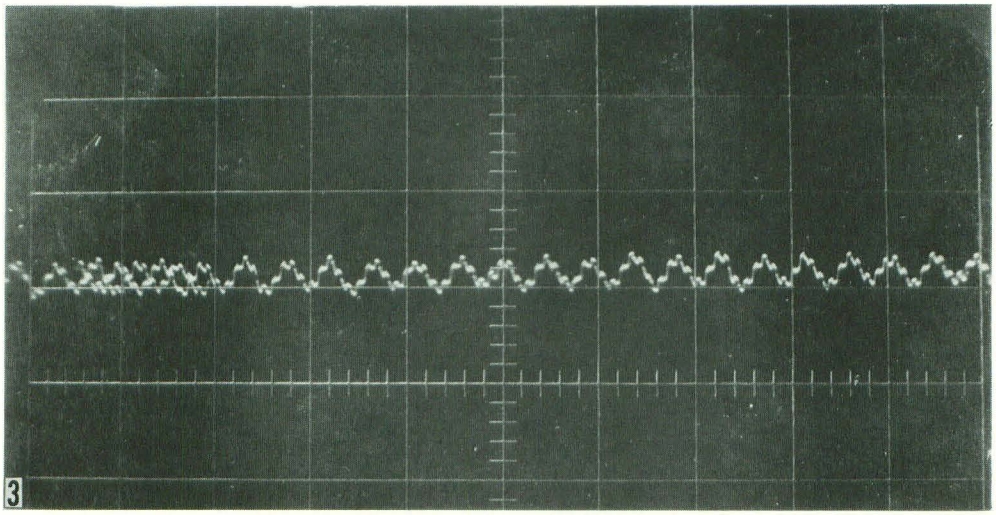

FIGURE 3.—Oscillogram of B-II rotor taken at about 30,000 rpm with modified experimental upper damping bearing. Vertical sensitivity, 0.005 in/cm; horizontal sweep rate, 0.02 sec/cm. The trace is monitoring the bottom end cap. High-amplitude frequency is 111 cps; low-amplitude frequency, about 30,000 rpm.

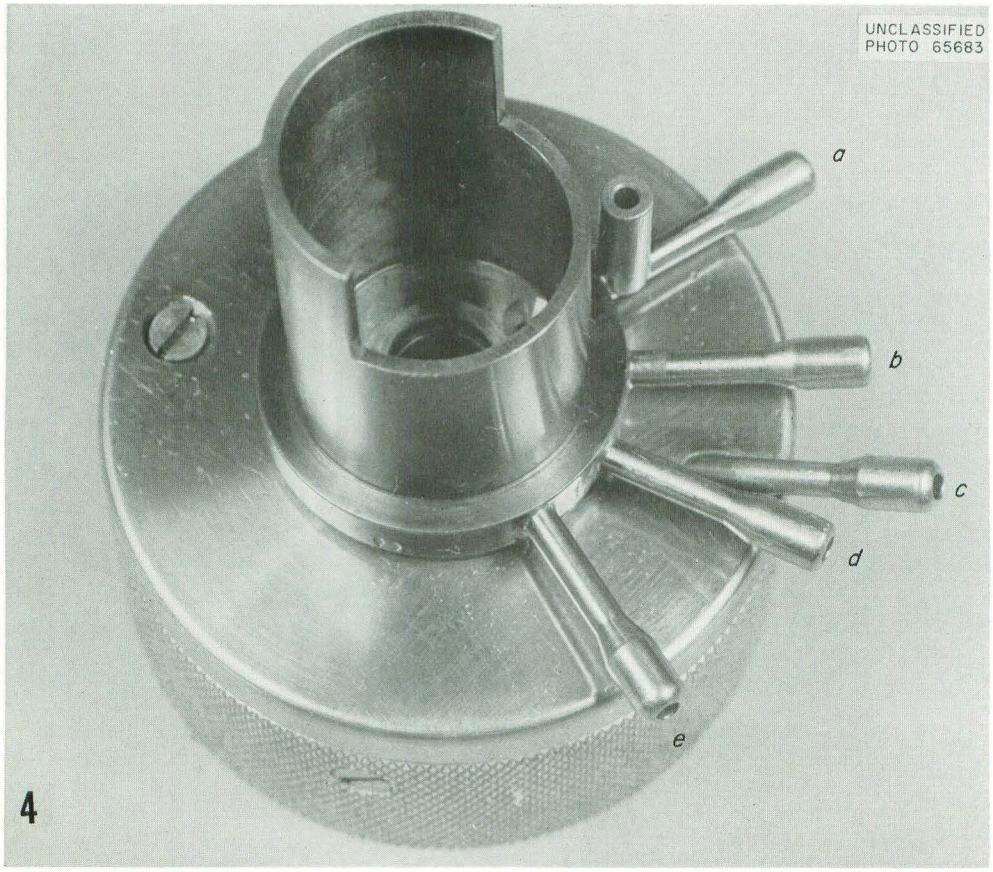

FIGURE 4.—Top damper bearing assembly for B-IV zonal centrifuge. (*a*) Overflow oil from bearing; (*b*) cooling water out; (*c* and *d*) oil lines into ball joint and bearing; and (*e*) cooling water in.

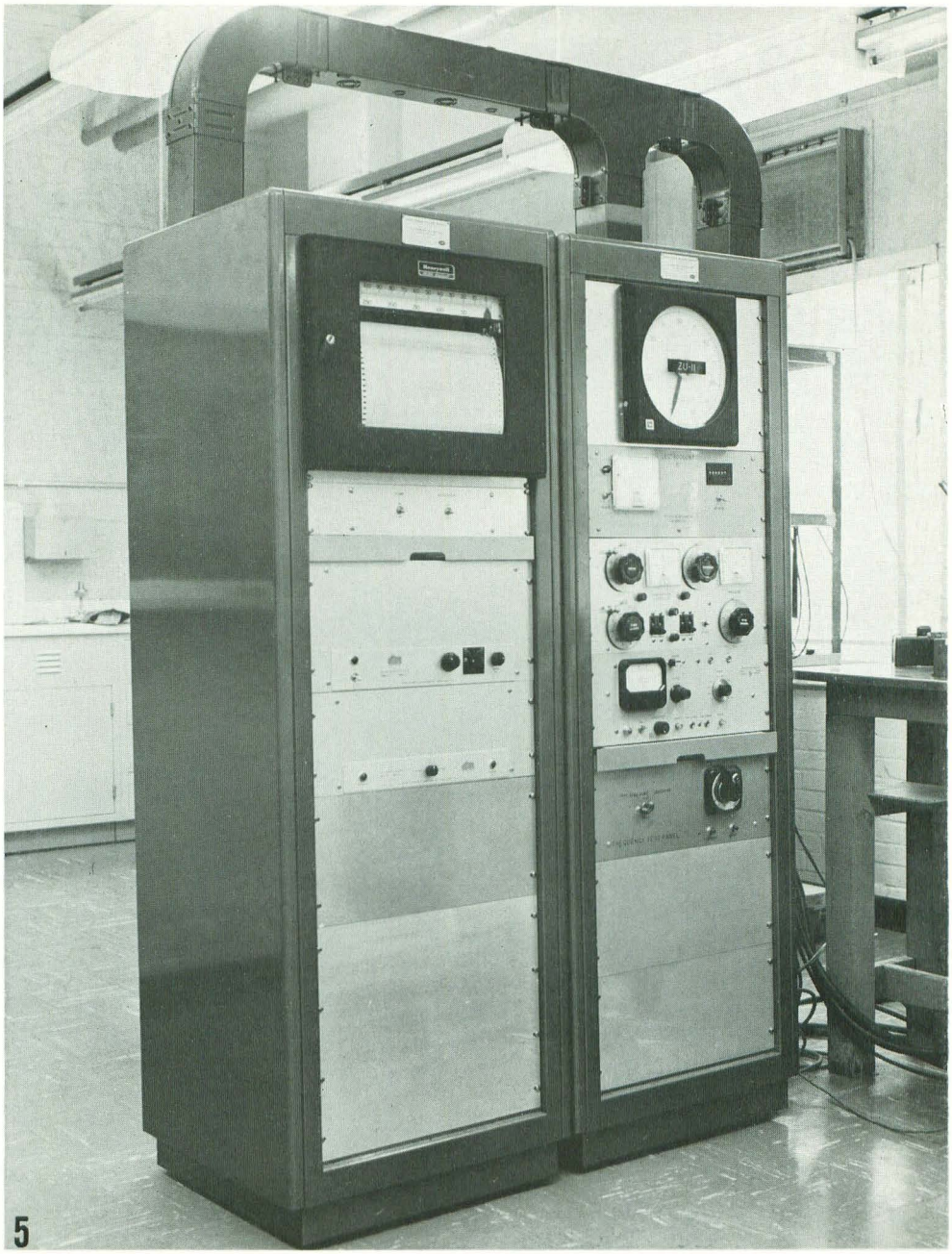

FIGURE 5.—Instrumentation for B- or C-series rotors. *Left* rack contains (*top to bottom*): 1) recorder, 2) switches for gradient pump and accessory, 3) optical density converter including range switch, and 4) hydrogen and tungsten lamp controls. *Right* rack has (*top to bottom*): 1) Speedomax rpm indicator, 2) Lectrocounter integrator panel with counter, 3) centrifuge controls (temperature, pressure, *above;* speed controls and on-off controls, *below*), 4) frequency meter, and 5) frequency test panel. The last two components are used to calibrate the rpm device.

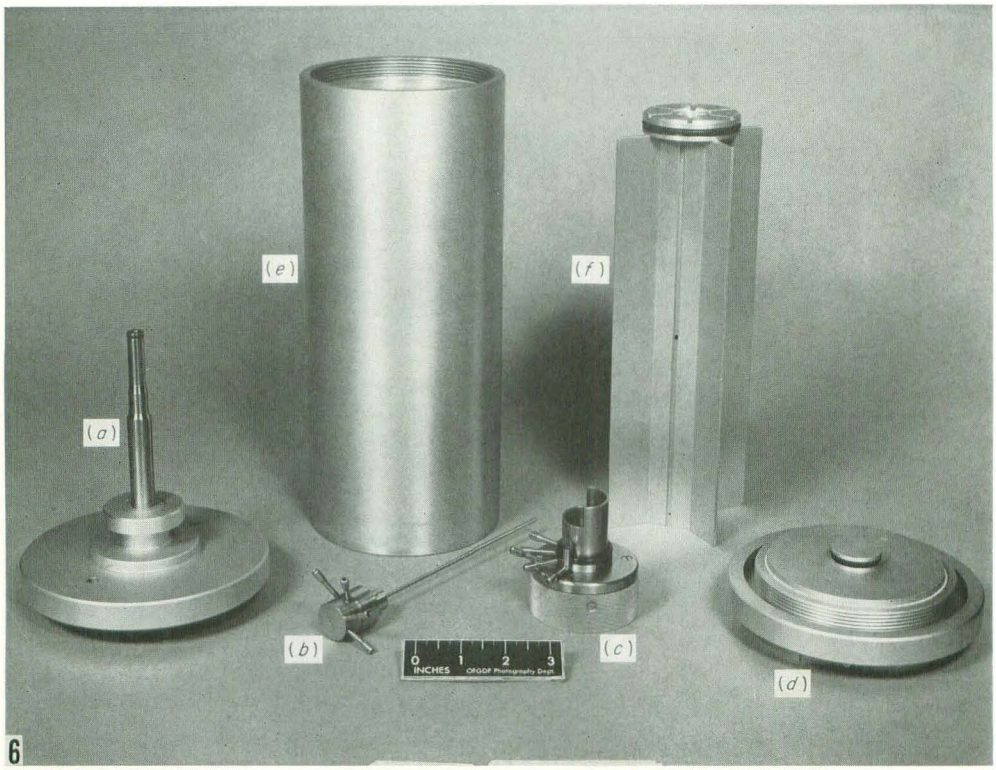

FIGURE 6.—Disassembled B-IV rotor with bearing and seal. (*a*) End cap; (*b*) seal; (*c*) bearing and damper; (*d*) bottom end cap; (*e*) rotor chamber; and (*f*) core.

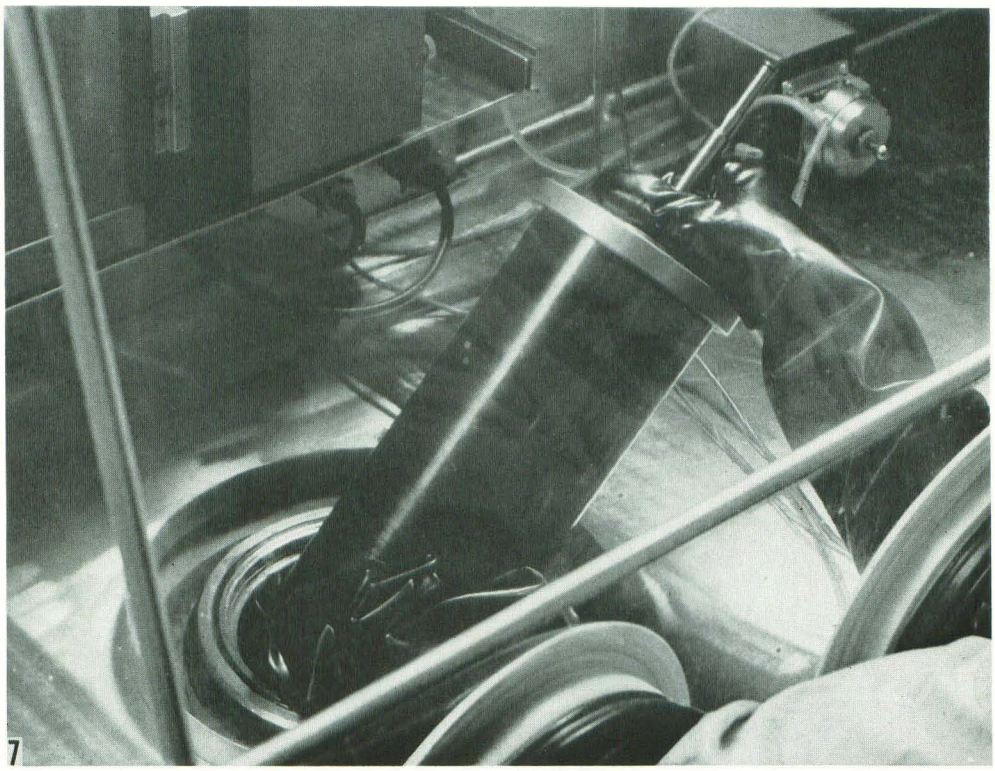

FIGURE 7.—Positioning of the B-IV rotor in centrifuge chamber located in glove box. Water pump in background (*right*).

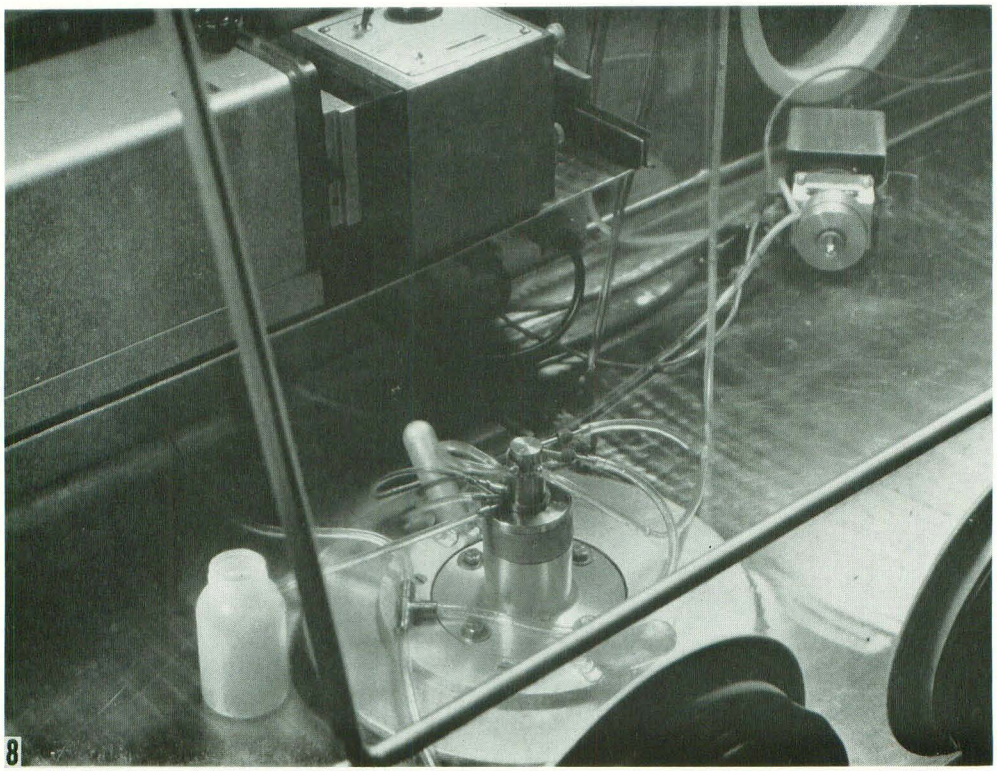

FIGURE 8.—Rotor assembly with cooling, oil, edge, and center lines attached.

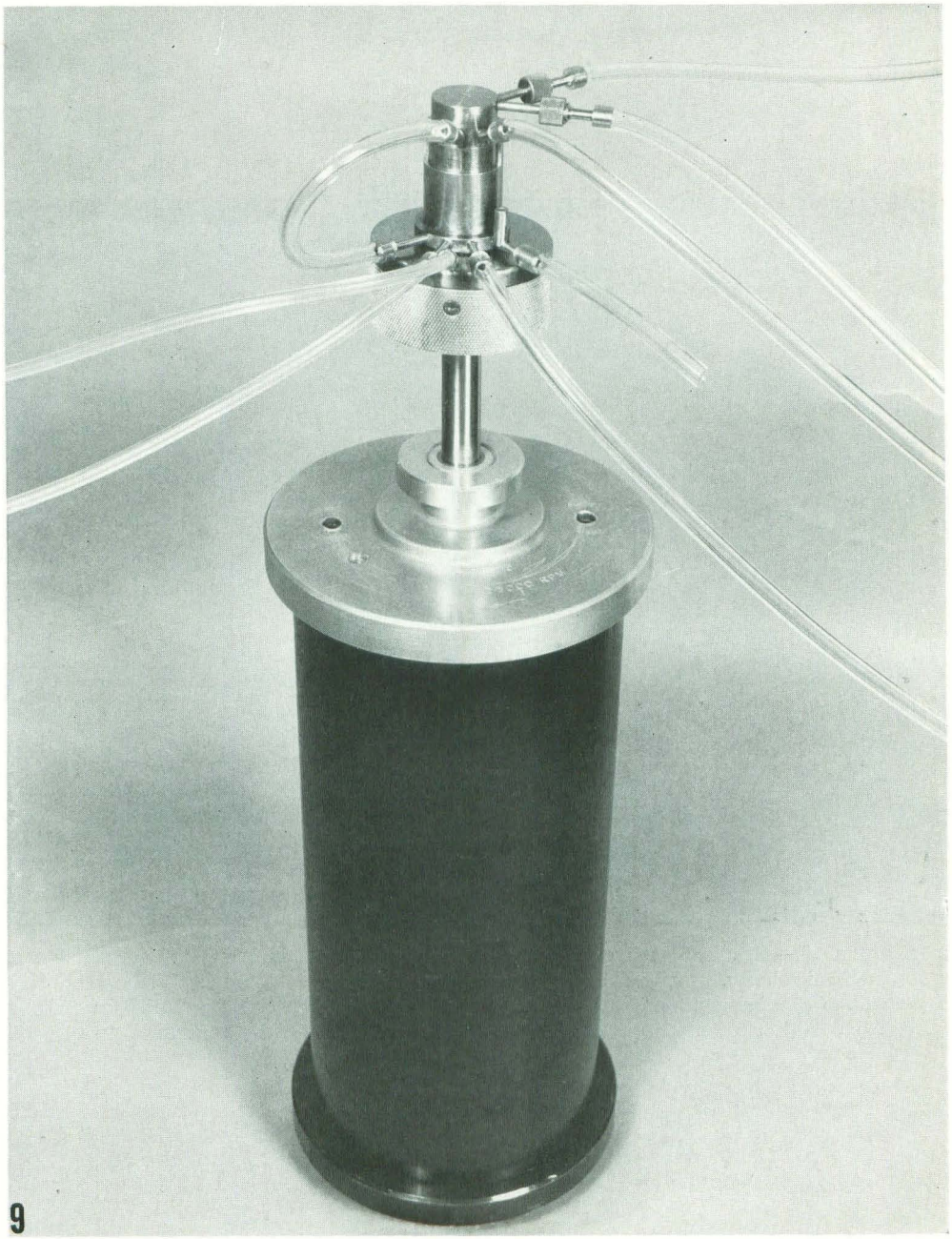

FIGURE 9.—Rotor assembled outside centrifuge, showing seal, damper bearing, and all lines attached.

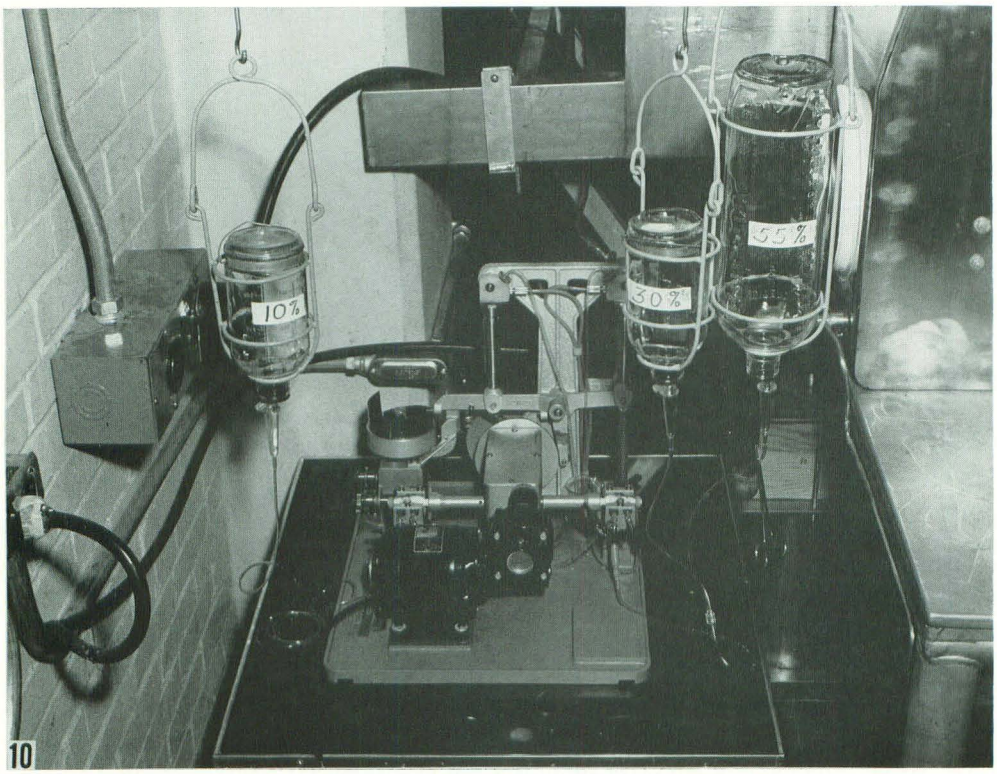

FIGURE 10.—Gradient pump assembly, at rear of glove box, with typical tissue gradient scheme. Program cam, *left rear;* glove box, *right.* Lines used for connection of gradient solution to pump are intravenous injection sets (for 10%, Cutter No. 860-01 or Abbott No. 4622; for 30-55%, Cutter No. 871-00 or Abbott No. 4656).