Zonal Rotors With Removable Seals: Rotors B-X and B-XI [1]

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SUMMARY

Two removable-seal zonal rotors, designated B-X and B-XI, have been built and successfully tested for large-volume rate-zonal or isopycnic-zonal centrifugation.—Nat Cancer Inst Monogr 21: 165–174, 1966.

THE INITIAL studies in the development of a new biophysical tool must of necessity be directed toward the exploration of principles and the construction of suitable test instruments. If these are successful, then simpler revisions of these tools having comparable resolution may be designed. This paper is concerned with the development of simple zonal rotors for use in existing commercial centrifuges. No modification of the instrument or close attendance during centrifugation is required.

In the previous intermediate-speed B series rotors, the attached upper seal required the use of a bearing, a continuous seal coolant, and an oil supply. The possibility of using a removable seal for loading and unloading the rotor at relatively low speed has been previously suggested (*1*). This paper describes two new removable-seal rotors (B-X and B-XI) designed for either rate-zonal or isopycnic-zonal centrifugation. They are not readily adaptable to continuous flow centrifugation, as is the case for the B-IV through B-IX centrifuges, except at relatively low speeds.

These rotors have been constructed from conventional aluminum alloy and from a new high-strength steel. The steel rotor is more efficient than the aluminum one but requires that a number of corrosion problems be examined and the feasibility of platings and other resistant finishes be explored.

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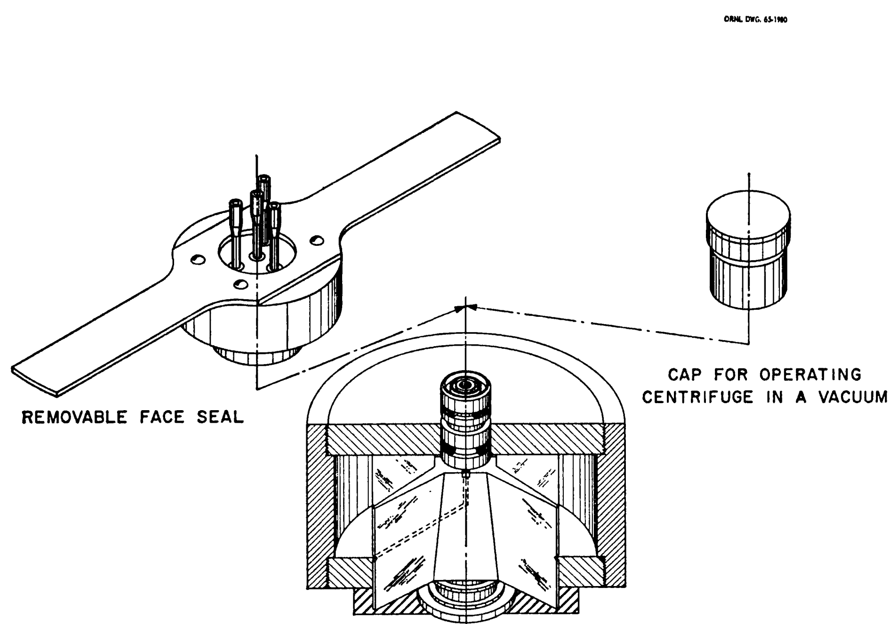
DESIGN CONSIDERATIONS

In the B-IV zonal centrifuge rotor (*2*, *3*), rotor diameter was limited by the yield strength of the aluminum rotor shell at 40,000 rpm. Greater capacity could be gained only by increasing the rotor length to give a configuration requiring an upper shaft and bearing (*4*). To eliminate the upper shaft and to make the rotor self-balancing, rotor diameter should be somewhat greater than rotor length (*4*). Under these conditions large capacity can only be attained by increasing the diameter (and proportionately the length), *i.e.*, by using stronger structural materials at the highest stress levels practical.

One-piece configurations having very desirable structural properties have been evolved (*5*), but fabrication is not practical at present. During operation of a centrifuge at high speed, the chamber wall expands radially more than the end caps, resulting in movement of one relative to the other. This movement can result in loss of alignment or concentricity of the rotor. In addition, the design must insure that a leaktight seal is maintained at all speeds. The basic configuration of the B-XI rotor, shown in text-figure 1, consists of a hollow cylinder with two end caps.

The core (text-fig. 1) comprises 4 tapered septa that divide the rotor chamber into sector-shaped compartments and contain flow lines to the



TEXT-FIGURE 1.—Isometric drawing of B-XI zonal rotor. Removable face seal is attached to the rotor during loading and unloading at low speed. Before acceleration to high speed, the seal is removed and replaced with the cap. The latter is removed before unloading is started at the completion of the run, and the seal replaced.

rotor edge. To channel the gradient into the center exit lines during unloading, the core has flat surfaces that slant in toward the axis of rotation. This core-face slope serves to deflect particle zones upward at the same time that they are concentrated toward the center line of the face due to the parabolic curvature of the zone. A given density zone, therefore, reaches the core face first at the lower corners, and moves up toward the center of the upper edge of the core face to the exit line. This core has no critical unloading speed and functions at a centrifugal force as low as $1 \times g$.

THE REMOVABLE SEAL

An "A" type seal (*6*) has been modified for attachment or removal from the rotor at speeds from 1000 to 7000 rpm (fig. 1). The seal slips over a central arbor, which extends through the rotor and core. The aligning ball bearing in the seal housing is accelerated to speed by friction, and a seal surface is obtained against the flat rotating Rulon[3] slides and the nonrotating metal stator. The seal housing is held in position by two flexible arms that attach to the centrifuge refrigeration chamber. All operations involving the seal and cap are performed while the rotor is spinning. After the gradient and sample have been introduced into the rotor, the seal is removed by hand and replaced by a cap that prevents evaporation from the rotor during operation in a vacuum. The rotor chamber is then closed and evacuated before acceleration to high speed. When centrifugation has been completed, the rotor is decelerated to unloading speed (500–3000 rpm) and the seal reattached for unloading of the rotor contents by pumping dense fluid to the rotor edge so that the rotor contents are displaced radially inward and then out through the seal.

ROTOR B-X

The ratio of $I_{spin}/I_{transverse}$ (*4*) in the B-X rotor is 1.35, similar to that for an angle-head rotor. The rotor chamber contains 685 ml of fluid when completely filled. The rotor components and the assembled rotor are shown in figures 2 and 3. The aluminum test model (7075 aluminum with a T6 heat treatment) may be spun to 31,200 rpm with sucrose gradients. The B-X rotors constructed from Maraging steel may be spun at 40,000 rpm ($119{,}000 \times g$ at $R_{max} = 6.67$ cm) with a cesium chloride gradient having a maximum density of 1.7. Other operating speeds are shown in table 1.

[3] Obtained from the Dixon Corporation, Bristol, R.I.

TABLE 1.—Maximum speeds for B-X and B-XI rotors

		7075-T6 aluminum				18% Nickel Maraging steel			
		Spin frequency (rpm)		g's at R_{max}		Spin frequency (rpm)		g's at R_{max}	
Medium	Density	B-X	B-XI	B-X	B-XI	B-X	B-XI	B-X	B-XI
CsCl	1. 7	30, 300	22, 200	68, 000	48, 700	40, 000	30, 000	119, 000	89, 500
Sucrose	1. 2	31, 200	23, 500	73, 500	54, 600	42, 000	30, 300	131, 000	95, 200
Water	1. 0	33, 200	24, 800	82, 100	61, 300	43, 000	31, 800	138, 000	101, 000

ROTOR B-XI

The B-XI rotor is comparable in volume to the B-IV zonal centrifuge rotor (*2*, *3*) and resembles the B-X in configuration, but has a larger sedimentation path than either. The aluminum version has been used in experimental studies (*7*) to 24,000 rpm with sucrose solutions. The Maraging steel version is designed for operation at 30,000 rpm (89,500 $\times$ g at $R_{max} = 8.89$ cm) with 1725 ml of a gradient having a maximum density of 1.7. Other operating speeds are shown in table 1. At top speed this rotor will store 590,000 ft-lb of energy. The components of the disassembled rotors are shown in figure 4.

CORROSION STUDIES

Stress corrosion has been observed in 7075-T6 aluminum in concentrated cesium chloride at room temperature after immersion for only a few hours. The corrosion resistance of several other materials has therefore been examined. Several samples of polished beta titanium (160,000 psi bending stress level), Kanigen-plated 18 percent nickel Maraging steel (230,000 psi bending stress level), and low phosphorous electroless nickel-plated 18 percent nickel Maraging steel (230,000 psi bending stress level) at stress levels of 95 percent of the estimated yield strengths have accumulated over 6,200 hours test time without failure.

The 18 percent nickel Maraging steel, attractive because of its high strength, is subject to stress corrosion in cesium chloride and therefore requires some type of protection. However, beta titanium is not subject to stress corrosion and needs no protective coating.

Kanigen plating is free from minute cracks that exist in other plating materials, and it readily conforms to contours without build-up at corners. Furthermore, commonly used biological materials do not attack or become poisoned by nickel plating.

TEMPERATURE CONTROL

Temperature control of the B-X and B-XI rotor is difficult because the rotor is operated in air initially. However, if the rotor is prechilled, the large rotor mass tends to reduce thermal gradients. A Lucite closure minimizes movement of air into and out of the centrifuge chamber during loading and unloading. The rotor chamber door also may be partially closed to leave a small opening for the tubing.

OPERATION

The loading and unloading procedures are similar to those described for the B-IV rotor (*2*) except that the seal is removed as soon as the overlay solution is in the rotor. Care is taken to see that both lines to the seal are clamped before the seal is removed, otherwise fluid will drain into the chamber when the seal surfaces part. The application of these rotors to specific biological separation problems is described in subsequent papers.

Note added in proof: Further experimental and design studies have resulted in additional simplification of rotors of this type. B-IX and X have therefore been redesigned as B-XIV and B-XV.

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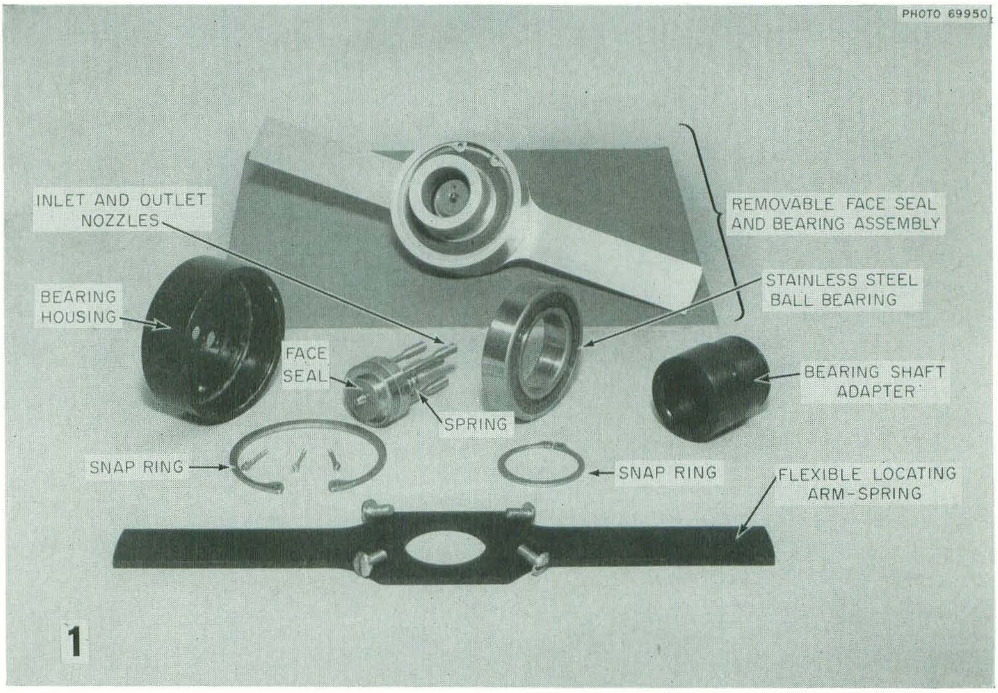

FIGURE 1.—Removable seal for B-X and B-XI rotors.

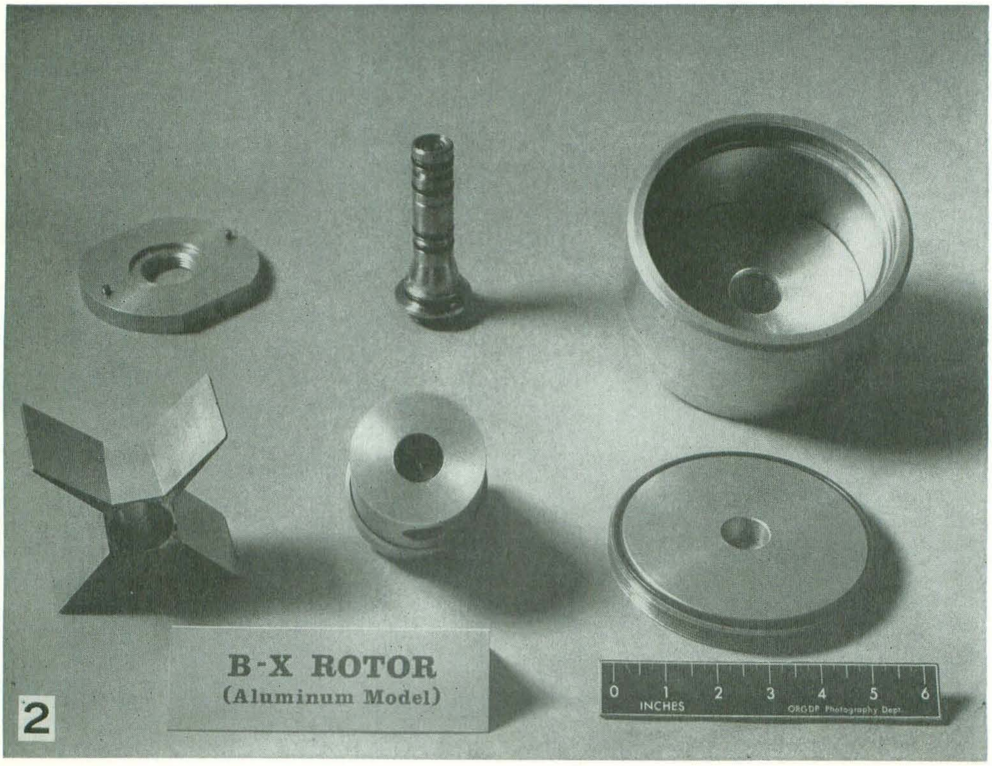

FIGURE 2.—Components of B-X rotor.

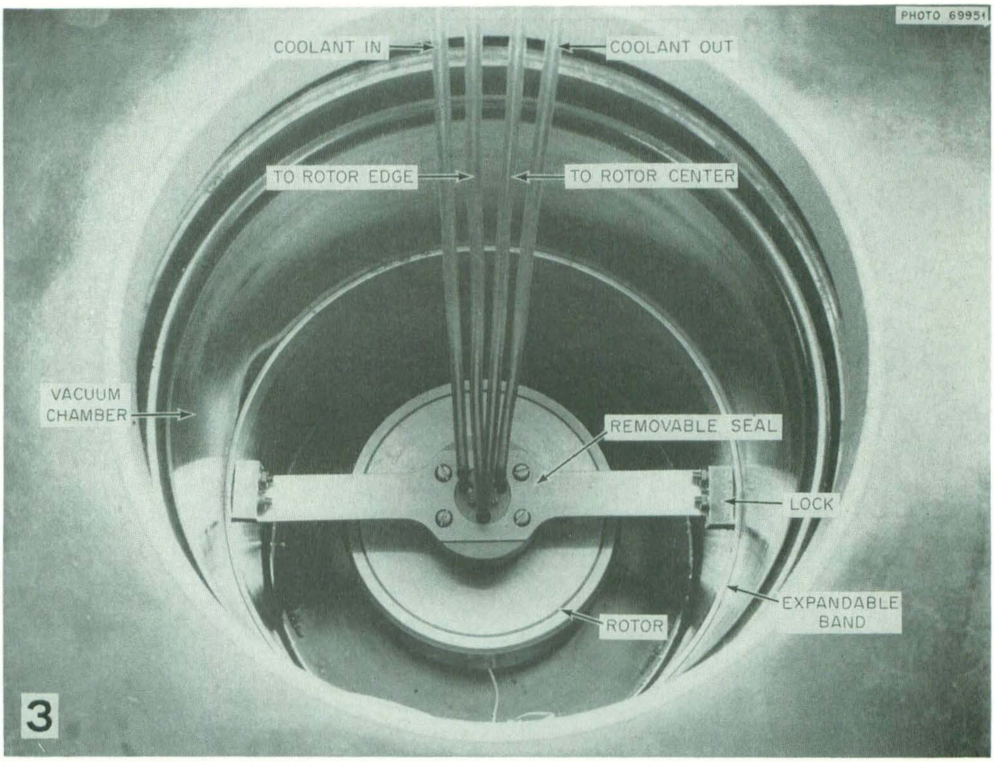

FIGURE 3.—Assembled B-X rotor and seal in centrifuge. The static portion of the seal is kept from rotating by attachment to a fixed band inside the centrifuge refrigeration chamber.

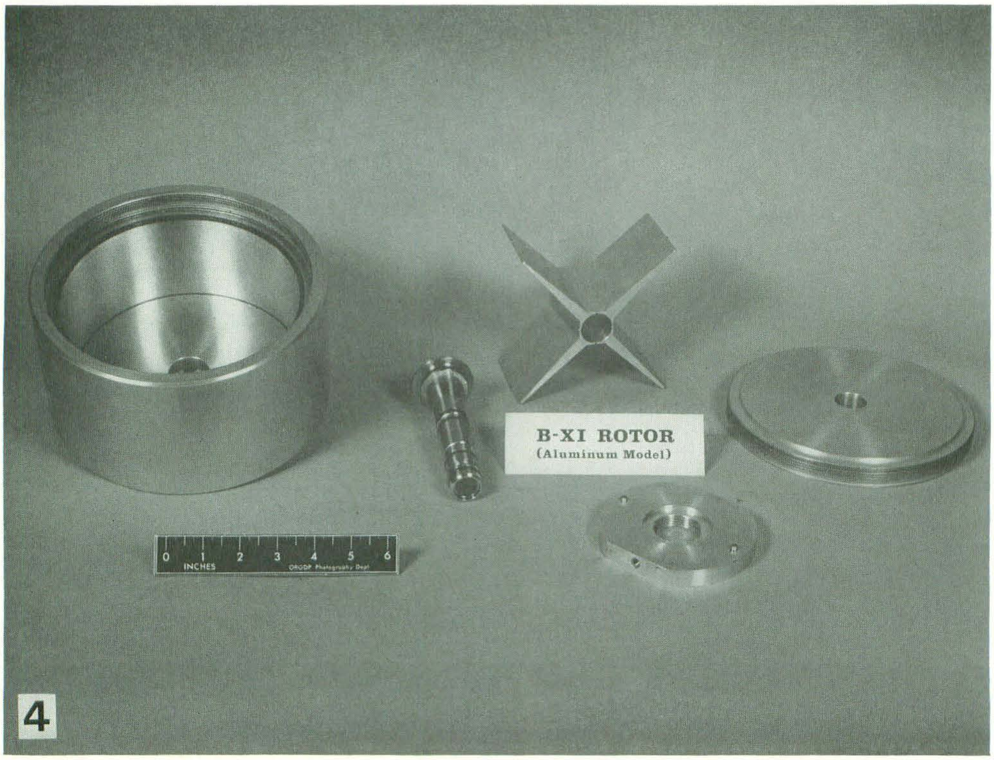

FIGURE 4.—Components of B-XI rotor.