AF-IM-2
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Beckman®

THE BECKMAN AIRFUGE™

Air-Driven Ultracentrifuge

Instruction Manual

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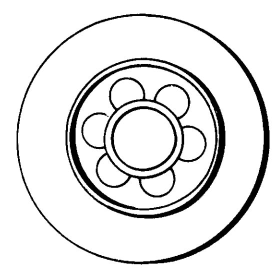

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References IN SECTIONS 1 AND 3

(1) Henriot, E. and Huguenard, E., *Comptes rendus 180,* 1389 (1925).

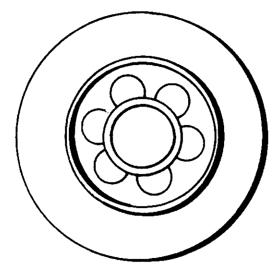
(2) Beams, J. W., and Pickels, E. G., *Rev. Sci. Inst. 6,* 299 (1935).

(3) Pickels, E. G., *Science 83,* 471 (1936).

(4) McBain, J. W., and Leyda, F. A., *J. Am. Chem. Soc. 60,* 2998 (1938).

(5) Hein, G. N., US Patent No. 3456875, issued 1969.

(6) Lindgren, F. T., Jensen, L. C., and Hatch, F. T. *in* Blood Lipids and Lipoproteins: Quantitation, Composition and Metabolism. *ed.* G. J. Nelson. Wiley-Interscience, New York, 1972, P. 181.



Labels on this page are those silkscreened at strategic locations on or inside the Airfuge Ultracentrifuge. They are shown here as a reminder of the precautions to observe when installing and operating the instrument. Other warnings and caution notes appear on various pages in this manual.

CAUTION
Let rotor stop before opening door

WARNING
Do not perform any maintenance on this equipment without first disconnecting from main power source

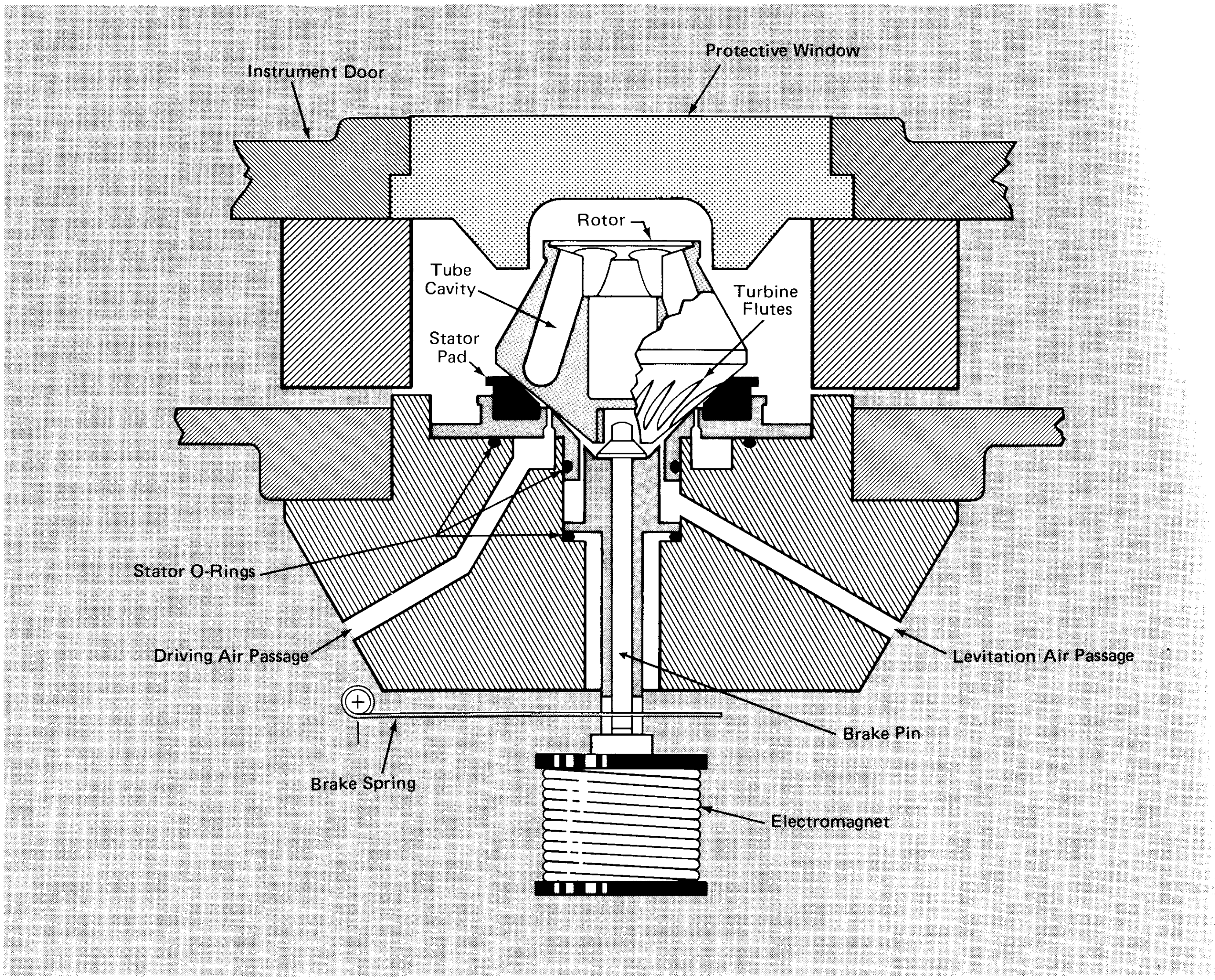
Maximum inlet pressure 75 psig

CAUTION
Insert rotor cap before operating

SAFETY INSTRUCTIONS

For your safety and for the safety of the instrument, please read the brief general instructions below. Also observe the precautionary notes that appear in the manual.

- **Locate** the instrument in a clean, safe uncluttered environment.
- **Instruction Manual** should be read before operating the instrument.
- **Maintenance** should be performed only by qualified, trained personnel.
- **Disconnect** the instrument from the power source before performing maintenance or troubleshooting in any area that requires the use of a tool to gain access.
- **Never Open** the instrument door while the rotor is turning. The Airfuge Ultracentrifuge is provided with a protective window to encourage the user to observe rotor action safely.


Protective Window
Instrument Door
Rotor
Tube
Cavity
Turbine
Flutes
Stator
Pad
Stator O-Rings
Driving Air Passage
Levitation Air Passage
Brake Pin
Brake Spring
Electromagnet

Section 1
General

BACKGROUND AND PURPOSE

Small air-driven centrifuges have a long and interesting history. First described in the technical literature in 1925 by Henriot and Huguenard (1), these "spinning top" centrifuges stimulated considerable interest and experimentation because of the high speeds and forces which could be generated in such a simple device. The centrifuge pioneer, Jesse Beams, published at least eight papers on the development and use of these centrifuges. (Ref. 2 is an example.) Edward Pickels, another pioneer and the developer of the familiar Spinco ultracentrifuges, worked with spinning top centrifuges early in his career (2, 3). McBain and his coworkers developed procedures for analytical measurements in simple spinning top centrifuges which gave sedimentation coefficients and molecular weights of respectable accuracy (4). But all of the pioneers, like McBain, were seeking centrifuges of analytical capability, and the simple tops were eventually passed by for sophisticated vacuum centrifuges.

Air-driven centrifuges were revived by Hein (5) with the development of a movable stator pad for increased stability and reliability.* The addition of a protective cover** and a rotor stopping system,** along with the use of tube-carrying fixed-angle rotors, made the air centrifuge an efficient laboratory instrument for fast separation of lipoproteins and other small-volume samples (requiring 1/10 the time needed for larger centrifuges).

*Patent No. 3,456,875
**Patents pending

DESCRIPTION AND PRINCIPLES OF OPERATION

The tabletop model Airfuge Ultracentrifuge is a miniature air turbine with a small, six-place rotor capable of reaching speeds in excess of 100 000 revolutions per minute and average centrifugal forces on the order of 165 000 times gravity. It takes only 30 seconds to reach these speeds and forces. The *k* factor is 11.

The rotor, which has machined turbine flutes on the bottom, floats on a cushion of air and is turned entirely by air. Six slanted jets (see Figure 1-1) in the stator below it provide air for forward motion (which is counterclockwise); six vertical jets provide vertical airstreams which support the rotor during deceleration. This constant flow of air also serves to keep rotor temperature fairly constant.

Because of the fixed relationship between rotor speed and applied air pressure, only a simple air-pressure regulator is required to control rotor speed. Rotor rpm is obtained by direct conversion of the air-pressure reading on a gauge.

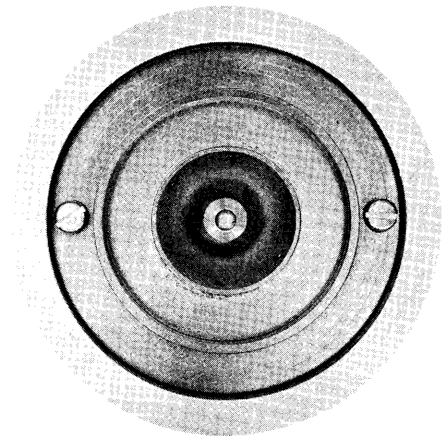
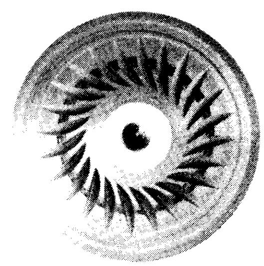


Figure 1-1. Driving Jets (a) and Levitation Jets (b). The latter operate only when driving air goes off.

The instrument is also exceptionally safe, with the rotor held in position over the stator by strong Bernoulli forces. Overspeed protection is provided by factory-set limitation of operating air pressure to approximately 240 kPa gauge (35 psig).

With its unique combination of small-volume tubes and high centrifugal forces, the Airfuge is a valuable tool in biological and clinical laboratories. Top performance, however, depends upon using care and good judgment in running it, and in maintaining it in a particularly clean condition.

A detailed description of the A-100 Fixed-Angle Aluminum Rotor for the Airfuge unit will be found on the rotor specifications pages in Section 3.



Rotor Bottom

Controls and Indicators

The pressure regulator controls the driving air pressure. It is set by the operator for the desired rotor speed by a knob on top of the instrument. This setting (which determines speed) is indicated by a

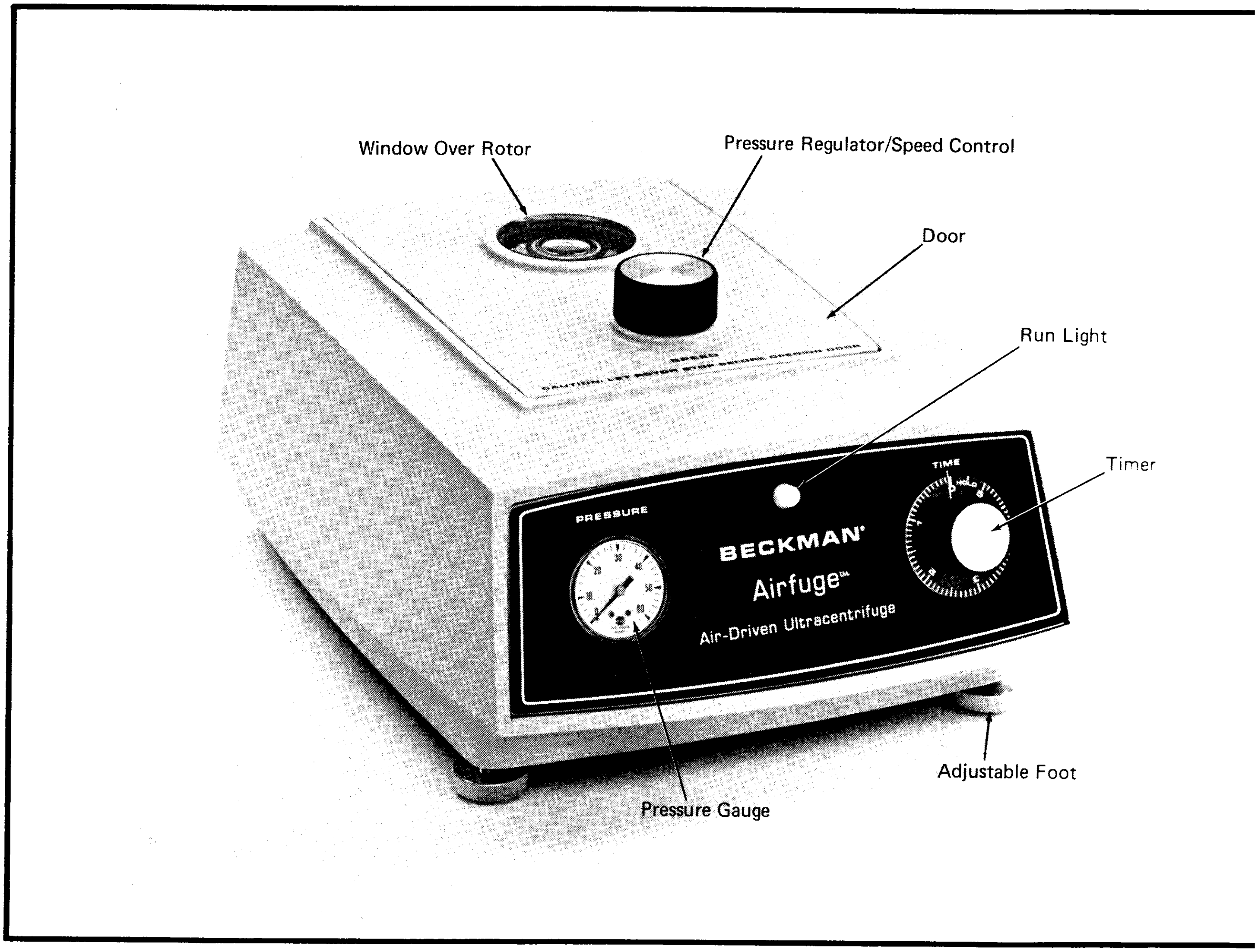


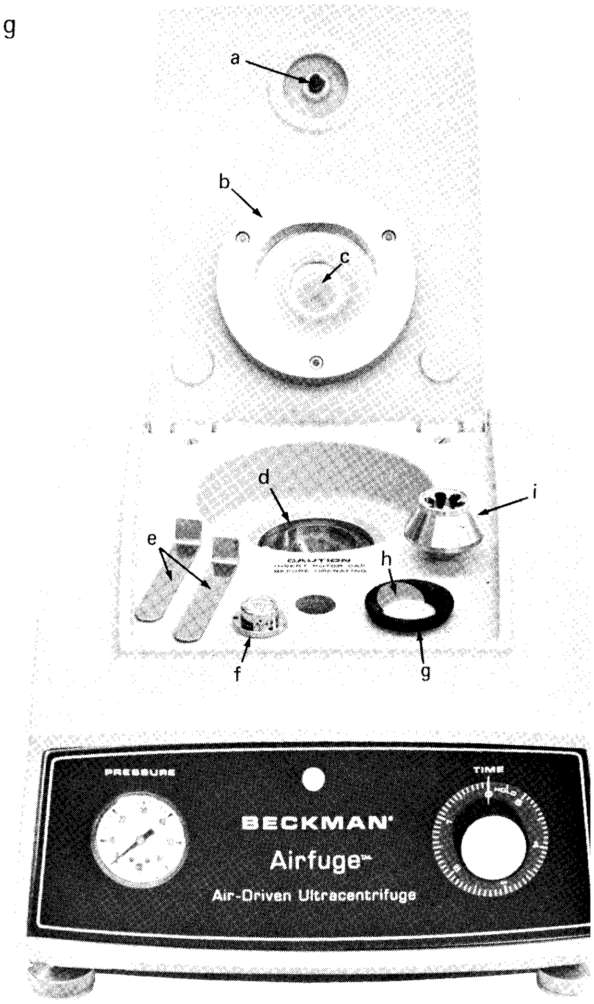
Figure 1-2. The Beckman Airfuge™ Ultracentrifuge

pressure gauge on the front of the centrifuge. The regulator provides a means of accelerating the rotor gradually, without disturbing tube contents, as the operator turns the knob. Pressing down on the knob and making four clockwise turns will close the metal door (with protective barrier) over the rotor. Turning the knob further will bring up air pressure to start the rotor's rotation.

Braking action to stop the run is provided by a movable pin set vertically on a spring in a hole in the center of the stator (Figures 1-1 and 2-3). An electromagnet controls the pin, holding it down when the run is in progress and releasing it when needed.

When the timer reaches zero at the end of the run, a time delay allows power to continue to the magnet for two minutes so the rotor can coast without interference. Then the magnet is de-energized, which allows the spring to push the brake up into a Teflon insert in the rotor bottom, gently stopping rotation. This also stabilizes the rotor at the end of the run.

An indicator light on the front panel goes on whenever the brake is retracted. That is, when the light goes out during deceleration, the brake has engaged the rotor. The time it takes to stop the rotor after the brake is applied should be about one minute to a minute and a half—if the instrument has been properly installed and checked out.



a Speed Control Knob Threads
b Rotor Chamber Wall
c Window
d Stator
e Prying Tools for Removing Stator
f Liquid Level
g Stator Pad
h Rotor Cap
i Rotor

Figure 1-3. Components and Accessories

Safety and Convenience Features

- Adjustable front legs and wide rear support permit leveling. (Use a liquid level.)

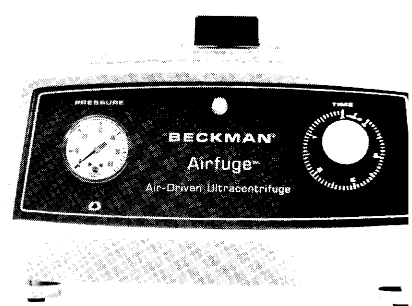
  NOTE: Make sure the instrument is properly leveled before using.

- A metal door encloses the moving rotor, protecting both operator and rotor.
- An interlock prevents bringing up air pressure until the door is secured.
- A protective plastic window in the door provides the operator a full view of rotor action (the door must not be opened until the rotor is completely stopped).
- Overspeed protection is inherent in the physical limitations of the air pressure system—very little speed increase is possible above 414 kPa (60 psig)—and factory-set limitation is approximately 240 kPa (35 psig).
- Sample temperatures, despite high rotor speeds, remain only a few degrees above that of the freely moving air surrounding the rotor.

NOTE: Section 3 describes how to make a cutting block for slicing tubes when necessary for quantitation.

INSTRUMENT SPECIFICATIONS

Power	120v, 60 Hz, 1 amp; 220v, 50 Hz, 1 amp
Air Pressure	103.5 kPa (15 psig) min.* 517 kPa (75 psig) max.
Factory-Regulated Air Pressure	240 kPa (35 psig)
Flow Rate	0.00118 m^3/s (2.5 cfm) at 207 kPa (30 psig)
Weight	10.4 kg (23 lb)
Dimensions	279 mm wide (11 in.) 182 mm high (7½ in.), incl. knob 381 mm long (15 in.), incl. fittings



*Low pressure will limit speed

Section 2
Installation and Trial Run

INSTALLATION

Work Surface

Select a sturdy table or bench for the Airfuge Ultracentrifuge that will not wobble or vibrate.

Location and Connections

Select a location near a supply of clean compressed air at 103.5 to 517 kPa gauge (15 to 75 psig) and a 120-volt convenience outlet (220 for 50 Hz) for 3-prong plugs. The unit will come with a suitable length of air hose with standard quick disconnects and a plain hose with clamps (see "Filter System" below). Your air outlet fitting for the plain hose should be a standard male 6-mm (¼-inch) barb fitting. (Although longer hoses could be used to reach a compressed air supply, it is preferable to have hard piping for this purpose.) Air usage in this instrument will be about .00118 cubic meters per second (2½ cfm) at 207 kilopascals gauge (30 psig).

Filter System

The filter supplied for keeping the air free of water and oil from the compressor is shipped with the Airfuge and MUST be used with it. It has excellent removal capability and the added advantage of indicating when it is saturated. The element *turns red,* at which time the entire element is replaced. Replacement elements are available from the filter manufacturer (see instructions with your filter).

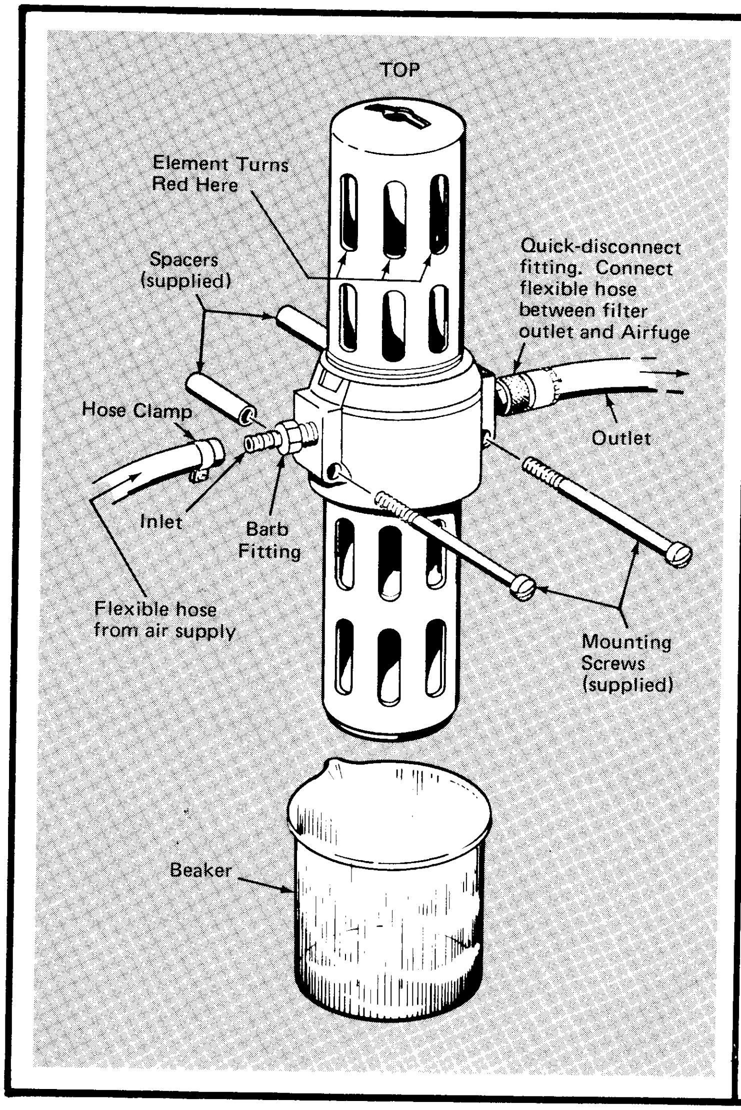


Figure 2-1. Wall-Mounted Filter

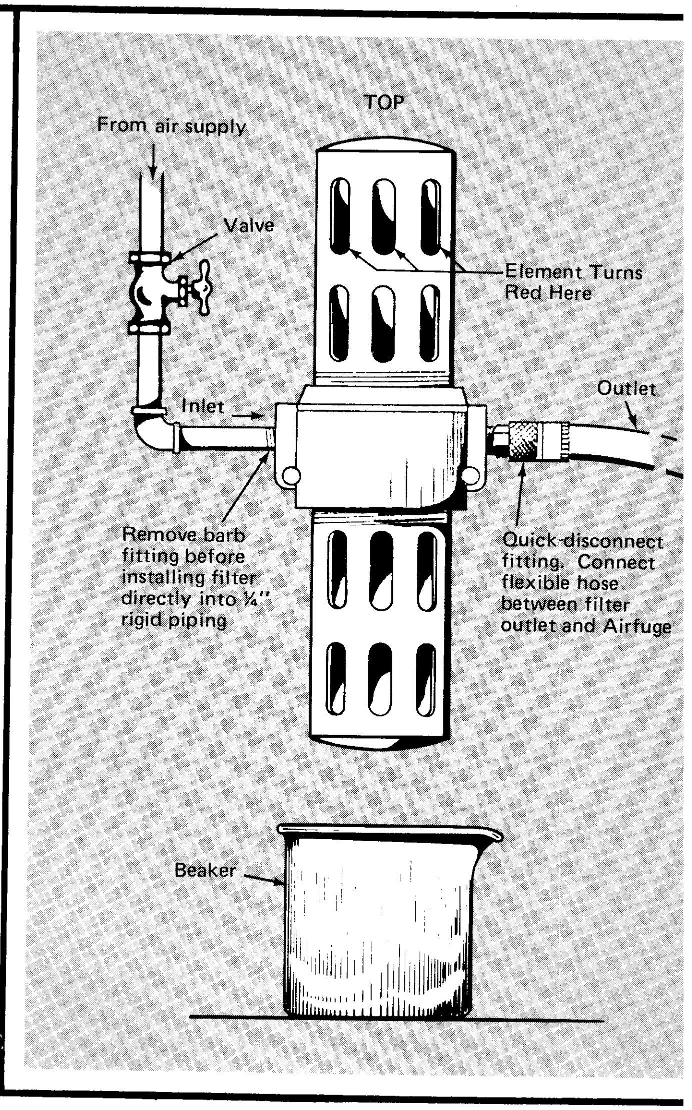


Figure 2-2. Free-Standing Filter

(NOTE: Shown is one type of filter used in the Airfuge; yours may differ slightly.)

Installation—The filter has a 6-mm barb fitting on the inlet (marked with an arrow or "IN"), and a quick-disconnect fitting on the outlet (with an arrow or "OUT"). The user may elect to install it in either of two ways, using ordinary hand tools:

1. *Wall-Mounted*—Screw the filter rigidly to the wall in vertical position (top and bottom are marked), using the screws and spacers supplied—or other fasteners suitable for your wall material. Figure 2-1 shows mounting holes. Attach the flexible hose with quick-disconnects* between the filter outlet and the Airfuge unit. Add hose clamps to the plain hose, then slip the ends of the plain hose over the barbs of the filter inlet and your air supply. Tighten clamps. *Place a beaker under the filter to collect small amounts of oil and water which will accumulate because of the self-purging action of the filter.*

2. *Free-Standing*—This method is recommended if rigid piping leads from your air supply and can be mated to standard ¼-inch pipe thread. Remove the barb fitting from the filter inlet and substitute rigid piping. The filter is installed in vertical position (Figure 2-2). The connection between the filter outlet and the Airfuge is the same as for the wall-mount method, using the hose with quick-disconnects.* *Place a beaker under the filter to collect small amounts of oil and water which will accumulate because of the self-purging action of the filter.*

NOTE: Make sure the time delay relay is socket after shipment. (See Figure 5-8.)

ADJUSTMENT

CAUTION

Do NOT connect power or air supply at this point.

Leveling

After the operating site has been selected, the instrument must be leveled in that location:

1. Remove the stator pad from the instrument. (The pad is shown in Figure 1-3.)

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*To use quick-disconnects: Attach by sliding back the spring-loaded sleeve on the female end. Push this end over the male end as far as it will go and release the sleeve. Remove by sliding back the sleeve. Attachment and removal may be done when the system is pressurized, but is easier when air pressure is off.

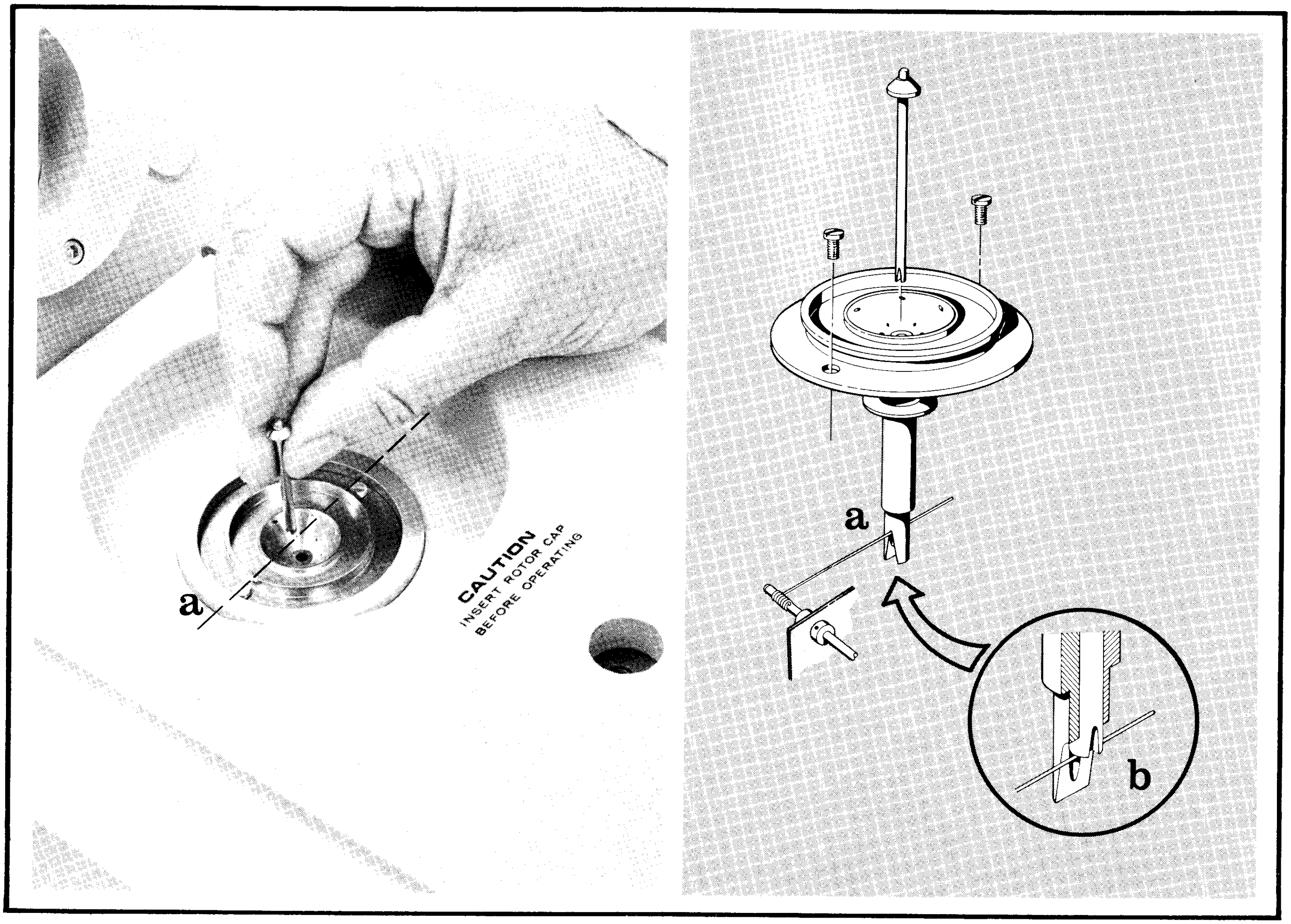

Figure 2-3. Aligning Brake Pin in Stator. The slot on the end of the pin (a) fits over the wire (not visible below the stator). Broken line shows wire direction (aligned with screws). When the tips of the slot stand on the wire (b) instead of straddling it, the pin will be too high, as in Figure 2-4 (b) and (c).

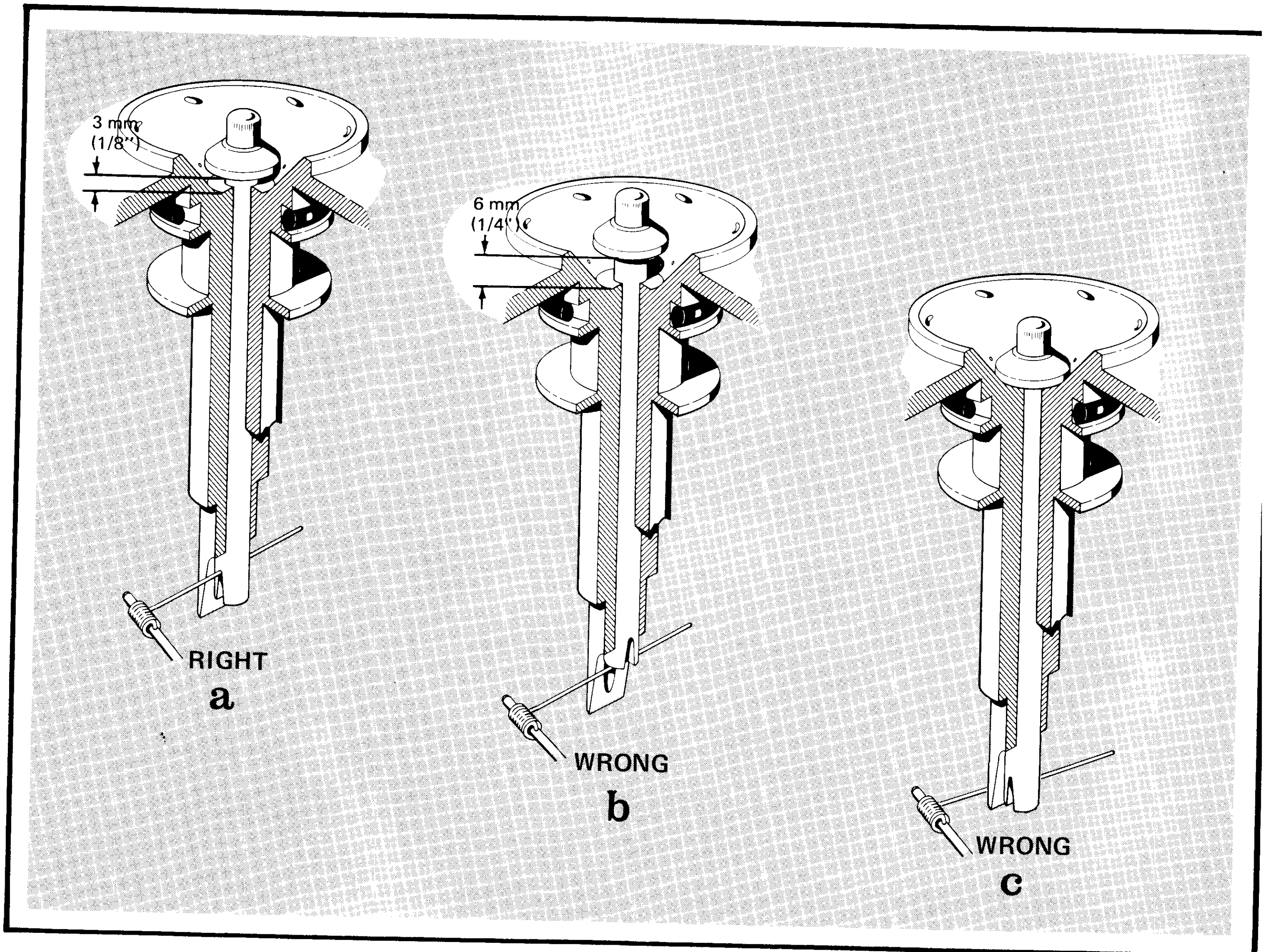

Figure 2-4. Pin Height, Right and Wrong. The bottom of the cone on the pin should be about 3 mm (1/8") above the stator surface when the pin is positioned correctly (a). If the pin rides on top of the wire instead of straddling it (b), the cone will be 6 mm (¼") above the stator. For pins that repeatedly fall flat on the surface (c), see Troubleshooting on the last two pages.

pin lightly with a finger. If it springs back up, it's properly mated with the wire. If the pin repeatedly falls down flat on the stator surface instead, refer to Troubleshooting in Section 5.

> **NOTE:** The brake pin will be held down flat to the stator surface by the electromagnet during acceleration and at operational speed. On deceleration, when the brake is in operation, the pin comes up above the stator to engage the rotor. If the pin is not mated with the spring as in Figure 2-4 (a), it can't operate.

3. At this point, the brake pin cone should be up at its normal position, 3 mm (1/8 inch) above the stator, minus the pad.
4. Place the black, ring-shaped stator pad on the stator. You are now ready for a trial run. (The pad is shown in Figure 1-3.)

TRIAL RUN

1. Connect the air hose and power cord.
2. Install a polyethylene rotor cap by rubbing it into place until it snaps firmly into the groove at the rotor rim. (Whether empty or loaded, the rotor *must* be run with a cap for aerodynamic reasons.)
3. Place the rotor on the stator pad.
4. Turn timer to HOLD position.
5. Close the door and secure it by turning the speed control knob clockwise. Push downward against the knob's spring pressure to engage the threads.
6. Gradually increase air pressure by continuing to turn the speed control knob. Watch the gauge and stop turning when pressure reaches 207 kPa (30 psig) or as close to it as building air supply permits.
7. Run 1 or 2 minutes, then turn the timer to zero. *Immediately start timing and measure the time it takes for the rotor to stop.* The run light should go off after about 2 minutes and the rotor should stop turning about 1 to 1½ minutes after that.* Any gross

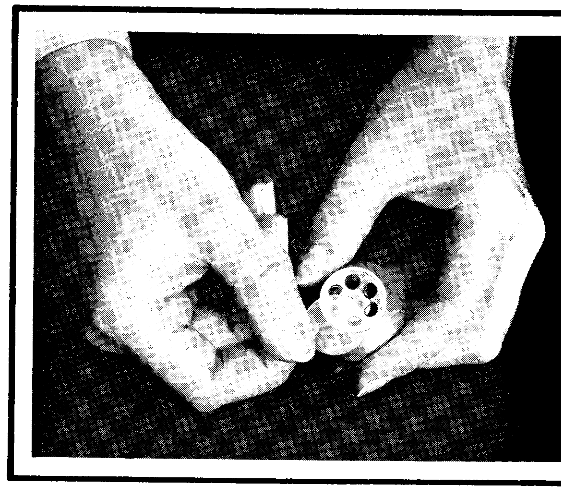

Figure 2-5. A Rotor Cap Is Necessary For Each Ru

*The steady, unwavering motion of a rotor at speed can fool the eye—if you CANNOT see individual rotor cavities, *the rotor is still moving.*

difference from this timing means that the brake and the levitation air need further adjustment. See instructions in Section 5 on adjusting the brake and levitation air.

8. *After the rotor has stopped,* turn the air pressure knob counter-clockwise until the gauge reading is zero. Keep turning until the door can be opened.*
9. Remove the rotor cap by pressing down in the center. This frees the edge from the groove in the rotor. Use a fingernail or tweezers to remove the cap.

IMPORTANT

When all steps are completed, return the pre-addressed, stamped warranty card shipped with the instrument. This will validate the instrument warranty and insure your receipt of further information regarding new accessories and modifications available for the instrument.

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*Do NOT screw the knob down again when the Airfuge is not in use, or the levitation air will flow.

Section 3
Operation

The run steps immediately below are repeated briefly, along with other tips to remember, on a plastic-coated wall chart included in this manual. The operator familiar with the instrument should be able to run and maintain it routinely with only the wall chart as a guide. If any difficulty should arise, however, a troubleshooting list is provided in Section 5.

NOTE: Please read the information concerning your rotor on pages 3-5 to 3-7 before attempting the first run. An understanding of rotor limits, capabilities and care is important for long service.

NORMAL RUN

(A lipoprotein separation method is given later in this section)

Loading the Tubes and Rotor

NOTE: Because the Airfuge rotor does not operate in a vacuum, the sedimentation process may not be entirely free of convection. Consequently, when solutes of low total concentration (1% or below) are pelleted, the sedimentation may be incomplete. This can be avoided in any of several ways: add sucrose or salt to the solution, carry out the run in a cold room, or operate at lower speeds. Lower speeds will improve pelleting.

1. *Tubes*—Use a suitable self-cleaning micropipette for filling the tubes, such as a Beckman Ultramicro pipette, an Eppendorf microliter pipette, an Oxford micro-sampler, or a microliter syringe. Fill the cellulose nitrate tubes from the bottom upward,

turn the dial past the 30-minute point, then back to the desired time.

3. *Close the door and secure it* by turning the speed control knob clockwise, pushing downward against the knob's spring pressure to engage the threads.

Be sure this is done *before* turning on air pressure (next steps), or the regulator will hum and the pressure gauge needle will vibrate.

4. *Continue turning until the rotor starts spinning and air pressure comes up* to the level for the desired run speed (see nomogram, Figure 3-1). Note that a slight amount of precession is normal at the beginning and end of the run. A whistling noise is also normal during acceleration.

CAUTION

If the rotor is unbalanced, or runs roughly for any other reason, abort the run by quickly turning the timer off. DO NOT LIFT THE DOOR UNTIL THE ROTOR HAS STOPPED! Rebalance the load or correct the problem before starting the run again.

Ending the Run

Stop the run with the timer:

1. When the timer is turned to zero or reaches zero automatically, the

Cut out, moisten, and place on page 3-3.

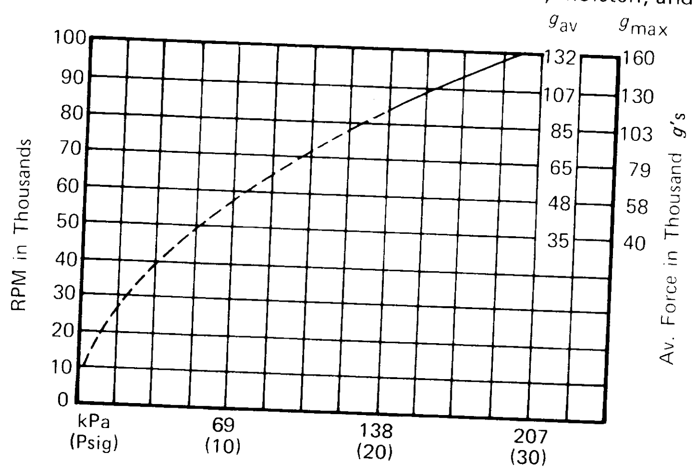


Figure 3-1. Rotor Speed Vs. Air Pressure

NOTE: To operate at pressures less than *138 kPa (20 psig),* call or write Airfuge Technical Support, Spinco Division, Beckman Instruments, Inc., 1117 California Avenue, Palo Alto, California 94304, 415-326-1970, for information on special adjustment procedures.

rotor will coast for about two minutes. The panel light will then go out, indicating that the brake is engaged. The rotor will stop about a minute to a minute and a half later. Again, DO NOT LIFT THE DOOR UNTIL THE ROTOR IS AT REST.* Rotor stop can be checked through the window on top.

CAUTION

If the coasting period seems unusually short—down to 30 seconds or so—the system must be cleaned before the next run. See Troubleshooting and Maintenance Section on cleaning the stator assembly.

2. After the rotor has stopped, turn the air pressure knob counter-clockwise until the gauge reads zero, the door can be opened, and the rotor removed. (Do not tighten knob between runs.)
3. Remove the rotor cap by pressing down in the center. This frees the edge from the groove in the rotor. Use a fingernail or tweezers to remove the cap.
4. Remove tubes with tweezers, *but be careful not to scratch the rotor.*
5. Set tubes in a suitable container, in order. Divide fractions by pipetting supernatant and scraping out pelleted material, or by aspiration or tube-cutting for less firm separations. Tubes may be cut with a razor blade, and if handled carefully, the liquid fractions will be held by surface tension in the cut tube pieces for further processing. For cutting tubes, you will need a single-edge razor blade and a block of aluminum, Lucite, or similar blade-resistant material. Drill cavities to fit the tubes at various fraction depths. (A small hole at the bottom of each cavity will aid in removing the bottom tube section.) Screw the cutting block to the bench or table. When cutting, hold the top of the tube firmly. After cutting, *hold the blade and cut piece of tube together with both hands and slide them off the bottom section of the cut tube. Then slide the cut piece of tube off the blade.* In that way, surface tension will keep the contents inside the cut tube.

*The steady, unwavering motion of the rotor can fool the eye—if you CANNOT see individual rotor cavities, *the rotor is still moving.*

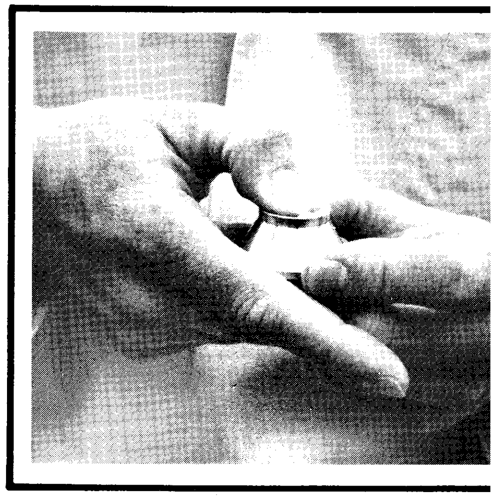


Figure 3-2. To Remove Rotor Cap Press D Center, Lift Edge

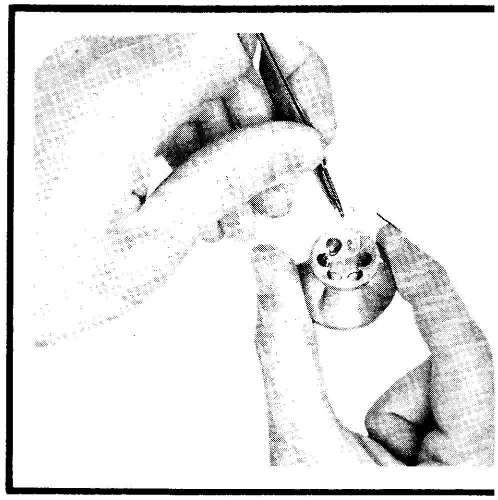


Figure 3-3. Remove Tubes With Tweez

SPECIFICATIONS AND INSTRUCTIONS FOR TYPE A-100 ALUMINUM FIXED-ANGLE ROTOR

Maximum speed for routine operation	100 000 rpm
Centrifugal force at maximum speed	(207 kPa [30 psig] air pressure)
At r_{min} (9.5 mm)	106 000 times gravity
At r_{av} (12.1 mm)	135 000 times gravity
At r_{max} (14.7 mm)	165 000 times gravity
Number of tubes	6
Capacity per tube (cellulose nitrate)	175 µl
Total capacity	1050 µl
Nominal tube diameter	4.8 mm (3/16 inch)
Tube length	19.9 mm (25/32 inch)
Approximate acceleration time	30 seconds or less
Approximate deceleration time	3.5 minutes

Figure 3-4. Operational Values, A-100 Rotor

Air Pressure		Rotor Speed	$\omega^2 \times 10^{-7}$	Relative Centrifugal Force*			Clearing Factor k**
kPa	psig			R_{min} 9.5 mm	R_{av} 12.1 mm	R_{max} 14.7 mm	
55	8	50 000	2.74	27 000	34 000	41 000	44
76	11	60 000	3.95	38 000	49 000	59 000	31
103	15	70 000	5.37	52 000	66 000	81 000	23
131	19	80 000	7.02	68 000	87 000	105 000	17
159	23	90 000	8.88	86 000	110 000	133 000	14
207	30	100 000	10.97	106 000	135 000	165 000	11

*Number times gravity. RCF = $\frac{\omega^2 r}{9800}$ where ω is angular velocity in rad/sec, or 0.10472 X rpm, and radius is given in millimeters.

**See next page on k factors.

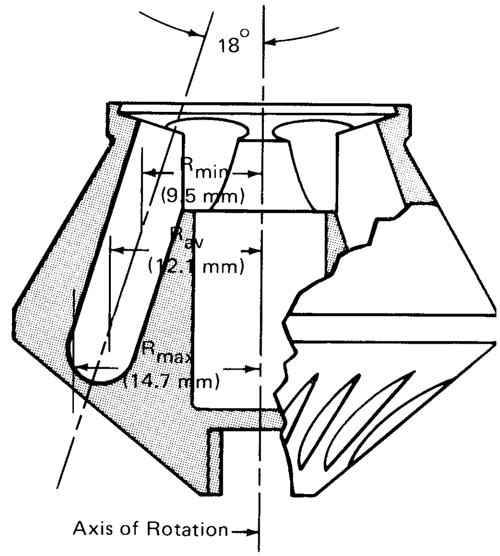


Figure 3-5. Rotor Cross-Section

Description

The A-100 has 6 rotor cavities for carrying 4.8 X 19.9 mm cellulose nitrate tubes, each with a capacity of 175 µl. The bottom of the rotor contains machined turbine flutes and a cavity lined with Teflon. The latter is engaged by the brake pin for deceleration.

Rotor material is anodized aluminum. Aluminum should not be used with strong acids or strong bases, or salts of heavy metals such as lead, silver, and mercury. Materials safe in aluminum include weak acids and their salts, weak bases, chlorides, organic chlorine compounds (free of heavy metals), dichromates, and nonelectrolytes (in the presence of air).

k Factor as an Index of Clearing Time

Clearing time is an estimate of the time required to clear a solution by centrifugation of material having a known sedimentation coefficient. The time is calculated by dividing a constant (k), which is different for each rotor type, by the sedimentation coefficient in Svedberg units (S).

$$t\ (\text{hrs}) = \frac{k}{S}$$

The clearing factor is calculated as follows:

$$k = \frac{\ln(r_{max}/r_{min})}{\omega^2} \times \frac{10^{13}}{3600}$$

where:

r_{max} = maximum radius from centrifugal axis
r_{min} = minimum radius from centrifugal axis
ω (angular velocity in rad/sec)
= 0.10472 X rpm

The value k/S represents the time required for a boundary of material to sediment from r_{min} to r_{max} in water at 20 °C, if no consideration is given to complicating factors of convection or variations of density or viscosity in the medium.

In actual practice, the Airfuge is often more efficient than indicated by its theoretical k factor. This is because all of the sedimenting material does not have to travel all the way to the bottom of the tube, only to the top of the pellet forming. A small distance at the bottom of the tube makes quite a difference in the calculated k factor. For example, for a pellet 0.01 mm deep on the bottom/side of the tube, the theoretical k factor would be 9.0, permitting a reduction of 20% in the time required for a separation.

Finding the k Factor at Less than Maximum Speed

If a rotor is run below its maximum speed, the k factor is higher and clearing time is increased. The k factor at a lower speed may be determined by multiplying the k factor shown in Figure 3-4 by the ratio of the squares of the two speeds.

For example, the k factor for a Type A-100 rotor at maximum speed is 11. The clearing time for 10S material in the rotor is 11/10, or 1.1 hours. The k factor for the same rotor at 50 000 rpm is $100\ 000)^2/(50\ 000)^2 \times 11$, or 44. Therefore the clearing time for the same material at 50 000 rpm would be 44/10, or 4.4 hours.

More details on using the k factor are given in Section 4, "Theory and Calculations."

Rotor Loading

Details on loading are given on page 3-1 under "Normal Run." In general, tubes must be balanced in weight within 10% (volume within 20 mg), balance being more critical with decreasing volumes. Two, three, four, or six tubes may be run if placed symmetrically in the rotor.

The small tubes need not be capped, but for aerodynamic reasons the rotor MUST be capped. Rotor caps are polyethylene discs which snap into place; a new cap is recommended for each run. Remove by pressing in the center and grasping the freed edge with fingers or tweezers. (See Figure 3-2.)

Rotor Care

The rotor must be cleaned regularly and rinsed immediately if corrosive substances have been run. Wash in a warm, dilute solution of mild detergent at nearly neutral pH, such as Beckman Solution 555™ rotor wash (Part No. 339555). Do not use strong laboratory cleaners.

Scrub rotor holes with a cotton-tipped swab. Rinse thoroughly and dry in an air stream or wipe with tissue. Store *without* a cap in place.

A SUGGESTED LIPOPROTEIN SEPARATION METHOD

This method for the Beckman Airfuge yields two lipoprotein fractions from human serum or plasma without density adjustment. The upper fraction is composed of chylomicrons and very low-density lipoproteins, and also non-lipid serum proteins.

Fill six centrifuge tubes with 175 μl of sample each, and run in the Airfuge for 2½ hours at the maximum speed available. At least 138 kPa (20 psig) air must be used; in most cases, the separations will be considerably cleaner if 207 kPa (30 psig) is used.

After the Lipoprotein Run

Remove the tubes from the rotor one at a time and transfer to a suitable cutting block as described earlier in this section. Cut with a clean razor blade at the 1/5 level (one-fifth of the volume in the upper part and 4/5 in the lower). Place each part of the tube in a small vial (fluid will remain in the cut portions of the tube with normal handling), and dilute each fraction back to 175 μl with background solution; that is, add 140 μl to the upper part and 35 μl to the lower part.

Each component in each fraction will now have the same concentration as in the original diluted sample. (Any cutting block may be calibrated for this by weighing the cut parts of the tubes filled and empty.)

Determine the relative amounts of lipoprotein components in each fraction by appropriate chemical or electrophoretic methods. Agarose gel electrophoresis should show only one lipoprotein band in the upper fraction (the pre-β component) and two in the lower fraction (the β and α components).

Run Steps in Brief

Loading Tubes and Rotor

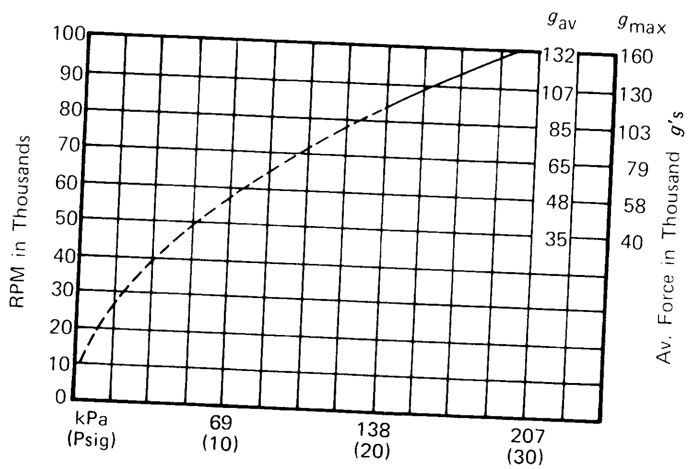
1. Use suitable pipette to fill with no more than 175 µl fluid. Allow no more than 10% difference in tube weight.
2. Run 2, 3, 4, or 6 tubes, positioned symmetrically for balance.
3. Install the new rotor cap firmly in its groove.

Centrifugation

(Start with clean brake, brake hole, stator, and pad; GENTLY test brake action.)

1. Place rotor on stator pad.
2. Set timer (past 30 min. and back for short runs, to HOLD for runs over 5 hrs).
3. Close door, pushing down and turning speed control knob clockwise.
4. Gradually bring air pressure to level on gauge for chosen run speed (below).

Cut out, moisten, and place on page 3-9.



Rotor Speed Vs. Air Pressure

(For running at pressures under 138 kPa (20 psig), contact the factory.)

Ending the Run

1. Let timer stop run—if on HOLD, turn timer to zero for automatic stop.
2. After rotor stops, turn speed knob counterclockwise to stop air and open door.
3. Push in center of rotor cap to free edge; remove it and tubes carefully with tweezers.

Points to Remember

1. **Never run without a rotor cap;** a new cap is recommended for each run.
2. Balance the rotor carefully. An unbalanced rotor will wobble or rattle when driving air is applied—abort such a run by turning the timer off.
3. Do not turn off air pressure or raise the door when the rotor is still moving.
4. Keep the rotor clean to avoid corrosion.
5. Keep oil mist out of the system by changing the filter element when it becomes red.
6. Keep the brake pin and its hole clean.
7. Keep the threads on the speed control knob shaft well lubricated.

Section 4
Theory and Calculations

GENERAL CENTRIFUGAL THEORY

The Centrifugal Force Field

The force exerted on a unit mass rotating at a steady angular velocity of ω radians per second at a distance of r centimeters about a fixed point is given by

$$\text{force field} = \omega^2 \, r \text{ dynes/gram}$$

The magnitude of the centrifugal force field is frequently expressed in terms of the earth's gravitational force field and is referred to as the "relative centrifugal force" (RCF), the "g field," or as the number times g.

$$\text{RCF} = \frac{\omega^2 r}{9800}$$

The angular velocity ω must commonly be computed from the rotational speed in rpm:

$$\omega = \frac{\pi}{30} \times \text{rpm}$$

A formula for the relative centrifugal force in terms of rpm is as follows:

$$\text{RCF} = 1.12 \left(\frac{\text{rpm}}{1000}\right)^2 r,$$

where r is in millimeters.

The Sedimentation Coefficient

The sedimentation coefficient is defined as the velocity of sedimentation per unit force field:

$$s = \frac{dr/dt}{\omega^2 r} \qquad (1)$$

The units of s are seconds. Biopolymers in general have sedimentation coefficients of 10^{-13} seconds or greater. For convenience in expressing sedimentation coefficients, the *Svedberg* unit, named in honor of The Svedberg, the originator of sedimentation analysis, has been defined as equal to 10^{-13} seconds. The Svedberg unit has the symbol S. Thus, a sedimenting species observed to have a sedimentation coefficient of 10×10^{-13} seconds is also referred to as having a sedimentation coefficient of 10S, or is called a "10S material."

Particles denser than the surrounding medium will have positive sedimentation coefficients while those less dense—that is, those that float in the medium—will have negative coefficients. A particle whose density is equal to the density of the medium will neither float nor sediment, has a sedimentation coefficient of zero, and is said to be "neutrally buoyant."

Clearing Time

An estimate of the time required to clear a solution of material of a known sedimentation coefficient can be obtained by assuming that a boundary moves from the smallest to the largest radial position in the tube, and that the rate of boundary motion is unaffected by the shape and orientation of the container. The properties of the medium are assumed to be the same throughout the container. The required expression is obtained by the integration of equation (1):

$$t = \frac{\ln(r_{max}/r_{min})}{s\omega^2} \qquad (2)$$

The clearing time for a given rotor is given at its maximum rate of rotation as follows:

$$t\ (\text{hr}) = \frac{k}{S} \qquad (3)$$

where S is the sedimentation coefficient in Svedberg units of the sedimenting material and the constant k has been computed as follows:

$$k = \frac{\ln(r_{max}/r_{min})}{\omega^2} \times \frac{10^{-13}}{3600} \qquad (4)$$

When the clearing time at a lower rate of rotation is desired, the factor k can be increased by the ratio of the square of the rates of rotation. (Examples are given in the rotor specifications pages in Sec. 3.)

The actual clearing time is likely to be somewhat less than the above estimate because the boundary can move faster in an angle tube under certain regimes of convection. The conditions that give rise to faster clearing in angle tubes appear to be that a fairly sharp boundary should form (i.e., that the diffusional spreading of the boundary be small), and that concentrations be substantial—for example, 1% or more.

It will be recognized, of course, that if no boundary forms during sedimentation, the clearing time cannot be reliably estimated by equations (2) or (3).

Additional information on centrifugation can be found in the following biennially published material from Spinco Division, Beckman Instruments, Inc., Palo Alto, California:

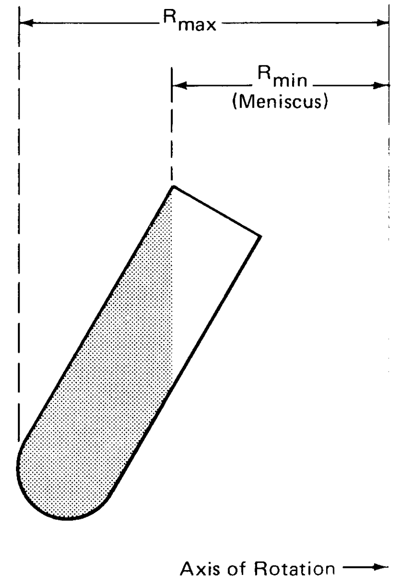


Figure 4-1. Tube Under Centrifugat...

Section 5
Troubleshooting and Maintenance

The following special procedures, routine maintenance instructions, and troubleshooting tips should keep your Airfuge unit in good repair. If the answer to a particular problem cannot be found here, U. S. and Canadian customers should telephone 415-326-1970 and ask for "Airfuge Technical Support." International customers should call their nearest Beckman representative.

NOTE: Troubleshooting tips are found on the last two pages.

SPECIAL PROCEDURES

Cleaning the Stator Assembly

WARNING

Disconnect electric cord and air supply for the following procedure.

1. Raise the door and lift off the stator pad. Remove the brake pin (lift straight out with tweezers).
2. Remove the stator as in Figures 5-1 and 5-2, using the two special prying tools provided.
3. If necessary, remove the three O-rings with a pointed wooden or plastic tool (to avoid scratches), including the one down the hole. Figure 5-3 shows O-ring locations. In most cases, they may be left in place.
4. Clean the brake pin and all passages and jets by immersing and agitating in denatured alcohol.

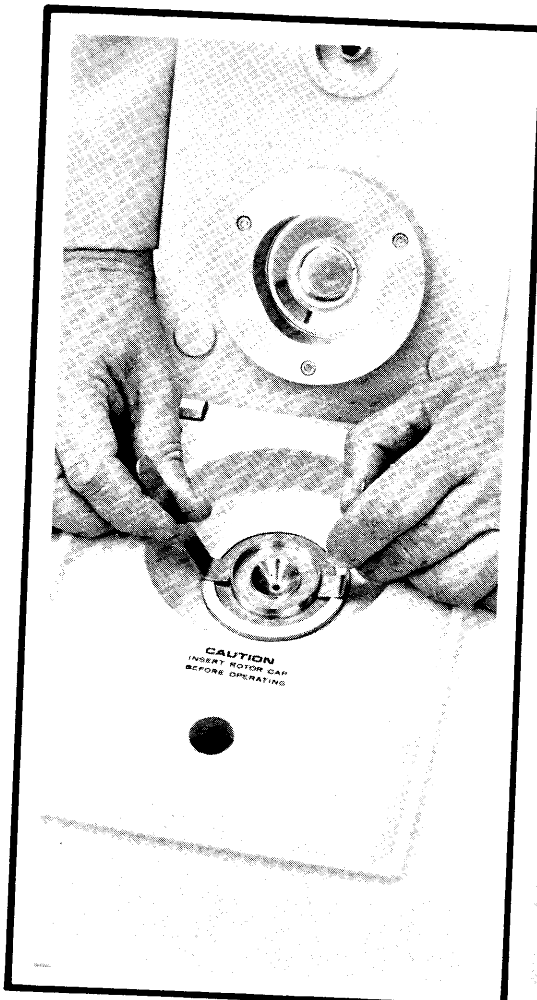


Figure 5-1. Prying Up Stator. Take out stator pad and brake; remove screws. Slip short ends of prying tools into shallow slots on outside of stator rim. Pry against base-plate until stator O-ring is free. Don't force it!

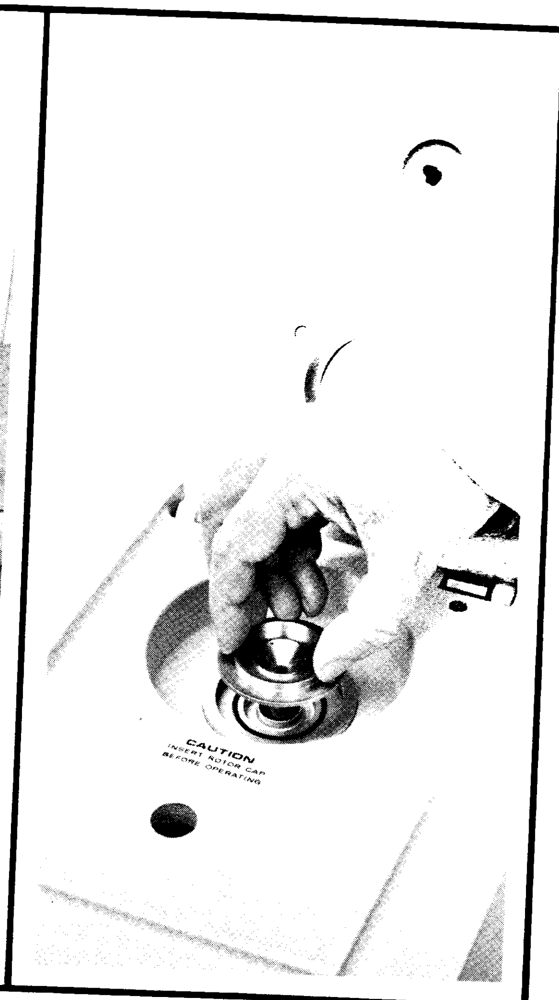


Figure 5-2. Lifting Out Stator. Lift with fingers, keeping stator level to prevent binding.

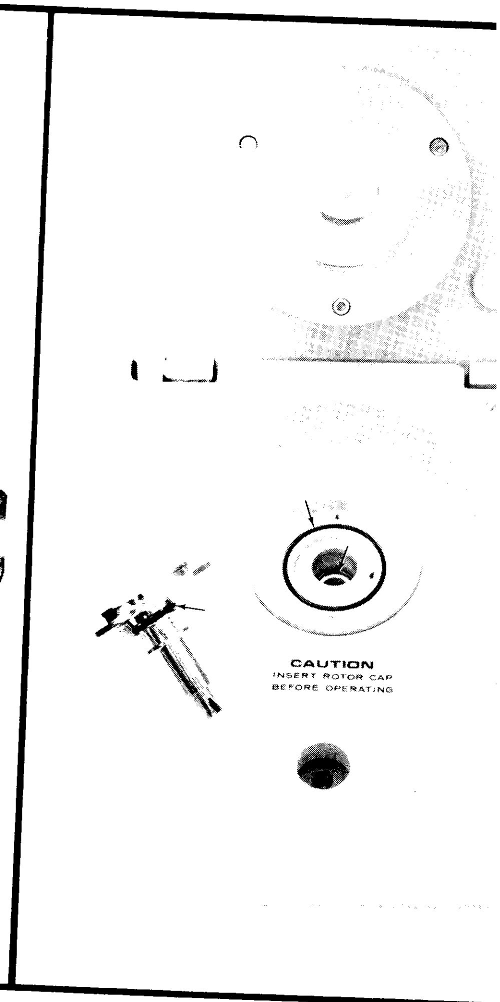


Figure 5-3. Stator O-Ring Positions. A third O-ring may be seen in the hole below.

5. If they were removed, lightly lubricate O-rings with vacuum grease and return them to their positions.
6. Press the stator back into place by hand. Reinstall the two screws securely.
7. Reinstall the brake pin, holding it securely so it won't drop down the hole, and aligning it as if the supporting wire ran between the stator screws.
8. If the cone on the pin is down flat on the stator surface, increase brake tension two full turns counterclockwise. The pin should now be up at least 3 mm off the stator (minus the pad). Light finger pressure should be able to move it freely up and down. If it doesn't move, the brake spring tension may not be adjusted (see CAUTION note after Step 9).

> **NOTE:** The three-part step below involves realigning the brake spring, and may be required when the stator is returned to the instrument.
>
> a. Lock the chamber door, turn the Airfuge on its side, and remove the bottom plate. Set plate aside.
>
> b. Note that the wire end of the spring (shown in Figures 5-4 and 5-9) runs on an imaginary line between stator screws. Reinstall the stator, aligning it so the wire runs through the slot on the bottom end as you insert it. Adjust the wire with a finger if necessary.
>
> **NOTE:** Wire position CANNOT be adjusted AFTER the stator is put in.
>
> c. Replace the bottom plate and turn the Airfuge rightside up.

9. Reconnect the air supply and level the Airfuge.

CAUTION

To be operable after the above steps, the Airfuge usually will require some finer adjustments of brake spring tension and levitation air flow. These procedures follow.

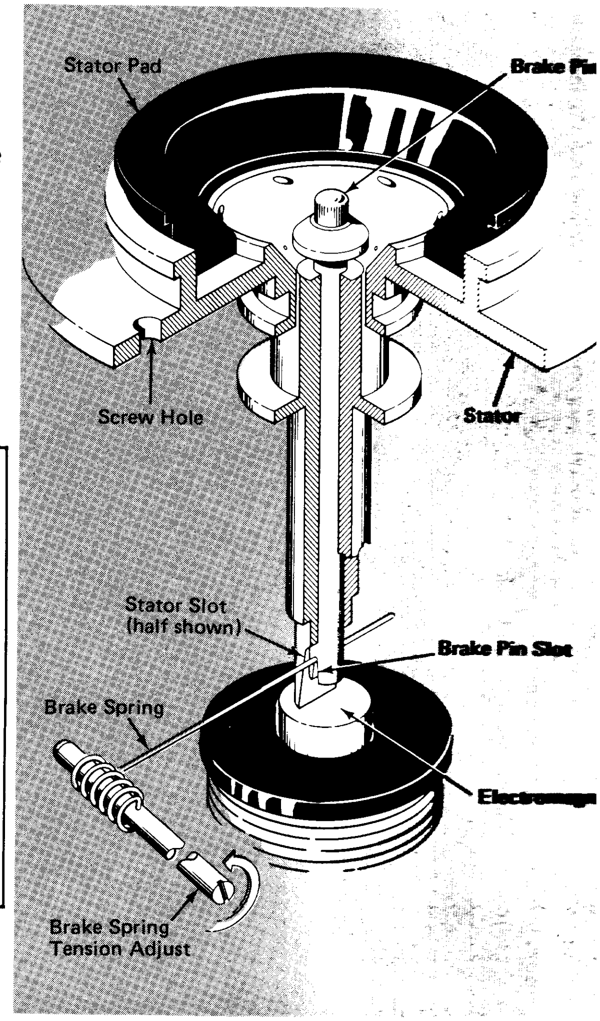


Figure 5-4. Detail of Brake Spring. The ... wire end must be visible across the ... bottom of the stator and pass through the ... on the bottom of both brake pin and stator.

WARNING

Make sure the electric cord is unplugged and that the speed control knob is turned all the way off (or the door is open) for the next step.

Adjusting the Brake (Brake may be adjusted without adjusting levitation air)

NOTE: The two adjustments which follow are done by reaching to the back of the Airfuge. If the instrument is turned to make the job easier, it must be LEVEL for the adjustments and RE-LEVELED after being turned back to operating position.

1. Remove the brake pin from the stator with tweezers and clean it with a pad moistened in denatured alcohol (if this has not already been done).
2. Reinstall the brake pin as in Figure 5-6 (a). Place the pin so that the slot on the bottom end is aligned on the spring wire. Follow instructions in Figures 5-6 and 5-7.
3. At this point, the brake pin cone should be up at its normal position, 3 mm (1/8") above the stator, with the pad removed. Now use a

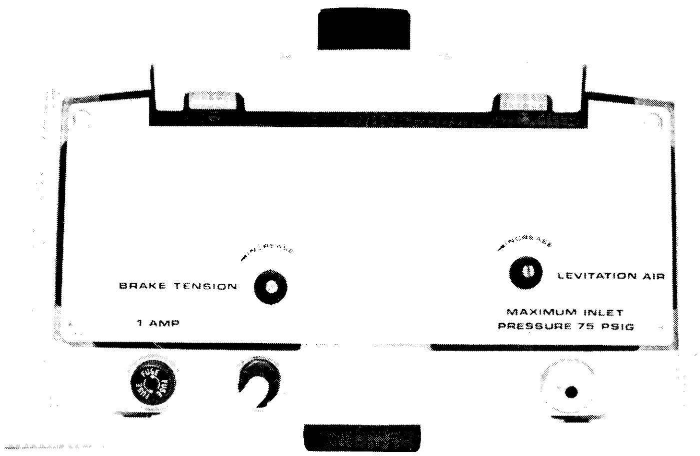
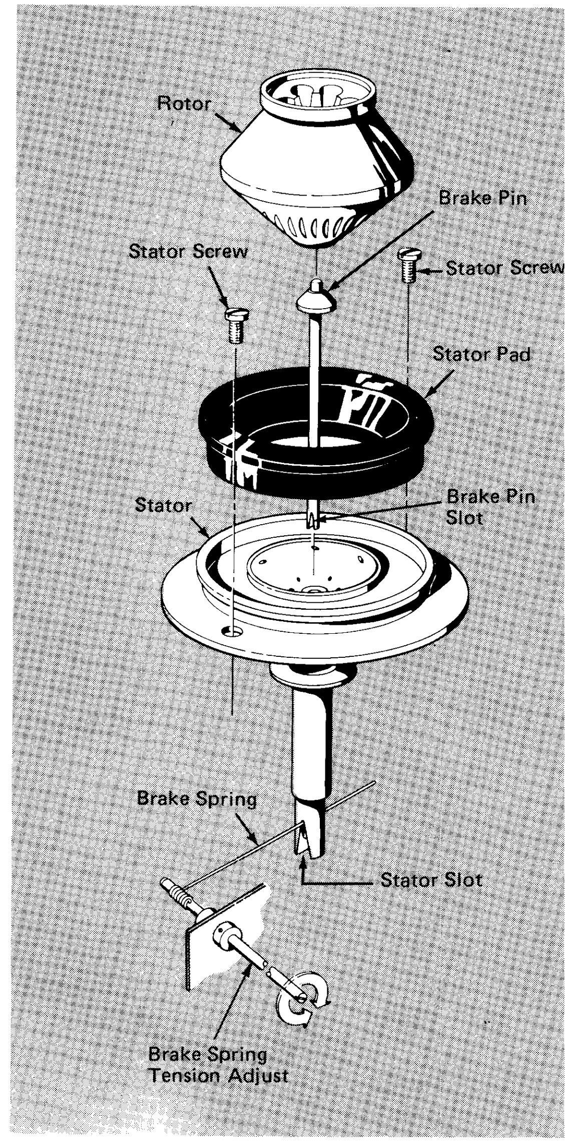


Figure 5-5. Rear Adjustment Controls



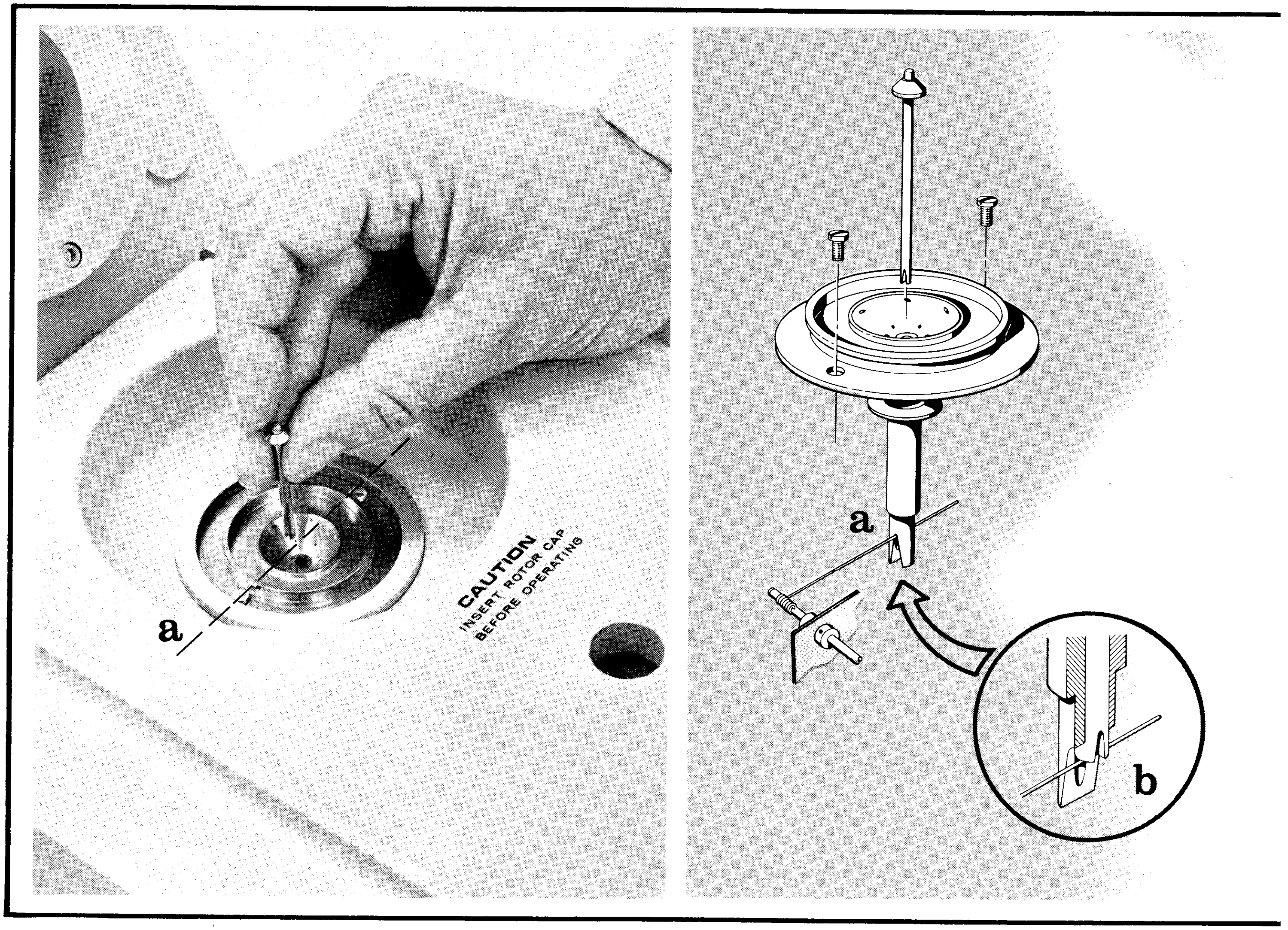


Figure 5-6. Aligning Brake Pin in Stator. The slot on the end of the pin (a) fits over the wire (not visible below the stator). The broken line shows wire direction (aligned with screws). When the tips of the slot stand on the wire (b) instead of straddling it, the pin will be too high, as in Figure 5-7 (b) and (c).

screwdriver to release brake spring tension: Turn *clockwise* as seen from the rear of the Airfuge, until the brake cone drops to the stator surface. (The tension control is shown in Figure 5-5.)

4. Starting with the brake cone down against the stator, increase brake spring tension (turn *counterclockwise* as seen from the instrument rear) until the pin comes back up to the normal 3 mm (1/8") position. Turn until light finger pressure (about the weight of a penny) moves the pin freely up and down. If this motion is sticky, the brake and its hole need further cleaning. If the pin moves easily and is at the right height, your brake is now in operating condition.

WARNING

The power cord should be unplugged for the following step.

Adjusting Levitation Air (Also includes brake adjustment, required when levitation air is adjusted)

1. With a screwdriver, release brake spring tension control shown in Figure 5-5. Turn clockwise (as seen from the rear of the Airfuge) until the brake cone drops to the stator surface.
2. Clean a stator pad (two are packed with the instrument) with denatured alcohol and place it on the stator.
3. Install a rotor cap. Rub the cap into place until it snaps firmly into the groove on the rotor rim.
4. Place the rotor on the stator pad.
5. Make sure the air supply to the Airfuge is connected and turned on.
6. Close the instrument door, then hold down and turn the air regulator knob (speed control) clockwise until the gauge indicates 207 kPa gauge (30 psig).
7. If the rotor wobbles, decrease levitation air: With a screwdriver, turn the control clockwise (as viewed from the instrument rear) until wobbling stops. The control is shown in Figure 5-5.
8. Slowly increase levitation air (turn *counterclockwise* about ¼ turn) until the rotor wobbles *slightly.* Note the angular position of the slot in the adjustment control when this occurs, as it will be used for the next step.

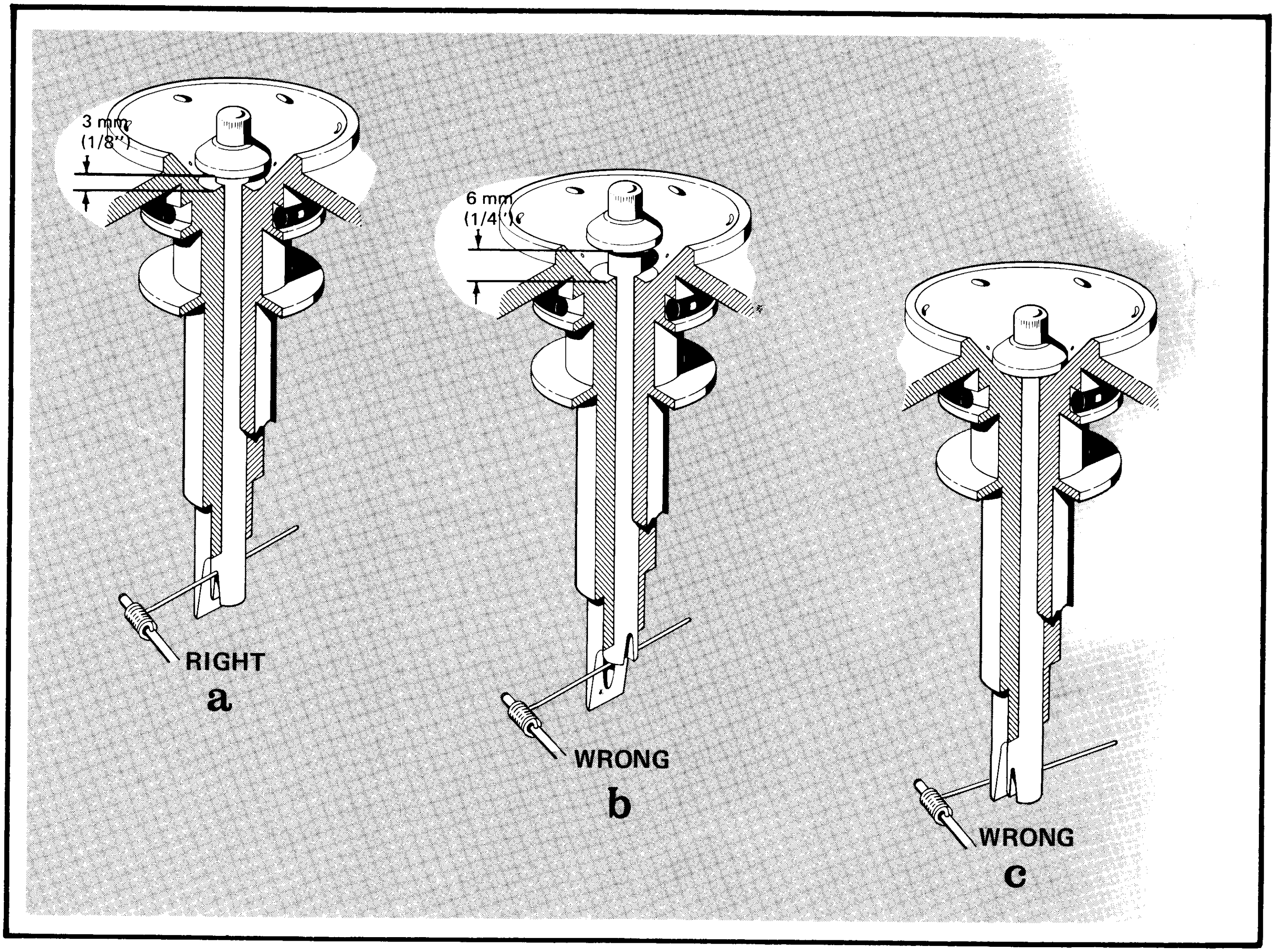


Figure 5-7. Pin Height, Right and Wrong. The bottom of the cone on the pin should be about 3 mm (1/8") above the stator surface when the pin is positioned correctly (a). If the pin rides on top of the wire instead of straddling it (b), the cone will be 6 mm (¼") above the stator. For pins that repeatedly fall flat on the surface (c), see Troubleshooting on the last two pages.

9. Now turn the levitation air control ¼ turn *further counter-clockwise* from the position reached in Step 8. Rotor wobble will increase and the rotor may be turning in either direction.

10. Starting with the brake cone still down against the stator, increase brake spring tension (turn *counterclockwise* as seen from the instrument rear) until the pin comes back up to the normal 3 mm (1/8") position. Turn until light finger pressure (about the weight of a penny) moves the pin freely up and down.

11. Return the stator pad to the instrument, then plug in the power cord. Your Airfuge is now adjusted for operation.

NOTE: Re-level if the instrument is moved.

Adjusting the Time Delay

WARNING
Disconnect electric cord and air supply for the following procedure.

The time delay period before braking begins may be lengthened or shortened for special circumstances. A new setting on the time delay control knob must be chosen empirically, however, as the numbers on the dial are arbitrary and represent no particular units. For instance, for the optimum 2-minute delay set at the factory, the pointer may be anywhere from "3¼" to "4."

To change this, lock the chamber door, turn the Airfuge on its side, and note the access holes in the bottom plate. Find the black knob and its numbered dial shown in Figure 5-8. Reset counterclockwise to shorten the coasting time; reset clockwise to lengthen this period. (The higher the setting, the longer but smoother the stop—better for difficult separations. At lower settings, the stop is faster but less smooth—better for pelleting.)

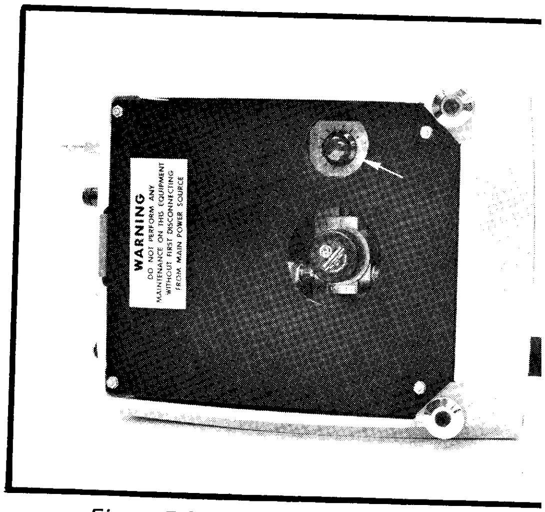


Figure 5-8. Time Delay Control Knob

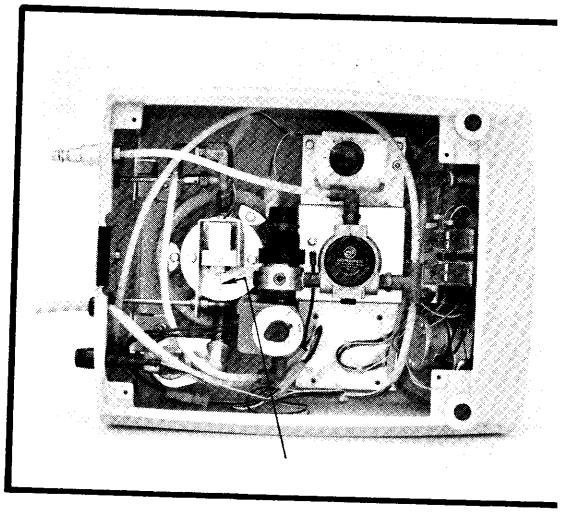


Figure 5-9. Location of Brake Spring Wire

ROUTINE MAINTENANCE

- To prevent accumulation of oil on interior surfaces, occasionally wipe off stator surfaces, stator pad, door and window, interior and brake pin. But better yet, prevent such accumulation by regular replacement of the filter element in the air line between compressor and Airfuge. Replace the filter as soon as it turns red—*before* clogging allows oil into the system. (Figures 2-1 and 2-2 tell you where to check it.)
- Keep the projecting threads on the speed control knob (pressure regulator) lubricated with Spinkote.
- The instrument's 1-amp fuse is next to the power cable at the back. To replace, *first disconnect power.* Twist the fuse-holder counterclockwise and pull out. Pull out the old fuse and insert a new one of the same amperage rating. Replace fuse-holder and twist clockwise into place.
- The polyurethane surface of the instrument can be kept clean by wiping with isopropanol, soap and water, or detergent solutions. If acetone is used, do not allow it to stand on the surface longer than 5 minutes or the paint can be softened.
- Use NO acetone, acids, salts or other caustics on the window; clean as above. To prevent scratches, use a good quality auto paste wax.

REPLACEMENT PARTS LIST

Cellulose nitrate tubes (100, 4.8 X 19.9 mm)	339647
Stator pad	339610
Prying tool (2 required)	339641
Polyethylene rotor caps (20)	339643
Brake pin	339611
A-100 six-place rotor, 18° angle	339640
Spinkote lubricant	306812
Vacuum grease	335148

(Turn page for Troubleshooting)

WARRANTY INFORMATION

The AIRFUGE™ instrument, components, a[illegible] the A-100 rotor are warranted against defects [illegible] materials and workmanship for one year after s[illegible]ment of the instrument. Spilled caustic materia[illegible] corrode the rotor and should be washed from [illegible] rotor after each run. For long rotor life, follow [illegible] care instructions in Section 3 of this manual.

PROBLEM	POSSIBLE CAUSE	SOLUTION
1) Brake pin keeps falling down on stator surface	1) The pin missed the brake spring completely; the wire end may have loosened in transit (or with use) and no longer runs under the hole	1) Look down the hole with a flashlight. If the wire is visible, realign it, following special steps a, b, and c under "Cleaning the Stator Assembly" in Section 5. If the wire is bent, damaged or won't run through the stator slot, call the numbers given on page 5-1.
2) The pin is too low (less than 3 mm off stator)	2) Not enough tension in brake spring	2) See "Adjusting the Brake" in Section 5.
3) Rotor won't spin	3) Air supply or power disconnected; fuse out; filter saturated; broken or missing stator pad	3) Check air and power connections; replace fuse; replace stator pad
4) Rotor runs erratically	4) Broken, damaged or missing stator pad; missing or damaged rotor cap; unbalanced load; dirty or clogged drive air jets	4) Replace stator pad (important for stability); replace rotor cap; review loading tips at beginning of Sec. 3; follow steps under "Cleaning the Stator Assembly" in Section 5.
5) Sample spillage	5) Overfill or tube collapse	5) Inspect rotor and clean well, especially if caustics were run. (See "Rotor Care" on rotor specifications pages, Section 3); use new tubes for subsequent runs.
6) Rotor unstable at end of run	6) Running without rotor cap or any of the possible causes listed under 4) above	6) See solution 4) above.
7) Poor fraction separations	7) a. Rotor stopping too fast BEFORE run light goes out (Not enough levitation air; broken stator pad; dirty stator; oil mist in system)	7) a. Check levitation air adjustment; check stator pad and replace if damaged; clean levitation jets if clogged; change filter element if clogged; clean system of oil (p. 5-1).
	b. Rotor stopping too fast AFTER run light goes out (Brake malfunction: rotor continues to wobble at end of run because brake pin has not come up to stabilize it. Brake may also be stuck in UP position or just sluggish in action)	b. Readjust brake and levitation air (instructions are in Section 5); clean the stator assembly (also in Section 5). If these steps do not solve the problem, telephone Palo Alto or your nearest Beckman office as directed.
	c. Delay timer set too long	c. Readjust delay timer (p. 5-8).
	d. Sucrose needed for sample stability	d. See NOTE, page 3-1.

TROUBLESHOOTING, continued

PROBLEM	POSSIBLE CAUSE	SOLUTION
8) Rotor hits stator pad after brake actuation	8) a. Brake tension low b. Burr on rotor bushing (Teflon liner) c. Bushing damaged after crash d. Stator pad damaged or dirty	8) a. Adjust brake (Section 5) b. Remove burr c. Return for repair d. Replace pad
9) Rotor won't accelerate	9) a. Stator locking screws loose b. No stator pad c. O-rings missing after cleaning	9) a. Tighten screws b. Replace pad c. Check and replace O-rings
10) Brake pin not actuating	10) a. Tension too low b. Shaft oily c. Pin damaged	10) a. Adjust b. Clean and adjust c. Replace
11) Oil visibly collecting on surfaces in the instrument	11) Clogged filter in air supply	11) Change filter element and clean oil from system. If very severe, return the instrument to Palo Alto for a complete flushing.
12) Teflon bushing on bottom of rotor is worn	12) With regular use of brake this will occur once or twice a year	12) Return rotor to Palo Alto for refitting.
13) Rotor fails to stop	13) Levitation air too high	13) Adjust levitation air (Section 5)
14) Air pressure regulator hums and pressure gauge needle vibrates	14) Air pressure was brought up before timer was set	14) Use proper starting procedure, pages 3-2 and 3-3.

PROBLEM	POSSIBLE CAUSE	SOLUTION
8) Rotor hits stator pad after brake actuation	8) a. Brake tension low b. Burr on rotor bushing (Teflon liner) c. Bushing damaged after crash d. Stator pad damaged or dirty	8) a. Adjust brake (Section 5) b. Remove burr c. Return for repair d. Replace pad
9) Rotor won't accelerate	9) a. Stator locking screws loose b. No stator pad c. O-rings missing after cleaning	9) a. Tighten screws b. Replace pad c. Check and replace O-rings
10) Brake pin not actuating	10) a. Tension too low b. Shaft oily c. Pin damaged	10) a. Adjust b. Clean and adjust c. Replace
11) Oil visibly collecting on surfaces in the instrument	11) Clogged filter in air supply	11) Change filter element and clean oil from system. If very severe, return the instrument to Palo Alto for a complete flushing.
12) Teflon bushing on bottom of rotor is worn	12) With regular use of brake this will occur once or twice a year	12) Return rotor to Palo Alto for refitting.
13) Rotor fails to stop	13) Levitation air too high	13) Adjust levitation air (Section 5)
14) Air pressure regulator hums and pressure gauge needle vibrates	14) Air pressure was brought up before timer was set	14) Use proper starting procedure, pages 3-2 and 3-3.