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K-Series Centrifuges

I. Development of the K-II Continuous-Sample-Flow-with-Banding Centrifuge System for Vaccine Purification

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Methods which will achieve both concentration and purification in a single step are required for the isolation of particulate antigens for viral or bacterial vaccine preparation. The technique of continuous-sample-flow-with-banding has been developed for this purpose (1–7); however, the rotors previously available were designed for research and were not readily adapted to large-scale efforts.[4] We therefore initiated development of a new group of centrifuges, designated the K-series, suitable for the purification of large quantities of virus and subcellular particles. The initial design parameters were based on the purification of influenza virus from chorioallantoic fluid, with the realization that the same centrifuge could, with suitable adaptations, be applied to other viruses and subcellular particles. A preliminary report of this work has appeared (8).

VACCINE PURIFICATION BY CENTRIFUGATION

Viral vaccines are prepared from suspensions which may contain a wide spectrum of particles ranging from whole cells, subcellular particles and debris, viruses, viral subunits, soluble proteins, nucleic acids, and

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

[4] Including eighteen A-rotor designs, thirty-four rotors in the B series, and a smaller number of C- and D-series rotors (9).

low molecular weight substances to insoluble precipitates which may be crystalline. These may be conveniently divided into four general classes of substances:

I. Immunoprotective antigens.
II. Nonimmunoprotective antigens.
III. Nonantigenic macromolecular or particulate material.
IV. Low molecular weight nonantigenic substances.

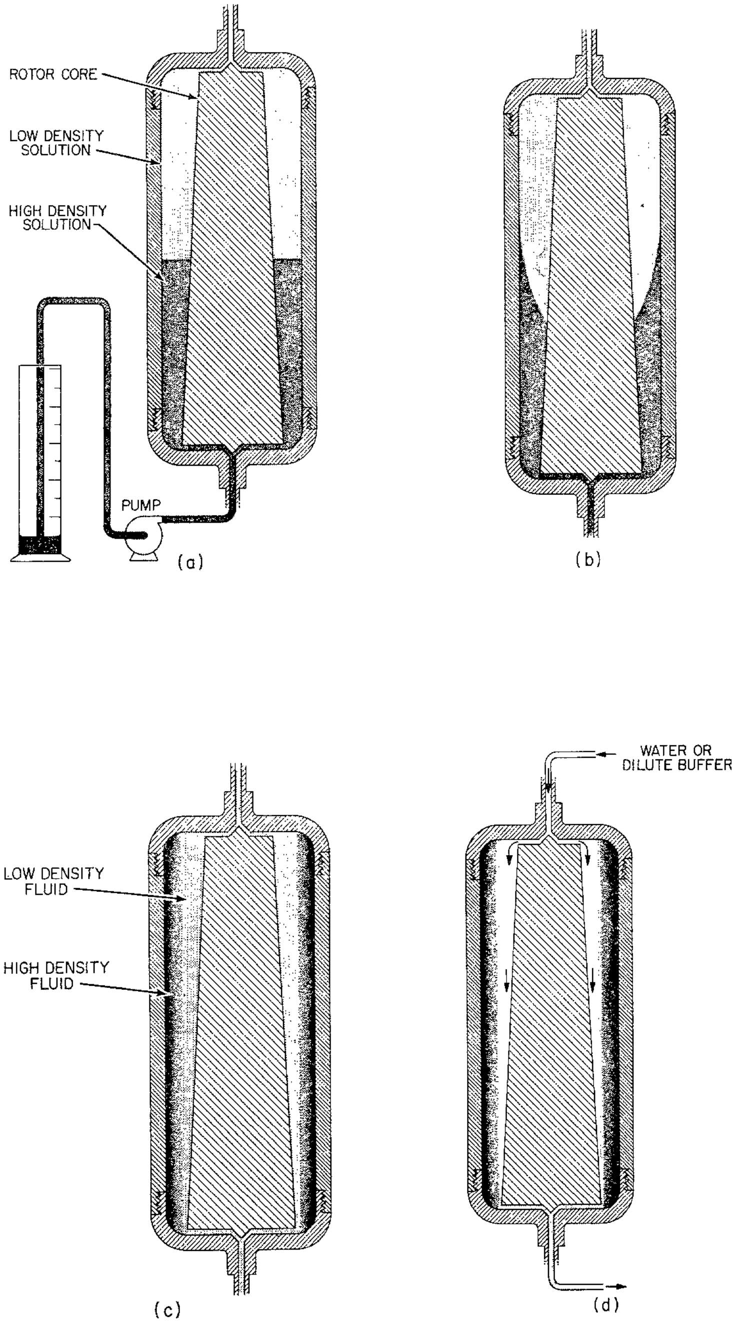
In class I are included intact virions, defective or empty virions, and certain viral protein subunits. These constitute the desired product.

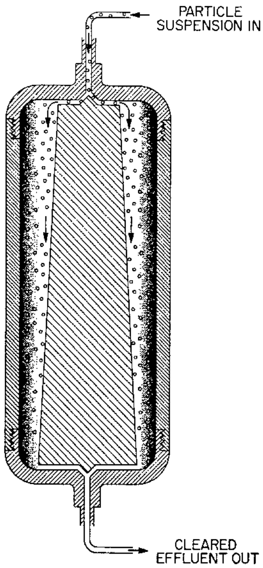
Substances in class II are clearly undesirable because the human immune system does not have limitless capacity for antibody production, because the ability to respond to antigenic stimuli declines with age (10), and because allergic sensitization may occur. If multiple-antigen vaccines containing large numbers of different viral antigens are contemplated, then clearly the amount of class II antigen contributed by each separate antigen preparation used to prepare the mixture must be kept very low if the sum of these contributions in the finished vaccine is to be kept within acceptable limits.

Substances of class III may be native to the vaccine or may be added as adjuvants, as may also substances of class IV. The latter group of materials has generally been of little concern, and includes salts, amino acids, sugars, cellular metabolites, etc. If they are retained during concentration, they may produce effects purely on the basis of hypertonicity in combined vaccines.

The production of biophysically homogeneous antigens requires, in many instances, that more than one purification technique be employed. However, in nearly all instances, the techniques of interest are not directly applicable to large fluid volumes. The systematic approach to purification requires the development of a spectrum of concentration and purification subsystems which may be employed sequentially, and which are matched to handle similar masses (though in dissimilar volumes) of antigen. Since some viruses are aggregated or inactivated by pelleting, *it is preferable to design systems that keep the virus in suspension at all times.*

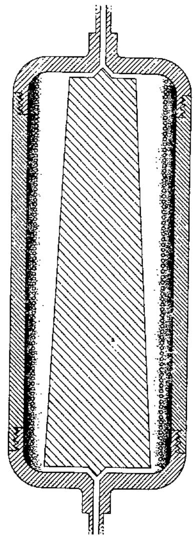
The principle of continuous-sample-flow-with-banding has been used in the B-VIII, B-IX, B-XVI, and B-XXVI rotors (2, 4–7). While these rotors are useful for orienting studies and for small- and intermediate-scale vaccine purification, in the commercially available versions sterility is difficult to maintain, cross-leakage between inlet and outlet lines may occur, and a high run-failure rate has been observed in practice largely due to manufacturers' modifications. However, since the original


ROTOR CORE
LOW DENSITY SOLUTION
HIGH DENSITY SOLUTION
PUMP
(a)
(b)
LOW DENSITY FLUID
HIGH DENSITY FLUID
(c)
WATER OR DILUTE BUFFER
(d)



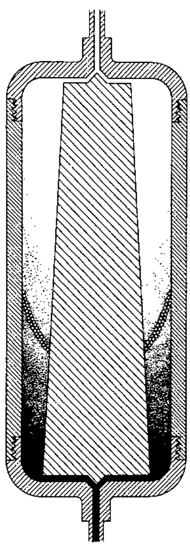
(e)

CONTINUOUS FLOW
(OPERATING SPEED BETWEEN
2000 AND 35,000 rpm)



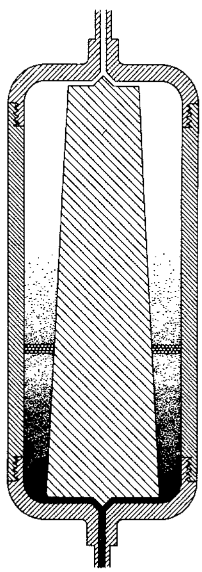
(f)

BANDING AFTER SAMPLE FLOW
IS TERMINATED
(OPERATING SPEED OR HIGHER)

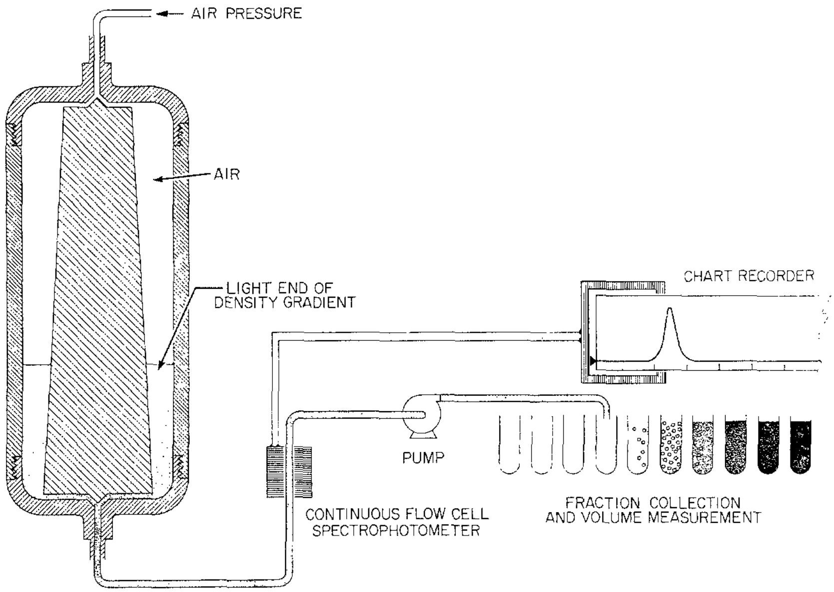


(g)

GRADIENT REORIENTATION
DURING DECELERATION
(SPEED 500 rpm)



(h)



(i)

FIG. 1. Schematic drawings of operation of K-II centrifuge using a static gradient loading and unloading procedure. (a) *Static loading of step gradient.* The rotor, at rest, contains water or dilute buffer in the upper annular space and a high-density sucrose solution in the lower space. (b) *Gradient reorientation during acceleration.* The density gradient changes from an axial to a radial orientation during acceleration from rest up to about 1600 rpm. Surfaces of isodensity are described by parabolas of revolution. The diagram shows gradient configuration for a speed of about 500 rpm. (c) *Gradient in spin configuration.* At speeds above 2000 rpm the density gradient is essentially in a radial orientation. Diffusion of the sucrose step gradient forms a continuous gradient within minutes. (d) *Initiation of flow.* Water or dilute buffer solution is pumped past the narrow end of the core and moves downward, displacing slightly denser solution out around the large lower end of the core. When the taper volume is completely filled with the light streaming fluid, the fluid continues out through the lower shaft and seal. Flow is maintained during acceleration to the chosen operating speed. (e) *Continuous-sample flow.* When the chosen operating speed is reached, the feed flow is switched from dilute buffer or water to the particle suspension to be purified. Depending on rotor speed, flow rate, and sedimentation rate of particles in the suspending fluid, a fraction of the particles entering the rotor sediment from the stream into the imprisoned gradient. In pratice, conditions are usually set to give 90–100% cleanout of the particles of interest. The gradient is sufficiently dense to allow the particles to form a band at their isopycnic level. (f) *Banding after sample flow is terminated.* After all the particle suspension has moved through the rotor, centrifugation is continued for an interval sufficient to allow all particles, including those which entered the rotor last, to band. (g) *Gradient reorientation during deceleration.* Reverse air flow through the turbine is used to brake the rotor to about 2000 rpm. A controlled deceleration program is then used to bring the rotor gradually to a stop, with very gradual reorientation of

designs and concepts have proved feasible, our attention was directed toward the development of higher-capacity systems.

OPERATION OF THE K-II CENTRIFUGE

The principle of continuous-sample-flow-with-banding is illustrated diagrammatically in Figure 1. The rotor contains a hollow core which occupies most of the internal rotor volume. Attached to it are vertical fins or septa which divide the annular space in the rotor into six sectors. Both static and dynamic loading and unloading have been used (4, 11); however, the former has been found preferable in practice because it gives higher resolution. As shown in Figure 1a, the rotor is initially partially filled with water or a dilute buffer at rest. A dense solution is usually then pumped in through the bottom to give a one-step "gradient." As the rotor is accelerated the gradient reorients from a vertical to a horizontal configuration (Figs. 1b and 1c). At this time a fluid of density less than that of the light end of the gradient, such as distilled water or a dilute buffer solution, is passed through to displace the denser gradient liquid from the taper volume of the rotor as it continues to accelerate (Fig. 1d). The displaced fluid flows over the large end of the core and out of the rotor. Since the taper volume is initially denser than the fluid displacing it, an unbalanced back-pressure is produced on the inlet line which is directly proportional to the square of the rotor speed. It is important, therefore, to maintain flow of light fluid through the rotor during the acceleration in order to obtain a good flow rate at top rotor speed. When operating speed is approached, the inlet stream is switched to the virus-containing fluid. The flow-through rate is adjusted for optimal removal of the virus particles from the flowing sample. This is continued until the continuous-flow portion of the experiment is completed (Fig. 1e). Virus sedimenting out of the stream is banded isopycnically in the gradient in a narrow band. Note that the gradient, while of large volume, is 1.15 cm thick in the spinning rotor. Diffusion therefore rapidly smooths out a preformed step gradient. A small amount of gradient solute is continuously washed out of the rotor in the flowing stream, thereby setting a limit to the total volume of fluid which may be processed.

When all the crude virus suspension has passsed through the rotor, it

the gradient to the rest configuration. (h) *Rotor at rest before unloading.* The concentrated particles are in a narrow band at their isopycnic level. (i) *Static unloading of gradient.* Using air pressure and a small peristaltic pump to control flow, the gradient and suspended particles are recovered as a series of discrete fractions. Unloading may also be done through the upper line using dense fluid to displace the rotor volume.

is allowed to continue to spin long enough to band the last virus particles sedimented from the stream (Fig. 1f).

The rotor is then allowed to come to rest under conditions such that the final deceleration stages are very smooth. The gradient reorients back to a vertical configuration (Figs. 1g and 1h) and is then recovered by either draining the gradient out the bottom (Fig. 1i) or by displacing it out the top with a dense fluid. The theoretical basis for the surprisingly good resolution obtained in reorienting gradient rotors (12) has recently been presented (13).

DESIGN OF THE K-II CENTRIFUGE

The design requirements for the K-II centrifuge were based on the purification of influenza virus at a rate which would make the instrument commercially feasible, i.e., up to 100 liters of fluid processed in an eight hour day. The conservative values used in arriving at the design are listed in Table 1.

TABLE 1
Design Requirements for the K-II Centrifuge

Flow rate	10 liters/hr
Sample zone viscosity	0.0152 poise, est.
Virus diameter	8.2×10^{-6} cm
Virus density	1.193 gm/cm^3
Sample fluid density	1.05 gm/cm^3
Clean-out	100%

Preliminary studies indicated that optimization of the mechanical design of the K-II system would result in as large a machine as would be practical to use industrially at this time.

The following considerations, in addition to the performance requirements, influenced centrifuge design. There were: (*a*) the rotor drive (compressed air or electrical power) should be compatible with facilities normally available in laboratories manufacturing vaccines, (*b*) the seal design should not allow cross-leakage between influent and effluent streams to occur, (*c*) the weight of the rotor should be limited to minimize special handling equipment, and (*d*) the system should be conservatively designed both to ensure the attainment of the vaccine requirements stated and to allow exploration of more difficult separations.

The requirement that there be no possibility of cross-leakage between the influent and effluent streams imposed a limitation upon the selection of the type of centrifuge drive. The only positive method for eliminating cross-leakage is to separate the seals completely by placing them at opposite ends of the rotor. This also implies that the one fluid passage

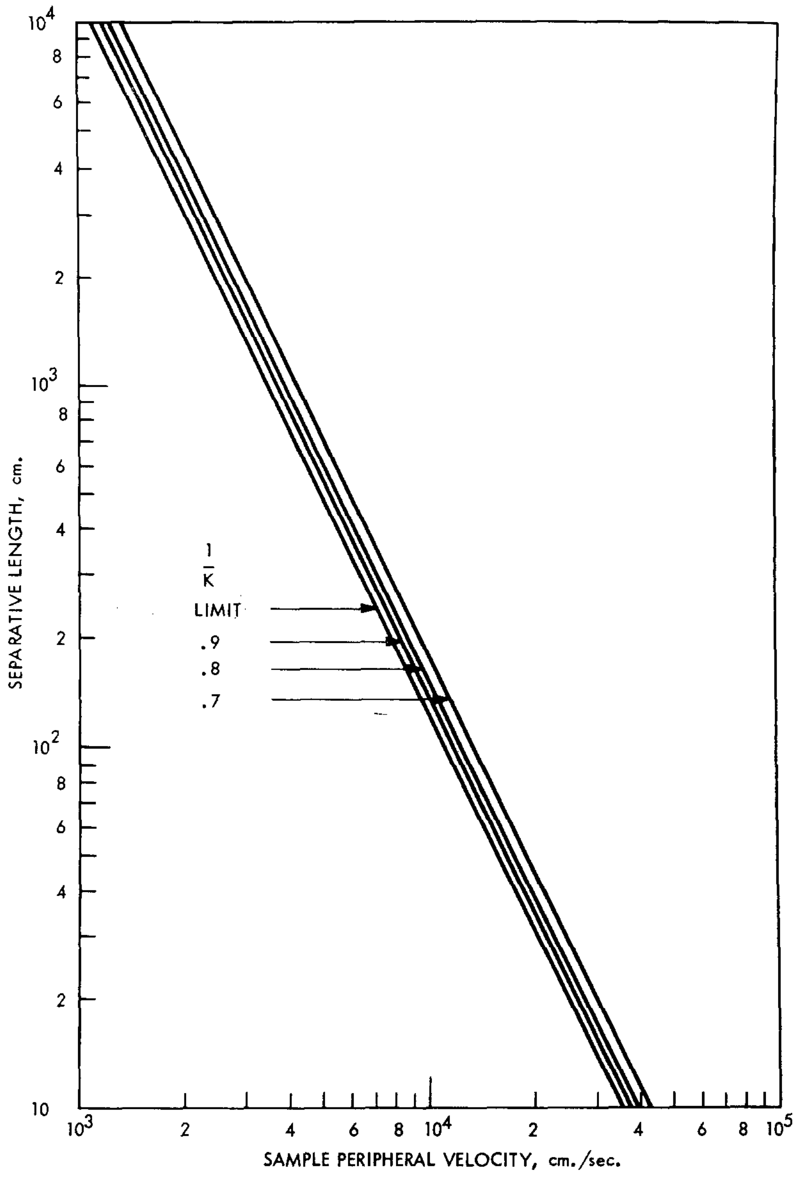


FIG. 2. Separative length required as function of sample peripheral velocity, where $K = r_s/r_c$ = outer radius of sample zone/average core radius.

must go through the centrifuge drive itself. A turbine drive was selected because of its simplicity and the fact that a central fluid passage within the drive could easily be arranged. The turbine drive has the added advantage that it will not heat the fluid within the flow passage as much as might be expected from an electrical drive. The weight of the rotor was arbitrarily limited initially to 70 lb to satisfy the requirement that peripheral handling equipment be kept to a minimum.

Design graphs for liquid-filled centrifuges based on the separational requirements specified in Table 1 are presented in Figures 2 and 3. Figure

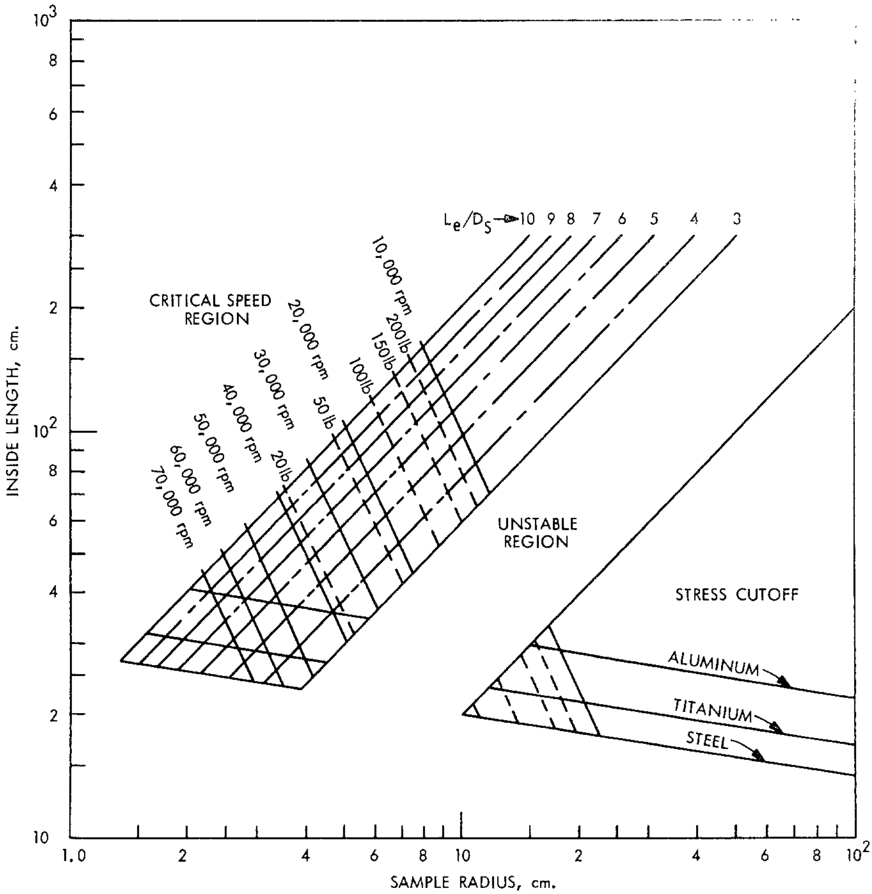


FIG. 3. Length, speed, average sample zone radius, and weight of rotor required for 100% cleanout of influenza virus at 10 liters/hr flow rate based on the equations developed by Berman (14) and Brooks (15). The particle and suspending fluid properties are assumed to be those given in Table 1.

2 presents an effective rotor length as a function of the peripheral velocity and annular clearance ratio of the rotor, K, in radial direction based on the separation duty specified in Table 1. K is the ratio of the outer radius of the sample zone to the average core radius and equals r_s/r_c.

The ratio K should be kept as close as practical to unity in order to minimize the effective length (L_e) required for the separation task at a given value of sample peripheral velocity. The physical implications of this are that the residence time of the virus particle within the sample zone will be minimized. For design purposes, a value of K equal to 0.9 was used in the remaining calculations. The values of L_e as a function of r_s and rotor speed in rpm are shown in the composite design curves in Figure 3.

The rotor stress, its flexural critical speed, its stability, and its weight impose physical limitations upon the allowable diameter and length of the rotor. These parameters can be considered separately in determining the allowable limits of the rotor design.

The stresses in a zonal centrifuge consist of *(1)* rotational load of the rotor material under a high centrifugal field, *(2)* a stress from the hydrostatic pressure developed by the fluid in the rotor, *(3)* bending stress due to rotor unbalance, and *(4)* mismatch stresses where end caps are joined to the rotor. With an ideal mechanical design, only the first two stresses are important in the stresses consideration.

By using the theory of the maximum shear stress as a criterion for failure, A. A. Brooks (15) obtained the maximum shear stress relating a cylindrical rotor to a zonal centrifuge by the superposition of the rotational stress in a cylindrical body with the hydraulic stress in a cylindrical body pressed from the inside. Then, the maximum shear stress is given by

$$\tau_{\text{opt}} = \frac{V^2{}_p}{2g} \{\delta + \rho + [(3 + \mu)\rho\delta]^{1/2}\} \tag{1}$$

where τ = the maximum allowable shear stress at optimum wall thickness,
V_p = the rotor peripheral speed (of inner rotor wall),
δ = metal density,
ρ = fluid density,
μ = Poisson's ratio for rotor material, and
g = gravitational constant.

On the other hand, the minimum shear stress for the rotor material can also be obtained in terms of the rotor wall thickness. Brooks *(15)* gave this optimum rotor wall thickness ($t = b - a$) as

$$t = b - a = a\left(\left\{1 + \left[\frac{4\rho}{(3+\mu)\delta}\right]^{1/2}\right\}^{1/2} - 1\right) \qquad (2)$$

where a = inside rotor radius and
b = outside rotor radius.

The stress cut off shown in Figure 3 was determined for 7075-T6 aluminum, 6A14V titanium and maraging[5] steel rotors with the inside radius of the rotor assumed to be 1.25 r_s.

The rotor must operate below its rotor flexural critical speed in order to limit the amplitude and balance requirements to a level suitable for mass production of the rotor. A maximum value of $L_e/2r_s$ or L_e/D_s of 10 was determined to be the suitable limiting value (where D_s is the maximum sample zone diameter).

The motor must operate below its rotor flexural critical speed in order the ratio of the polar moment of inertia (I_p) to the transverse moment of inertia (I_t) lies in the region,

$$I_p/I_t \geq 1.2, \qquad I_p/I_t \leq \tfrac{1}{4}$$

This does not imply that a rotor cannot be operated within the unstable region shown in Figure 3, but merely indicates that it would be more difficult to design suspensions to attenuate the whirl frequencies of the rotor within this region.

Finally, the weight of the rotor filled with fluid can be approximated by the formula

$$\text{weight} = \delta\pi(1.69r_s^2 + 1.67r_s t)L_e + 0.94r_s^3 \qquad (3)$$

where δ = rotor material effective density (including fluid),
t = rotor thickness,
r_s = sample zone radius, and
L_e = rotor effective length.

At values of L_e/D_s above four, this reduces to

$$\text{weight} \simeq 2.1\delta\pi r_s^2 L_e \qquad (4)$$

The design region for the rotor system with all mechanical factors considered as well as the biological requirements lies in the region bounded by

$$3 \leq L_e/D_s \leq 10$$

and

$$20{,}000 \leq \text{rpm} \leq 40{,}000$$

where the limit of 20,000 rpm is determined by the maximum allowable

[5] The term "maraging" refers to a process for aging steel after transformation to martensite to attain high strength.

rotor weight and the 40,000 rpm is the present upper limit of proved reliable seal design. All disc-type rotors for which $I_p/I_t \geqslant 1$ are eliminated by either the rotor stress or the rotor weight.

The actual K-II rotor ($L_e/D_s \approx 8$) was designed in the upper half of this region for two reasons: (*a*) the L_e/D_s was made as large as possible to reduce the shafting requirements to, and the damping requirements of, the supension systems, but (*b*) the L_e/D_s was limited to a value below 10, and r_s was limited to 4.94 cm to allow overspeed operation above the initial speed of 26,500 to 35,000 rpm without encountering any problems due to either the rotor flexural critical speed or overstress in the rotor.

The pressure-vessel type end-cap design and the method of securing the end caps to the bowl with buttress threads are identical to those of the B-XIV and B-XV rotors (17). Reliability of this end-cap design was proved under extremely high stress conditions imposed during a destructive test (see section below on destruction test). Except for the following differences, the caps are identical: (*a*) the types of connections for top and bottom shafts are different, and (*b*) the bottom cap is fitted with two pins which lock the core against rotation inside the bowl. Two spanner-wrench notches in each end of the core fit the locking pins so that the tapered core is completely reversible. This means that the direction of sample fluid flow may be either up or down, depending on core orientation. Maximum hoop stress (at the inside bowl wall) was calculated as a function of rotor speed for various densities of rotor fluid contents to determine the limitations on operating speed. In practice, operating speed is limited by the density gradient used.

The core runout relative to the spin axis of the rotor increases during operation as the fundamental flexural critical frequency of the core is approached. This core deflection would produce serious imbalance if left unattenuated. The problem was solved by using six septa to give the required radial support.

Manifold plugs on each end cap serve to center and anchor the core inside the bowl. Sample fluid flowing through the rotor comes out through radial grooves in the end of the core, then flows up (or down) the tapered core periphery and back in through radial grooves on the other end.

The rotor stability was enhanced as much as possible within the restrictions imposed by the separation task. This choice was in part dictated by the desire to use simple quick disconnect locking devices to hold the shafts to the rotor. In other words, if an L_e/D_s of three or four were chosen, much stiffer shafts would be required to couple the rotor to the suspension and damping systems in order to attenuate the natural whirl frequencies of the rotor; the use of stiff shafting would preclude the possibility of using a nut locking device for the shaft. Since the natural stability of the rotor was in a sense maximized, relatively flexible shafting could be used which would allow operation of a more unbalanced rotor without causing runout at the seals (since at operating

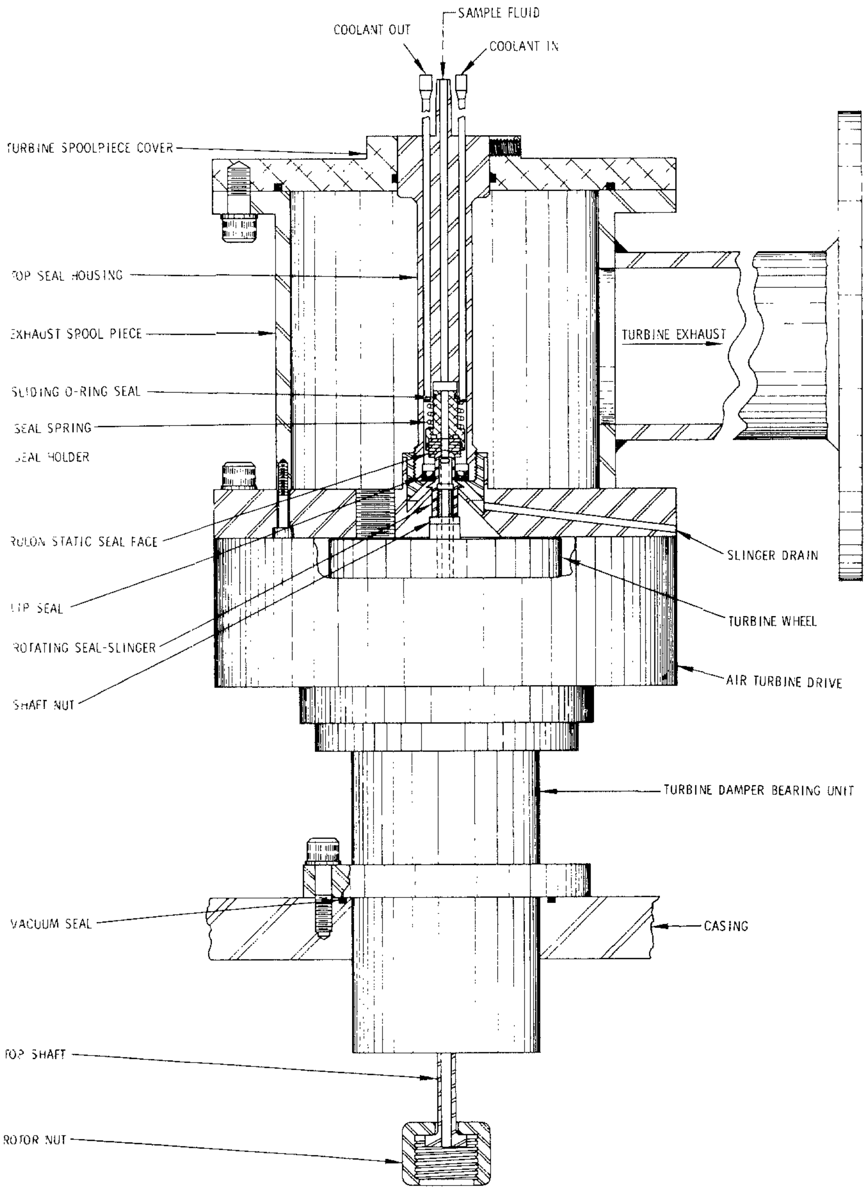


FIG. 4. Diagrammatic presentation of upper part of K-II centrifuge.

speed the dampers would be essentially "seismic"). In actual operation, no stability problems whatsoever have been encountered.

The K-II drive is shown diagrammatically in Figure 4 and the rotor

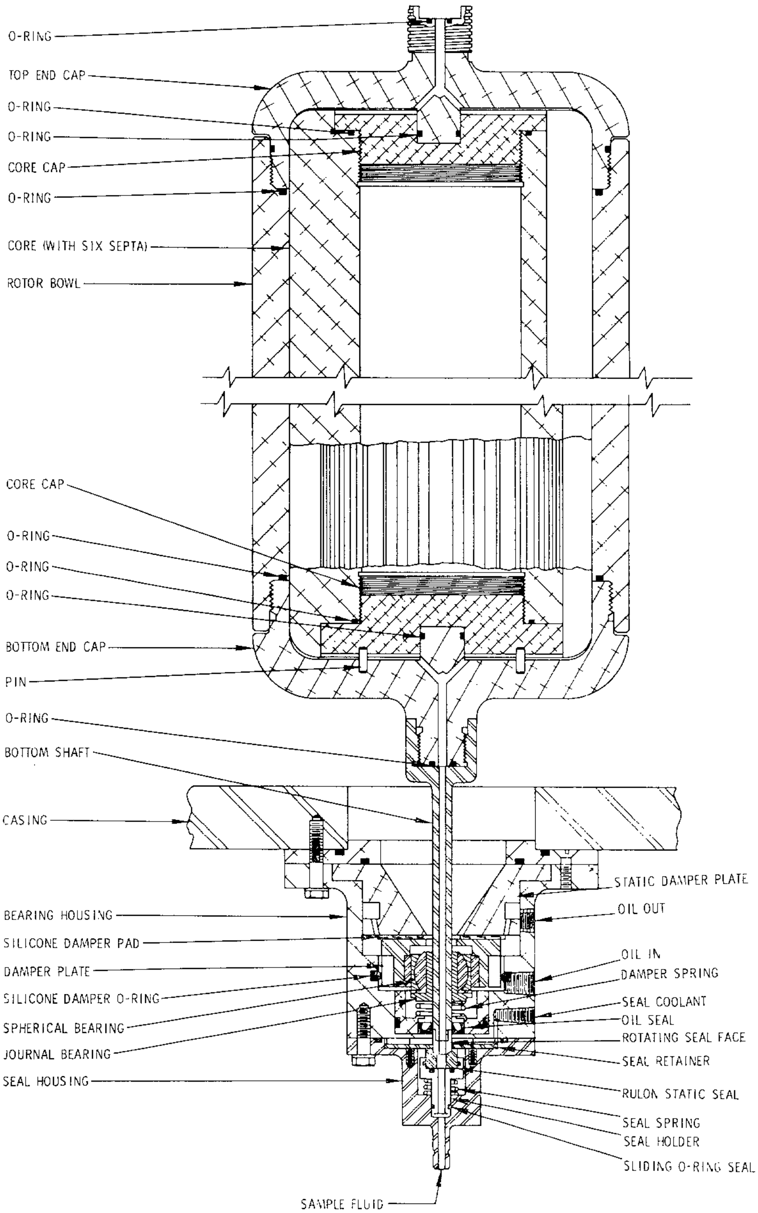


FIG. 5. Diagrammatic presentation of lower part of K-II centrifuge.

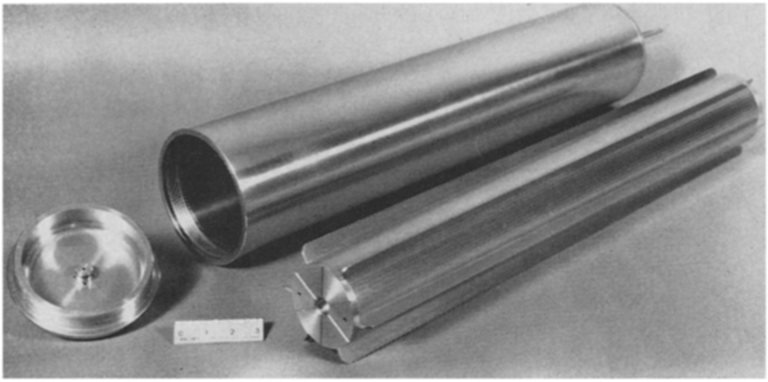


FIG. 6. Disassembled K-II centrifuge rotor. The tubular centrifuge bowl is shown above the tapered six-vaned rotor core. The upper end cap is shown at the left.

in Figures 5 and 6. Table 2 lists the salient features of the rotor. Note that the gradient during rotation extends radially only 1.15 cm.

A clear plastic version of the K-II was also constructed so that actual flow paths and other phenomena inside the spinning rotor could be observed.

TABLE 2
Aluminum K-II Rotor

Assembled weight (empty)	52 lb
Total volume with core	3600 cm^3
Taper volume	700 cm^3
Initial operating speed	28,000 rpm
Force (g) at initial operating speed	53,400
Maximum safe speed	35,000 rpm
Force (g) at safe speed (maximum)	83,440
Inside length	76.20 cm
Bowl inside radius	6.09 cm
Core radius (large end)	4.94 cm
Core radius (small end)	4.29 cm
Rotor flexural critical speed	approx. 60,000 rpm

THE K-II DRIVE SYSTEM

The turbine chosen[6] used the diameter shaft needed for the rotor, had the peak of its horsepower curve in the range of anticipated operating speed, and had a maximum safe listed operating speed of 60,000 rpm. Air consumption at 27,000 rpm under normal rotor operating conditions has been measured at ~80 standard cubic feet per minute (scfm) at

[6] Model 2501, 4 in. vertical turbine available from Barbour-Stockwell Company, Cambridge, Massachusetts.

~25 psi inlet pressure. At 35,000 rpm, air consumption is in the range of 90–100 scfm.

From deceleration rates, the power required to spin the full rotor at 27,000 rpm was computed to be ~1 hp. The machine uses four journal bearings and two ball bearings. Three of the journal bearings are located in the turbine damper unit, one is used in the lower damper bearing, and the two ball bearings are used to mount the turbine wheel. The turbine damper unit was altered by mounting the bronze bearings inside spherical bearings so that misalignment could be more easily accommodated. The hollow drive spindle is easily installed and is supported by a nut on the top of the turbine wheel.

Lubrication of the turbine wheel ball bearings comes from an air mist lubricator on the turbine control manifold. The turbine damper bearing unit is filled with oil, which is recirculated by the same oil pump that lubricates the lower damper bearing.

A modification of the turbine exhaust spoolpiece was required to accept the top seal mounting flange. Exhaust air from the turbine, which normally goes straight up through the spoolpiece, was routed out the side of the spoolpiece so that the top seal would be more accessible.

A magnetic pickup unit furnished with the turbine is connected into the centrifuge control panel speed indicator and overspeed circuitry.

Due to the extremely high-pitched noise created by the turbine, the use of a muffler was necessary to protect operators. To prevent an aerosol oil mist from filling the room, piping of exhaust air outside was found to be desirable.

DESTRUCTIVE TEST OF TURBINE

The maximum safe operating turbine speed according to the manufacturer is 60,000 rpm. At the air pressures which might be used, the maximum turbine speed with no load is estimated to be 70,000 rpm; and stress calculations show the turbine wheel to be safe at this speed, assuming no material defects. However, since the turbine is at head level (5–6 feet from the floor), a new armored turbine housing was designed and subjected to burst test.

Two radial slots were machined in the SAE-4340 steel turbine wheel 180° apart to the minimum depth calculated to effect destruction in the 60,000 rpm range. The turbine was driven to 62,500 rpm (the maximum speed with existing air supply) without failure. The slot depth was increased by 3 to 85% of the wheel radius, and part of the hub was undercut so that the actual conditions more closely resembled the disc configuration assumed in the calculations. Under these conditions, the wheel flew apart (Fig. 7) at 60,200 rpm. At this speed the calculated

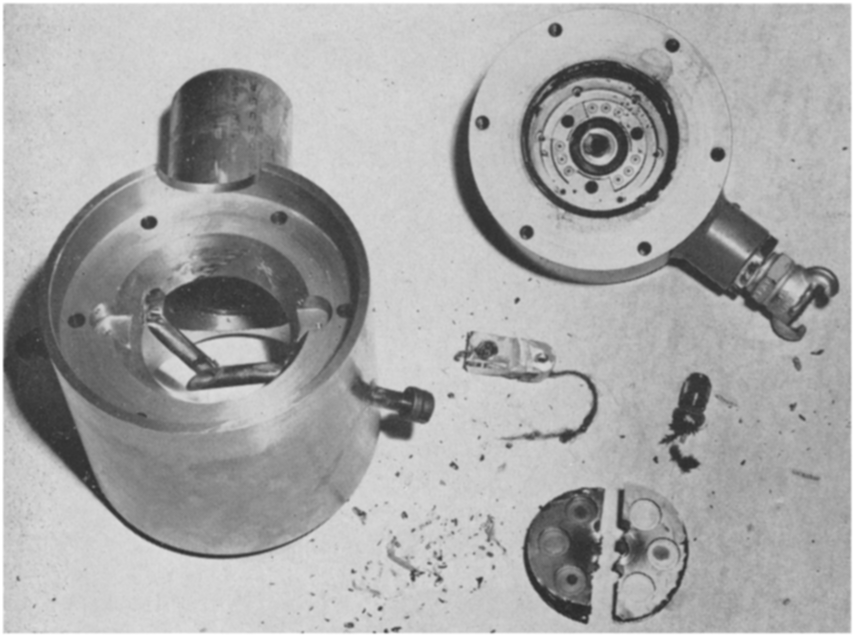


FIG. 7. Destructive test of turbine.

kinetic energy per unit length was almost twice that of the K-II rotor at its burst speed.

The impact of the two halves of the wheel did not damage to the redesigned armored housing and no cracking or penetration occurred. An overhanging ledge designed into the housing prevented pieces of metal from flying out the exhaust line. The large safety margin of the turbine wheel and the increased armor ensure safe operation under all circumstances.

SEAL DESIGN AND TESTING

Two identical single-path seals are used; one is mounted on top of the turbine drive and the other is mounted under the lower damper bearing. With this straight-through design, the sample fluid flowing through the rotating system enters and leaves at approximately the same radius; therefore, there is no "pumping effect" to limit the direction of flow. Neglecting the effects of core orientation (since the core is reversible), there is little or no difference in the inlet pressure required to pump through the rotor from either end unless gradient materials diffuse into the stream. It is evident that fluids are subjected to less shearing action with the straight-through seal than with the previously developed two-path seals (4).

During the initial stages of development, several seal configurations were tested. The first seal was a rounded convex static seal, face spring-loaded against a concave Rulon[7] face on the end of a rotating shaft. This design was modified a number of times but still proved to be too susceptible to vibration and separation of the seal faces at high speed. The upper seal performed more reliably since its rotating part was mounted directly on top of the turbine drive rotor (the rotor being mounted rigidly on ball bearings). Due to the nature of this suspension, very little rotor vibration was transmitted to the seal and only the runout of the dynamic seal face itself was a problem. The rotary seal face on the bottom seal had a more flexible suspension in the bottom damper bearing. In addition rotor runout and precessional motion were transmitted by the bottom shaft to the seal face. It was noted that the conical Rulon seal face had to be machined perfectly concentric and aligned with the shaft, and the loading spring had to be replaced with soft rubber washers to prevent the onset of separation. After considerable testing and modification it was decided that this design was unsatisfactory. A much more reliable design was found by reversing the seal faces so that a conical concave Rulon static seal face was spring-loaded against the rounded convex end of the rotary member. Testing showed that this seal did not require precision alignment and vibration damping as the previously described seal did.

The fluid, which is circulated around the seal, carries away much of the heat generated by friction and also catches any material that might leak past the seal faces. During centrifugation of hazardous material, a disinfectant may be used as the coolant, providing a better secondary seal. Tap water has been used as the coolant in most of the runs thus far. A slinger-catcher unit is used on the upper seal to prevent leakage of coolant into the turbine if a leak past the lip seal should occur.

The upper seal now in use is shown in Figure 8, the bottom damper bearing in Figure 9, and the lower seal assembly in Figure 10. As of this writing, at least 4000 hours of accumulated run time has been recorded using these seals, and reliability has been generally good. Wear characteristics have been acceptable even with the somewhat gritty sample solutions used during evaluation. Seal life will be greatly improved by heat-treating (hardening) the rotating seal faces. Although most of the seal reliability data has been accumulated at 27,000 to

[7] Available from The Dixon Company, Bristol, Rhode Island.

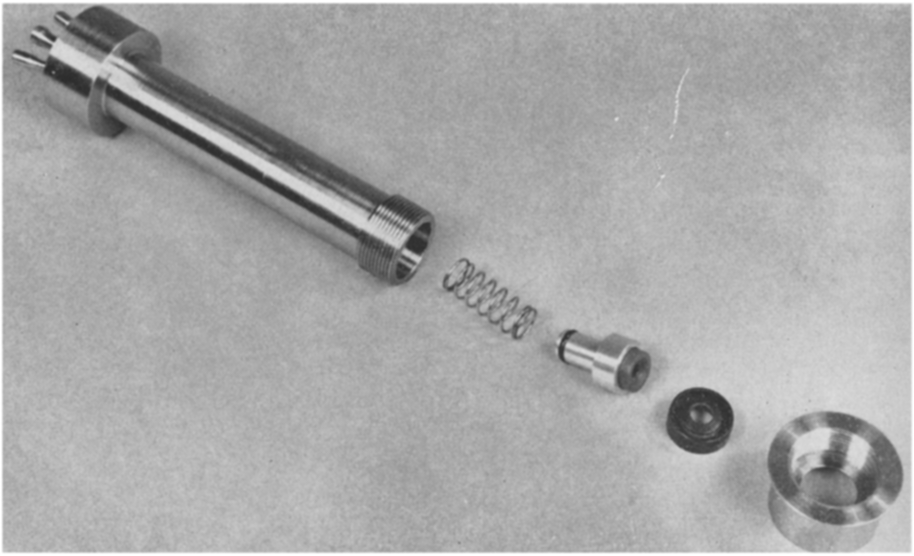


FIG. 8. Upper seal assembly for K-II centrifuge system.

30,000 rpm, the seals perform satisfactorily at the maximum safe rotor speed of 35,000 rpm.

CRASH SHIELD DESIGN

The design of a safe rotor chamber or crash shield was one of the most difficult problems of the K-II machine development. Two casing designs (K-IIA and K-IIB) consisting of partial cylinders with hinged,

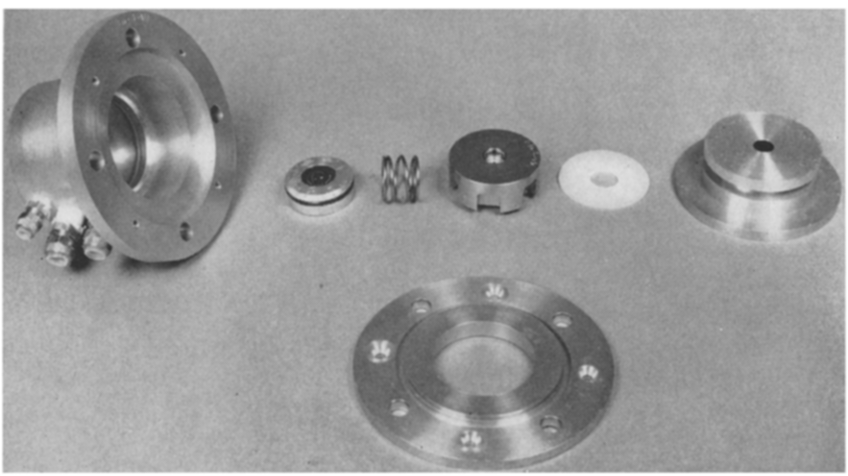


Fig. 9. Bottom damper bearing.

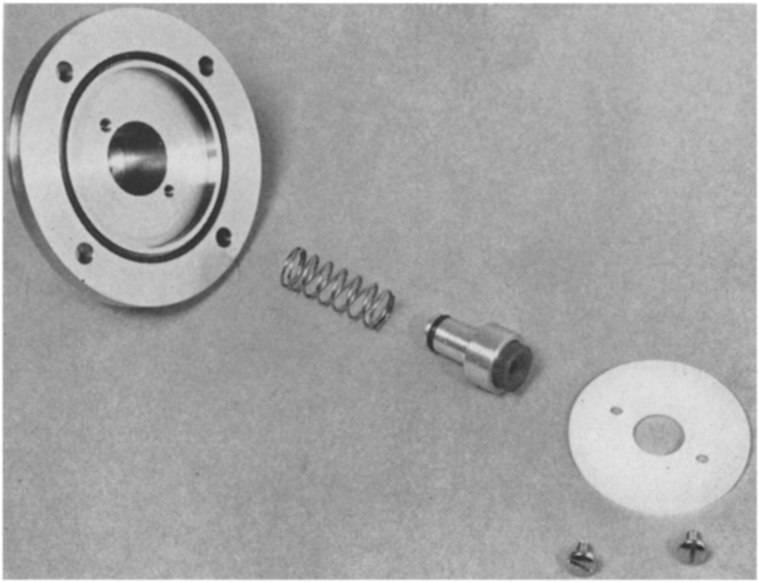


FIG. 10. Lower seal assembly.

flat doors were used initially. High-speed rotor burst tests have pointed out weaknesses in both types. The second design differed from the first in the manner of latching the door shut (Figs. 11–13). Although the problem of holding the door shut under impact was solved, the selection of armor plate material itself came under scrutiny as a result of the burst tests.

The typical mechanical properties of materials (especially ductility as measured by per cent elongation, and the area under a tensile test diagram) are of little value in determining the impact toughness. Such properties might be used if one was certain that the material had the desired microstructure (one in which brittle fracture below yield strength would be unlikely to be initiated under given impact). One type of test that can be useful is the impact test at low temperature ($\sim -120°F$). It has been found that the effect of very high impact velocities may be simulated by standard Charpy tests carried out at such low temperatures (18). The notion of a critical impact velocity also appears to be of interest (19). No clearcut theoretical approach has been found for problems similar to this, and experimental tests are the best source of design data.

The K-IIC casing was designed to give adequate insurance against penetration with both the aluminum rotors described and the titanium rotors under construction. A cylindrical configuration was adopted which

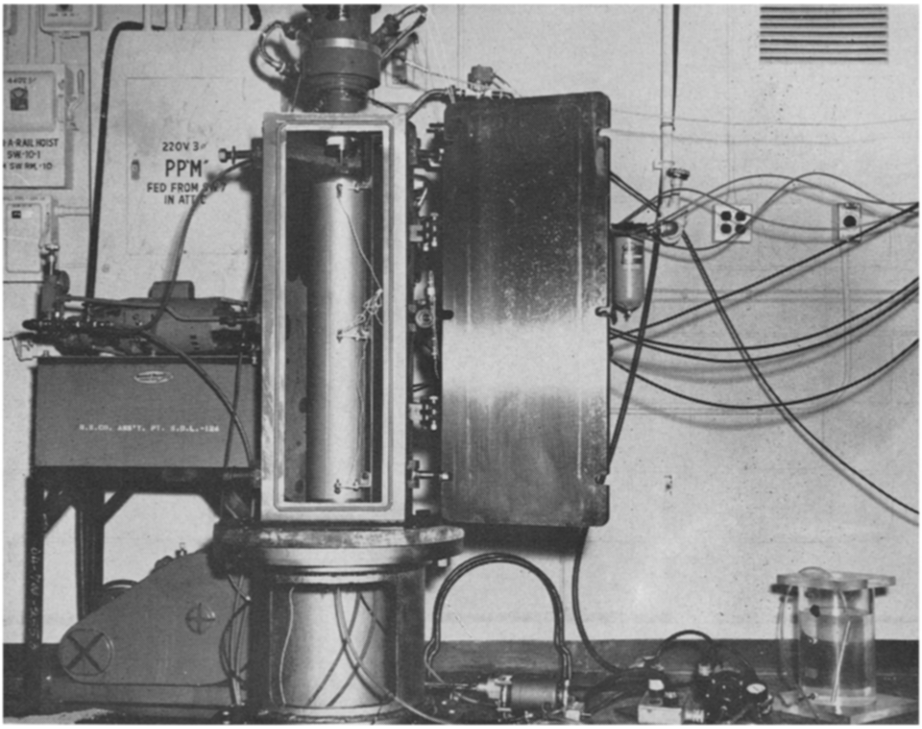


FIG. 11. K-IIA shield arranged as test stand for initial burst tests.

requires a hydraulic hoist to lift the turbine-rotor in and out of the machine, and to minimize manual handling and possible rotor damage. The armor consists of (*a*) an outer vacuum shell of schedule 80, 16″ steel pipe, (*b*) an inner forged cylinder of HY-80 armor plate steel (minimum Charpy "V" impact value of 50 ft lb at —120°F), 10 in. inside diameter and 2 in. thick, and (*c*) a cylindrical refrigeration jacket 8.5 in. i.d. and ⅛ in. thick of brass. All three were concentrically mounted, and the armor cylinder was free to spin under impact inside the vacuum chamber. The complete K-IIC centrifuge is shown in Figure 14. Burst tests with the three casings listed are described in a subsequent section.

THE K-II CENTRIFUGE CONTROL SYSTEM

The control system consists of a main panel containing relays, switches, pressure regulator, vacuum gage, and electronic circuitry, and an air manifold containing manual valves, solenoid valves, control valve, critical flow orifice, an oil mist lubricator, and appropriate pressure taps and quick-disconnect couplings. Air flow to the turbine is effected

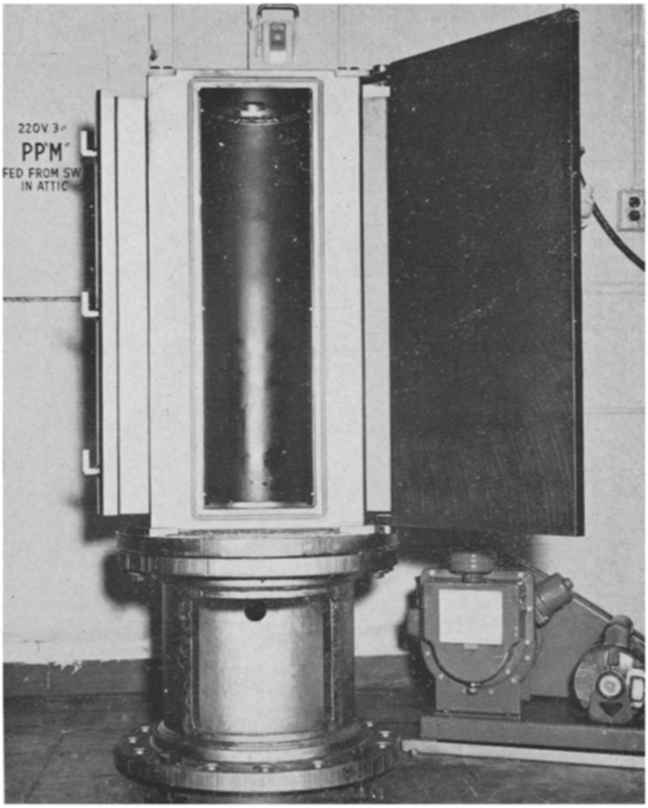


FIG. 12. K-IIB armor shield showing clamping arrangements for securing door.

by manipulation of the solenoid valves and the control valve from the panel which may be located remotely from the centrifuge. Speed is registered on a digital readout device. A photograph of the control panel and digital device is shown in Figure 15, and the manifold is shown in Figure 16.

A manual valve on the air manifold admits air pressure to the main air solenoid and the brake solenoid. At the same time air is admitted to the oil mist system to ensure that the turbine is not operated without adequate lubrication. When the power switch on the panel is actuated the oil pump supplying oil to the rotor bearings is energized. Thus, the rotor cannot be run without adequate lubrication.

Redundancy is provided between the air switch and the start switch. Both must be activated before the main air solenoid can be operated. However, these switches serve two separate purposes. The air switch provides a means of manually controlling the air to the turbine if it

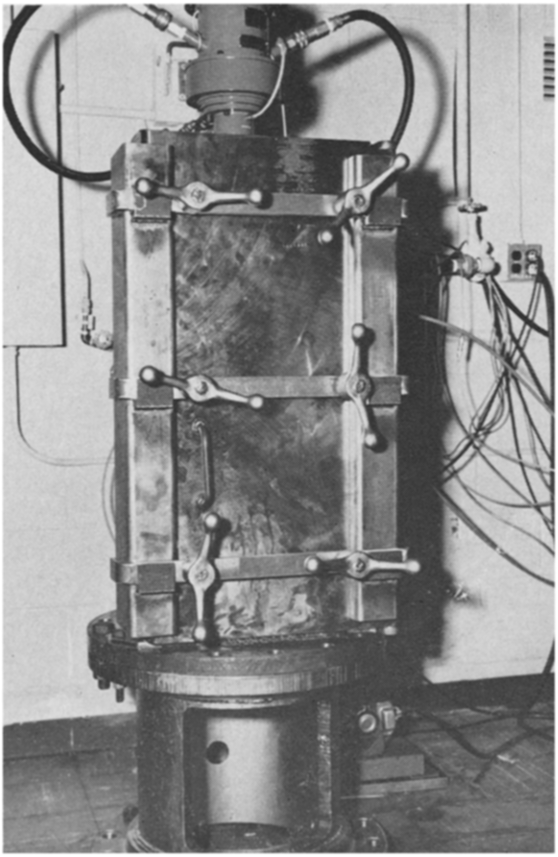


FIG. 13. K-IIB centrifuge closed ready for operation.

is desired to allow the system to coast, or to make adjustments, etc., without shutting down the lubrication system. The start button allows reactivation of the air supply in the event of an overspeed cutout.

Speed is controlled by adjusting pneumatic pressure to a control valve. This is effected through the throttle (pressure regulator) and pressure indicator. The pressure regulator is an internal feedback type such that output pressure is maintained constant regardless of changes in upstream pressure. The control valve is a self-balancing type maintaining constant flow for a given control setting. The pressure indicator is useful as an accelerometer or as a rough speed indicator. Speed may be controlled reliably during either acceleration or deceleration. If it is desired to program the system, this can be done quite readily by gearing the throttle to an electric motor driven from a timing or controlled unit.

FIG. 14. Completed K-IIC centrifuge system.

Three methods are available for deceleration. These are: (*a*) coasting—by shutting off the air switch and allowing the system to decelerate due to bearing friction, etc., (*b*) controlled deceleration—readjusting throttle setting for lower speeds, usually for slower deceleration than would be obtained by coasting, and (*c*) braking—by cutting off the air supply to the turbine and applying air to the brake. The brake is activated by depressing the "stop" button. The system may be restarted after depressing the stop botton by depressing the reset button and then "start." Thus one can make rather rapid decelerations during normal operation, or in the event of emergencies. Also it is possible to restart without coming to a full halt or cutting off lubrication.

Three main safety features remain and are somewhat independent of the controls thus far mentioned. An electronic circuit automatically cuts the turbine air off in the event the system exceeds a preselected speed. This prevents operation of the rotor above safe design speed, and provides a "circuit-breaker" for speed. The system may be restarted after the speed has again dropped to the safe operating level.

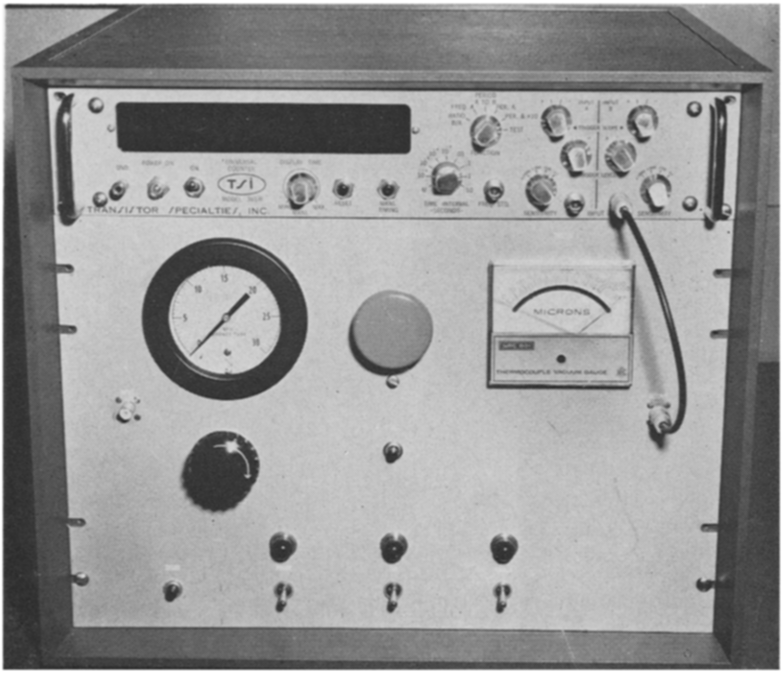


FIG. 15. Control panel for K-IIB centrifuge.

Provision is made on the air manifold for insertion of a critical flow orifice in installations in which air pressure may be exceptionally high or may fluctuate excessively. This limits the upper speed attainable to a safe level.

Provision is made on the control panel for activation of the vacuum solenoid and the monitoring of system pressure. These circuits are independent of the speed control.

Square wave signals are derived, using suitable circuitry, from the electronic overspeed circuit and a proximity pickup in the turbine. A conventional counter is used as a speed register. All circuitry is solid-state. Conventional operational amplifiers are used for low maintenance and ready replacement. Relays are plug-in type units, and the solenoids have replaceable coils and plungers.

INITIAL DESTRUCTIVE TESTS

In an attempt to prove that the rotor chamber would contain the impact energy created by a high-speed rotor failure, a remotely controlled burst test was conducted in the K-IIA shield. Two grooves were machined 180° apart on the outside periphery of a standard K-II

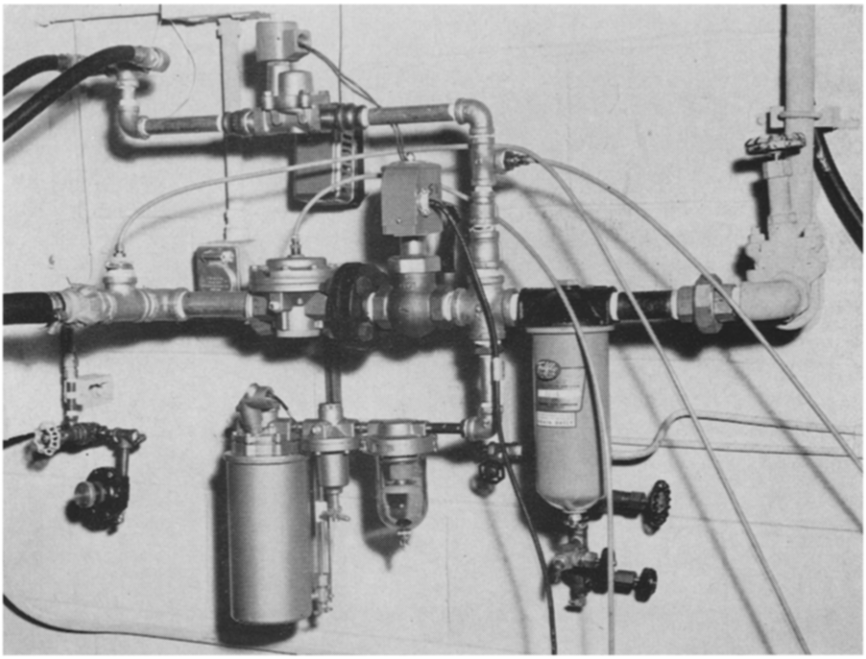


FIG. 16. Air control valves and filter system for K-IIB centrifuge.

rotor bowl so that the bowl should pull apart in two halves at about 45,000 rpm. The rotor was filled with sucrose and water to give the added stress. The test stand used in these experiments is shown in Figure 11.

On the initial run the rotor was driven to approximately 46,000 rpm and appeared to be quite near the rupture point when it suddenly began whirling at large amplitudes ($\sim$30 mils). This occurred because the rotor was approaching its flexural critical frequency. Evidently the oil seals had failed since the bottom of the shield contained several inches of oil. This was attributed to the high runout (7 mils) of the shafting which occurred when the rotor yielded. The oil pool in turn caused the precession (whirl) with the same 25 cycle per second translational whirl frequency that had been observed previously at lower speeds with improper damping. Enough of the rotational energy of the system was dissipated by the large amplitude whirl so that it could not be driven further with the available air supply. Surprisingly, the rotor decelerated and the whirl gradually disappeared without shaft breakage.

For the next attempt the depth of the radial grooves was increased in the hope that the rotor would burst before the onset of whirl. The rotor was filled with cesium chloride and water and accelerated while stability and growth were checked with proximity probes. This time the rotor was driven to about 45,000 rpm, when the whirl suddenly appeared again for the reasons previously stated and caused deceleration without failure. Since this was the second time the bowl had been stressed *past* yield strength, it was feared that if the next attempt failed the bowl would be too elliptical and unbalanced to run again. The groove depth was then increased to 66% of the wall thickness and a denser cesium chloride solution having a density of ~1.8 gm/cm³ was used in the rotor. Rotor failure finally occurred at about 17,000 rpm and the remains of the rotor are shown in Figure 17. Neglecting stress concentration around the bottom of the groove, the average stress across the section between the inside bowl wall and the bottom of the groove was calculated to be about 32,000 psi. Since the ultimate strength of the aluminum bowl material was about 81,000 psi. a stress concentration factor of at least 2.5 was required to cause failure. It is known that deep, sharp grooves perpendicular to the direction of tension produce high stress concentration around the bottom of

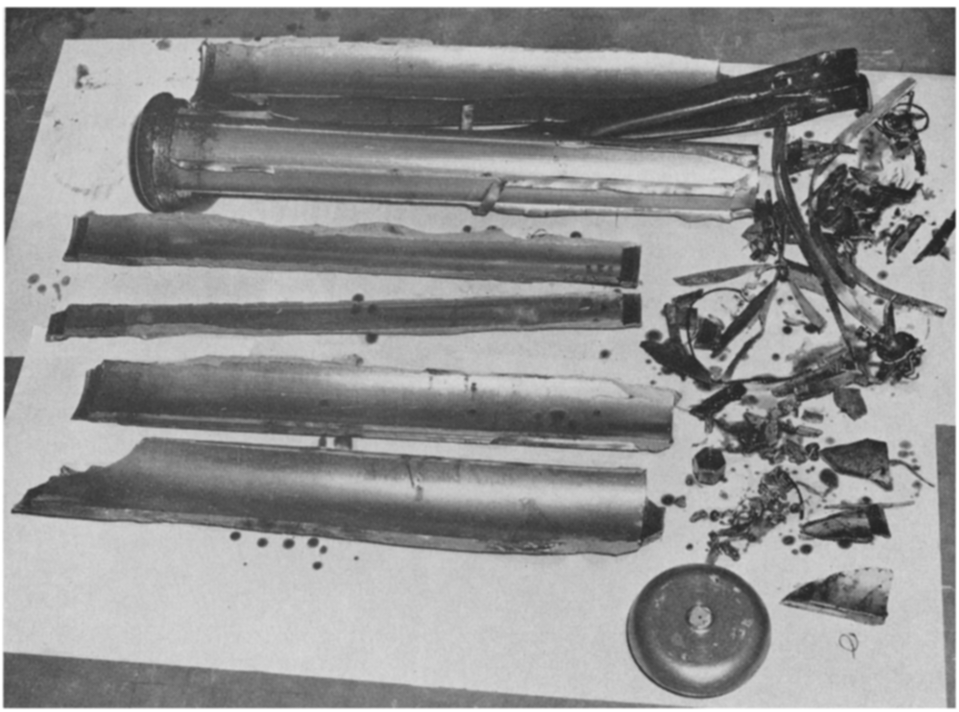


FIG. 17. Fragments of K-II rotor recovered after initial burst test.

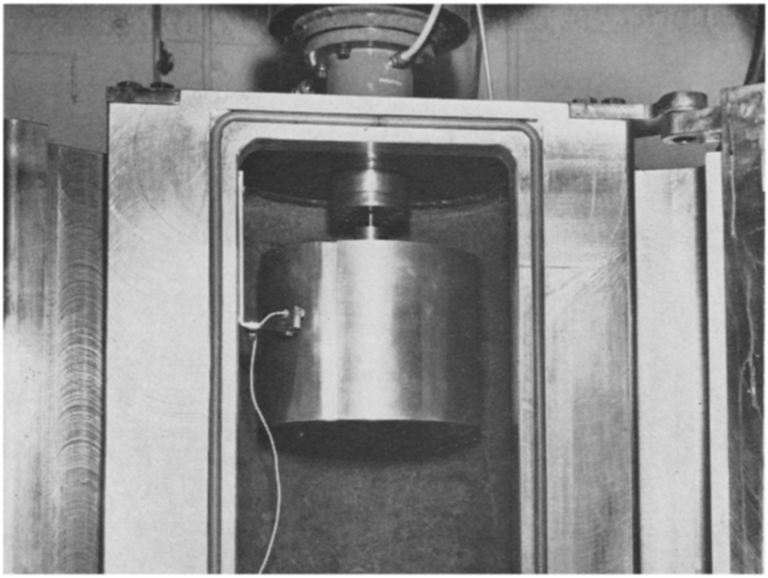


FIG. 18. Rotor simulator for burst tests in K-IIB centrifuge shield.

the grooves. By extrapolation of data taken on grooved bar specimens (20), a stress intensity factor of approximately 2.5 was calculated. Later burst rotors were designed with a radius at the bottom of the grooves to prevent such high stress concentration.

The K-IIA casing showed no evidence of penetration or cracking although the door latch bolts were loosened by the impact. Steel washers behind the latchbolt nuts were extruded by the impact, leaving the bolts free to swing away, indicating that better ways of latching the door should be investigated.

The K-IIB casing was designed to provide better door closure and less chance of shrapnel development in case of rotor failure at high speed. It was tested with a specially designed burst rotor. In designing the burst rotor, the primary concern was to build a stable rotor with about *two* times the energy per unit length of the K-II bowl at 40,000 rpm. The rotor developed was a hollow alumnium cylinder, 6-in. long by 8-in. o.d. and 6-in i.d., as shown in Figure 18. It was grooved along the length at 180° to facilitate failure in two halves. The drive disc was constructed of brass and was threaded into the center of the rotor to prevent the rotor from growing away from the drive disc. The burst rotor was designed to burst at 30,000 rpm and contain 176,000 ft lb of energy at failure.

The rotor was supported at the top of the casing by the turbine shaft and the brass drive disc. Actual burst speed of the rotor (the last printing on the electronic counter) was 30,900 rpm. Energy contained in the rotor at this speed was 185,000 ft lb, which is 44% of the energy of the longer K-II bowl at 40,000 rpm. A single probe mounted at the center of the rotor (see Fig. 18) indicated the average growth. Total growth just before burst was 0.017 in.; 0.010 in. growth was detected from 25,900 rpm to burst. Figure 19 shows the remains of the rotor in the bottom of the casing. Examination of the parts indicated that failure was at the grooves, as expected.

The casing being tested was damaged by the impact. The door was

FIG. 19. Simulator rotor fragments after burst in K-IIB centrifuge armor.

bowed slightly and small fragments of aluminum were found on the flange outside the O-ring gasket. The ends of the top safety bar failed when the door bowed, but the door remained closed. The 14 in. diameter schedule 160 steel pipe was cracked in the area of impact (Fig. 20); and the top plate of the casing was pulled away from the weld above the impact area.

FIG. 20. Crack in armor casing of K-IIB centrifuge armor after rotor simulator burst test.

With full realization of the severity of this test, a study was initiated to provide a stronger casing, which resulted in the K-IIC design.

The full-length rotor simulator used in the K-IIC had a larger diameter than the K-II rotor and was designed to burst at 28,800 rpm with kinetic energy equivalent to a K-II aluminum rotor bursting at 45,000 rpm. The rotor failed at 28,600 rpm on the first run. No cracking or penetration was observed in the armor cylinder or vacuum chamber; however, the refrigeration jacket was "explosively formed" to the inside of the armor cylinder. The impact rotated the armor at least 180°, and produced a maximum ellipticity of only 0.008 in. Both rotor shafts snapped off in such a way that no damage was done to the damper bearings. These tests provided the necessary information to ensure safe operation of the K-II rotors in the C casing at 35,000 rpm (67,600*g* at the outer edge of the fluid stream, and 83,440*g* at the outer edge of the gradient).

THEORETICAL PERFORMANCE

Using the equations developed by Berman (14), the flow rate as a function of the particle sedimentation rate in a given fluid with several different rotor speeds is given in Figure 21. It is evident that the rotor

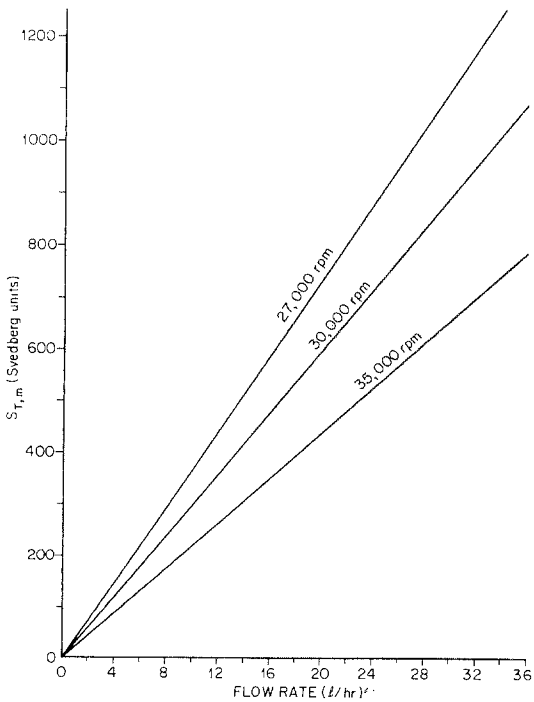


FIG. 21. Theoretical performance curves for K-II centrifuge rotor. Calculated from equation of Berman (14), assuming 100% cleanout.

may be efficiently used with particles having sedimentation rates ranging from that of poliovirus (152 S) to large particles such as poxvirus or bacterial cells. The sedimentation rate of influenza virus measured in chorioallantoic fluid has been reported as 700–800 S extrapolated to 20°C in water (20). From a theoretical viewpoint, the K-II centrifuge should therefore be suitable for the isolation of a variety of different types of virus.

DISCUSSION

A spectrum of new vaccines of high purity may now be prepared using the K-II continuous-sample-flow-with-banding centrifuge. Previously most vaccines have been prepared using empirical methods in which a variety of procedures are applied sequentially and specific activity (activity per unit of mass, nitrogen, or protein) is followed. The problem is to know what purification could theoretically be achieved. This is only known when pure reference standards are available. Results are usually expressed in terms of the concentration and titer of the starting material, i.e., as a 10-fold concentration or purification. It is evident that this does not indicate whether the final product is 100 or 0.01% pure. Expressions of enrichment relative only to the starting material are therefore not satisfactory indications of purity.

The present approach to vaccine purification is to characterize biophysically all classes of particles present, using analytical techniques that have preparative counterparts. Purity may then be assessed by a number of different criteria, which could include measurement of size, sedimentation rate, buoyant density, solubility, and surface charge. Nearly all the analytical tools required are now available. Not all of their preparative counterparts are developed to the stage at which they may be a applied on a large scale, however.

It is one purpose of this program to examine the basic problems concerned with vaccine purification and to present the results of efforts to develop those techniques and large-scale separation systems not presently available.

Few fractionation methods are applicable to large volumes of very dilute antigen. The first problem, therefore, is to concentrate the antigen sufficiently so that a variety of fractionation methods may be used. The concentration methods which have been used include selective adsorption, precipitation, filtration, concentration by phase separation, and either batch or continuous-flow centrifugation. Of these, continuous-flow centrifugation has potentially the widest application. The disadvantages have been the small flow rate, bacteriological contamination, the batchwise recovery of a pellet of virus, and aggregation or inactivation during pelleting.

The combination of continuous-flow centrifugation with isopycnic banding allows virus particles to be concentrated, separated from particulate contaminants having different densities, and recovered in suspension—all in one operation.

The K-IIB has been used for commercial influenza vaccine purification for approximately two years (21). It should be stressed that the initial design specifications were for fluids of higher density and viscosity than are encountered with many other virus suspensions. In addition, the published procedures (21) used a concentrated citrate solution as the suspending medium for flow through the centrifuge, reducing the flow rate considerably. As expected, very much higher flow rates may be used when influenza virus is recovered from tissue-culture-grown preparations (22). The effectiveness of purified vaccine has been demonstrated experimentally (23, 24).

In subsequent papers the performance of the K-II rotor and modifications of it will be discussed. Each rotor modification is given a different Roman numeral as has been the practice with each previous rotor series.

The contrary and anachronistic view that more extensive biological testing of impure materials is more important than actually developing methods for purifying them has been presented recently (25). If "the existing rabies vaccines represent the worst biological products ever injected into the human body" (26), it is difficult to see the advantage of additional testing of such a material in preference to purification.[8]

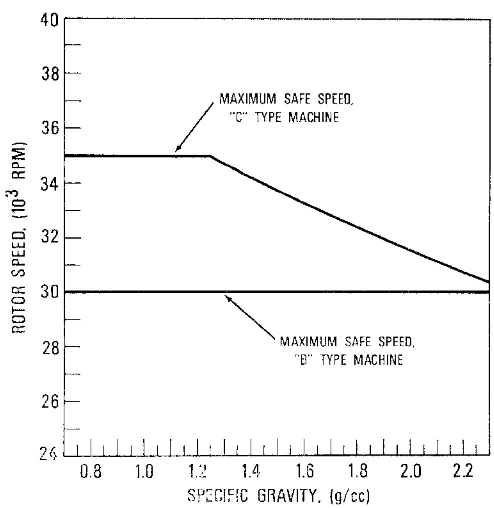


FIG. 22. Operating speed of aluminum K-II rotor as function of fluid density.

[8] The K-IIC centrifuge is available from Electro-Nucleonics, Inc., 368 Passaic Avenue, Fairfield, New Jersey 07006.

SUMMARY

The K-II continuous-sample-flow-with-banding[9] zonal centrifuge has been developed for large-scale virus isolation. The cylindrical aluminum rotor (capacity 3600 ml) contains a 3 liter gradient and has a 700 ml stream volume. Fluid line seals are located on opposite ends of the rotor, eliminating the possibility of cross-leakage. An air turbine drive is used to accelerate the rotor to 35,000 rpm (83,440*g*, maximum). The development of safe armor and control systems is described.

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[9] The phrase "continuous-sample-flow-with-banding," while technically correct, is unwieldy and should be replaced by "*c*ontinuous *f*low *b*anding" or CFB, with the understanding that the sample is flowing and not the collecting gradient. Should it prove feasible to flow a gradient through a rotor continuously, the method would be distinguished as *c*ontinuous *g*radient *f*low with *b*anding (CGFB). If both the sample and gradient flow through the rotor continuously, the phrase "*c*ontinuous *s*ample *a*nd *g*radient *f*low with *b*anding" (CSAGFB) would apply.

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