Title
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Publication Date
1961-08-17

UCRL 9767

UNIVERSITY OF CALIFORNIA

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Radiation Laboratory

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Research and Development

UCRL-9767
UC-37 Instruments
TID-3500 (16th Ed.)

UNIVERSITY OF CALIFORNIA

Lawrence Radiation Laboratory
Berkeley, California

Contract No. W-7405-eng-48

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Printed in USA. Price 50 cents. Available from the
Office of Technical Services
U. S. Department of Commerce
Washington 25, D.C.

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ABSTRACT

A four-place swinging-bucket-type rotor, designed for use with either Spinco Model L or E ultracentrifuges, is described which employs full-size 6-ml and 9-ml 1/2-inch preparative tubes. Operation with the 9-ml preparative tube provides a full 8-cm radial path for achieving either sedimentation or flotation under nearly ideal conditions. Useful application of this rotor may be anticipated up to rotor speeds of 20,000 rpm (mean force of 27,000 $\times$ g) with a safety factor of 3.5.

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INTRODUCTION

Although swinging-bucket-type rotors have been designed[1] or are available commercially, such as the Spinco (SW39L, SW 25.1, and K6) and Serval (HS) rotors, none provide for use of both full-size 6-ml and 9-ml 1/2-inch preparative tubes. Use of these convenient full-length tubes, in particular the 9-ml tubes, would present a long radial path (8 cm) in which to achieve nearly ideal sedimentation or flotation. Because of the widespread use of Spinco-type equipment it seemed desirable to design a swinging-bucket type of rotor that would operate in either the standard Model L or Model E ultracentrifuge. Since Spinco-type equipment provides for high-vacuum operation, a requirement for the swinging-bucket assembly would be for an effective vacuum seal to prevent possible escape of fluid from the centrifuge preparative tube into the vacuum chamber during operation. Another potentially desirable feature includes sealing these preparative tubes with standard preparative cap assemblies to achieve an additional safety vacuum seal as well as for ease of removal of the preparative tube from the centrifuge bucket. These features have been incorporated in a low-speed swinging-bucket rotor described below.

CONSTRUCTION AND TESTING

Because of the convenience of either two- or four- place operation it seemed advantageous to design a four-place rotor employing 90 deg symmetry. The rotor assembly shown in Figs. 1 and 2 consists of three parts: a rotor base, the cylindrical rotor, and a locking cylinder which also serves as a suspension coupling for use on the Model E ultracentrifuge. The rotor base allowing operation in the Spinco Model L ultracentrifuge was turned down from an old Spinco preparative rotor which had been retired after prolonged use at its rated speed. The cylindrical rotor was made from a forged billet of 7075 aluminum. After the cylindrical rotor had been machined slightly oversize it was subjected to a T-6 heat treatment. This process consisted of maintaining the rotor at 880°F for 1 hour followed by a 125°F water quench. The rotor was then reheated to 250°F for 24 hours and air quenched. A final machining of the tempered rotor was made to the specified tolerance (±.002 in. for all dimensions).

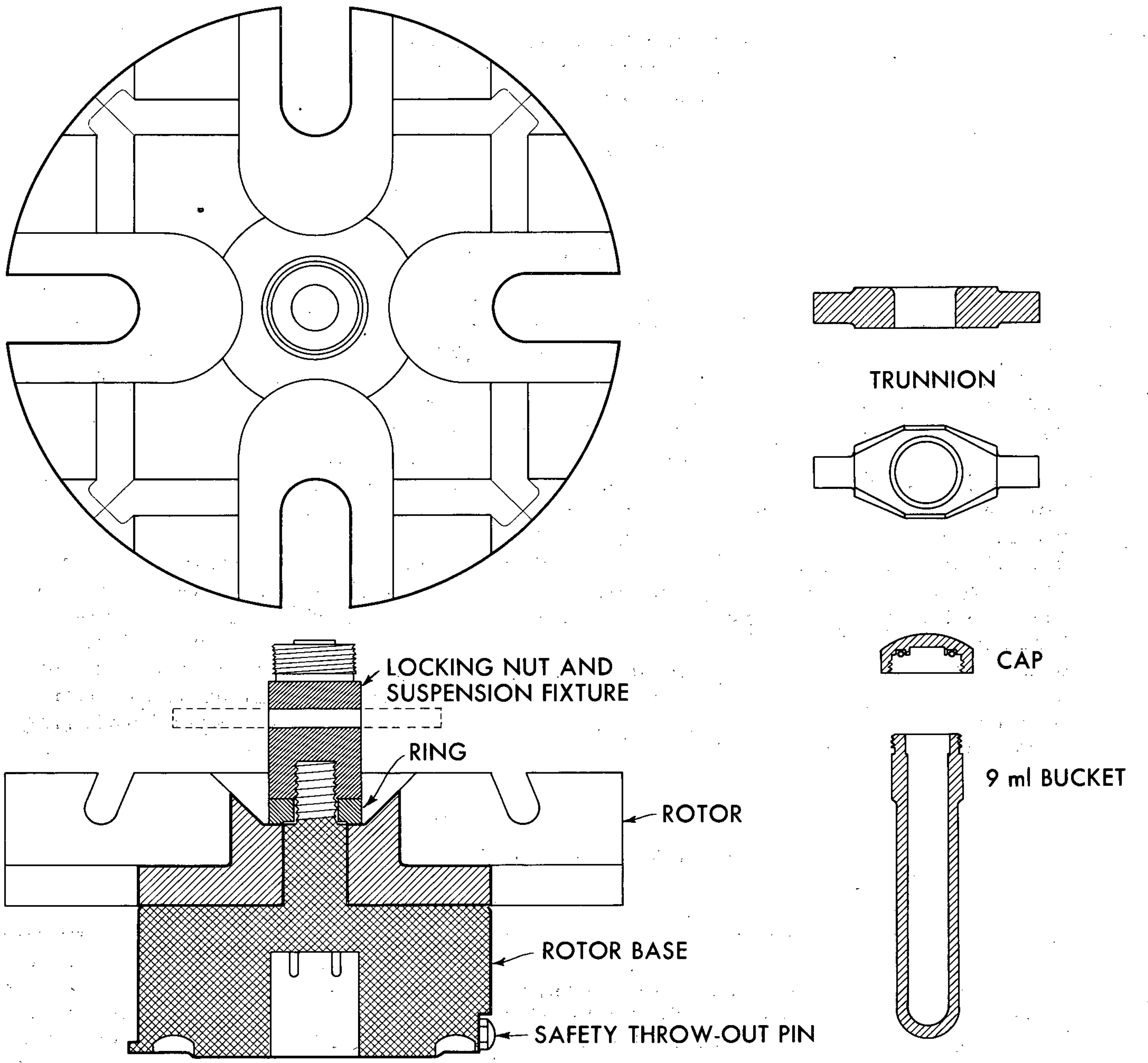

Fig. 1. Scale drawing of the rotor assembly, including a trunnion and 9-ml swinging-bucket assembly. The outside diameter of the cylindrical rotor is ~~4~~.47 in.
7.47

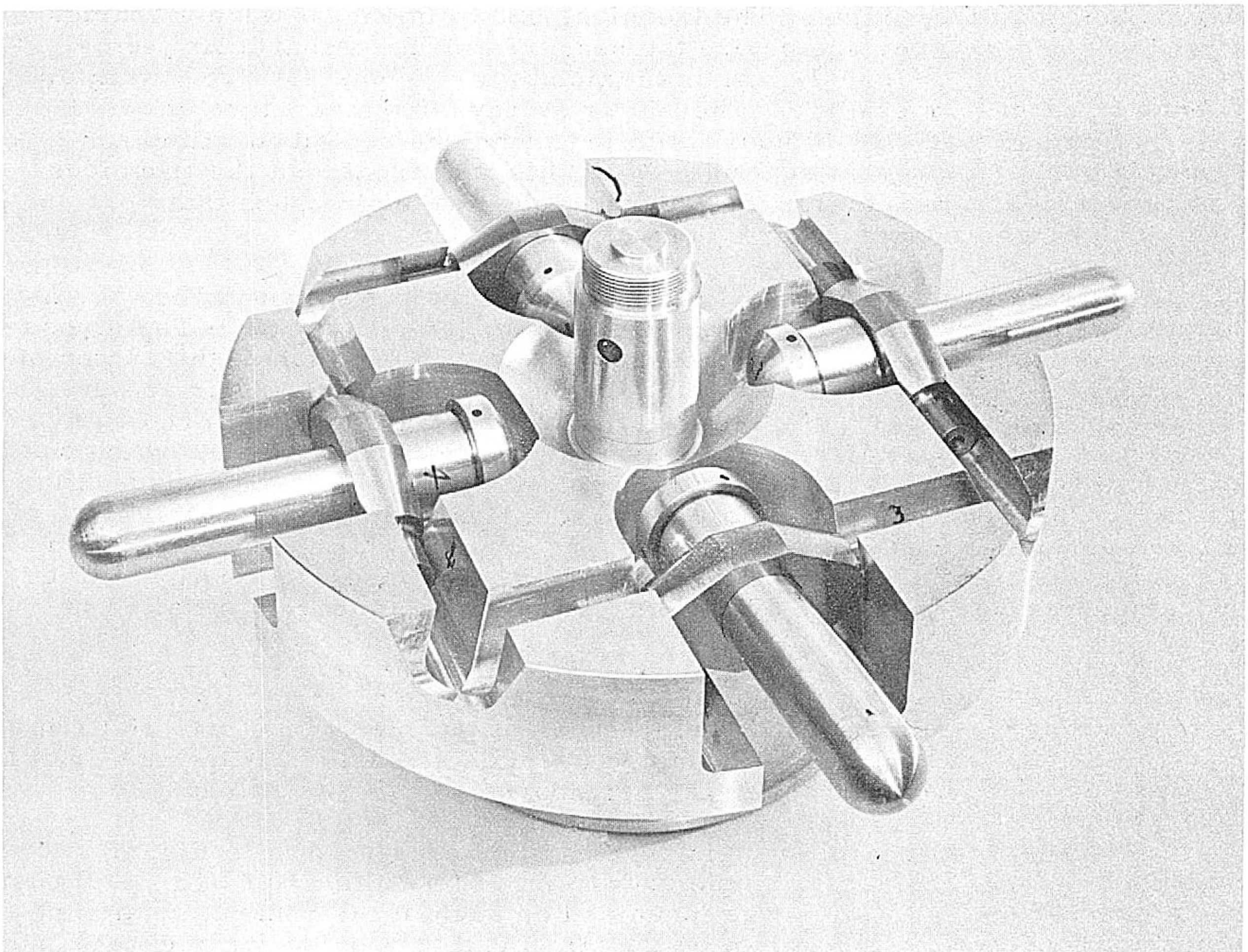

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Fig. 2. The complete rotor equipped with 9-ml swinging buckets in operational position.

Perhaps the most critical parts of this centrifuge rotor assembly are the trunnions, which should not be excessively massive yet must support not only their own weight but also that of the preparative tube assemblies in the centrifugal field. For this reason a special silico-manganese type of steel (Allegheny Ludlam 609) was selected for constructing these trunnions, because of its high elastic limit and its ability to withstand severe stress before bending or breaking. The trunnions shown in Fig. 1 were machined first to 0.010-in. oversize before the heat treatment. A hardening temperature of 1550° to 1650°F was maintained for 1/2 hour followed by an oil quench at room temperature. Thereafter a drawing temperature of 800°F was maintained for 12 to 18 hours with the trunnions contained within a "neutral pack" to prevent scaling. After this heat treatment the trunnions were machined to full tolerance, which included balancing such that no trunnion differed from another by more than ±0.020 gram.

Each trunnion was tested in a special fixture shown in Fig. 3. A hydraulic press carried each trunnion to a full 20,000 lb stress. The stress-strain relationship as determined by the downward deflection of the center of the trunnion is shown in Fig. 4. Also shown along the stress axis are the comparable operational rotor speeds using the fully loaded 9 ml preparative tube - trunnion assemblies. One trunnion was carried to failure, which occurred at a loading of 33,200 lb. Although a radial cross section through the center of the preparative tube hole of the trunnion was approximately 90% of the combined area of the two trunnion support points, failure occurred simultaneously across these latter two support points. Close examination of the trunnion subjected to failure (Fig. 5) suggests that the near pivot failed in shear, whereas the far pivot failed as a result of the severe bending movement developed in the region of failure. Thus it would appear from this failure (at approximately 150,000 lb/in.^{2}) that enlarging the radius in the region joining the support pivot and the body of trunnion would potentially increase trunnion strength. The expected ultimate strength of Allegheny Ludlam 609 with our heat treatment is in the neighborhood of 300,000 lb/in.^{2}

The 6- and 9-ml swinging buckets and caps (shown in Fig. 6) were machined out of ST24 duraluminum and balanced to within ±0.020 g. A thrust shoulder of 3/32-in. radius was machined on each of the buckets, matching a complementary supporting rim on the trunnions to provide for accurate and reproducible centering of the trunnion assembly during operation. Failure to provide such centering could lead to potentially detrimental rotating couples during operation. Each bucket cap is fitted with an O-ring type of seal.

APPLICATION

Practical operation of this rotor thus far has been confined to rotor speeds of from 4000 to 10,000 rpm (1,200 to 6,800 × g). However, useful operation at from 15,000 to 20,000 rpm (corresponding to a mean force of 15,000 to 27,000 × g) can be expected with safety factors of approximately 6.4 and 3.5, respectively. Unfortunately, because of the reduced chamber diameter of the Model E ultracentrifuge (relative to the model L), only the 6-ml swinging buckets can be used in this machine. Thus far this rotor has

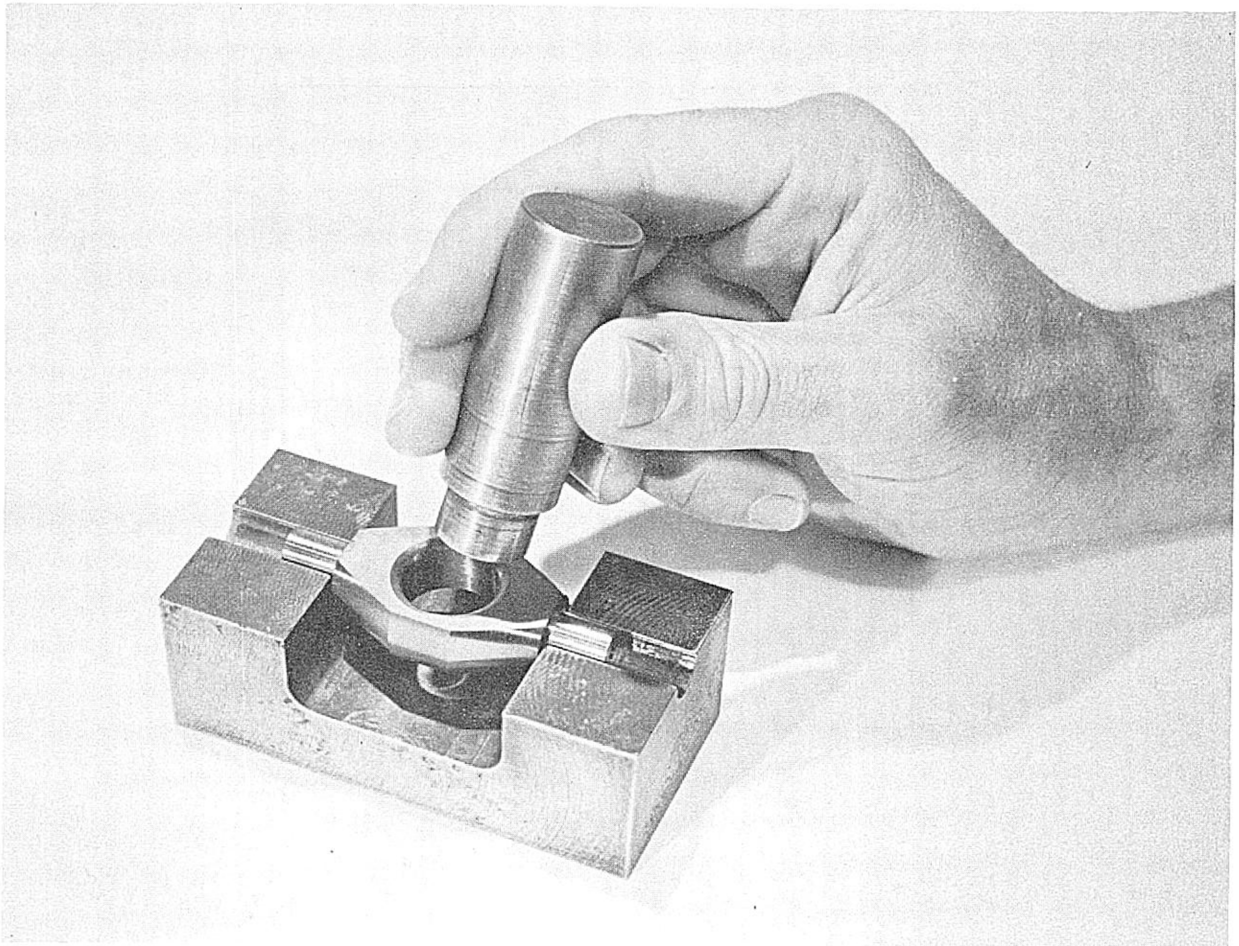

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Fig. 3. Support fixture and plunger for testing trunnion.

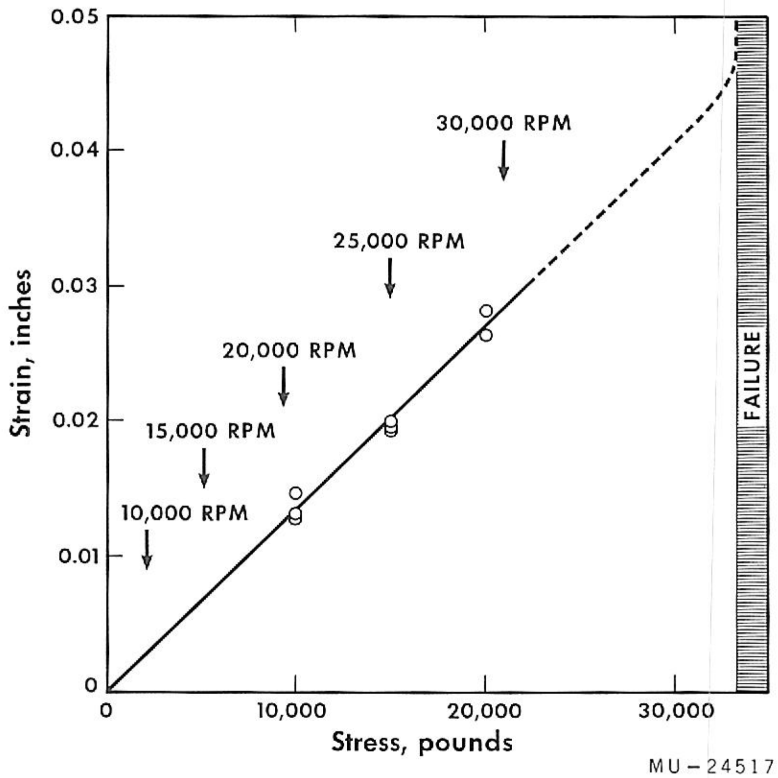

Fig. 4. Strain-stress relationship obtained from trunnion test, including failure at 33,200 lb stress. Comparable operational stress at various rotor speeds is also shown. All tests were made with Baldwin Southwark 200,000-lb hydraulic testing machine.

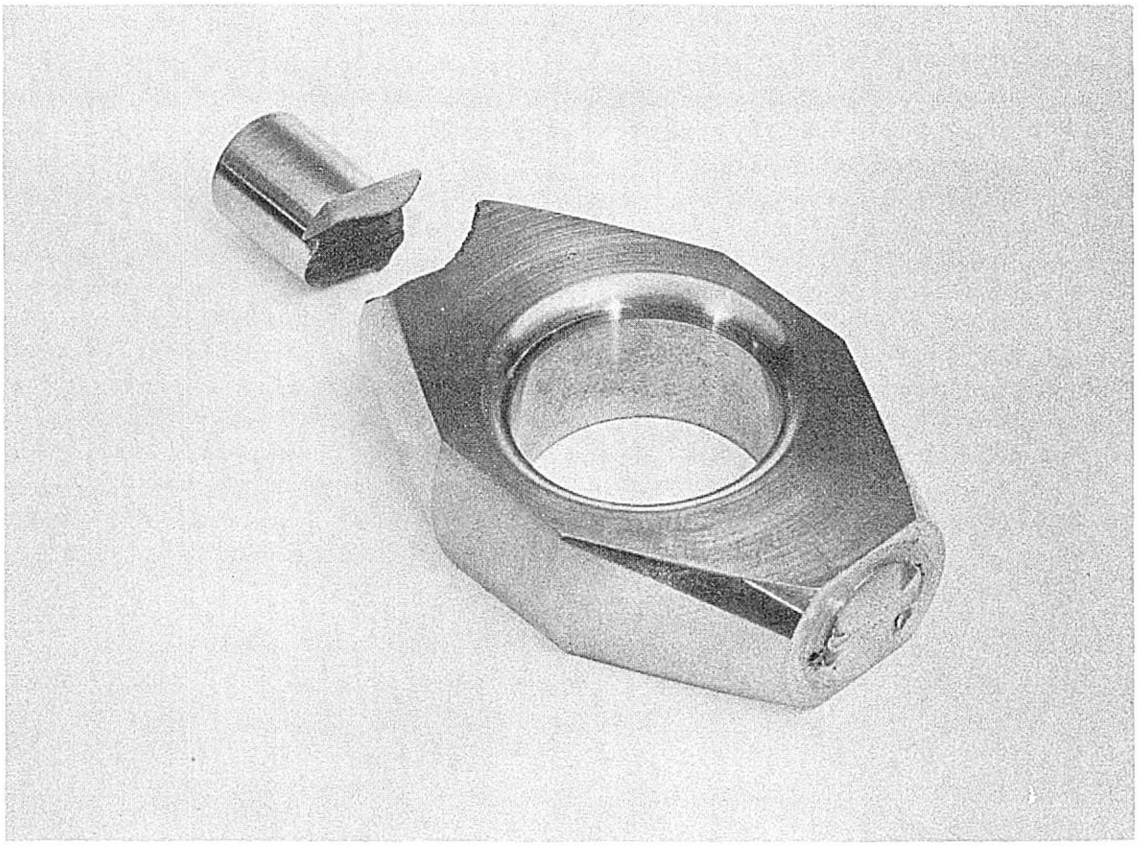

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Fig. 5. Trunnion carried to failure, illustrating shear failure (right support) and failure resulting from bending-moment stress (left support).

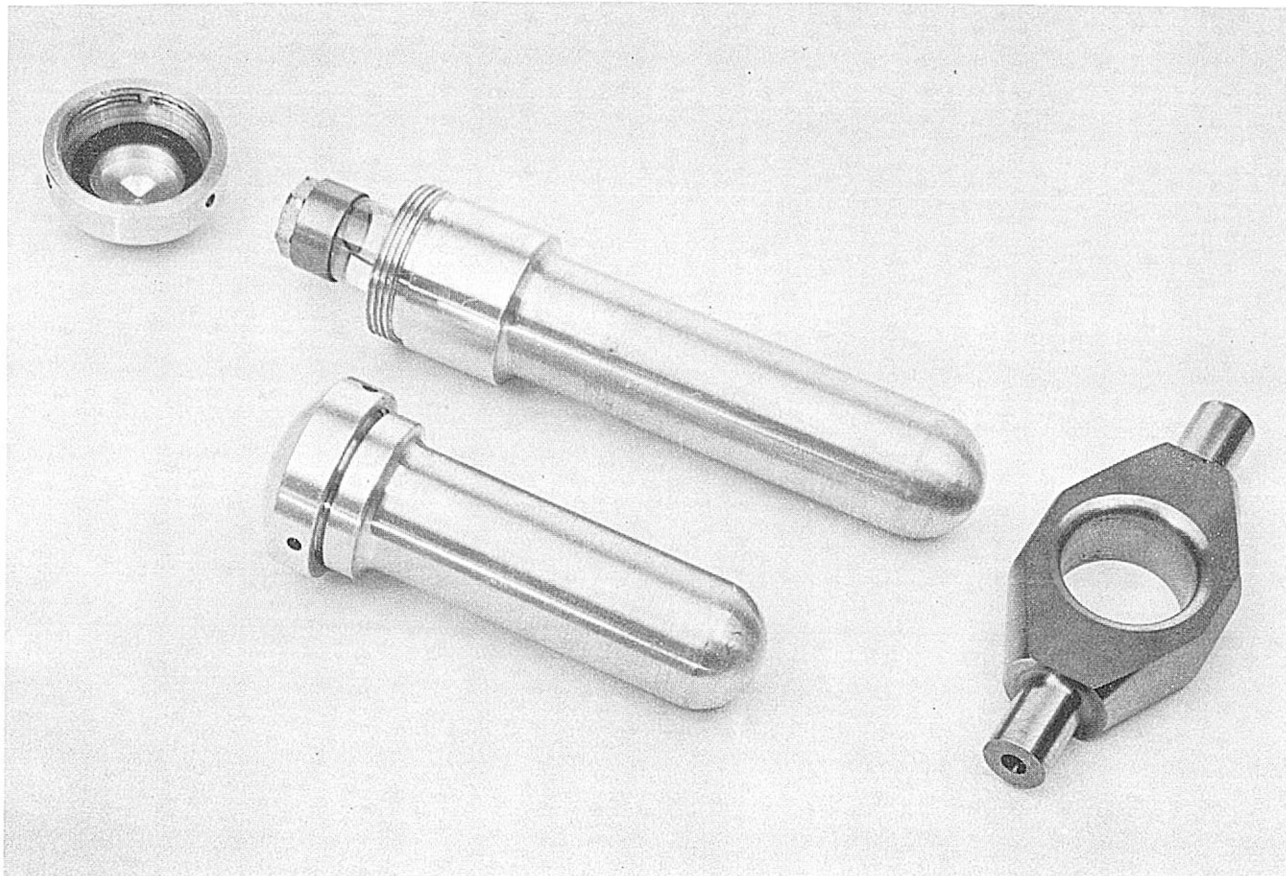

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Fig. 6. Trunnion, swinging buckets (9- and 6-ml capacity) and cap. Swinging buckets and cap allow use of full-size standard 1/2-in.-diam Spinco preparative tubes (of 6 ml and 9 ml capacity) and 1/2-in. Spinco cap assemblies.

has been used to fractionate,[2] by means of density-gradient techniques, certain of the human serum lipoproteins of the chylomicron class (above S_f400). However, potential applications include ultracentrifugal fractionation of subcellular components, including microsomes, mitochondria, and such granules or substructural granules as are found in mass cells and eosinophils.

ACKNOWLEDGMENT

I thank Mr. Leslie O. Seaborn of the Inorganic Materials Research Laboratories (Lawrence Radiation Laboratory, Berkeley) for carrying out the stress and failure tests on the trunnions, as well as Mr. George E. Jolis and Mr. Jack L. Hill (Lawrence Radiation Laboratory, Livermore) for radiographs and ultrasonic inspection of the rotor assembly. I particularly wish to thank Mr. Pete Dowling and Mr. Jack Kroll (Lawrence Radiation Laboratory, Berkeley) for their helpful suggestions and craftsmanship during the construction of the complete rotor assembly and test fixtures. Also, I much appreciate the helpful analysis made by Dr. Karl S. Pister (University of California Engineering Department, Berkeley) of the bending moment developed during stress of the trunnion.

This work was done under the auspices of the U. S. Atomic Energy Commission.

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