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PREPARATIVE ULTRACENTRIFUGATION AND ANALYTIC ULTRACENTRIFUGATION OF PLASMA LIPOPROTEINS

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PREPARATIVE ULTRACENTRIFUGATION AND ANALYTIC ULTRACENTRIFUGATION OF PLASMA LIPOPROTEINS

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INTRODUCTION

There are several chapters[1-6] and a book[7] dealing with both preparative and analytical ultracentrifugation (AnUC) of plasma lipoproteins. However, what we would like to present are the procedures as currently done here at Donner Laboratory. They have been modified and improved since the earliest lipoprotein flotation was demonstrated in 1949, and this will present a combined, practical up-date of our chapter[4] on AnUC in *Blood Lipids and Lipoproteins* (1972) and our chapter[5] on preparative procedures in the AOCS book, *Analysis of Lipids and Lipoproteins* (1975). This information is intended as a practical laboratory guide to anyone who wishes to do preparative and AnUC of plasma lipoproteins.

Fasting blood is normally drawn in 10-20 ml evacuated containers that contain enough concentrated EDTA solution (1/100 dilution; w/v 15% K_3 EDTA; 1.5 mg/ml blood) to prevent clotting. These blood tubes should be placed in crushed ice and then centrifuged to prepare plasma at 1,500 rpm for 30 min in a 4 C refrigerated centrifuge. Plasma samples are then stored at 4 C prior to lipoprotein fractionation. Some plasma samples may require special precautions to prevent bacterial contamination. If so, this may be accomplished by adding an appropriate amount of 13% E-amino-n-caproic acid (1/100 plasma dilution) and a 1/100 plasma dilution of 1% Garamyacin. Both the above procedures will dilute the plasma approximately 2-3%, but this dilution can be calculated and corrected, if desired. Plasma can conveniently be stored in either 9-ml or 15-ml glass vials with air-tight caps that employ inert teflon gaskets. Alternatively, clean, inert, air-tight plastic containers may be used.

PREPARATIVE ULTRACENTRIFUGATION

Since lipoproteins vary in density from approximately 0.92-1.16 g/ml, they are conveniently separated by flotation in salt solutions of varying density. Figure 1 illustrates the size and density of the major lipoprotein classes, namely, chylomicrons (Chylos), very low density lipoproteins (VLDL, $0.92 < d < 1.0063$ g/ml), intermediate density lipoproteins (IDL, $1.0063 < d < 1.019$ g/ml), low density lipoproteins

(LDL, 1.019<d<1.063 g/ml), and high density lipoproteins (HDL, 1.063<d<1.200 g/ml). Normally we fractionate the total low-density spectrum, d<1.0651 g/ml, and the total lipoprotein spectrum, d<1.216 g/ml. These procedures require a minimum of 2.5-6.5 ml of plasma, preferably a larger volume, especially if other analyses are also to be done such as chemical and apolipoprotein analyses, agarose or gradient gel electrophoresis. Density manipulation can be accomplished either by adding solid salt or by mixing precise volumes of serum with appropriate volumes of high-density salt solutions. If highly concentrated fractions of lipoproteins are needed, the solid salt procedure is recommended. For routine preparation of low and high density lipoprotein fractions, we use a Beckman 40.3 or 50.3 rotor (18 tubes of 6-ml capacity). This involves mixing 2-ml plasma with 4-ml NaCl or NaBr solutions to achieve a background salt density of ρ_{26} = 1.0651 g/ml (ρ_{20} = 1.0630) and ρ_{26} = 1.2128 g/ml (ρ_{20} = 1.2160 g/ml) for the low and high density runs, respectively.

Preparation of Salt Solutions for Lipoprotein Fractionation

We recommend the use of NaCl or NaBr (Mallinckrodt, reagent grade) which has been calcinated at 550 C overnight in a muffle furnace to remove dust and water. Storage thereafter should be in teflon-sealed jars in a desiccator. Appropriate distilled H_2O is conveniently prepared using a Millipore filter system and then stored

in Nalgene 1-gallon containers (Cole-Parmer Inst. Co., Chicago, IL). It is convenient to prepare first a large NaCl stock solution of the small molecule background density of plasma, namely ρ_{20} = 1.0063 g/ml. Since laboratory manipulations occur over a range of 20-26 C and both refractometry and AnUC are recommended at 26 C, our two salt tables contain values at both 20 and 26 C. Table 1 (NaCl) and Table 2 (NaBr) contain ρ_{20},ρ_{26}, η_{20}, η_{26}, g salt/l at 20 C, g H_2O/l at 20 C, g salt/g H_2O, molality, molarity, wt% salt, n_D^{26} and ΔS (Precision Abbe refractometric scale increment above a distilled H_2O reference). An expanded version of this table is recommended if precise density manipulations are required. For this a computer can interpolate quadratically three points at a time with convenient incrementation of ρ_{20} values to one part in the fourth decimal place.

Our two tables for NaCl and NaBr represent a merging of the International Critical Tables [8] salt data with our laboratory-determined pycnometry and precision refractometric data as well as more recent viscosity data[9]. Other data on NaCl, NaBr, and other salt solutions are available in the *CRC Handbook of Chemistry and Physics*, 70th edition[9], although n_D data are given only to the 4th place.

In all density manipulations, plasma may be considered to consist of 6% macromolecules by volume and 94% NaCl background solution (ρ_{20} = 1.0063 g/ml) by volume. In all solutions we recommend incorporating 100 mg/l of EDTA. Thus, the main solutions as used for preparative and AnUC lipoprotein work are as follows:

1. Plasma small molecule background: 0.196 m NaCl, ρ_{20} = 1.0063 g/ml (n_D^{26} = 1.33435, Δn^{26} = 0.00196, ΔS = 0.355)
   34.32 g NaCl plus 300 mg K_3 EDTA plus 3000 ml H_2O
2. Total low density lipoprotein (TLDL) plasma solution: 2.505 m NaCl, ρ_{20} = 1.0915 g/ml (n_D^{26} = 1.35451, Δn^{26} = 0.02211, ΔS = 4.135)
   33.69 g NaCl plus 250-ml Solution 1
3. Uncentrifuged background (TLDL) for the above run: 1.744 m NaCl, ρ_{20} = 1.0651 g/ml (n_D^{26} = 1.34834, Δn^{26} = 0.01594, ΔS = 2.945)
   22.46 g NaCl plus 250.0 ml solution 1
4. Centrifuged background (40,000 rpm for 18 hr, 18 C) of the above corresponding to the top milliliter, i.e., the background used for AnUC of the D run: ρ_{26} 1.0611 g/ml (ρ_{20} = 1.0632 g/ml), 1.690 m NaCl (n_D = 1.34790, Δn^{26} = 0.01550, ΔS = 2.86)
   100-ml Solution 3 plus 3.04-ml H_2O

5. Total plasma lipoprotein solution: 0.196 m NaCl, 4.507 m NaBr, ρ_{20} = 1.3104 g/ml, ρ_{26} = 1.3066 g/ml (n_D^{26} = 1.38596, Δn^{26} = 0.05356, ΔS = 10.45)

   115.83-g NaBr plus 250-ml Solution 1

6. Uncentrifuged background for the total high density (G) run: 0.196 m NaCl, 3.065 m NaBr, ρ_{20} 1.2160 g/ml, ρ_{26} = 1.2128 g/ml (n_D^{26} = 1.37023, Δn^{26} = 0.03873, ΔS = 7.20)

   75.97 g NaBr plus 250-ml Solution 1

7. Centrifuged background (40,000 rpm for 24 hr, 18 C) corresponding to the top milliliter, i.e., the baseline used for AnUC of the G run: 0.190 m NaCl, 2.866 m NaBr, ρ_{20} = 1.2032 g/ml, ρ_{26} = 1.2000 g/ml (n_D^{26} = 1.36809, Δn^{26} = 0.03569, ΔS = 6.77)

   100-ml Solution 6 plus 6.40 ml H_2O

These salt solutions should be monitored after preparation by refractometry n_D^{26} or by an appropriate fourth-place calculating digital density meter (Paar/Mettler, Model DMA46). It is convenient to set the thermoelectric temperature control system to either 20 C or 26 C, and this should be controlled to ±0.02 C. Since some errors are inherent in salt solution preparation (volumetric, weight, some H_2O, inert inclusions in the salt, etc.), adjustment may be needed to achieve the appropriate final density, r_f, accurate to the fourth place.

If the density is too high for solution 1, then appropriate dilution with distilled H_2O is needed. If any of the other solutions are high, appropriate dilution with the correct solution 1 is needed. Conversely, if any of the solutions are too low in density, addition of more NaCl (solutions 1-4) or NaBr (solutions 5-7) is required. Examples are as follows:

1. Example for solution 1 where a higher density is obtained, i.e.,

   $\rho_H = 1.0066$ g/ml or $\Delta\rho_H = 1.0066 - 0.9982\ (H_2O) = 0.0084$ g/ml

   $\Delta\rho_1 = 1.0063 - 0.9982\ (H_2O) = 0.0081$ g/ml

   The total corrected H_2O volume is $\frac{0.0084}{0.0081}\ (3{,}000\ \text{ml}) = 3{,}111$ ml

   Therefore, add 111-ml distilled H_2O, mix thoroughly and reread density (or Δn).

2. Example for solution 2 where $\rho_L = 1.0910$ g/ml

   $\Delta\rho_L = 1.0910 - 0.9982 = 0.0928$ g/ml (water in salt?)

   $\Delta\rho_2 = 1.0915 - 0.9982 = 0.0933$ g/ml

   a corrected salt mass = $\frac{0.0933}{0.0928} \times 33.69 = 33.87$ g NaCl

   Therefore, add 0.182 g NaCl and reread density (or Δn).

After final preparation of the above solutions we normally recommend filtering through a coarse glass sintered filter and then storing in teflon-gasketed bottles. For all solution except solution 1, pint whisky bottles with 28-mm teflon-gasketed caps are convenient,

compact, and inexpensive containers. Before putting the cap on, be sure that there are no chipped surfaces on the bottle top as evaporation, especially from concentrated solutions, can be an insidious problem if undetected.

Standard TLD lipoprotein and high density fractionation normally utilize 2-ml plasma plus 4-ml of solutions 2 and 5, respectively. Under high centrifugal force and prolonged centrifugation, redistribution of the salts occurs, yielding a salt density gradient in the preparative tubes. This occurs if plasma is centrifuged 18 hr, 40,000 rpm, at a background density (ρ_{20} = 1.0063 g/ml) to remove VLDL, for 18 hr to prepare the TLD lipoprotein fraction, initially at ρ_{20} = 1.0651 g/ml and for the 24-hr run to prepare a total lipoprotein fraction, including the total HDL spectra (ρ_{20} = 1.2160 g/ml). The extent of this redistribution is shown in Figure 2 and the values for the initial and final top milliliter background density for both the D and G runs are given in the properties of solutions 3 and 4 (D run) and 6 and 7 (G run), respectively. Solutions 4 and 7 are used as appropriate background solutions in the D and G AnUC runs. However, because preparation of the individual fractions involves errors in the volumetric pipettes as well as differences in the protein content of each plasma, individual background densities will vary somewhat from the mean values of solution 4 and 7. The details of preparing low and high density lipoprotein fractions, as well as

calculating individual top milliliter background densities will be given next.

Preparation of Standard Low and High Density Lipoprotein Fractions

After preparing plasma samples that will be processed for AnUC, they should be logged in a standard laboratory book that is lined and has numbered pages. This book should have a nonredundant numbering system that, where appropriate, should have entered the sample number, the subject or patient's name, the date the blood was drawn, and the date of logging. Other useful information such as diagnosis or study involved should also be included on the left open page. It is also helpful to log on the right side all the analyses to be performed and any remarks. Where several people are involved in the analyses this book is a valuable reference and later can be incorporated into a computer data base for convenient retrieval of needed information and results.

Working with human blood, plasma and serum naturally places one at increased risk of exposure to blood-born diseases. As a precaution we recommend using vinyl gloves while manipulating any blood component (TRU-TOUCH stretch vinyl medical gloves, #102215, Becton Dickinson and Company, Rutherford, NJ 07070) . Pipetting by mouth is strictly avoided by relying on a pipetting bulb

when pipetting plasma and other solutions (S/P Pipet Bulb #P5305-3, American Scientific Products, 1430 Waukegan Rd., McGaw Park, IL 60085-6787). Wearing safety glasses while hand-pipetting lipoprotein fractions is recommended as occasionally a pipet will break and splash one's face with a few droplets of serum.

Since labeled vials, 6-ml prep tubes, and prep caps require some time to prepare, they are best set up the afternoon before the G and D runs are prepared. The following morning, the high density G run is pipetted and set up first as it is a full 24-hr run. As described earlier, exactly 2 ml of each plasma sample is pipetted (volumetric drain pipettes) with 4 ml of solution 5 into the 6 ml prep tubes. These tubes should be marked with the last two digits of the sample number and D or G on each tube (using a black felt marker) to avoid sample mix-up. After firmly tightening the prep caps, all prep tubes are inverted at least 6 times in the loaded rotor to insure thorough mixing.

Another useful record is the "prep sheet", which should identify the prep machine used, time up to speed, and shut off time. Since the 40.3 and 50.3 rotors have 18 holes, the plasma sample numbers in each hole should be identified. This is another safeguard against sample mix-up. This 24-hr G (high density run) at 18 C is normally put on about mid-morning or earlier as it will come off

about an hour or so later the following day. When finished, this run needs to be pipetted as soon as possible to yield a 1-ml top fraction containing all plasma lipoproteins d ≤ 1.20 g/ml concentrated two-fold, and a second half-ml fraction for density reference. The latter density is conveniently monitored by precision 5th place refractometry as soon as convenient after pipetting. It is convenient to store all fractions in vials (properly labeled, with date) that have an air-tight teflon-gasketed seal. The preparative machine (or machines) used should have a log book containing machine number, date, run number, oil level before and after the run, counter reading (in thousands) and a space on the opposite right page for remarks.

Normally, the TLD plasma lipoprotein fractionation is similar to the G run in that labeled vials, prep tubes, and caps are set up prior to pipetting the 2-ml plasma samples and 4-ml of solution 2 into each labeled tube. Again, after firmly tightening the prep caps, the tubes are inverted several times in the loaded rotor for thorough mixing. Since this is an 18-hr, 40,000 rpm run at 18 C, it should be put on about 1-2 pm for convenient shut-down about 8-9 am the following day. Again, after centrifugation the samples are pipetted to yield a 1-ml TLD lipoprotein top fraction (concentrated two-fold over plasma) and a 1-ml second fraction that will be a density reference. After pipetting, both the top 1-ml $2D_1^0$ and the second 1-ml $2D_2^1$

fraction are measured by precision refractometry. This also allows measurement of total low density lipoprotein content. If excessively high, i.e., $\Delta n_{26} > 0.00283$, appropriate dilution with solution 4 avoids an expensive AnUC rerun. In the case of the D run, the extrapolated $2D_2^1$ density to the $2D_1^0$ is -0.043 precision refractometric units. This corresponds to a lower background $2D_1^0$ density than $2D_2^1$ by Dr = 0.0011 g/ml. Similarly, the $2G_1^0$ background density is lower than the $2G_{1.5}^1$ by 0.094 precision refractometric units or $\Delta\rho$ = 0.0035 g/ml.

Although a 50.3 Beckman rotor may be used for these two D and G runs, there would be no changes in the procedures and results if done at 40,000 rpm, as it is the exact dimensions of the 40.3 except that it is titanium. However, we have found that there are some disadvantages using the 50.3, particularly at 50,000 rpm. First, the 50.3 titanium rotor cannot safely be run in any older LH-V type machines. Secondly, if run at 50,000 rpm, the total up-to-speed time would be reduced to 64% of the time of the 40.3 runs, namely, 11.5 hr and 15.4 hr for the D and G runs, respectively. This might result in a more inconvenient work cycle. Also, the salt redistribution would be different and would have to be evaluated. An even more important factor would be an increased frequency of prep tube leaks requiring inconvenient delay and rerunning, only possible if enough plasma is available.

Rotor, Prep Tube, and Prep Cap Care

Rotors should be kept clean and the two "O" rings treated frequently with silicone grease. If there is a prep tube leak, the rotor should be thoroughly washed with cold water and a nonabrasive brush, and then dried, but not in an oven. The best procedure is to drain the water and allow the rotor to stand upside-down overnight. We recommend the use of stainless-steel prep cap stems and duraluminum crowns and nuts. When assembling the prep cap and rubber gasket, put a small amount of Lubriplate only on the inside threads of the nut. Some maintenance of these prep cap parts is periodically needed. For example, a stiff or hard rubber gasket should be replaced, minimizing future prep tube leaks. Depending on prep cap usage (about yearly) we rethread the nuts with a 5/16"-32 tap and the stainless-steel stems with a 5/16"-32 die. When you can feel that the nut is becoming loose on the stem, it should be discarded. Failure to do this may result in stripping the nut threads while running with the prep stem impacting the bottom of the prep tube with possible severe rotor damage.

Other more routine precautions are essential for safe preparative runs. Although it is obvious that the loaded rotor must be balanced, this should be checked carefully when loaded. If less than 18 samples (of the same density) are loaded they must be

opposite each other. This can be easily checked by rotating a pencil around the rotor center. Many rotor explosions have resulted from a partially filled rotor that was 1 tube off 180° symmetry. Also, if for any reason two or more prep tubes of different densities are run in the same rotor, each density class should be opposite each other. If an odd number of a given density is used, an appropriate "uncentrifuged" salt background tube, such as solution 3 or 6, should be included as a balance.

Pipetting or Collecting the Lipoprotein Fractions

There are two principal means of collecting the lipoprotein top fractions, namely, by tube slicing or by pipetting. For certain applications, the former may be more convenient and preferred. However, with tube slicing an exact volume cannot be collected, except by dilution to a higher constant volume. Secondly, the shock of the tube slicer stirs up the bottom contents of the prep tube eliminating the possibility of a proper second-ml density reference fraction. We therefore recommend the use of a special capillary pipette and a pipetting fixture as shown in Figure 3. This allows full inspection of the prep tube, with collimated illumination from above allowing visualization of the top fraction by light scattering (in a semi-darkened room). We use a specially fabricated capillary pipette of 0.3-0.4 mm inside-bore diameter (General Glass Blowing Co.,

Richmond, CA). If the pipette tip is jagged, a scratch with a diamond stylus with breakage allows restoring the tip to an optimum normal configuration. First inspect the cap to insure no lipoprotein fraction has been lost. Then ring the inside of the prep tube to free and dissolve any pellicle-like material from the wall. The capillary bulb is slightly depressed to allow sucking up of, say, approximately 0.2 ml fraction volumes at a time, with repositioning of the pipette tip around the periphery and at the surface of the top fraction. Each 0.2 ml is pipetted with slight bubbling into a calibrated 1-ml volumetric and, when almost 1 ml has been collected, the volumetric is stoppered and spun in a table-top centrifuge to remove bubbles. The fraction is then brought up to 1-ml volume and transferred to appropriately labeled teflon-stoppered vials. The second fraction is similarly collected and transferred to the appropriate vial.

AnUC of the D (Low Density) and G (High Density) Lipoprotein Fractions

Since the lipoprotein fractions will have considerable scientific value and the AnUC cells are extremely expensive, great care is needed to avoid or minimize cell leaks. Therefore, cell maintenance and meticulous care in cell assembly, final torquing, and sample introduction are essential. We fabricate all our cell parts except the quartz windows, which are untwinned quartz with the surfaces

normal to the Z optical axis. For the X or the vertically displaced G run schlieren pattern, we use a 49' wedge which has a matching 49' sector cup. This allows uniform parallel surface centerpieces. However, since the base of the cell must be unambiguous in both the D and G runs, which are run simultaneously, we use a standard $2^{1/2}°$ double-sector centerpiece mold and a 0.020" offset centerpiece mold. Great care is needed to fabricate these two types of epon-aluminum powder-filled centerpieces. Another feature of our centerpieces is a cylindrical reservoir (diameter = 0.028", #70 drill) between the sectors with a 0.001" scratch across the inner edge of the reservoir on both surfaces, connecting the two sectors (see Figure 4). Each calibrated syringe delivers 0.420 ml, which will fill each sector slightly above the scribe mark. As the rotor accelerates to full speed, each sector meniscus drains to the scratch level, allowing near perfect meniscus and baseline match. A mismatch of the baseline and sample will lead to serious baseline errors, particularly in the high density G run.

Another feature of our system shown in Figure 5 is the use of pinned rotors and notched cell housings, allowing consistent radial alignment to less than 0.001". In conventional scribe-line alignment, there is a tendency for the cells to rotate slightly during acceleration. If a cell is misaligned, the schlieren pattern develops vertical striations leading to errors in lipoprotein quantification. The scribe

lines on the rotor and cell normally do not match perfectly, and the correct alignment is to adjust the cell so that both scribe lines on the cell and rotor are either coincident or, more frequently, exactly parallel. A small amount of heavy silicone grease will tend to prevent cell rotation during acceleration. However, this will prevent evaluation of cell micro-leakage by weighing each cell before and after each run. This, of course, is possible with our slotted cell housing pinned rotor system. Our cells are weighted to 0.1 mg and the routine acceptable cell leakage is 1-3 μl (1-3 mg).

AnUC Calibration for the Low and High Density Lipoprotein Classes

We employ a Beckman Model E schlieren system that has a phase-plate wire combination, set at approximately 53°. Two factors are involved in relating the schlieren patterns (which are a plot of dn/dr) to lipoprotein concentrations within the low and high density subfractions. First, the area on the film or on an appropriate magnified tracing must be related to the integral of dn/dx or Δn. The most accurate way of monitoring this calibration is with the Beckman Calibration Cell (Part No. 306386). This is a wedge quartz window of angle Θ (ours is 0.00918 radians) on which are scribed two parallel lines 0.9984 cm apart. The schlieren pattern of this cell run with a reference cell results in a deflected and undeflected horizontal line

and two vertical lines giving a rectangular pattern corresponding to the following relationship[10]:

$$\frac{\Delta n}{\text{area calibration cell}} = \frac{\Theta x \Delta r}{T(\text{cell})} = \frac{(0.00918 \text{ rad})(0.9984 \text{ cm})}{1.20 \text{ cm}}$$

A 5x enlargement of the film produces a total magnification of the cell = 11.62 x. Our E3 magnified rectangle = 115.77 cm^2 corresponds to 0.007638 Δn.

The original specific refractive increments expressed as $\Delta n/g/100$ ml were 0.00154 for LDL[1] and 0.00149 for HDL. Some revisions of these factors have been evaluated and they are: 0.001417 for total LDL and 0.001347 for total HDL[10]. Lipoprotein area corrected to base of cell $(\frac{xb}{xi})^2$ provides the conversion to Δn and to original lipoprotein concentration in the cell. Thus, as an example for E3, a 1% concentration of LDL in the cell would correspond to 20.42 cm^2 on the 11.62x magnified tracing. Plasma lipoprotein levels must be corrected by the concentration factor, usually $2c_o$. Linear radial magnification, which is involved with measuring flotation rates, is related to the Δr of the calibration cell times the schlieren horizontal optical magnification times the magnification of the enlarger. (In our E3 machine, this is 0.9984 x 2.320 x 5 = 11.62 x on the projected tracing). If Beckman spectroscopic glass plates are used, a scribed circle of 2 cm diameter on an unexposed and developed plate can conveniently be made with a sharp drafting protractor. A white thin posterboard card

scribed with an intermittant circle exactly 5-fold (10 cm) greater in diameter allows adjustment of the 5x-magnified tracing to within ±0.2%.

If no Beckman calibration cell is available, an alternate calibration accurate to ±0.00002 Δn can be made using a sucrose solution and a boundary cell as described previously[10].

Whenever the Model E optical system is cleaned, manipulated, adjusted, or the drive is changed, a new calibration run should be made. These calibration factors may be incorporated as input factors to a computer program that calculates with appropriate corrections all the lipoprotein subfraction concentrations. Such calibration factors have been maintained to within ±0.5% over the past 24 years.

Standard Low and High Density Lipoprotein AnUC Runs

AnUC runs are performed routinely at Donner Laboratory to quantify the major lipoprotein classes with respect to amount of material present (in mg/dl) and flotation rate characterization (in S_f^o values for low density lipoproteins and $F^o_{1.20}$ for high density lipoprotein characterization). Additionally, the molecular weight, density, diameter, and more accurate moving boundary flotation rate are calculated for the principal component of LDL.

To produce these data, we must simultaneously run a high density preparation (G run) and a low density (D run) of the same plasma. A standard AN-D rotor with two holes accommodates the cells for these runs. Since each cell is composed of at least 17 separate parts which must withstand forces > 200,000 x g and a vacuum < 1 μ for 70 min, great care must be exercised in cleaning, assembling, torquing, and filling the cells if leaks and cell breakage are to be minimized.

Assembling Cells

Cell parts to be assembled must be thoroughly clean, dry and cooled to room temperature. The same complement of nondisposable parts should be used to compose a cell each time it is used, unless some part is known to be defective. Each part should bear a mark identifying its place in a cell. For example, cell F1 might have the lower window and sector cup marked F1-1 and the upper window and sector cup marked F1-2, the screw-ring and housing marked F1, and the centerpiece marked F1-regular with an arrow indicating its upper face.

Some of the tools indispensable for cell assembly are as follows: an ear syringe; a fine-tipped forceps; a dissecting probe; a hand-held screw-ring driver; lint-free tissues; a dial indicator reading in 0.0001-in

increments and mounted vertically on a stage; a can of Release Agent/Dry Lubricant (MS-122); a fine wire brush; a plastic rod 0.6 in x 4 in; and dry compressed air.

The housing threads should be brushed gently with a wire brush and blown clean with dry compressed air. Rusty keys should be removed and cleaned with crocus cloth before use. Screw-rings and gaskets should be stacked in order on a plastic rod, then brushed and blown free of particles with compressed air. Finally, the rings should be treated with a fine spray of dry lubricant and returned to the assembly tray.

Each sector cup should be blown free of dust and salt deposits with the ear syringe and fitted with a new window gasket (0.007-in vinylite, Beckman part # 327021). A bakelite window-liner should be placed in the sector cup with its bend nearest to the keyway. The matching window should be polished with a lint-free Kimwipe (Kimberly-Clark Corp.), blown dust free with an ear syringe, and placed in its sector cup. A small mark with a permanent pen or diamond stylus on the window chamfer helps to assure identical window placement from run to run.

Placement of X windows requires the utmost care as they are about three times more likely to break than flat windows. The square-cut face of an X window must face up when placed in its cup so that the beveled face fits the angle cut in the bottom of the sector cup. To check for a correct fit, rotate the sector cup and window assembly under a dial indicator and reposition the X window until the best parallel fit is obtained, usually within 0.0003-0.0005 in. Mark the chamfered edge of the X window adjacent to a scribe on the sector cup and draw an arrow on the window edge pointing toward the square-cut face to expedite reassembly. An upside-down X window will still sit parallel in its sector cup; however, a high-pressure point will be formed against the thick side of the window as up-to-speed forces are obtained. This condition leads to window and/or centerpiece breakage and should be avoided.

With the appropriate cell housing standing upright, place the lower flat window/sector cup assembly in the housing and press its keyway over the key. Blow the window free of dust and press it down with a centerpiece until its keyway engages the key. Avoid fingerprinting the centerpiece by pressing only on its down-stepped edge. Similarly, the upper sector cup/window assembly is pressed down without rotating the window to engage the key and keyway. Bottom all internal parts by pressing them down firmly with a plastic

rod. Place a bakelite gasket and screw-ring in position and hand tighten with a screw-ring driver. Never store assembled cells under full torque or window life will be reduced.

Preparing AnUC Cells for a Run

Many AnUC operators prefer to turn on the vacuum pump, diffusion pump, and mercury lamp of the E-machine(s) prior to torquing and filling the cells, in order to warm the diffusion pump oil, test the vacuum system, and monitor the lamp and viewing screen for problems before the runs begin. While the machines are pumping down, the empty cells are weighed to 0.1 mg and their weights recorded on a data entry form along with the cell numbers, run numbers, and date. The current weights are compared to the weights from a previous successful run to check that the correct parts have been assembled. Variations greater than 10 mg should be explained prior to torquing the cells. The empty X cell should be lighter than its matching F cell by 0.050-0.100 g. When the cells are filled, the denser G solutions will offset the difference in weights. Adjustments in cell weights are conveniently made by changing screw-rings or by adding more or removing bakelite gaskets. Balancing cells is a safeguard against rotor explosion and serves to lengthen the life of the centrifuge drive.

Cell Torquing

After proper cell assembly, the most critical step for a successful AnUC run is torquing. Over-tightening the screw-ring may break a window while under-torquing permits excessive leakage. For some cells, this margin of error is only ± 0.5 ft-lbs. Window life is inversely proportional to the degree of torquing and the length of time that it is compressed. Ideally, a cell is torqued the minimum amount necessary to prevent leakage greater than 4 mg during the run. A loss greater than 4 mg may distort the schlieren patterns and meniscus lines, necessitating a rerun.

Before torquing an X cell, check it for upward refraction of a horizontal line as seen through the sectors when the screw-plug hole is facing upward. Downward deflection indicates 180° misorientation of the X sector cup. Push a cell to the rear of the torque-wrench collet with the ring driver, and secure the cell by tightening the collet handle. Turn the wrench clockwise until a torque of 5-10 ft-lbs is obtained. The screw-ring should glide smoothly during torquing. Sticking or roughness indicates dirty threads, inadequate lubrication, or a poor fit of screw-ring and housing. A cell is sufficiently torqued when the centerpiece can be felt to stiffly spring back in response to being compressed between the windows. Remove the torqued cell from the collet and check the "seal" between the septum and the

window for a homogeneously stippled appearance. A small circle with a dot at its center indicates that dust particles are disturbing the "seal". The centerpiece and window should be cleaned before proceeding. After the torqued cells have sat for about 5 min, they should be carefully torqued again to take up any slack which may have developed in their parts.

Filling the Cells

AnUC cells are filled after torquing by means of 1.00-ml ground-glass tuberculin syringes fitted with #23 stainless-steel needles whose barrels have been cut off squarely to 1/2 in. Each numbered syringe is carefully calibrated prior to use to determine the precise position (say, 0.411 ml) at which its numbered plunger will deliver a volume of 0.420 ml. An 8-ml vial, topped with parafilm, is weighed to 0.1 mg and reweighed each time an aliquot of distilled water, believed to be 0.420 ml, is added by the syringe being tested. When the weighed volume is equal to 0.420 ml, or 0.419 g, the calibration value and syringe number are recorded for later reference.

Syringes must be clean, dry and at room temperature before use. A separate syringe is used for each baseline and for each serum preparation. As each syringe is used, its number, calibration value, the sample number, and the number of the cell being filled are

recorded. Later, if a sample must be refilled or saved after a run, the correct syringe can be easily identified.

Our convention is to fill baselines first in the right-hand sector of each cell: G baseline (p_{20} = 1.2032) to the X cells and D baseline (p_{20} = 1.0632) to the F cells. The high density preparation, G run, is loaded into the left sector of the X cell and the low density preparation, D run, is loaded into the left sector of the F cell. The filled syringe and needle combination must be free of all bubbles or its calibration will be compromised. Holding a filled syringe upward and snapping the base of the needle with a finger of the free hand usually dislodges the smallest bubbles. Drawing the fluid column downward with the plunger will allow bubbles to be absorbed by the meniscus. Pushing the fluid column upward until clear fluid appears at the tip of the needle assures an air-free syringe. Fluid must be delivered slowly and smoothly into a cell to keep it from surging out through the fill hole.

Within minutes of being filled, a pair of vinyl gaskets, 0.004 in x 5/32 in diameter, followed by one teflon gasket 0.007 x 5/32 in diameter, should be inserted in the fill-hole recess. The gaskets are compressed with a new screwplug made of delrin rod threaded to 8-32 and cut squarely to 3/32 in lengths. The screwplugs are conveniently

picked up and started in the housing with a screw-holding driver (#5-3, H.J.J. Co., Oakland, CA). Plugs are tightened firmly, short of stripping, with a small screwdriver, then trimmed to the level of the housing with a single-edged razorblade, taking care not to cut the housing or one's fingers.

The fully loaded cells are next weighted to 0.1 mg and the weights recorded and compared to those from a previous filling. The difference in weights between the X and F cells should be 100 mg or less. Greater variations in weight may cause an imbalance in the rotor.

Starting a Run

By the time that the cells are filled, the E machine should be fully pumped down. The diffusion pump is turned off a few minutes before opening the chamber air valve and a rotor is removed from the 26.5 C oven to cool. We monitor rotor temperature with a thermocouple thermometer, reading to ±0.01 C, which has been calibrated at 26.00 C against a Bureau of Standards thermometer. With the room temperature at 20 C, the rotor will gradually cool to run temperature of 26.00 C. Before the rotor temperature reaches 26.3 C, the filled cells should be placed in it so that the sample temperature will have time to equilibrate to 26.00 C. To be consistent,

we always load the X cell in hole #1 and the F cell in hole #2. Cells are pressed in place with a screw-ring driver while turning slightly clockwise to engage the housing notch and rotor pin. Rotors are inspected visually from beneath to affirm that housings have been fully seated.

Before the rotor reaches 26.00 C, be sure that the vacuum chamber is open, but never open a chamber under pressure. Also, inspect the view screen for light intensity and striations. Vertical striations can usually be eliminated by cleaning the collimating and condensing lenses in the chamber. Horizontal striations are usually traced to light source problems and may be corrected during pumpdown. Light intensity can usually be changed by turning the lamp or by changing the slit width.

To attach the rotor to the drive, first slide the drive coupling up and place the rotor under it, then push the rotor up to the coupling and turn it to engage the threads. Stabilize the drive coupling with a fork wrench and turn the rotor until it just seats against the coupling. Snug the rotor firmly against the coupling using both hands but avoid over-tightening the rotor as it tends to tighten during braking. A loose rotor, however, can become disconnected during acceleration. By all means, remember to remove the fork wrench!

Close the chamber and air valve, turn on the vacuum pump, and wait until a pressure of 200 μ or less is obtained before turning on the diffusion pump. The machine should not be started until a vacuum <1 μ is obtained or a high end-of-run temperature may result. Pumpdown normally takes about 15 min, so this time is best spent recording run data in log books and preparing the film.

We record the run number, rpm, cell number, baselines, rotor number, start time, length of run, rotor temperature in and out, and data pertaining to the automated acceleration phase. Additionally, for each E machine, notes should be taken describing any problems during the run, maintenance performed, and the final rpm counter reading.

At Donner Laboratory, we have developed an AnUC facility of three E-machines whose start times, photo sequencing, and end times are automated through a digital clock and integrated circuitry. The acceleration phase for each machine is controlled by motor-driven variacs to uniformly accelerate the rotor at 10,000 rpm/m until nearly up to speed, at which time a frequency counter, working through a magnetic pickup and geartooth synchronous to the drive, shuts the accelerating current from 15 amps down to 3-4 amps as determined by the anti-hunt speed control mechanism. Rpm and photograph times

are monitored and recorded every 20 sec during the runs. Later, another program uses these records to calculate mean rpm ± SD during the up-to-speed portion of each run and to integrate w^2dt as an addition to the total run length. The g force experienced by the sample and its flotation during linear acceleration is equivalent to 1/3 of the up-to-speed time at 52,640 rpm. After increasing the drive current to 14 amps at 2000 rpm, the 5.20' acceleration time is equivalent to approximately 1.73-1.83' of equivalent up-to-speed (UTS) at 52,640 rpm.

Each E machine at Donner Lab is fitted with a camera specially made to use Kodax Tri-X Ortho Film in 4" x 100' rolls (cat. # 156-0259). Before each run, a data card is photographed which includes the run number, sample number, baselines used, cell numbers, rotor number, machine number, operator initials, and the beginning rotor temperature. Following a run, the card is photographed again to include the end-of-run temperature and the start time. A small, clear plastic tab with the machine number printed on it is fixed behind the shutter so that the machine is identified in every photograph.

Although spectroscopic plates are no longer used routinely at Donner Lab, other AnUC operators report success with cutting film and fitting it in inserts inside of plate holders. Leaving one frame

unexposed would create a blank on the developed film for writing data pertinent to that run.

For manual operation with the proper run speed selected, 52,460 rpm for D and G runs, the vacuum <1μ, and the timer set at run length or longer, the run may be started. Turn the variac clockwise until 3-4 amps are read on the ammeter, then increase the current steadily over the next 30 sec until 15 amps are applied to the drive motor. By maintaining 15 amps over the next 5 min, the rotor should accelerate at the rate of about 10,000 rpm. Continue accelerating the rotor until it is nearly up to speed, 52,000 rpm, then steadily reduce the drive current to 3-4 amps to avoid overspeeding. Record the time at which the rotor reaches UTS velocity and start timing the run with an accurate timer. The run speed may be checked by timing the number of seconds required for the rpm counter to increase by 52.6 counts, assuming 1000 rev/count. Exactly 60 sec are required for the true speed whereas a longer time indicates underspeeding and a shorter time confirms overspeeding.

In order to have photographs of moving boundaries which conform in flotation velocity to Donner templates, photos must be taken at full (0 min) UTS time and then 2, 6, 30, and 64 min later. For more accurate moving boundary flotation rates and rho intercept

analysis, it is recommended that additional photos be taken at 8, 14, 22, and 48 min UTS.

After running 64 min at 52,640 rpm the HDL schlieren pattern will be fully resolved and the run may be ended. With braking set to "rapid", turn the timer to "hold" and return the variac to zero position. The lamp may also be turned off and allowed to cool for 10 min before turning off the water. During braking the diffusion pump should be shut off and allowed to cool. The rotor will come to a complete stop after about 15 min of rapid braking. The chamber should return to atmospheric pressure within 1 min of opening the air valve, and the rotor should be immediately removed for an end-of-run temperature measurement. The odometer reading is routinely recorded in an E machine log book after each run.

The cells can usually be removed from the rotor with finger pressure; however, tight cells may be pressed out with a plastic rod (4" x 3/4" diam) mounted vertically in a plastic stand. The cells should be weighed to 0.1 mg to determine leakage during the run, and the mg lost entered on the cell torquing record. It is recommended that any cell leaking more than 4 mg per run in two successive runs be thoroughly cleaned and tested before reuse. A cell which has leaked during a run must be replaced with another cell of

equal weight before being rerun with the matching but nonleaking cell.

Occasionally a sample must be rerun for reasons other than cell leaks, i.e., lamp outage, high end-of-run temperature, problematic film development, etc. The filled cells may be rerun, barring excessive leakage, after thoroughly shaking and resuspending the lipoprotein material and re-equilibrating the rotor temperature. To shake up a sample, it is recommended that the cell be shaken in the direction of the sector axis while bumping it gently against the palm of the free hand. This technique minimizes the chance of sector-to-sector leakage. It is strongly recommended that films or spectroscopic plates from the runs be developed before the cells are emptied in case an undetected problem necessitates a rerun.

Disassembling and Cleaning the Cells

After a run, the delrin screwplugs are removed from the cells and discarded. Fillhole gaskets are easily removed with a dissecting probe. The windows and centerpieces are placed in distilled water and the other nondisposable parts are placed on a paper-lined tray in an orderly array. Parts are cleaned only with distilled water since detergents could leave a residue which would react with the lipoproteins in the next samples. Thorough cleaning of centerpieces

is done with pipecleaners and a wash bottle. Centerpieces are blown free of water with an ear syringe and dried for 15 min under the heat of a hairdryer. Any parts being dried under a hairdryer should be covered with tissues to prevent contamination with oil and brush carbon from the dryer motor. The quartz windows are placed in systematic order on wet paper towels between lint-free tissues and kept wet until reassembled. Each window is dried and polished with Kimwipes before being placed in its sector cup.

To clean the ground glass syringes, first remove the needles and force a stream of distilled water from a wash bottle through each needle for about 15 sec. Blow the needles free of distilled water with an ear syringe and cover them with tissues. Hold the tip of a syringe in a beaker of distilled water and move the plunger rapidly up and down at least 30 times, then remove the plunger and force distilled water through the barrel for 15 sec with a wash bottle. Squeeze the glass plunger between the thumb and forefinger of one hand while moving it back and forth with the other hand under a stream of distilled water until the plunger feels "squeaky clean". Needles, syringe barrels, and plungers should be set vertically in a rack and dried for 15 min with hot air from the hairdryer. Barrels and plungers should not be reassembled until cool.

Tracing Schlieren Patterns

Films or plates 2" x 10" are magnified 5 x in an enlarger and traced in a darkroom. The enlarger is calibrated by projecting the enlarged image of a 2-cm diameter circle onto a discontinuous 10-cm diameter circle drawn on a thin card. Since the image of the cell is magnified about 2.33 x by the schlieren optical system, the total magnification of the traced image is 11.64. Donner templates are specially printed with calibration marks defining the flotation intervals for the 0-, 2- (or 6-), and 30-min UTS pictures of the D run and the 64-min UTS picture for the G run (see Figure 7 for an appropriate schlieren film). We recommend to anyone attempting schlieren analysis of lipoproteins according to Donner protocols that Donner templates be used for the tracings. On a trial basis, tracings sent to Donner Laboratory could be analyzed by our sonic digitizer measuring and VAX station 3200 analysis, with final data returned to the sender. To make Donner templates compatible with other E machines is would be necessary to make a calibration run using a standard Beckman calibration cell with scribed lines 1 cm apart. The phase-plate wire angle should not be changed (we use 53°). Once the magnification factor of the E machine to be used is determined, the appropriate enlarger magnification can be determined by dividing the total E-2 magnification factor for Donner templates (11.64 x) by the E machine magnification factor in question.

A vertical broken line is provided on the templates for each photograph traced to align the base-of-cell with the correct flotation interval on the template. Patterns are traced by hand with a sharpened # 7H-lead drafting pencil. The patterns are traced directly down the center of the light bands produced by the schlieren pattern as shown in Figure 8. A separate line is similarly traced for the baseline. Both schlieren lines are traced on the templates between the vertical lines which define the flotation interval 100-400 for the 0-min frame, 20-100 for the 2-min (or 6-min) frame, and 0-20 for the 30-min frame of the D run and the 0-9 interval for the 64-min picture of the G run. Now we conveniently read the schlieren tracings with a sonic digitizer as shown in Figure 9, and the results go directly into our VAX station 3200.

Peak points of the main LDL component are traced for both the G run and the D run using UTS photos 0-, 2-, 6-, 8-, 14-, and 22-min of the G run and UTS photos 8-, 14-, 22-, 30-, 48- and 64-min for the D run peak points. A vertical line from the left side of the appropriate template is positioned vertically through the point of an LDL peak and a tiny dot is marked on the knife-edge for that frame. A plot of LnX of these distances as a function of w^2t should define a straight line whose slope is the uncorrected moving boundary flotation rate of the LDL peak in the high or low density run.

Figure 10 shows schematically how our three programs, schlieren analysis, moving boundary F rate of the major LDL component and the final S_f^o, σ and molecular weight calculations are interrelated. In addition to numerical output, a convenient corrected graphical presentation of both the low and high density lipoprotein spectra is presented in Figure 11.

Because this chapter is intended as an introduction to both preparative and AnUC of plasma lipoproteins and there are text limitations, the full details and theory of AnUC analysis cannot be included here. However, the complete technical details of the lipoprotein schlieren analysis are given in reference 4.

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Figure Legends

Figure 1. The major classes of human plasma lipoproteins with comparison of size, flotation rate, and density.

Figure 2. Salt redistribution for VLDL and LDL 18-hr run. Note the more severe redistribution in the HDL 24-hr run. Reprinted with permission from Figure 1 in J. Lipid Res. 5, 68-74 (1964).

Figure 3. Pipette and pipetting fixture. Reprinted with permission from Figure 3, Lindgren, F.T., in Analysis of Lipids and Lipoproteins, Perkins, E.G., ed., American Oil Chemists' Society, Champaign, IL, 1975, pp. 204-224.

Figure 4. Centerpiece with reservoir (both standard and 0.020" offset for unambiguous identifications of base of cell in both flat and X cells. Reprinted with permission from Figure 2, Lindgren, F.T., Jensen, L.C., and Hatch, F.T., in Blood Lipids and Lipoproteins, Nelson, G.J., ed., John Wiley and Sons-Interscience, New York, 1972, pp. 181-272.

Figure 5. Slotted cell housing and pinned rotor. Reprinted with permission from Figure 3, Lindgren, F.T., Jensen, L.C., and Hatch, F.T., in Blood Lipids and Lipoproteins, Nelson, G.J., ed., John Wiley and Sons-Interscience, New York, 1972, pp. 181-272.

Figure 6. Set-up for a typical preparative run. Note the sizing gauge (1), tube puller (2), vise for prep tube (3), pipetting fixture (4), capillary pipette wash jar (5), plasma and sample block (6), and refrigerator storage boxes. Reprinted with permission from Figure 2, Lindgren, F.T., in Analysis of Lipids and Lipoproteins, Perkins, E.G., ed., American Oil Chemists' Society, Champaign, IL, 1975, pp. 204-224.

Figure 7. Typical AnUC schlieren film of a D and G run. Shown are the card photo and the 0-, 2-, 6-, 30-, and 64-min UTS frames. Reprinted with permission from Figure 6, Fundamentals of Lipid Chemistry, 2nd edition, Burton, R.M. and Guerro, F.C., eds. Bi-Science Publication, Webster Grove, MO, 1974, pp. 475-510.

Figure 8. Tracing on the low density (0-, 6-, and 30-min) UTS templates and on the 64-min UTS high density template. Note the measured height between the pattern and baseline which used to serve as input to our AnUC computation program. Reprinted with permission from Figure 7, Fundamentals of Lipid Chemistry, 2nd edition, Burton, R.M. and Guerro, F.C., eds. Bi-Science Publication, Webster Grove, MO, 1974, pp. 475-510.

Figure 9. Schematic revised schlieren tracing showing the use of a sonic digitizer.

Figure 10. Interrelationships among the three major programs that run together and give the indicated results. Reprinted with permission from Figure 14, Lindgren, F.T., Jensen, L.C., and Hatch, F.T., in Blood Lipids and Lipoproteins, Nelson, G.J., ed., John Wiley and Sons-Interscience, New York, 1972, pp. 181-272.

Figure 11. Typical graphical output of the low and high density spectra. Shown are lipoprotein concentrations within the S_f^o 0-12, S_f^o 12-20, S_f^o 20-100, and S_f^o 100-400 low density intervals and $F_{1.20}^o$ 3.5-9 and $F_{1.20}^o$ 0-3.5 high density intervals.

Table I

NaCl (mol wt = 58.44) salt table[a]

ρ_{20}	ρ_{26}	η_{20}	η_{26}	wt%	NaCl (g/l)	H_2O (g/l)	NaCl H_2O	Molality	Molarity	ΔS	n_D^{26}
0.99823	0.99683	1.0021	0.8750	0.	0.	998.23	0.	0.	0.	0.	1.33240
1.00534	1.00386	1.0194	0.8876	1.00	10.05	995.29	0.01010	0.173	0.172	0.311	1.33412
1.00630	1.00481	1.0217	0.8893	1.13	11.42	994.88	0.01148	0.196	0.195	0.354	1.33435
1.01246	1.01090	1.0360	0.9005	2.00	20.25	992.21	0.02041	0.349	0.346	0.627	1.33585
1.02680	1.02509	1.0688	0.9280	4.00	41.07	985.73	0.04167	0.713	0.703	1.255	1.33927
1.04127	1.03941	1.1038	0.9588	6.00	62.48	978.79	0.06383	1.092	1.069	1.895	1.34272
1.05589	1.05388	1.1444	0.9944	8.00	84.47	971.42	0.08696	1.488	1.445	2.540	1.34618
1.07068	1.06852	1.1930	1.0364	10.00	107.07	963.61	0.11111	1.901	1.832	3.194	1.34965
1.08566	1.08335	1.2506	1.0864	12.00	130.28	955.38	0.13636	2.333	2.229	3.860	1.35316
1.10085	1.09839	1.3164	1.1436	14.00	154.12	946.73	0.16279	2.785	2.637	4.534	1.35667
1.11621	1.11362	1.3877	1.2056	16.00	178.59	937.62	0.19048	3.259	3.056	5.230	1.36026
1.13190	1.12919	1.4649	1.2727	18.00	203.74	928.16	0.21951	3.756	3.486	5.943	1.36391
1.14779	1.14498	1.5574	1.3530	20.00	229.56	918.23	0.25000	4.277	3.928	6.668	1.36757
1.16395	1.16106	1.6758	1.4558	22.00	256.07	907.88	0.28205	4.826	4.381	7.430	1.37137
1.18040	1.17741	1.8209	1.5819	24.00	283.30	897.10	0.31579	5.403	4.847	8.197	1.37516
1.19717	1.19407	1.9897	1.7285	26.00	311.26	885.91	0.35136	6.012	5.325	8.963	1.37890

[a] ΔS values above a distilled water reference are given as obtained with a series 716 sugar refractometer. n_D^{26} values are relative to air as measured by the reflection method described in text.

Table II

NaBr (mol wt = 102.91) salt table[a]

ρ_{20}	ρ_{26}	η_{20}	η_{26}	wt%	NaBr (g/l)	H_2O (g/l)	NaBr H_2O	Molality	Molarity	ΔS	n_D^{26}	ΔS^*	n_D^{26*}
0.99823	0.99681	1.0020	0.8781	0.	0.	998.23	0.	0.	0.	0.	1.33240	0.	1.33240
1.00599	1.00451	1.0074	0.8805	1.00	10.06	995.94	0.01010	0.098	0.098	0.251	1.33379		
1.00630	1.00482	1.0076	0.8805	1.04	10.46	995.85	0.01051	0.103	0.103	0.261	1.33384	0.354	1.33435
1.01385	1.01229	1.1026	0.8832	2.00	20.28	993.62	0.02041	0.198	0.197	0.503	1.33517	0.589	1.33564
1.02984	1.02811	1.0231	0.8899	4.00	41.19	988.61	0.04167	0.405	0.400	1.011	1.33794	1.096	1.33840
1.04623	1.04433	1.0341	0.8984	6.00	62.77	983.43	0.06383	0.620	0.610	1.534	1.34078	1.618	1.34123
1.06305	1.06099	1.0462	0.9087	8.00	85.05	978.05	0.08696	0.845	0.827	2.075	1.34369	2.157	1.34414
1.08033	1.07810	1.0601	0.9210	10.00	108.00	972.30	0.11108	1.080	1.050	2.625	1.34663	2.714	1.34708
1.09810	1.09570	1.0768	0.9355	12.00	131.80	966.30	0.13640	1.326	1.281	3.205	1.34971	3.291	1.35016
1.11638	1.11381	1.0962	0.9523	14.00	156.30	960.10	0.16280	1.582	1.519	3.803	1.35268	3.888	1.35331
1.13519	1.13247	1.1185	0.9717	16.00	181.60	953.60	0.19044	1.851	1.765	4.420	1.35608	4.506	1.35652
1.15455	1.15169	1.1442	0.9941	18.00	207.80	946.80	0.21948	2.133	2.019	5.063	1.35941	5.148	1.35984
1.17449	1.17159	1.1739	1.0198	20.00	234.90	939.60	0.25000	2.430	2.281	5.730	1.36282	5.813	1.36326
1.19505	1.19198	1.2073	1.0489	22.00	262.90	932.20	0.28202	2.741	2.555	6.417	1.36631	6.502	1.36673
1.21627	1.21307	1.2444	1.0811	24.00	291.90	924.40	0.31577	3.069	2.837	7.123	1.36985	7.210	1.37027
1.23817	1.23482	1.2865	1.1177	26.00	321.90	916.30	0.35130	3.414	3.128	7.863	1.37352	7.955	1.37393
1.26078	1.25728	1.3356	1.1603	28.00	353.00	907.80	0.38885	3.779	3.431	8.639	1.37732	8.730	1.37774
1.28413	1.28046	1.3946	1.2116	30.00	385.20	898.90	0.42852	4.164	3.743	9.452	1.38126	9.536	1.38158
1.34615	1.34209	1.5923	1.3834	35.00	471.20	875.00	0.53851	5.233	4.570	11.621	1.39150	11.708	1.39190
1.41384	1.40944	1.8659	1.6210	40.00	565.50	848.30	0.66663	6.478	5.496	14.062	1.40256	14.149	1.40296
1.48785	1.48311	2.2172	1.9260	45.00	669.43	818.40	0.81797	7.950	6.506	16.832	1.41454	16.928	1.41494

[a] ΔS^* and n_D^{26*} correspond to an aqueous NaBr system in which a constant 0.196 *m* NaCl content is maintained.

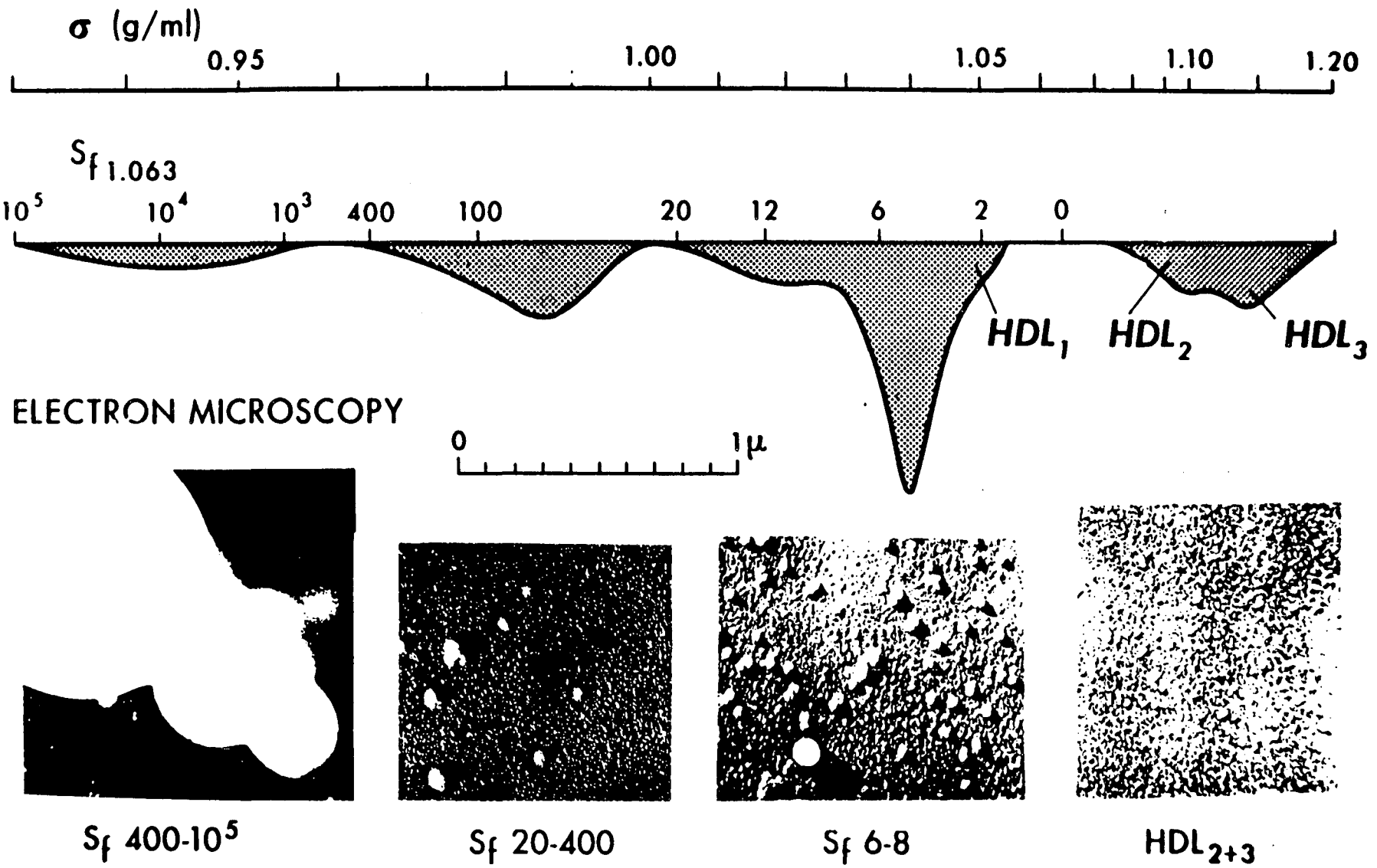

ULTRACENTRIFUGAL ANALYSIS
σ (g/ml)
0.95
1.00
1.05
1.10
1.20
Sf 1.063
10^5
10^4
10^3
400
100
20
12
6
2
0
HDL1
HDL2
HDL3
ELECTRON MICROSCOPY
0
1μ
Sf 400-10^5
Sf 20-400
Sf 6-8
HDL2+3

Figure 1

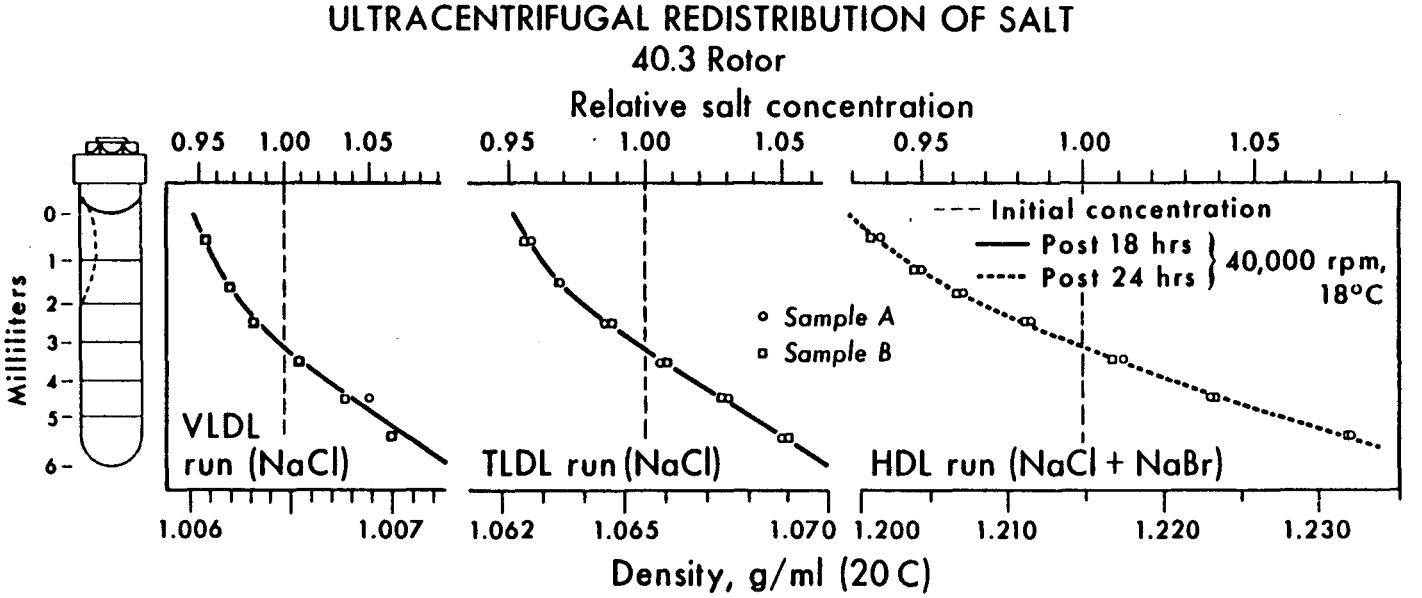
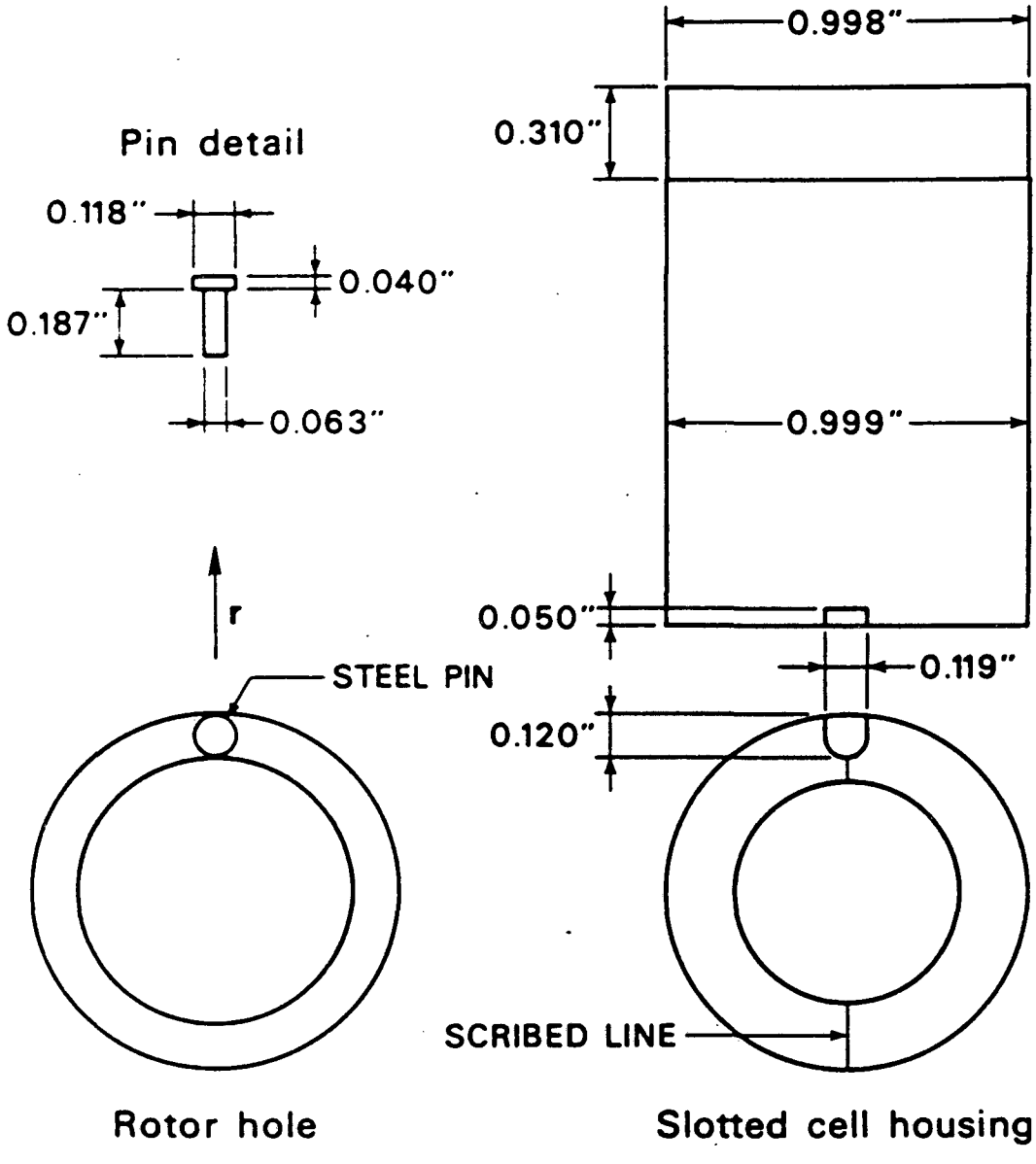

ULTRACENTRIFUGAL REDISTRIBUTION OF SALT
40.3 Rotor
Relative salt concentration
0.95
1.00
1.05
Milliliters
0
1
2
3
4
5
6
VLDL
run (NaCl)
TLDL run (NaCl)
HDL run (NaCl + NaBr)
Initial concentration
Post 18 hrs
Post 24 hrs
40,000 rpm,
18°C
Sample A
Sample B
1.006
1.007
1.062
1.065
1.070
1.200
1.210
1.220
1.230
Density, g/ml (20 C)
DBL 701-5545

Figure 2

Figure 3



DBL 6912-5244

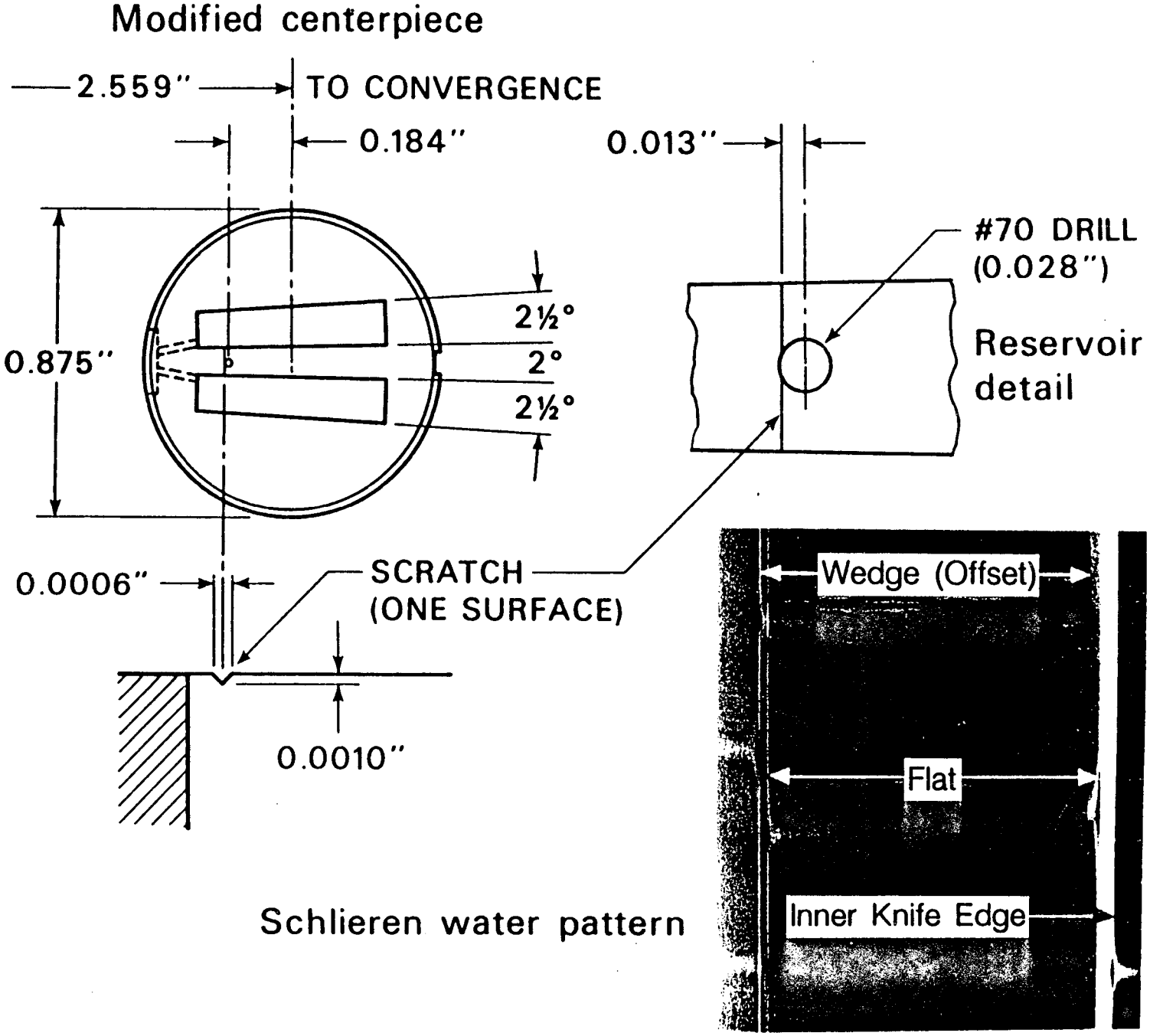
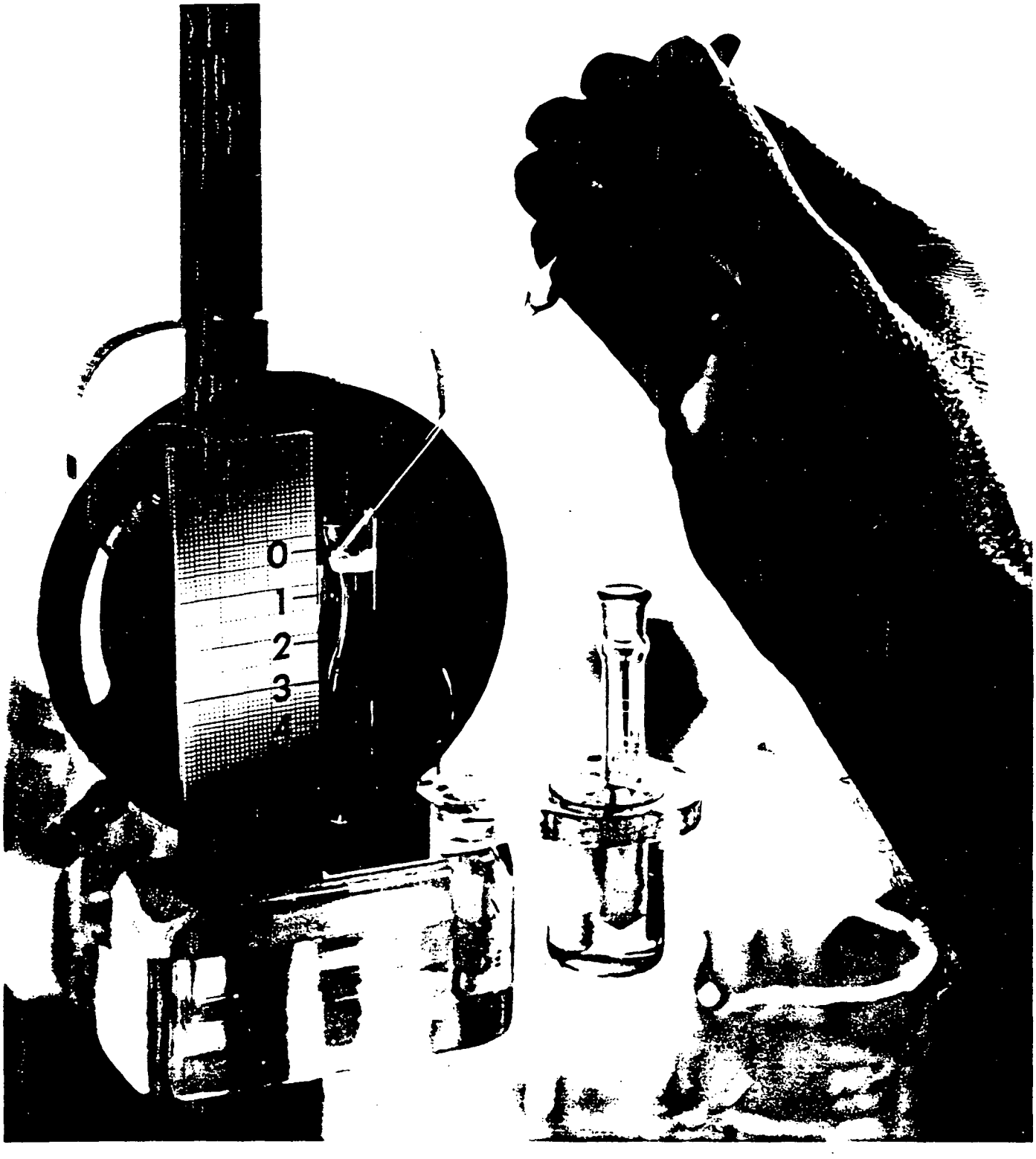

Modified centerpiece
2.559"
TO CONVERGENCE
0.184"
0.013"
#70 DRILL
(0.028")
Reservoir
detail
0.875"
2½°
2°
2½°
0.0006"
SCRATCH
(ONE SURFACE)
0.0010"
Wedge (Offset)
Flat
Inner Knife Edge
Schlieren water pattern

Figure 4

Figure 5



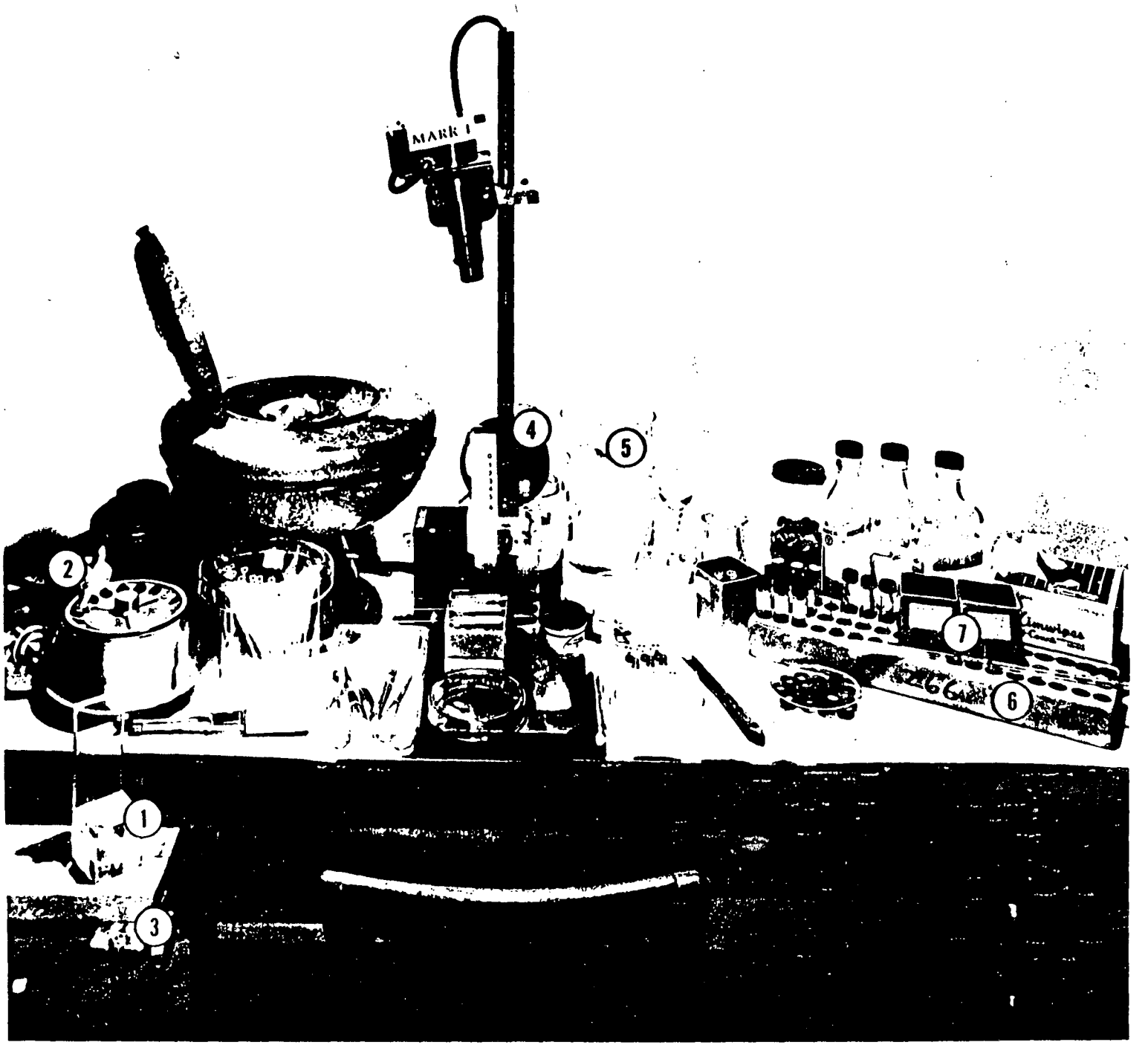


Figure 6

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