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OPERATION OF THE ISO-DALT SYSTEM
SEVENTH EDITION

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PREFACE

The ISO-DALT system of two-dimensional electrophoresis was developed in our laboratory as a series of modifications of the original technique of O'Farrell (J. Biol. Chem. **250**, 4007-4021, 1975). Since the appearance of the first two open literature publications describing the ISO-DALT system in 1978 (Anderson, N. G., and N. L. Anderson, Anal. Biochem. **85**, 331-340, 1978 and Anderson, N. L., and N. G. Anderson, Anal. Biochem. **85**, 341-354, 1978), the technique has continually been refined. As a supplement to these first references and to those that have followed (see Bibliography at the end of this report), we have written a series of recipes and more detailed laboratory procedures that incorporate refinements and "tricks of the trade" as they have developed during our use of the system. The present collection is the seventh version in this constantly evolving series and represents the state of art as of May 1984.

The first four versions of this manual were untitled and unauthored, prepared for our own use and for the use of all who came to consult and to learn our techniques. The fifth version was enlarged and more formal; it was published as Argonne National Laboratory Report ANL-BIM-79-2 so that our techniques could receive wider distribution and be properly cited and acknowledged. This, the seventh edition, has been prepared to incorporate the changes that we have found helpful, as well as to add a few new sections such as silver staining, transfer gels, ACIDOs, and BASOs.

Although these directions are specific for our laboratory, we hope that they will be helpful in other laboratories as well.

Although some disagreement still exists on this point, we recommend that gel patterns be oriented with acid isoelectric points (pIs) to the left and high sodium dodecyl sulfate (SDS) molecular weights at the top. This results in a system of pI and molecular weight values that run according to the Cartesian convention and facilitates the use of the charge and molecular weight standards described herein.

The cover illustration is a computer map of a two-dimensional protein spot analysis underneath a drawing from De Humani Corporis Fabrica by Andreas Vesalius, published by Oparinus, Basel, in 1543.

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FIGURES

THE ISO SYSTEM

Preparation of samples for isoelectric focusing:

A sample to be prepared for two-dimensional (2-D) electrophoresis using the ISO-DALT system is first mixed with a solubilizing agent, a "mix," before separation in the first dimension by pI (isoelectric point). We are currently using four methods of sample preparation, depending upon the type of sample. You may wish to experiment with these methods to establish the optimum solubilization of your own sample. The mixes listed below are frozen at -70°C in microfuge tubes as 1 mL aliquots. The recipes for the mixes are listed on pages 37-44.

1. Sodium dodecyl sulfate (SDS) Mix (recipe No. 17; blue tubes). This mix seems to be best for serum, plasma, or amniotic fluid. To 10 µL sample add ~ 20-30 µL SDS mix (making a 1:3 or 1:4 dilution). The sample plus mix may be heated on a 95°C heating block for 5 min to solubilize it and, if possible, inactivate proteolytic enzymes.

2. Urea mix (recipe No. 18; pink tubes). This mix works well with solid tissue samples and some tissue culture cells such as fibroblasts. Use from 30-100 µL of this mix for each 10 mg of tissue. Homogenize and centrifuge (and/or sonicate) the sample. From 10 to 30 µL of the urea mix may also be mixed with 10 µL of a concentrated liquid protein sample. DO NOT heat the urea mix. This mix is adjusted to pH 9.5.

3. Urea mix for urine (recipe No. 19; yellow tubes). This preparation for solubilizing urine contains no NP-40 (Nonidet P-40, a nonionic detergent) and gives best results (fewer streaks) when 10 mg lyophilized urinary proteins are put into 100 µL mix and about 10 µL (or less) are loaded onto the ISO gel.

4. Urea mix for muscle samples (recipe No. 20). This preparation, which solubilizes muscle and other tissues well, contains dithioerythritol (DTE) instead of mercaptoethanol and is adjusted to pH 9.5.

Often tissue samples contain DNA and cell debris, so you might want to centrifuge the sample, analyze the supernatant, and discard the pellet. We centrifuge tissue samples for 1 hour at 40,000 rpm in a Beckman ultracentrifuge using a type-42 titanium rotor.

From 5 to 30 µL of sample are layered on the ISO system. Obviously these are minimal volumes and do not completely fill the ISO tubes. If larger initial protein samples are available, proportionately larger samples can be prepared with the mix. Samples prepared in SDS or urea mix may be stored at -80°C and reused.

If your sample potentially contains only a very small amount of protein, you may want to radiolabel the proteins, dry your gels, then autoradiograph them. The following example describes the preparation of fibroblasts labeled with L-[^{35}S]-methionine. (The procedure for labeling tissue sections is described in Clin. Chem. 27: 1918-1921, 1981.)

1. The L-[^{35}S]-methionine will arrive frozen on dry ice. Before it is used, thaw it and divide it into 250 µCi aliquots. Store these aliquots at -70°C and thaw each one immediately before use.

2. Start cultures in multiwell plates with approximately 5 x 10^4 cells per well, using complete minimum essential medium (MEM). Allow the cultures to incubate for 24 to 48 hours, until the cultures are semiconfluent.

3. Remove the medium and replace it with 400 µL of labeling medium (such as RPMI 1640) containing 5% fetal bovine serum, 2% glutamine, and no methionine (made from a Gibco Selectamine Kit, for instance). Add 25 µCi of L-[^{35}S]-methionine to each well. The actual volume added will vary with the batch of [^{35}S]-methionine used, but it is normally 2-4 µL per well). Incubate the plates for 16-18 hours.

4. Remove medium and discard it into the liquid radioactive waste container.

5. Add 50 µL of urea-NP-40 mix to one well at a time and rinse the bottom of the well once or twice with the solution. Then place the solution containing the solubilized fibroblast proteins into microfuge tubes.

6. Freeze the samples at -80°C. Microfuge the sample after it is thawed and before it is loaded into the ISO tube. The volume loaded is usually 20 µL.

Preparation of ISO equipment:

The entire system should be clean and dry. ISO tubes are cleaned by soaking them in a tank of chromic acid (Chromerge) at room temperature for at least 24 hours or in a tank of hot chromic acid for at least 1 hour. The Teflon chromic acid container may be heated to 90°C in the microwave oven for 50 seconds before the ISO setup is introduced. Be sure the level of acid is adjusted so only the tubes and not the lucite holder touch the acid. The microwave oven and all cleaning apparatus should be in a suitable fume hood. The assembly should be rinsed thoroughly in tap distilled water and each tube should be flushed with glass distilled water from a squirt bottle and then dried by air aspiration through the tubes. (Tap distilled water refers to the once distilled water from a central building system; this water may be used from a faucet. Glass distilled water refers to tap distilled water that we have distilled one more time in glass.)

The large chamber of the ISO system is filled to the LOAD mark (approximately 2 L) with tap distilled water. To prepare the electrofocusing gel, use a 150 mL lyophilization flask and measure into it:

Urea	8.25 g
Ampholytes (usual range pH 3-10)	0.75-0.8 mL
30% acrylamide with 1.8% N,N'-methylene bisacrylamide (bis) (recipe No. 1)	1.6-2 mL
Glass distilled H_2O	6.4-6.0 mL

Dissolution, which is an endothermic process with urea, is aided by warming the flask and its contents in warm water because the solution becomes cold on mixing and heating will bring the solution back up to room temperature. Do not overheat! Degas the solution briefly on the lyophilizer. If you degas it too long, however, you will find the urea coming out of solution. If this happens, warm the sample slightly until the urea goes back in solution.

Add 0.3 mL NP-40 (nonionic detergent) for all samples except urine (add only ~ 2 drops for urine) and stir slowly or swirl to mix. Then use Eppendorf pipettes and add carefully:

50-65 µL 10% ammonium persulfate (catalyst)

5-10 µL TEMED (N,N-tetraethylene methylene diamine) (accelerator)

The above amounts are given as a range to allow for slight variations between batches of ammonium persulfate and TEMED or for the temperature variations of the room. The polymerization proceeds faster as the temperature of the room increases. In a hot laboratory, use less TEMED. Mix the solution carefully, then pour it into the clear lucite gel-loading trough. Position the trough on the base of the removable support piece, and place the upper chamber with the metal gel retainer with the glass tubes extending into the acrylamide. Water may be carefully layered in the trough to bring the fluid level up to the very top. Then lower the entire assembly carefully into the large vessel containing an appropriate volume of water (load mark) to allow the acrylamide to rise evenly by displacement to the desired level (6.5 inches) in all tubes. Allow the gels to polymerize for at least 1 hour. The isoelectric focusing apparatus is shown in Figure 1.

Prefocusing:

Remove the entire assembly from the large buffer box, then lift the upper buffer chamber so that the glass tubes and the metal gel retainer come out of the gel-casting trough. The lower buffer box holds 2 L. Empty the water and replace it with 200 mL of 0.85% phosphoric acid (marked 10X). The 200 mL mark

on the box provides a sufficiently accurate measure. Then add glass distilled water to the 2 L mark. With a fresh razor blade, cut between the end of the tubes and the metal gel retainer, then remove the retainer and rinse the outside of the tubes. Place the excess acrylamide gel in the trash can and not in the sink. Then reinsert the upper chamber with attached tubes into the box containing the acid solution.

The upper electrode solution is prepared as follows: 200 mL of glass distilled water is placed in a 600 mL Virtis lyophilization flask and degassed using the lyophilizer. (The flask should be swirled to help outgassing.) To this water add 0.4 mL of 10N NaOH, mix, and pour the mixture into the upper chamber. Remove the air bubbles in each tube with a Hamilton syringe containing the buffer in the upper electrode chamber or with a 1 mL syringe fitted with a 2-inch 22-gauge needle. Be careful not to disturb the top of each gel.

Prefocus the ISO setup for ~ 1 hour at 200 volts.

Isoelectric focusing:

Add up to 30 µL of sample to each tube. Keep a record of actual sample numbers (such as 121, 122, 123, etc.) on a master protocol sheet such as that illustrated in Figure 2 because tubes read only from 1 to 20. For some samples, especially those that are radiolabeled, add 2 µL of the creatine kinase (CK) charge standards mix (recipe No. 21) to each tube above the sample.

For most samples, run the setup at an appropriate voltage overnight until you have reached ~ 14,000 volt-hours. For example, run the setup 17.5 hours at 800 volts. Some samples, such as plasma or amniotic fluid, may require a different run (~ 10,000 volt-hours). It is best to do a time study to determine optimum running time when you are running a new kind of sample.

ISO-gel recovery:

Turn down the voltage, then turn off the power supply. Remove jacks from the electrodes and remove the ISO tube holder. Drain the upper buffer solution into the sink and place the ISO tube holder on the unloading rack.

Use a cut-off yellow Eppendorf tip on a 1 mL syringe to expel the gels slowly with pumped water (or by manual pressure) into numbered vials containing equilibration buffer (3-5 mL in each tube) (recipe No. 8). If necessary, loosen the top of the gels by carefully moving the needle of an empty Hamilton syringe around the top edge.

Rock the tubes gently in the buffer for ~ 15-30 minutes. Then either apply the ISO gels to DALT slabs or freeze them at -70°C, as the proteins will diffuse if the gels sit too long at room temperature. Frozen gels give the same results as fresh gels, but they are thinner and somewhat more fragile to

handle. If we are going to load and run the ISOs on DALT plates within a day or two, we sometimes store the ISOs in a -20°C freezer. Otherwise we store them at -70°C.

THE DALT SYSTEM

Gel casting:

The casting of the gels for the second dimension electrophoresis is an operation that requires planning and care. (The idea is to cast 10 or 23 gradient gels at a time and to do so reproducibly). The apparatus is shown in Figure 3.

The slab gel holders are made from 7 inch glass squares, held together by strips of red silicone rubber fastened along one edge. WE MAKE THESE so be as careful as you can with them. The 1.5 mm spacers are glued in position. Note any defective holders and set them aside for repair.

From the rack on the sink you will need (a) a bottom plate that has an angle cut at the edge for the caster box, (b) a plastic air bag for the back of the box, and (c) a front plate.

Stack either 10 or 23 plates into the gel casting apparatus (depending on the box size) with the red strips to the right and vertical. (We pour 23 plates for a 20 sample run to allow for a few bad slabs with air bubbles or for rounded gel edges.) Prepare your gel serial numbers (typed with carbon typing ribbon on No. 1 filter paper) for the plates. Numbers should be cut and placed in order in front of gel casting box before you begin to pour.

Checklist for DALT procedure:

1. Be sure the entire gel casting system is clean, dry, and free from any polymerized acrylamide. Under our running conditions, most polymerized acrylamide from a previous run will be blue if it is past the entry port for the displacing solution, which is 35% sucrose dyed with a trace of bromophenol blue or methylene blue. You may use 50% glycerol as your displacing fluid but glycerol is much more expensive than sucrose.

2. Run blue displacing solution to the T in the feed line and CLAMP.

3. Shut off the vacuum line and make sure the clamp is closed.

4. Be sure that the gradient-maker lines are clamped off, with one clamp on the heavy (20%) line just as it leaves the gradient maker and the other on the main line just beyond the mixer.

5. Set gel casting chamber to a 45° angle counterclockwise from vertical.

6. The acrylamide solutions can be made up from the stock mixes as follows: Degas each solution after the acrylamide and buffer L (recipe Nos. 6 & 7) are mixed. (Note: Amounts of persulfate and TEMED

required may vary with different batches of acrylamide or the temperature of the laboratory.

	27% Acryl 0.8% Bis (mL)	Buffer L10 (recipe No. 6) (mL)	Buffer L20 (recipe No. 7) (mL)	10% SDS (mL)	Amm. Persulf. (recipe No. 3) (mL)	TEMED (µL)
FOR 10 PLATES:						
9%	80	160	---	2.4	4	70
18%	240	---	120	3.6	2	10
FOR 23 PLATES:						
9%	192	384	---	5.7	6	70
18%	440	---	220	6.6	3.5	10

Mix each solution as the ammonium persulfate and TEMED are added. You will have about 15 minutes to make the plates before the gels begin to solidify at the top.

To make 10 plates (actually room for 11 plates plus air bag):

7. Pour in the light (9%) solution up to about the 21 mark on the gradient maker. Open the line beyond the mixer and let the solution run down to fill the mixer. Then reclamp.

8. Add the heavy (18%) solution to about the 21 mark. Carefully open the clamp on the line from the chamber and let the heavy solution run to the T. Then bleed out any air bubbles in the line.

To make 20 plates (actually room for 23 plates plus air bag):

7a. Pour all the light (9%) solution into the right side of the gradient maker. Open the line beyond the mixer to fill the tubes and mixer up to the gel casting box so that the light solution level is about at the 27 mark.

8a. Add the heavy (18%) solution to just below the 27 mark, taking care not to overfill (the foam settles down to be liquid). You probably will not use all the 18% solution. Bleed out the air bubbles in the line and remove the clamp on the 18% line and the clamp above the mixer to allow solution to run into the "funnel" formed by the V in the gel holder.

9. Watch level in gel holders and slowly rotate gel caster to vertical as filling is completed. Then begin adding labels. Move quickly. Use a level to make sure plates are completely level.

10. Clamp the line between vacuum and blue sucrose inlets.

11. Open the line with the blue displacing fluid and begin adding the blue fluid almost all the way to the bottom of the glass plates.

12. While the blue fluid is flowing in, open the vacuum line, being sure the water suction is on.

13. Add water to both chambers of the gradient maker, making sure you get about 2 L through the system at this stage.

14. Keep your eye on the blue displacing fluid level.

15. While the displacing fluid is reaching the appropriate level, continue adding labels to the lower right corner of each plate, then immediately (and very gently) add ~ 1 mL of water-saturated sec-butanol to each holder. This solution may be added with a pipette or a misting squirt bottle. (The Photoflo overlay [recipe No. 13b] instead of the sec butanol may be used if acrylamide recipe No. 2b is used; this is our preferred method.) Use of Photoflo avoids the unpleasant fumes of the alcohol. It is very important to adjust the displacing fluid container up or down on the ring stand so that the blue level remains just below the bottom of the plates without clamping the line. This placement allows slightly more displacing fluid to be drawn in as the gels polymerize.

16. Continue flushing ~ 2 L more water through the gradient maker and tubing. Drain the system and drain the mechanical mixer by inverting it (as the outlet tubes are at the top of the mixer). If you are using the nonmechanical mixer, you do not need to invert it.

17. Allow gels to polymerize for at least 1 hour. It is important for the polymerization to proceed from the top down, or the plates will contain swirls or lines.

18. Carefully disassemble the apparatus, using a razor blade to separate the plates. Wash the plates carefully with warm water, rinse the top surface of the gel with tap distilled H_2O, and stack the plates in the dish rack sideways (with the rubber hinges up) to drain excess water away from the surface of the gel. Examine the plates at this time for air spaces. Discard any that contain air (looks like Christmas tree branches) and replace each defective plate with one of the three extra plates. If all plates are good, put the extras in buffer L diluted 1:4 at 4°C to use for an odd run or when more than three plates are bad in a single batch

(which rarely happens). Also it is possible to store extra plates by wrapping them in plastic wrap and putting them with a wet paper towel in a sealed plastic stain box.

19. Load ISO gels on to the DALT plates as soon as possible because the acrylamide tends to dry out and form air bubbles at the bottom of the plates.

Addition of a stacking gel:

Most of the time a stacking gel is not necessary, as the ISO gel acts as its own stacker. However, on rare occasions, a stacker might be desired on top of the running gel. If so, proceed through the following steps 1-5.

1. After the gels have polymerized, add excess running or separation gel buffer to each holder and rock the apparatus back and forth to rinse the top of the gel. Then pour off all supernatant fluid into the enamel tray. Blot the excess on top.

2. For 10 plates, add 3 mL of the stacking gel preparation to each plate:

Stacking gel mix (recipe No. 15)	40 mL
10% ammonium persulfate	800 µL
TEMED	30 µL

For 20 plates, double the stacking gel mix and ammonium persulfate but use only 50 µL TEMED.

3. Use 1.0 mL water-saturated sec-butanol or 0.1% Photoflo and gently overlay it on the plates.

4. Allow 15-20 minutes for polymerization.

5. Pour off the overlay and proceed to step 19 above.

Loading DALT gel plates with the ISO gels:

The ISO samples, if they are frozen, should be removed from the freezer about 15 minutes before you are ready to disassemble the plates.

Pour the first sample onto the sieve (plastic tea strainer), letting excess equilibration fluid drain through.

Then pour out the gel cylinder onto the top of the loading lecturn. Position the ISO gel so that the enlarged SDS "bulb" is at the left (acid) side. There will be no bulb if the samples were prepared in urea, so orient the ISO gel with the razor rough-cut end to the left (acid) side and the smooth tapered end to the right (basic) side.

Place DALT gel in position on front of lecturn with the red rubber strip to the right and the label in the lower right corner. Add a few drops of buffer L10 diluted 2:3 with tap-distilled water from a squirt bottle and roll the ISO gel into position in the DALT gel holder. Smooth the gel into position with a spatula and eliminate any air bubbles. Turn the plate upside down on a towel to drain off any excess fluid before overlaying the gel with agarose (recipe No. 11)

Overlay the gel with ~ 0.5 mL melted running buffer agarose. The agarose is stored in the freezer in 100 mL portions and can be liquefied in the microwave oven, but be sure the cap of the flask is loose or off during microwaving! Solutions often superheat when they are microwaved so that they boil over when disturbed or when a pipette is added. Caution should be taken to direct the flask away from you when you start to use the agarose. Make sure that there are no bubbles in the agarose on your plates! Place the gel holder back in the rack with the sample in a horizontal position.

Repeat this procedure for each of the samples.

Running the DALT electrophoresis system:

The refrigeration units and buffer pump circulators remain on at all times to keep the tanks chilled and to reduce possible bacterial growth.

The old-style DALT tank holds 36.7 L of buffer (recipe No. 9) made with tap-distilled water. The newer 20-gel convertible tank contains 26 L of buffer (recipe No. 9), and the new ENI 10-place tank contains 6.5 L.

Note on the tank label when you change the tank buffer. Formerly, we used each tank for 1-3 runs, but we now change the buffer in our tanks before every run and find that we get better results with fresh buffer. Occasionally, for preliminary results, we will use the tank a second time before changing the buffer, but when we are planning to silver stain the gels we will always use fresh buffer. When a tank is not going to be used for several days, we pump out the buffer and rinse the tank with distilled water, allowing the tank to sit empty. This procedure will eliminate bacterial growth in the old buffer.

Insert holders carefully with ISO gel (blue sample) along the left side and with the red rubber along the bottom using a slight leftwise sliding motion so that gasket flaps open to left. Slide holders firmly to the bottom. It will help to dip the plate in the tank first, as it will slide down into place better when it is wet. Be careful, as the plates slip easily; use both hands. Note plates being loaded into the tank in Figure 4. Note the buffer level after the plates are in position; the level should be even with the spacer of the plate and not above the top of the plates.

Close the lid on the tank. Attach the electrodes to make proper electrical contact with the power supply. Turn on the power supply and run

the system until the blue tracking dye reaches the right side of the gel holder (actually the bottom of the gel). We now run the tanks slowly at ~ 100-150 volts (and < 0.6 amperes) for a convenient overnight run of about 16 hours. We have found that we get better spot patterns by running the tanks slowly so the tank stays completely chilled (< 10°C). Most of our runs are now done this way, so our samples are removed the following morning.

When you are ready to remove the samples, TURN OFF CURRENT. The interlock should interrupt the current but turn off the power supply anyway so that there is not a large surge of power the next time the power supply is used.

Remove plates carefully, one at a time, using fingers, rubber gloves, or the paraplate remover. The plates will be very slippery.

Place holders on the unloading lecturn and pry open the plates with a screwdriver or the DALT opener tool. Use a razor blade to free the gel alongside the spacers.

Peel the gel from the glass carefully and place it in the stain solution. In case of a rip in the gel, remove the ripped section last; work toward the rip. Remove the 20% end of the gel first, it possible, because it is less fragile to handle. Gels to be silver stained should be put directly into the fixative solution (see page 23). Silver stained gels will show fingerprints, so wear gloves when handling these particular gels.

Cleaning the plates:

Soak the used plates in distilled water with a small amount of acetic acid added (~ 1%) until you can wash them. The acetic acid cuts down on bacterial growth. Do not use detergent, as the used plates already have detergent (SDS) on them. Rinse the plates thoroughly in warm water, going over all surfaces with a teflon "Tuffy;" finally rinse the plates with tap distilled water and air dry them in the open position in our drying rack. Store the plates in an enclosed cabinet to keep them free from dust.

Staining with Coomassie Blue:

Put 10 gels into 1 L of stain, which is run into the mixing chamber to the 1 L mark, and open the hemostat to allow the stain to drain into a plastic staining box. The stain solution is made up of ~ 0.2% Coomassie Blue in phosphoric acid and ethanol (recipe No. 16). We no longer use an acetic acid-ethanol stain. We stopped using acetic acid-ethanol stain because two parts of the stain had to be mixed at the last minute. By using the Coomassie Blue stain, we have reduced the number of solutions used and eliminated unpleasant lab fumes, while still achieving the same results. The gels are stained for at least 6 hours (or overnight) on the reciprocal shaker. Then they are destained about four times in 20% ethanol/H_2O. If the gels are removed in the morning after an overnight run, we stain all day and begin destaining in 20%

ethanol in late afternoon, leaving the gels in 20% ethanol overnight. The next morning, we continue destaining in 20% ethanol until the backgrounds are clear, and then put the gels into tap distilled water about 30 minutes before we photograph them. This procedure usually gives excellent results, as the background of the gels will be clear. We store the gels in 10% ethanol if we want to save them, as the gels often become moldy if stored in tap distilled water for an extended time.

For autoradiography, the gels do not need to be completely destained, so two to three changes of destaining solution are enough.

To speed up the destaining process, we sometimes put a piece of undyed raw wool (from wool socks) into the box of destaining solution. The wool takes up the dye; thus, one overnight destaining treatment is ample before the gels are dried for autoradiography.

PHOTOGRAPHIC TECHNIQUES

Photography of gels:

1. Turn on both light boxes. Place a slab gel on the translucent glass plate containing the photographic step wedge at the top. Orient the slab so the top of the gel is against the step wedge and the number is in the lower right corner. Place a piece of Scotch mending tape at the bottom of the gel and write the number clearly with a felt marking pen, as the small number in the slab does not always show up on the negative. Be sure to remove all air bubbles carefully by pushing them out from under the gel when you slide your wet finger over the top of the gel because bubbles show up as gray spots on your pictures.

2. Focus the camera by using a hand magnifier to focus the numbers on the step wedge when the camera setting is on "B" with the shutter open.

3. Place the gel on the glass plate, oriented so the Kodak step wedge is at the top of the gel. Place the glass plate containing the gel slab between marks on the light box so that the illuminated area encompasses the gel and step wedge.

4. The film we use is a special high-contrast 4 x 5 inch film CPP (Kodak Contrast Process Pan catalogue no. 168 7755). Photographs are taken for 1 second on setting f16 or f11, through an interference filter (550 nm narrow band; Baird-Atomic, Inc.). For densitometry or for photographing autoradiographs for publication, we use Plus-X film (catalogue no. 144 3167), which is a lower-contrast film.

5. The 4 x 5 inch film holders each contain two sheets of film. The dark slides (which protect the film from light) have a silver side and a black side. When the silver side is out, the holder is loaded and the film is unexposed. Insert the film holder in the spring-loaded track on top of the camera. With the unexposed film down, remove the lower dark slide, and turn it over. Take the picture and reinsert the dark slide. The black edge is now showing, indicating that the film is exposed. Remove the film holder and turn it over to expose the other side. Then remove the film holder and insert a new one on the camera.

6. Continue taking pictures until all 10 gels in the box have been photographed. You will need five film holders. As you finish each gel, put it with the "finished" gels into a separate box of water.

7. Photograph the second 10 gels of your experiment.

8. When you are finished photographing, close the top of the camera to keep out the dust.

Development by using the Kodak Versamat Film Processor Model 5A-N:

1. In the main photo lab, remove the top cover of the Versamat and take the flat evaporation cover off the developer tank next to the wall. Rinse the cover at the sink. Put the two sets of rollers in the developer tank by first turning them right side up (they have been draining upside down at the sink overnight). Position them so that the gears on the rollers mesh with the gears on the left side of the machine and the set with black rubber rollers (marked Dev #1) is next to the wall. Replace the top cover.

2. Start the Versamat by going in the inner dark room and turning on the appropriate water valve and flipping the control switch to "operate." Check to make sure that the speed is set on 1 3/4-2 feet/second. Press the red pump test switch, which starts the replenisher pump to replace any developer lost over the inactive period, and fill the replenisher tubing with solution. It takes about 20 minutes for the Versamat to warm up, so it is a good idea to turn it on before you begin photographing your gels.

3. Take two Kodak Roller Transport Cleanup 4955 sheets from the box and cut them lengthwise so you will have four long narrow sheets to feed through the machine. Put the first sheet into the machine and feel the rollers pulling it out of your hands. When the bell sounds, feed the second one in, etc.

4. It is probably a good idea to take a test exposure and check the optical density (O.D.) of the film before sending all the film through at perhaps a bad setting. Therefore, it is good practice to take a picture of your first gel at 1 second and 0.5 second and send the film through the machine. When it comes out, check the O.D. on the densitometer. The most desirable O.D. for the background is between 1.0 and 1.4 (optimum for printing is ~ 1.2). Adjust the speed of the Versamat if necessary to obtain the correct O.D. Otherwise, you can change the f stop on the camera accordingly.

5. To develop the film, go into the inner dark room with your exposed film in the film holders. Shut the outer door and the inner black curtain. Your box of film (CPP) is on the shelf at your right as you face the Versamat. Stack your film holders there and be seated facing the Versamat.

6. Turn off the main light by the switch on the wall at your right. In total darkness (no safe light), remove one dark slide with your left hand and take the piece of film out with your right hand.

7. Turn over the piece of film so the emulsion side is down, and feed it into the Versamat so that the rollers will "grab" it from your hand. A "ready" bell will ring in about 15 seconds.

8. Take another exposed film holder and remove the film and feed it into the Versamat after the bell has rung. It is best to feed the film alternately between the right and left sides of the feed tray so that the rollers of the machine will have even wear. If you are inexperienced in photography, do not attempt to reload cassettes as you are feeding them into the machine, as we have had problems with scratched film and improperly loaded cassettes.

9. You may reload film holders with fresh film as you are developing only after you have had a lot of practice loading film. To reload the holders, open the box of film on the shelf and remove two boxes. Then take out the stiff envelope and tear off the end (if it is a new package). The film will be protected by a piece of cardboard on each side. Put the top piece of cardboard under the bottom. Orient the film so the notches are at the lower right corner when the 5-inch side is horizontal (4-inch side vertical).

10. Pick up one piece of film at the notched area and slide it into the film cartridge, guiding it into the track, and snap the end in place with your thumb. Make certain that the dark slide is turned so the rough edge (silver) will be facing out and push the slide in until it is completely closed.

11. Turn the cartridge over and remove the dark slide from the second piece of film. If the "ready" bell has sounded, turn the second piece of exposed film over and feed it into the Versamat.

12. Reload a new piece of film into the film holder, making sure the dark slide is turned over. At this stage, the film holder should contain two new pieces of unexposed film, and the rough edges should be facing out. Place the completed film holder on your lap or on the shelf behind you.

13. Continue feeding exposed film into the machine and reloading unexposed film until you have finished.

14. Put the unused film away by placing the pieces of cardboard around it and slipping it back into the envelope. Close the box, making sure all three parts are properly closed.

15. You may turn on the room light after the bell has rung to indicate that another piece of film may be introduced. After about 11 minutes, the finished negatives will drop down in the tray at the end of the machine.

16. Remove the finished negatives from the collection tray, place them in glassine envelopes, and mark the numbers on the upper right corners of the envelopes so that they can be filed easily.

17. If you plan to use the machine again during the day, simply turn the switch from "operate" to "standby" so the rollers will not be turning. If you do not plan to use the machine again, turn the switch to "off" and shut off the water supply.

18. Again remove the top cover of the Versamat, lift out one set of rollers at a time from the developer tank, and place them in the splash tray provided in the sink. At the sink, rinse off the rollers thoroughly with warm water, and lightly scour the rollers with Scotchbrite to remove any deposited silver. Rinse the rollers thoroughly and turn them upside down to drain on a towel at the sink, or keep them immersed in a tank of water.

19. Replace the flat evaporation cover on the developer tank. Replace the top cover on the Versamat.

Printing with the Kodak Royalprint Processor Model 417:

1. Printing is done in room C113. Start with the room lights on.

2. Take the top and side pieces off the processor and put aside the clear plastic door support strip, which holds the lid up overnight. Close the water drain valve and bring the hose over from the wall faucet. Turn on the valve and fill the water chamber to the fill line, and top off the fixer solution with water only to the appropriate mark. Shut the water off and hang the hose back on the wall. Turn on the refill water faucet on the wall behind the sink.

3. Replace the lid and side cover on the processor; turn on the power switch and flip the second switch from "standby" to "operate."

4. Send two uncut pieces of Kodak Roller Transport Cleanup Sheets 4955 through the machine. These sheets can be put back in the box to be reused unless they appear very streaked or dirty.

5. Turn machine back to "standby" and wait 15-20 minutes for the solutions to warm up to about 25°C.

6. Turn off the room light, turn on the two safe lights, push up on the exhaust fan handle, and turn the processor back to "operate." Turn on the enlarger light ("focus" switch).

7. Snap a negative in the holder with the emulsion side down. The notch will be in the upper right corner. Place the holder up in the enlarger just under the large condenser lenses.

8. Adjust the size of the picture with the appropriate knob, and focus with the other knob by looking through the "Magnisight" at the step wedge numbers with the diaphram wide open. After you focus the enlarger, set the diaphragm stop to f8. Adjust the opening on the enlarger using the light meter so that the reading on the meter is 16 when the photo cell is placed in a "typical" background area on the picture, usually slightly farther than halfway down the gel. If you don't use the light meter, take a few test exposures until you find the setting that gives the best exposure (see direction 12 below).

9. Turn the focus switch to "time" and take a sheet of Kodabrome II 8 x 10 F H (equivalent to #3) paper (for most work) from the paper safe box on the shelf at your right. The lid slides up to reveal three shelves of paper, and H is located on the bottom shelf. The lid will slide back down by itself. Refill paper in the safe box from the stock supply on the shelves. Please do not leave the paper box empty. We also use E H (#4) and U H (#5) paper for very high contrast prints for publication. Sometimes the photographs of the autoradiographs will print better on S (1) or M (2) lower-contrast paper. Set the timer for ~ 15 seconds.

10. Slip the paper into the easel guide with the emulsion (shiny) side up and press the timer button on the right of the control box.

11. When the light goes off, slide the paper out to the right side and start it going through the Royalprint Processor with the shiny side down.

12. For a check on the quality of the first print, lift the front of the processor lid and the print will come out of the machine within 10 seconds, before it is fixed or washed. Look at it in the safe lights and if the print is satisfactory, place it back into the machine with the print side down for final processing. The photographic emulsion responds to exposure in a logarithmic manner. Therefore, if the print is too dark, you may decrease the light by using (a) one-half the previous time or (b) the same time at the next smaller f stop (which is a higher f-stop number).

13. Continue printing all your negatives. They take 52 seconds each to be processed through the Royalprint Processor.

14. When you have printed all your negatives, make sure all the paper is put away in the safe box and that the box is closed before you turn on the room lights.

15. Turn off the printer and remove the top and side. Open the water drain and allow all the water to flow out to prevent growth of algae in the tank. Squirt the gears with water from the water bottle on the shelf to remove encrusted fixer.

16. Replace top of the printer, but leave the lid propped open with the clear plastic door support. Turn off the water behind the sink.

17. The Royalprint Processor functions best when it is maintained properly. We dispose of all solutions and thoroughly clean the machine about every 2 weeks, depending on the amount of use.

AUTORADIOGRAPHY

In many instances, samples must be radioactively labeled to increase the sensitivity of the two-dimensional analysis, as mentioned in the section describing the ISO system. When it is possible to label cell samples, ^{35}S, ^{14}C, or ^{32}P are preferred to ^{3}H because they can be detected more efficiently. The two-dimensional ISO-DALT procedure for labeled samples is the same as for unlabeled samples. Gels are stained in the Coomassie Blue stain and destained through the first three steps as described earlier. Next, the gels are soaked in 2% glycerol for 30 minutes (this solution is changed once). The gels are then ready to be dried. (The glycerol solution prevents the gels from cracking badly when they are dried.)

Operation of the gel dryer:

1. Remove all water from the defrosted lyophilizer and plug the drainage tubing.

2. Turn on the lyophilizer's refrigeration unit, and allow it to cool the apparatus to below -40°C (~ 15 min).

3. Make sure all vacuum ports are closed.

4. Turn on the Virtis vacuum switch and then flip the kick switch on the large vacuum pump. Check to ensure that the lyophilizer is pumping a vacuum.

5. Clean all pieces of dried gel off of the metal screen and the silicone rubber cover with a damp cloth. Center the bottom permanent filter paper over the screen of the drying plate.

6. Place four gels upside down (number facing down) on the Mylar sheet. Place fresh filter paper over the gels so the edges of the Mylar and the filter paper match.

7. Flip the "gel sandwich" over and place it directly on top of the reusable filter paper on the drying plate.

8. Cover the gels with the silicone rubber sheet and open the appropriate vacuum port. Check to be sure that there are no leaks and that a vacuum is being pulled. Because the silicone rubber attracts dust, which causes leakage when it is present on the sealing rim, it is very important that the sealing (gel) side of the rubber be cleaned with a damp towel just before each drying run.

10. All 20 gels should be loaded on the dryer as described above.

11. If you want to dry the gels in 2-3 hours, turn the main heater switch to a setting of 40 and flip the switches for the drying plates being used. However, if you dry the gels overnight, no heat is necessary. Excessive amounts of heat or prolonged exposure to heat cause the dried gels to crack.

Unloading the gel dryer:

1. First, release the vacuum on the lyophilizer by opening the right front port, then turn off the vacuum switch on the lyophilizer and turn off the vacuum pump.

   Never turn off the pump before releasing the vacuum. The result of such a mistake is oil everywhere!

2. Turn off the lyophilizer refrigeration.

3. Open the drainage tube and insert it into the waste bottle.

4. Remove the dried gels.

Loading gels on film:

1. Cut the four dried gels from each tray apart and label each with the appropriate number in radioactive ink (^{14}C) found in the X-Omat/Versamat darkroom. The ink is made by adding 10 µCi ^{14}C-glucose/100 mL ink. The specific activity of the glucose is 6 µCi/g.

2. Insert the dried gel between the film and the loose paper of the ready packs (XAR-2 - Catalogue No. 165 1579). Five to ten ready-pack films can be placed in each yellow Kodak exposure holder.

3. Place each exposure holder in an empty slot of the gel squasher and open the valve attached to its air bag. Exposure holder and contents should be squashed flat.

4. Record appropriate information in the log book (date, time, sample, and initials).

5. Autoradiograph gels for 1-3 weeks depending upon the isotope label used and the cpm in the sample.

Developing the film:

1. Develop the autoradiographs in the X-Omat model M6AW. Turn the machine on at the main power switch in the small dark room (left hand side of the machine).

2. Close the water drain and turn on the water flow (large red valve).

3. Check the fixer and developer replenishing bins.

4. Close the outside tank cover lid.

5. Allow machine to warm up for ~ 30 minutes (until developer temperature reads 35°C).

6. Activate the transport by pressing the "standby" switch at the left and underneath the feed tray. Wipe off the feed tray.

7. Feed two uncut sheets of Kodak Roller Transport Cleanup Sheets 4955 through the machine to clean the rollers. These sheets may be put back in the box to be reused if they are not streaked.

8. Turn on the inner darkroom exhaust fan switch, located on the wall to the right and above the outer darkroom sink.

9. Turn on the green safe light, which can stay on during the entire process. Turn off the room light. Press the "standby" switch to start the rollers. Start the film through the machine. Two films can be fed through the machine side by side (8-inch edge first) to save wear on the rollers. Make sure that the films do not touch.

10. Feed the next films through the machine when the ready bell sounds. Continue until all the film has been fed through the machine and wait until the next ready bell sounds before you turn the room light on. Turn off the safe light.

11. After the film has been processed, turn off the main switch and the red water valve. Open the valve cover in the dark room and drain the water from the machine.

12. Open the top cover in the main room and take out the two center sections of rollers covered with a plastic top. Wash the rollers and lightly scrub them with Scotchbrite. Rinse thoroughly.

13. Replace the rollers and the plastic cover. Put the cover back on the machine, but leave it open about 2 inches to allow air circulation when the machine is not being used.

14. If you intend to save your dried gels for possible future use, put them tightly into a file drawer so they do not curl and crack.

FLUOROGRAPHY

This procedure is used when the sample has been radioactively labeled with ^{3}H, ^{14}C, or low levels of ^{35}S.

1. Run gels as usual and destain them, draining off final destaining solution completely.

2. Put gels (five to a box, maximum) into dimethyl sulfoxide (DMSO I) at 250 mL/gel and rock the apparatus for 30 minutes. Return the DMSO I to the original DMSO I container. DMSO I should be labeled "waste" after the third use.

3. Put gels into DMSO II (250 mL/gel) and rock the apparatus for 30 minutes. Return the DMSO II to the original container. DMSO II should be labeled "DMSO I" after the third use.

4. Put gels in 13% 2,5-diphenyloxazole (PPO) made up in DMSO. Rock the apparatus for 3 hours. Return PPO solution to the original container. This solution can be used eight times. After that the PPO must be recovered and the DMSO put into the organic chemical waste container. The PPO must not be put down the drain because it precipitates with water.

5. Put the gels into distilled water (250 mL/gel) and rock them for 15 minutes.

6. Change the water and rock the gels again for 15 minutes.

7. Discard water. Put gels in 2% glycerol (250 mL/gel). Rock them for 15 minutes.

8. Change glycerol and rock the gels an additional 15 minutes.

9. Dry gels on the gel dryer for either 2-3 hours with heat (rheostat setting of 40) or overnight without heat.

10. Put gels on XAR-2 film that has been flashed (exposed to a quick flash of light to expose the film sufficiently to linearize the photographic response). Place gels with films inside black plastic bags, evacuate the bags, and heat seal them. Store sealed bags at -80°C throughout the exposure time. Thaw the gels and develop the film in the X-Omat.

SILVER STAINING

Several methods of silver staining have been tried in this laboratory, with varying degrees of success. A major factor in achieving success is the use of very fresh and pure chemicals and solutions. All water must be glass distilled. The use of tap distilled water will produce a somewhat cloudy background. The DALT tank must be very clean and must have fresh buffer. Keep all solutions covered so that dust does not fall in them. Make sure that there are no fingerprints or dust on the DALT plates. At present we are using the methed described by Guevara et al., Electrophoresis 3, 197-205, 1982, as modified by Sharron Nance.

We get best results if we do not stain our gels first with Coomassie Blue, as the backgrounds will be higher; it is possible, however, to silver stain gels that have previously been stained with Coomassie Blue.

Use gloves for all steps including unloading of DALT plates (do not touch the gels). Shake the gels during each step. Allow 200 mL of solution/each 7 x 7 inch x 1.5 mm gel.

We also use a plastic mallet the same size as the gels with a handle for draining gels so we do not touch them. It is also important to place as little pressure as possible against the mallet while retaining the gels in the staining box when pouring off used solutions. This step should be performed quickly. If excessive pressure is used or if the surface of the gel is allowed to dry in the air, there will be large staining artifacts in the gels.

1. Fixation

   a. Fix freshly run DALT gels (five gels to a box) for > 4 hours in 1 L of either of the following freshly made solutions. We have had success with both. Perhaps you should try both fixatives to see which one works best with your samples.

      1. 50% EtOH/0.1% formaldehyde (1 mL of 37% formaldehyde).

      2. 2.5% sulfosalicylic acid/5 % acetic acid in 20% EtOH. Be sure to clean the top of the ethanol drum before pouring from it.

   b. Drain gels after at least 4 hours of fixing and put them into 20% EtOH overnight.

   c. Rinse the gels the next morning in 20% EtOH $\geq$ 1 hour (3 changes for $\geq$ 20 min each).

NOTE: Some methods specify that gels be fixed in glutaraldehyde but our backgrounds are too high when we fix by this method. Also surface contaminants or dry gel surfaces increase the background.

2. Silver diamine staining

It is very important to make this solution in the following order:

a. 740 mL H_2O

b. 200 mL EtOH

c. 2.35 mL 6N NaOH (or 3.53 mL 4N NaOH)

d. 10.5 mL NH_4OH (add just before use). The stock (NH_4OH concentration is 60% (30% NH_3).

e. Immediately before use, slowly add 5 g $AgNO_3$ dissolved in 50 mL H_2O to the above solution while stirring vigorously. A transient brown precipitate will appear as the $AgNO_3$ is added.

f. Shake gels in this solution for exactly 1 hour.

3. Washing

a. Rinse briefly with distilled H_2O.

b. Wash exactly 1 hr with 20% EtOH (20 minutes per wash, three washes).

4. Development

Mix in order:

a. 800 mL H_2O

b. 200 mL EtOH

c. 100 mg citric acid. Citric acid may be added as a freshly weighed solid, or, if convenient, maintained as a stock 100 mg/mL solution in water.

d. 1.0 mL formaldehyde (add immediately before use). Shake gels in this solution until the low molecular weight spots are developed. Then pour off this solution. The development step is temperature sensitive. A simple rule of thumb for the time it seems to take for the developing process is 50 minutes minus the temperature of the lab. (If the lab temperature is 25°C, 50 - 25 = 25 minutes developing time.) Note: Occasionally a slightly altered concentration of either citric acid or fomaldehyde will give better results.

5. Stop

   Mix 5 mL glacial acetic acid in 1 L water and put the solution on the gels while the gels are shaking; continue shaking them only about a minute. If left too long in this solution, gels will begin to bleach.

6. Wash

   Rinse the gels (3-4 times) with tap distilled water for 45-60 minutes.

7. Shake the gels overnight in 20% EtOH to prepare them for photography. Then store the gels in 20% EtOH (changed daily) until photography is completed. If the background was too dark on the gels, you can shake them overnight in water, and the gels will become lighter.

SILVER STAIN PHOTOGRAPHY - XRD IMAGE INSTRUCTIONS
(This method was developed by Harold H. Harrison and is described in Clin. Chem. **29**(8): 1566-1567, 1983.)

This procedure outlines the method for making positive black and white transparency image transfers from silver-stained gels onto Kodak X-OMat Duplicating Film (DUP, Cat. No. 163 7842). Protein spots come out black in these images regardless of their color in the silver-stained gel. The general procedure assumes a bit of basic photographic knowledge and is, in outline, as follows: The wet two-dimensional slab gels are sandwiched between two thin, transparent supports (we use Kodak Roller Transport Cleaning Sheets) and then placed onto an 8 x 10 inch sheet of X-OMat Duplicating Film. A white or blue light exposure is then made and the film is developed. The result is a positive image transfer in an essentially 1:1 scale "contact print" format. Instructions for producing these films in the large darkroom, C-114, follow.

1. Turn on the RP X-OMat automatic film processor to warm up, as previously outlined on page 20.

2. Set-up the enlarger and projection table.

   a. The Beseler 43M color enlarger is used without color filters and without the projection lens essentially as a tungsten filament white light projection source. If the color filters (slot above bellows) or projection lens are in place, remove them.

   b. Adjust the height of the enlarger head so that the projection lens attachment/safelight filter slide carrier is 50-90 cm above the wooden base table. This height ensures even light distribution over an area centered on the projection grid that is pasted to the base table. There is a spring-loaded toggle switch that controls the elevation motor on the right-hand side of the projection head support frame; the "up" position drives the frame upwards and the "down" position does the opposite. The frame drive motor power is controlled through the exposure timer, so if the drive motor does not go on when you move the toggle switch, flip the rocker switch on the timer to the "focus" position and try the toggle again.

   c. Check the safelight filter slide carrier at the base of the enlarger to be sure that it slides freely and that the RED safe filter is in place.

   d. Check that the enlarger lamp and controls are functioning properly. Set the rocker switch on the enlarger to the "focus" position. The enlarger lamp should turn on. If it does not, check the slide switch that hangs from the right-hand side of the head frame. If neither position of this slide switch turns on the enlarger lamp, check all electrical cord connections and try again. If the lamp still does

not go on, rotate the rheostat (marked "resistrol" with dial scale 0 to 150) to check that this control functions properly. Light intensity should increase with clockwise rotation of the knob. Set the timer switch to the "time" position and activate the "print" button for a brief exposure, e.g. 5 seconds, to ensure proper timer function. Familiarize yourself with the time set control knobs and multiplier switch on the timer; the maximum exposure duration achievable is 99 seconds.

e. Set up a support block for film exposure centered on the target grid of the enlarger table. There is usually a 9 x 9 x 0.5 inch plexiglass block for this purpose already in place under the enlarger. A sheet of glass on top of this plexiglass further allows for easy film loading and removal. Any equivalent set-up is acceptable; the important elements are to provide a centering support for the gel and film and to make easy the loading and removal of film from under the gel after it is in place.

3. Set-up darkroom and safelights.

a. Make sure that there is enough DUP film on hand for the work you plan to do. You should plan to use about three sheets of film per gel. There is an operating supply in the drawer directly beneath the Beseler enlarger and a reserve supply in the two-door cabinet under the right-hand bench in the 60°F cool room adjacent to the darkroom.

b. Turn on the safelights in the inner darkroom (through-cord switch for circular safelight on the shelf) and the outer darkroom (pull-chain switch on the rectangular safelight on the wall next to the transillumination gel photography apparatus).

c. Convert the flat fluorescent view box to a large safelight work table for gel loading and image checking by placing six multicolored transparency assemblies on top of the illumination box in overlapping fashion. These assemblies are stored in a manila file marked "XRD Image Kit" in the drawer below the Beseler enlarger. Be sure that the view box is clean and <u>dry</u> before putting down the filters.

d. Locate the wall switches for the overhead fluorescent room lights and the darkroom-in-use warning light on the wall between the door and the transillumination photo set-up.

e. Locate the switch for the fluorescent transillumination light source on the wall to the left of this set-up. While making the XRD images, you may wish to examine how they will look on the transillumination set-up for subsequent photography. This light is not "safe," so if you do use it, be sure that all undeveloped film is protected before you turn on the switch.

4. Production of XRD images

The materials you will need for this step are (1) an adequate supply of X-OMat DUP film, (2) your silver-stained gels, (3) a box of fresh water into which you will transfer gels after image work, (4) five or six roller transport clean-up sheets (Kodak No. 4955) 8 x 10 inches or 11 x 14 inches, and an indelible felt-tip marking pen. A flashlight may also come in handy for reading the gel number labels. Plan on 2-3 hours work per batch of 20 gels.

a. Run the cleaner sheets through the X-Omat. Change loading locations to ensure coverage of the entire roller width. Inspect the cleaner sheets after they run through the X-OMat. Those that are dirty, smudged, or scratched should be used for roller clean-up only. If the clean-up sheets all come through smudged or streaked, check the X-OMat chemical reserves, tanks, rollers, and temperature. Streaky development may be caused by low levels of chemicals or exhausted chemicals and shows up prominently in subsequent photographs.

b. Select several cleaner sheets that are dry, clear, and without smudges or scratches to use as support sheets to interpose between the wet, stained gels and the DUP film. You may wish to mark the corners with a small "x" to make recognition of the sheet boundary easy during gel placement and handling.

c. Place a clean and DRY clean-up sheet in a convenient place on the safelight work table. Usually an area of the light box where one edge of the cleaner sheet can be easily lifted is a handy place. It is also helpful to use a clean glass support plate for the clean-up sheet to avoid picking up dirt from the filter assemblies and to make wiping up any water spills easier.

d. Carefully pick up a gel by the corners and allow excess water (or storage solvent) to run back into the storage bin.

e. Very carefully center the gel onto the prearranged gel support cleaner sheet. Orient the gel with the number plate in the lower right-hand corner and gently eliminate any air bubbles within the gel frame. IT IS ABSOLUTELY CRITICAL AT THIS STEP TO KEEP THE BOTTOM OF THE GEL SUPPORT SHEET DRY. Any moisture on the bottom of the gel support sheet will result in artifactual film "exposure" and will either generally ruin the image or will create fictitious spots that may be subsequently interpreted as proteins. Be sure to check for this problem before transferring the support sheet to the enlarger table; a drop of water at the edge of the clean-up sheet will spread rapidly on the underside via capillary action. Small amounts of

moisture may be blotted up with an absorbent towel, but all moisture must be thoroughly removed. Cover the gel with another gel support sheet.

f. Dry your hands thoroughly. Wet fingerprints also create problems.

g. Very carefully lift and transfer the gel and support sheet to the enlarger table. The gels tend to slide around on the cleaner sheets; gently flexing the support sheet concave to center can help avoid this sliding. Center the gel on the support block under the enlarger light with the safe filter "on."

h. Carefully slide a sheet of X-OMat DUP film emulsion side up (notch in the lower right hand corner, gray-violet surface up) between the gel support sheet and the support block.

i. If a calibrated photographic step wedge is to be included, insert it now between the gel support sheet and the film.

j. With the safe filter "on" and the timer set to "focus," make sure that the gel and film are centered under the light.

k. Make an exposure by setting the timer to the "time" position, sliding out the safe filter (move all the way to the left with the carrier slide knob), and pushing the "print" button on the timer.

Exposure guide: Typical exposure times are 20-30 seconds at 120-150 W (resistrol setting) and 50-90 cm enlarger height. Factors affecting development are primarily related to gel background stain, but there are also batch-to-batch variances due to X-OMat chemical status and film sensitivities. You will need to run a few test shots for each group of gels.

Run a few test shots of an average gel from your batch. If exposure times of 20, 30, 45, 60, and 90 seconds at a constant enlarger height and resistrol setting vary from underexposed to overexposed, then you are in a good range. If they are consistently under- or overexposed, adjust the exposure intensity accordingly. There are three ways to increase exposure intensity: increase the exposure duration, lower the light source height, or increase the resistrol setting. To decrease exposure intensity, do the opposite. It has worked out best so far to stay within the height and Resistrol boundaries mentioned in the exposure guide above.

1. Immediately after the exposure, feed the film into the X-OMat and compare the developed film image with the original gel to determine if the exposure was satisfactory. Due to gel-to-gel staining

differences, you may need to validate each image individually, rather than rely on a uniform setting as with Coomassie stain photography.

m. On each XRD image record the gel number, the exposure duration, the resistrol setting, and the enlarger height.

n. After you have finished with each gel, transfer the gel to a "finished" water box. Dry the cleaner sheets with a Kay dry towel before the next gels are placed on them. The cleaner sheets tend to pick up some of the silver stain after a number of runs so you should inspect each one before using it again and discard the sheet if it is badly stained.

o. When you have finished, turn off the X-OMat, turn off the safelights, put away the cleaner sheets and filter assemblies, turn the enlarger off, and generally clean up the work area.

5. Handling and further processing of XRD images

a. Duplication: The XRD images may be duplicated on the BXR Mk II Blu-Ray radiograph duplicator by a process similar to that for autoradiograph duplication or by contact duplication underneath the enlarger lamp.

b. Photography: XRD images are somewhat easier to photograph in black and white than the original gels due to better background balance. The set-up used for Coomassie stain photography is used. Plus-X film generally gives a better result than CPP, and no green filter is used.

c. Storage: XRD images are stable positive transparencies and may be handled and filed as regular x-rays or negatives would. If yours are destined for computer analysis, keep them clean and scratch-free in glassine envelopes.

PROTEINS IMMOBILIZED ON NITROCELLULOSE (TRANSFER GELS)

This technique is based on one described in a paper by N. L. Anderson et al. in Electrophoresis 3, 135-142, 1982. Our transfer tanks are modified from our old style DALT tank (40 L); they hold six transfer gels running perpendicular to the front of the tank on two transfer plate assemblies. The transfer assembly consists of two sheets of plastic "egg crate" lattice material (normally used as a diffuser in fluorescent light fixtures) between which a sandwich of foam rubber-nitrocellulose gel-foam rubber is compressed by means of nylon screws. A gel is placed on the cathodic side of each nitrocellulose sheet, which has the number of the gel stamped on it. Current is passed through the gel-nitrocellulose sandwiches by means of the platinum electrodes permanently mounted on the long side walls of the DALT tank. During transfer, the DALT tank is filled with a buffer consisting of 27mM Trizma base and 207.5mM glycine (recipe No. 24). Although the proteins have been denatured in SDS and separated into subunits and run on 2-D gels before being transferred to nitrocellulose, the nitrocellulose-bound molecules still react with appropriate specific antisera even after storage of the transfer in air at room temperature for several months.

1. Wear gloves when handling gels and nitrocellulose paper. Transfer 2-D gel proteins to 6-1/4 x 6-1/4 inch BA-85 nitrocellulose (Schleicher and Schuell) paper in the DALT tank for ~ 500 volt hours (~ 120 volts for 4 hours). Remove the assembly and discard the DALT gels.

2. Block the nitrocellulose transfers with 3% bovine serum albumin (BSA) 10mM Tris/saline, pH 7.4-7.6 (recipe No. 27), for 1 hour at 40°C (or 2 hours at room temperature or overnight at 5°C).

3. Pour off blocking solution and save to dilute antisera.

4. Dilute primary antibody (Ab) in blocking solution (1:100 dilution or greater, depending on Ab) and pour over the transfer. At least 50 mL is required for each transfer for rocking on the shaker. Leave on the shaker for about 1 hour, depending on the strength of the Ab.

5. Pour off Ab. Save and freeze at -20°C for future use, if desired, and rinse transfer five times with saline to wash off all traces of unbound Ab.

6. Add 1:000 anti-IgG-peroxidase (Miles) diluted in blocking solution. For example, if the primary Ab was rabbit anti-human, then use goat anti-rabbit IgG-peroxidase. Concentrated solutions of these conjugates are in the -20°C freezer. One microfuge tube containing 250 mL of a 1:2.5 dilution will make 100 mL. Rock the transfer in this solution for 1 hour. Rinse five times with saline.

7. Staining solution for 2-3 transfers:

   Prepare: 60 mg of 4-chloro-1-napthol (peroxidase reagent) in 20 mL cold methanol (dark and fresh).

   Prepare: 60 µL 30% H_2O_2 in 100 mL cold 20mM Tris/saline, pH 7.5.

   Mix the above solutions at room temperature and use immediately. Do not freeze the mixture, as a precipitate will form. Pour the mixture over the transfer and rock it until staining is adequate (blue spots with white or light blue background); pour stain solution onto next gel and rinse stained gel thoroughly with water.

8. Photograph the wet transfer gels using the 550 nm filter and reflected light from the two floodlights. Use about a 0.5 second exposure with f16 opening and the CPP film we use for photographing DALT gels. The darker the transfer background, the longer the exposure you will need. The stained transfer may be air dried and stored indefinitely; however, the stained spots fade with time.

RUNNING AN ACIDO (MAUP) SETUP

Urine contains a very acid protein, which always piles up at or goes off of the left end of an ISO gel. This most acid urinary protein (MAUP), can be resolved through an ACIDO run (as distinguished from an ISO or BASO run). α-1-Acid glycoprotein also runs nicely on an ACIDO. Only the conditions that differ from an ISO run will be cited here.

ACIDO recipe for setting up the first-dimension run:

8.25 g urea
2.0 mL 30% stock acrylamide
1 mL 2.5-4 ampholyte (LKB)
0.3 mL 2-11 ampholyte (Serva)
5.5 mL H_2O

Degas and add:

0.3 mL NP40
90 µL 10% ammonium persulfate
10 µL TEMED

Allow the gel setup to polymerize 1 hour. Prepare lower chamber buffer with 3 mL concentrated H_2SO_4 added to 2 L H_2O. Make the upper buffer by degassing 40 mL H_2O and adding 1 mL 2-11 ampholyte and mixing. Debubble the solution carefully, as the top buffer barely covers the tubes. We use this very small volume because the ampholyte is expensive. Prefocus the setup for 1 hour and load ~ 20 µL sample. Run the set up for ~ 3600-4000 volt-hours. You can run this off in one day, or else run at 200 volts for 18-19 hours overnight. At the top of the tubes you will find a "stacked" white material, which consists of the more basic proteins that never enter the gel. This material will wash out when the gels are unloaded. Run these ACIDO gels on normal DALT plates or use a 7-14% gradient if you wish to further spread out the high-molecular-weight proteins.

RUNNING A BASO SETUP

Basic proteins always streak off the right side of the gel. Therefore, we include this section on running a BASO under nonequilibrium conditions. This technique is based upon the methods worked out by modifying the O'Farrell NEPHGE system (Willard et al. in Anal. Biochem. 100, 289, 1979).

The first-dimension gels are cast in the ISO apparatus using the same recipes as for running ISOs except the ampholyte used is Servalyt 2-11. After the gels have polymerized, the lower tank reservoir is filled with 2 L of degassed 20mM NaOH. BASO gels are not prefocused, and the samples are applied with a Hamilton syringe directly to the upper gel surface. To protect the proteins from the acid used in the upper reservoir, samples may be overlaid with 4M urea (degassed), filling the remainder of the focusing gel tube. The upper reservoir is then filled with 250 mL of 10mM H_3PO_4. For running BASO gels on proteins from seeds, it is not necessary to overlay the sample with urea. The upper buffer (H_3PO_4) is simply injected into each tube to debubble the tube before the sample is loaded.

Note that the lower reservoir now contains the catholyte and the upper reservoir the anolyte, the reverse of the ISO system. The electrode wires are also reversed (the black wire goes to the red electrode, etc.), and the focusing is started at 400 volts for 1 hour and then turned up to 800 volts for 4-5 hours, stopping the run at ~ 4000 volt-hours for most samples. It is still advisable to do a time study because we recently found that some of our samples need a longer time. The gels are extruded as usual and then loaded on DALT plates "backwards," with the jagged end to the right and the smooth tapered end to the left. In this run the smooth tapered end is the acid end, so the gels will still finally be oriented the same way as ISO gels, with the acid end to the left. This orientation will facilitate the preparation of a composite map at the end of an ISO and a BASO run by matching the overlapping spots that are common to both patterns.

AMPHOLYTE EVALUATION
20 Tube ISO Recipe

This section of ampholyte evaluation is included to give some idea of an approach to use for checking different ampholyte mixtures before proceeding in depth into research on your samples. There is no one perfect ampholyte that produces perfect results for all kinds of samples. We have modified an ISO apparatus, so that there are two rows of 10 ISO tubes, each spaced to allow a plastic centrifuge tube to fit over the bottom of each two ISO tubes. Thus, each of the 10 centrifuge tubes can contain a different ampholyte mixture.

1. Set up apparatus and tubes.

2. Mix in lyophilization or sidearm flask

   22.0 g urea
   16.8 mL glass distilled water
    4.6 mL 30% acrylamide/1.8% bis

3. Warm flask to room temperature to dissolve urea; swirl to mix.

4. DEGAS.

5. Add 0.8 mL NP-40, while swirling.

6. Aliquot 3.5 mL into each of the 10 mixing tubes.

7. Add 188 µL ampholyte mixture per tube, using one type of ampholyte or a mixture of more than one brand, or a narrow-range plus a wide-range ampholyte, etc.

8. Add 11.7 µL ammonium persulfate per tube and mix.

9. Add 3.0 µL TEMED dilution.*

10. Immediately mix by inversion and pour into the tubes attached to ISO apparatus.

11. Place ISO apparatus on electrode strand.

12. Carefully overlay acylamide solutions with glass-distilled water until each tube is filled.

*In microfuge tube place 30 µL glass-distilled water.
Add 15 µL TEMED directly into center of water (not on sides of tube).
Mix.

13. Gently lower entire apparatus into ISO chamber filled with tap-distilled water.

14. Polymerize for 1.5 hour.

15. Remove tubes, clean and prefocus as usual.

16. Load samples. Preferably load the same samples onto each different ampholyte mix so that you can compare results. For example, if we pour 10 mixtures of ampholyte into our 20 tubes, we load only two samples, such as a body fluid sample (plasma) plus a tissue sample. Thus, we can compare 10 examples of plasma and 10 examples of a tissue with 10 different ampholytes.

RECIPES FOR ISO-DALT SOLUTIONS*

1. 30% acrylamide with 1.8% bis (for ISO setup)

    30 g acrylamide (Bio-Rad)

    1.8 g bis

    Bring volume up to 100 mL. Filter. Store in refrigerator.

2a. 30% acrylamide with 0.8% N,N'-methylene bisacrylamide (bis) (for DALT plates)

    300 g acrylamide (Serva)

    8 g bis (Serva or Bio Rad)

    Mix on magnetic stirrer in glass distilled water and bring up to 1 L. Store in refrigerator. Make 10 L at a time.

2b. Our current recipe for No. 2 - acrylamide mix

    27% stock acrylamide

    300 g acrylamide

    8 g bis

    300 mL glycerol

    H_2O to 1.1 L. Filter with Gelman 3 mμ filter.

    If this recipe is used for an acrylamide solution, then note recipe No. 7b and 13b.

3. 10% ammonium persulfate (Bio Rad)

    Empty 1 small 10 g bottle into brown bottle. Add 100 mL water. Store in refrigerator. This solution will last about 2 weeks.

4. Buffer "II" - 375 mL recipe

    11.25 g Trizma base (Sigma)

    0.75 g SDS (Serva)

*Unless otherwise specified, glass distilled water is used in these recipes.

Start with about 300 mL water. Mix the solution on magnetic stirrer and add 6N HCl until pH is 6.8. Bring volume up to 375 mL.

5. Buffer "L" - 3 L recipe

   400 g Trizma base

   200 g Trizma HCl

   Approx. 120 mL 6N HCl

   Adjust pH to 8.5-8.6 at room temperature with 6N HCl. Fill water to 3 L.

6. Buffer "L10"

   3 parts Buffer "L"

   5 parts water

7a. Buffer "L20"

   3 parts Buffer "L"

   1 part glycerol

7b. Buffer "L20" when recipe 2a is used.

   3 parts Buffer "L"

   1 part water

8. Equilibration buffer = 750 mL recipe

   75 mL glycerol

   375 mL Buffer "II"

   300 mL water

   15 g SDS

   1.0 g dithioerythritol (DTE) or 1 g dithiothreitol (DTT) or 37.5 mL β-mercaptoethanol (= 5% mercaptoethanol)

   Small spatula tip of bromophenol blue

   Adjust pH to 6.8.

   Put above ingredients directly into dispenser. Mix ~ 0.5 hour on magnetic stirrer.

9. DALT buffer tank mix (made into dry packets in Ziplock® bags for storage)

36.7 L tank	26 L tank	6.5 L ENI tank
110.0 g Trizma base (24 mM)	84.5 g Trizma base	17.5 g Trizma base
528.5 g glycine (0.2 M)	375 g glycine	86.9 g glycine
36.7 g SDS (3.5 mM)	26 g SDS	6.5 g SDS

Mix the above into approximately 3 L deionized water and stir with a magnetic stirrer until dissolved. Add to empty, clean DALT tank and fill with deionized water to mark.

10. H_3PO_4 1:100 dilution for 0.85% H_3PO_4 (marked 10X on bottle)

20 mL H_3PO_4

1980 mL water

11. Running buffer agarose (when stacker is not used)

3 g Trizma base

14.4 g glycine

1 g SDS

5 g agarose

Fill to 1 L with water. Microwave to dissolve. Freeze in 100 mL portions for use.

12. Sample overlay agarose (when stacker is used)

0.5 g agarose

1 g SDS

50 mL water

50 mL Buffer "II"

Microwave to heat and dissolve.

13a. Water saturated sec-butanol

Mix approx. 350 mL sec-butanol: 100 mL water.

Let stand and butanol will become clear. Make sure that there is a small water layer on the bottom. Otherwise add more water or sec-butanol as appropriate.

13b. 0.1% Photoflo made in 2 parts buffer L10 and 1 part water. This overlay may be used to avoid using sec-butanol if acrylamide recipe No. 2b is used.

14. Separation gel rinse solution

Dilute Buffer "L" 1 part in 3 parts water

i.e.: 50 mL "L"

150 mL water

15. Stacking gel mix

480 mL Buffer "II"

380 mL water

140 mL 30% acrylamide with 0.8% bis

16. Coomassie Blue stain solution in EtOH

Mix 40 g of Bio Rad Coomassie Blue or 25 g of Serva Blue R in 9.5 L glass distilled water. Add a 1 pint bottle of phosphoric acid. Add 10 L of 95% ethanol (from a 5 gallon drum), and mix thoroughly. We use a motor driven stirrer for ~ 0.5 hour.

17. ISO preparation - "SDS mix" solubilizing agent - Blue vials

CHES (cyclohyexlamino ethane sulfonic acid)	0.05M, pH 9.5	100 mg
SDS	2%	200 mg
DTT	1%	100 mg
glycerol	10%	1 mL

Add water to make up a final volume of 10 mL.

18. ISO preparation - "Urea mix" solubilizing agent - Pink vials

9M urea	54 g
4% NP40	4 mL
2% ampholyte (9-11 range)	10 mL (20% w/v stock used)
2% mercaptoethanol	2 mL

Add water to make up a final volume of 100 mL. Adjust if necessary with NaOH. pH should be $\geq$ 9.5.

19. ISO preparation - "Urea mix for urine samples" solubilizing agent - Yellow vials

9M urea	54 g
2% ampholyte (3-10 range)	5 mL
5% mercaptoethanol	5 mL

Add water to make up a final volume of 100 mL.

20. ISO preparation - "Urea mix with DTE" for muscle samples

9M urea	54 g
4% NP40	4 mL
2% ampholyte (3.5-10 range)	5 mL (40% w/v stock used)
1% DTE	1 g

water; quantity sufficient to 100 mL. pH to 9.5 with NaOH.

21. Preparation of creatine kinase (CK) charge standards*

    a. Dissolve 5 mg of rabbit muscle creatine phosphokinase (Sigma) in 1 mL of a solution that is 8M urea and 1% mercaptoethanol, to give a concentration of 5 mg CK/mL. We use 110 mg CK/22 mL of above mix to make a 6 months' supply of standard.

    b. Aliquot the above mixture into seven tubes.

*See example of charge standards in Figure 11.

c. Do not heat the first tube. Heat each of the remaining six tubes for 4, 6, 8, 10, 12, and 15 minutes at 95°C in a heating block. At the end of each time period, place the appropriate tube in an ice bucket.

d. These seven tubes are then mixed together and 50 µL mix is put into small microfuge tubes for storage at -70°C.

e. Thaw out a tube for each experiment and load 2 µL CK mix on top of each ISO tube containing the protein sample to be run.

22. Preparation of rat heart molecular weight standards*

a. Sacrifice the rat by decapitation and perfuse heart with cold phosphate-buffered saline (PBS).

b. Cut out the heart, drop it into a tared beaker containing cold PBS and, weigh it.

c. Dry heart with blotter and place it into SDS homogenization buffer (1% SDS, 0.5% DTT, 0.125M Tris-HCl, pH 6.8) and mince it with scissors.

d. Mix the minced tissue with additional buffer to yield a 5% homogenate (5 g of tissue per 100 mL homogenate) and mix at 4°C for 30 seconds at full speed in a chilled Waring blender.

e. Filter the homogenate through two layers of cheese cloth and mix with a stock agarose solution (1.4% agarose in the SDS homogenization buffer) to yield mixtures containing from 2.5 to 20 mg tissue/mL of 0.7% agarose.

f. These preparations are frozen in 5 mL aliquots and stored at -20° or -70°C.

*See example of heart standards in Figure 12.

g. For use, thaw in a microwave oven and draw the sample up in a 0.2 mL or 0.5 mL pipette, holding the pipette upside down. Run cold water on the pipette until the agarose standard solidifies and blow the contents of the pipette out on a piece of glass (blow from the tapered end of the pipette). Cut the solidified agarose into small sections (~ 1 cm) with a razor blade and load a section on each gel at one side on top of the loaded ISO gel before overlaying with agarose.

NOTE: We are now using rabbit psoas (skeletal) muscle standards in addition to rat heart molecular weight standards, since many more rabbit proteins have been sequenced and studied.

23. Separation gel mixes (for 23 plates)

a. 10% acrylamide mix

192 mL of 30% acrylamide with 0.8% bis (recipe No. 1)

384 mL buffer L10 (recipe No. 6)

5.7 mL 10% SDS

6 mL 10% ammonium persulfate (recipe No. 3)

80 µL TEMED

b. 20% acrylamide mix

440 mL of 30% acrylamide with 0.8% bis

220 mL Buffer L20 (recipe No. 7)

6.6 mL 10% SDS

3.0 mL ammonium persulfate

10 µL TEMED

The following recipes are prepared for use with transfer gels:

24. DALT buffer tank mix containing no SDS - 40 L tank

    121 g Trizma base (27 mM)

    576.8 g glycine (207.5 mM)

25. 1.0M Tris buffer, ~ pH 7.5 when diluted to working concentrations:

    38.5 mL 1M Tris HCl

    + 11.5 mL 1M Tris Base

26. Saline solution = 90 gm NaCl/10 L

27. BSA/saline/Tris for blocking:

    5 mL 1.0M Tris buffer

    50 mL 30% BSA

    Add saline to make up a final volume of 500-600 mL

    pH 7.4 to 7.6 (check when starting new bottle of 30% BSA.)

ISO-DALT CHEMICALS

Chemical	Supplier	Amount Ordered
Acrylamide	Serva	10 kg
Agarose powder	Bio Rad or Serva	50 g
Ammonium persulfate	Bio Rad	Ten 10-g bottles
Ampholytesb	LKB	Assortment of
	Serva	ranges
	Pharmacia	
Bromophenol blue	Serva or Bio Rad	10 g
CHES (cyclohexylaminoethane sulfonic acid)	Calbiochem	10 g
Chromerge	Fisher Scientific	
Coomassie Brilliant Blue R250	Serva or Bio Rad	100 g
Dithiothreitol (DTT)	Bio Rad	25 g
Dithioerythritol (DTE)	Sigma	100 g
Ethanol - 95%		
Glycerol	Fisher Scientific	Four 8-pt bottles
Glycine	Sigma	25 kg
2-Mercaptoethanol	Bio Rad	50 mL
Bis-acrylamide (N,N'-methylene bisacrylamide, bis)	Serva or Bio Rad	500 g
Nonidet P-40 (NP-40)	Particle Data Laboratories, Elmhurst, IL	1 pint bottle
Phosphoric acid (85%)	Fisher Scientific	12 - 1 pint bottles
Sec-butanol	Fisher or Eastman	1 L
Sodium hydroxide	Fisher Scientific	
Sodium dodecyl sulfate (SDS)	Serva	2 kg
N,N-tetraethylene methylene diamine (TEMED)	Bio Rad or Serva	10 mL
Trizma base	Sigma	5 kg
Trizma HCl	Sigma	1 kg
Urea	Bio Rad or Serva	500 g

SILVER STAIN CHEMICALS

Ammonium hydroxide	Fisher Scientific	1 pint bottle
Acetic acid	Fisher Scientific	5 lb
Formaldehyde	J. T. Baker Chem. Co.	500 mL
Silver nitrate	Mallinkrodt	1 lb
Citric acid monohydrate	Fisher Scientific	1 lb
5-Sulfosalicylic acid	Sigma Chemical Co.	500 g

PAPERS ON TWO-DIMENSIONAL ELECTROPHORESIS FROM THE MOLECULAR ANATOMY PROGRAM
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Argonne National Laboratory
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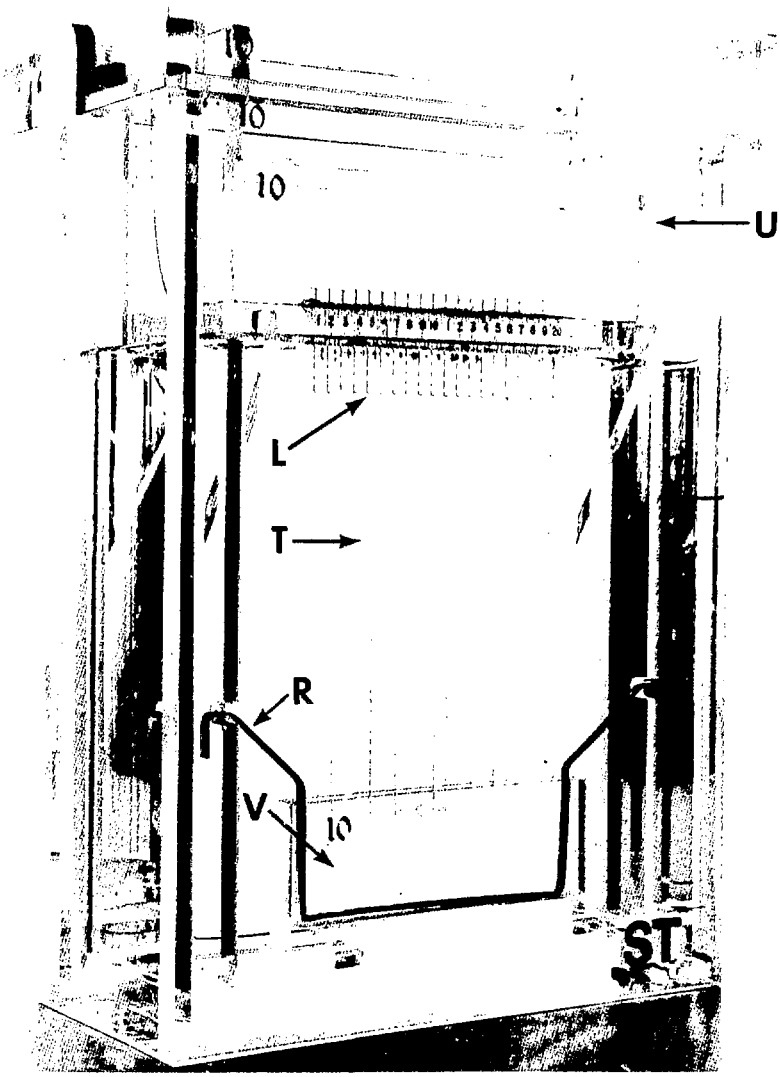
Figure 1. Isoelectric focusing apparatus



The acrylamide monomer solution plus catalyst, persulfate, urea and the nonionic detergent NP-40 are placed in the small casting vessel (V) at the lower end of the twenty precision-bore glass tubes (T), and displaced up into the tubes to level L with water, which is contained in the external vessel. The photograph shows the apparatus at this stage in the loading process. When the gel has set, the casting vessel (V) and the metal gel retainer (R) are removed. The protein samples are introduced into the numbered glass tubes from above, and an alkaline solution is placed in the upper electrode vessel (U), while an acid solution is placed in the lower (outer) electrode vessel in preparation for isoelectric focusing

FIGURE 2. Protocol sheet

Iso - Dalt

Experiment No. _____

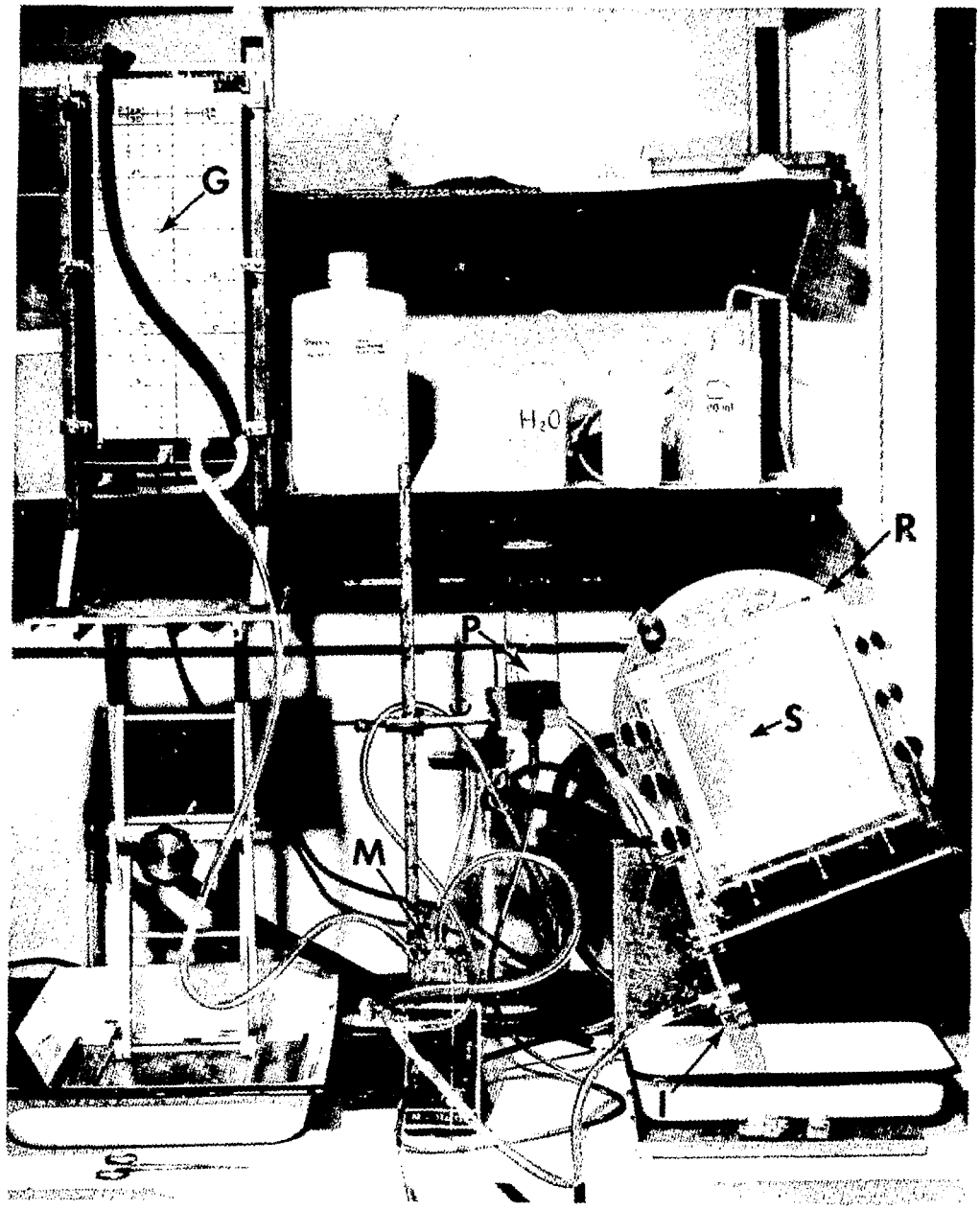
Date _____

	Time	
	Iso	Dalt
Made		
Start		
Stop		

Dalt No.	Layered	Iso Tube No.	Sample	Pretreatment
		1		
		2		
		3		
		4		
		5		
		6		
		7		
		8		
		9		
		10		
		11		
		12		
		13		
		14		
		15		
		16		
		17		
		18		
		19		
		20		

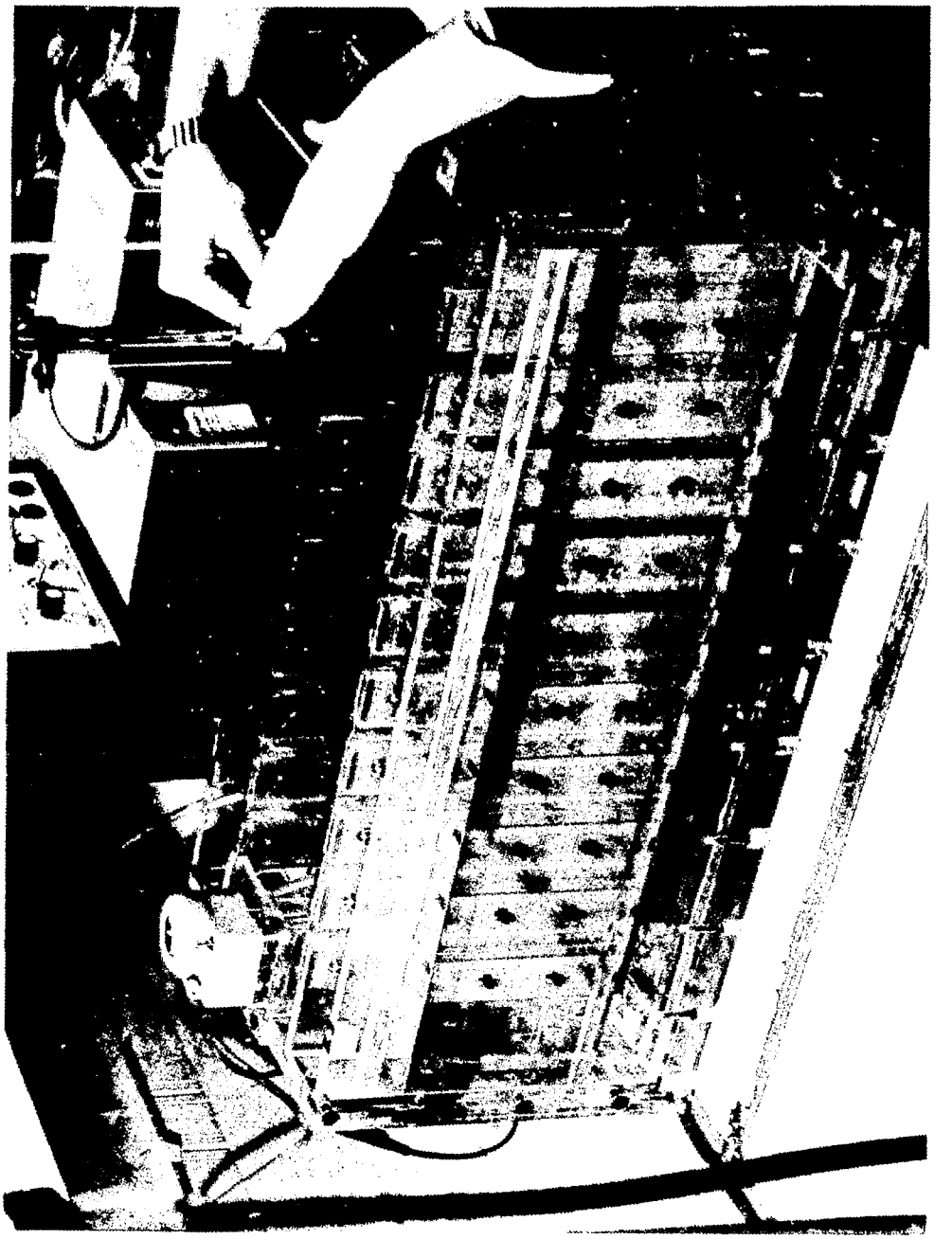
COMMENTS:

Figure 3. Apparatus for casting the slab gels for the second dimension electrophoresis using the DALT system for casting 2-D gels.



The two chambered gradient apparatus (G) generates a gradient whose shape may be readily modified. The gradient flows through magnetic mixer (M) into a V-shaped input (I) where the velocity of flow is decreased in preparation for flow into slab gel casting chamber S which holds twenty pairs of hinged slab gel plates. The slab gel holder can be rotated so that the spaces between the slab gels form a V shaped compartment for additional flow control. When the holders are nearly full, the chamber is slowly rotated through the position shown to horizontal. Acrylamide is displaced out the input lines with displacing fluid P.

Figure 4.



Dalt tank for electrophoresis of 10 slab gels simultaneously. The tank is divided into three compartments which are the electrode compartments on either side, and the cooling compartment in the center. The slabs are run horizontally, and are inserted into slits in silicone-rubber septa as shown to insure electrical isolation of the two ends of the gels. The buffer in the center chamber is rapidly circulated by a small pump and cooled by cooling coils.

Figure 5 - 2-D pattern of human plasma protein

Two-dimensional separation of human plasma proteins using the ISO-DALT system. Note that the low pH (acid) end of the isoelectric focusing gel is the left. While some workers have placed the acid end on the right, we have adopted the convention of placing it on the left so that if actual pH values are plotted, the low numbers would start from the left in keeping with the universally adopted conventions of Cartesian coordinates.

Figure 6. Diagram of 2-D pattern of human plasma proteins.

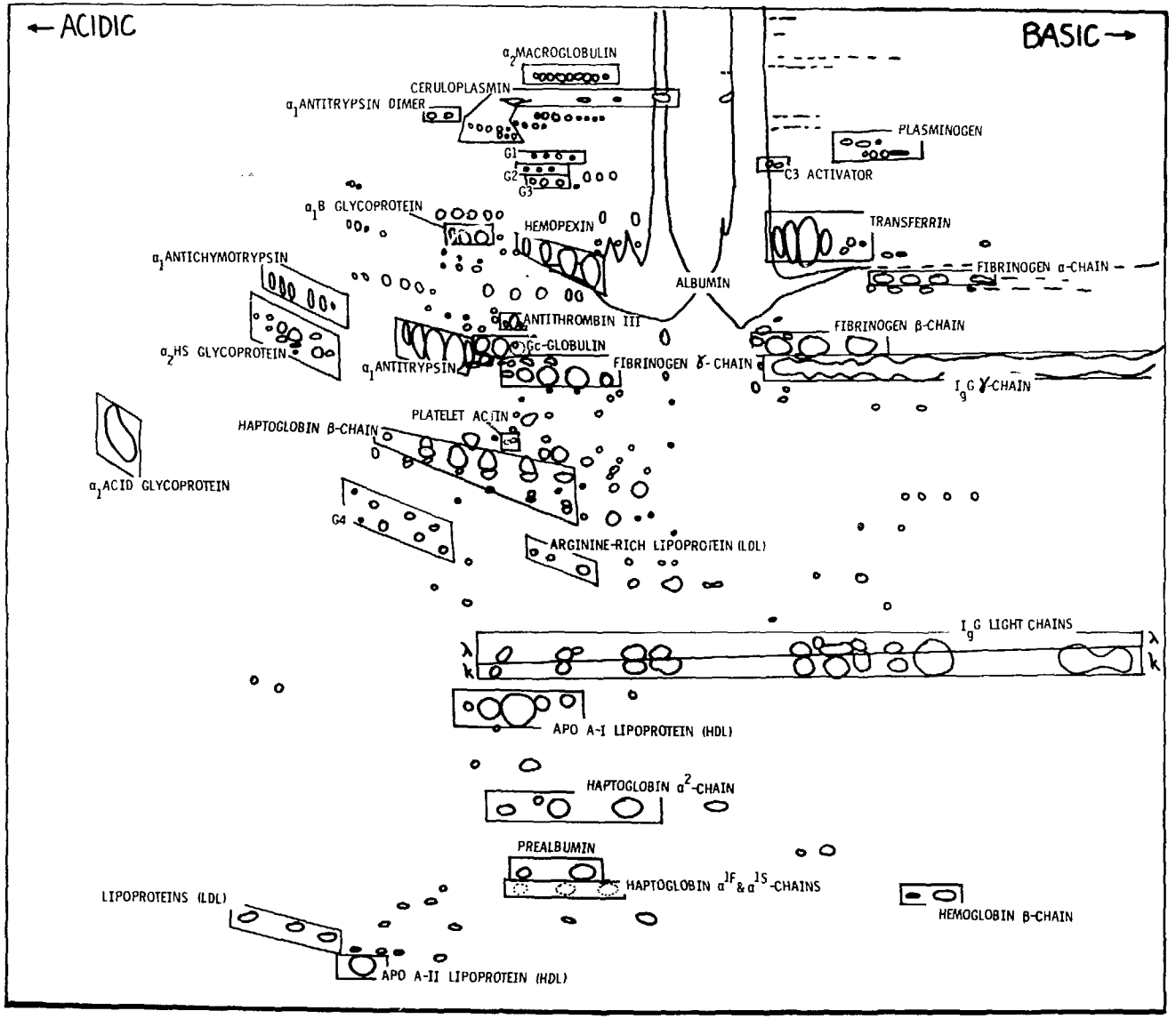


Figure 7. 2-D pattern of human skeletal muscle.

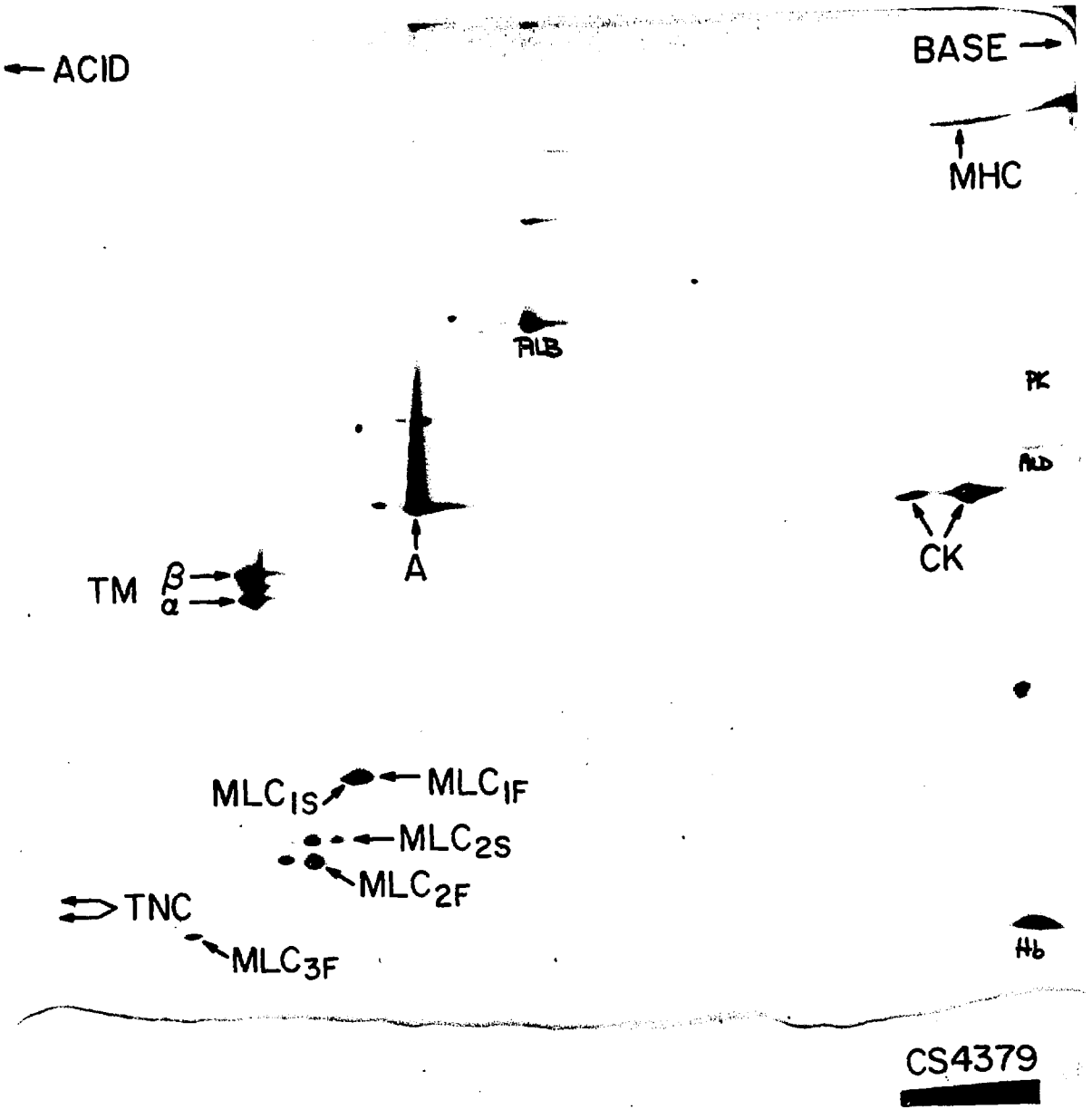


Figure 8 - 2-D pattern of rabbit psoas muscle whole homogenate

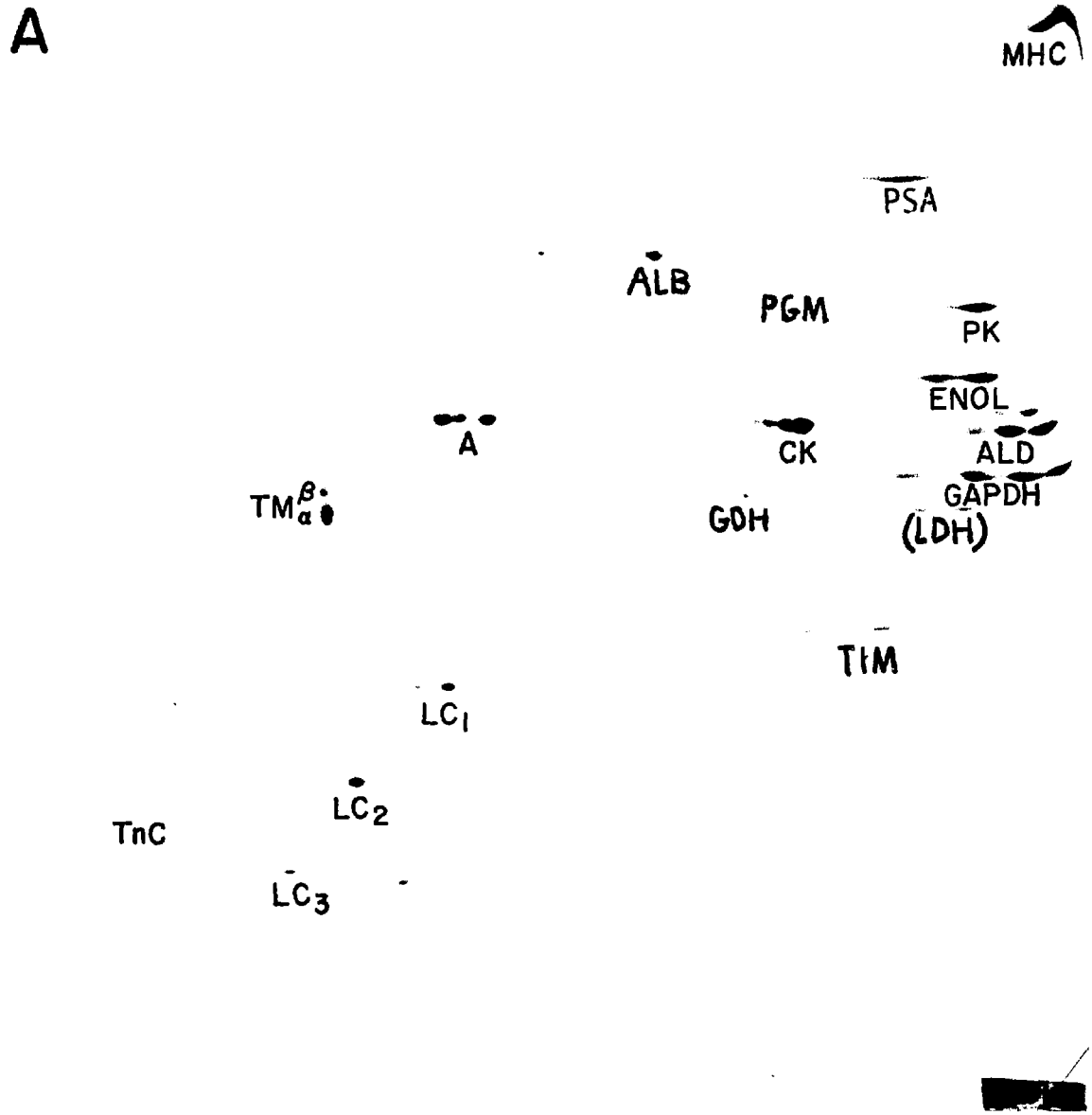


Figure 9. Diagram of 2-D patterns of rabbit muscle.

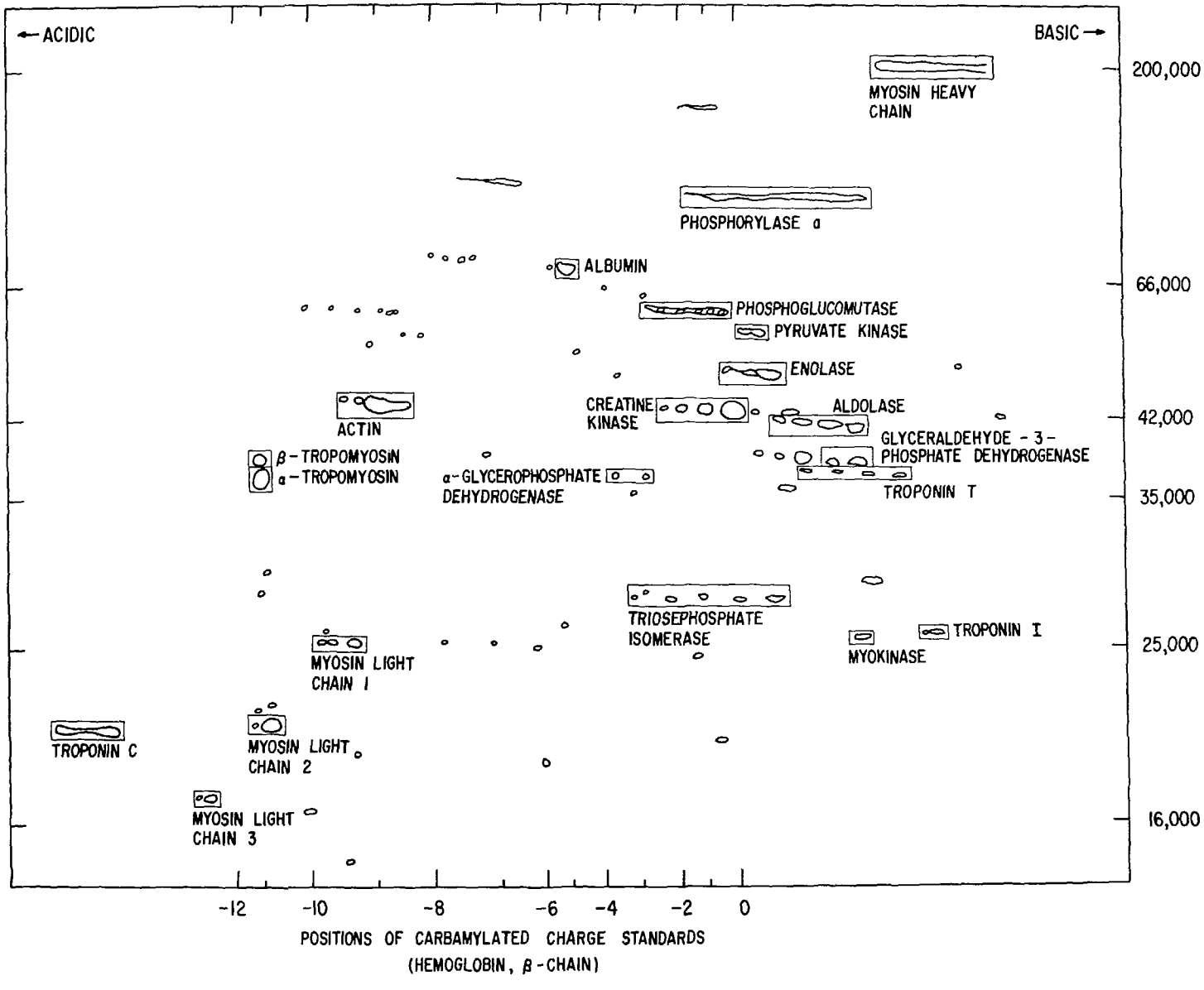


Figure 10 - Photograph of an autoradiograph of human lymphocytes

Figure 11. Human lymphocyte 2-D pattern with CK charge standards.

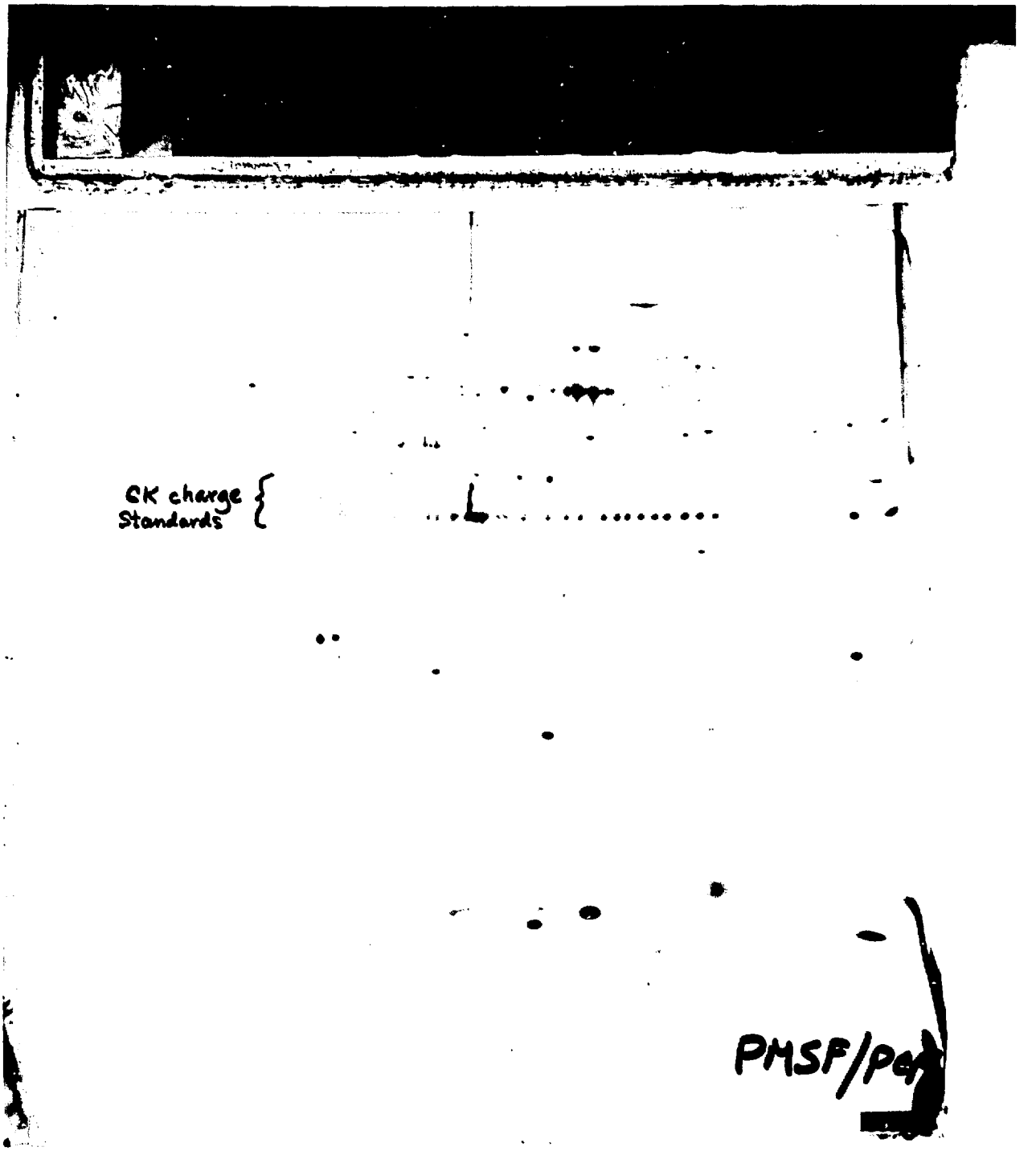


Figure 12 - 2-D pattern of normal human male urine

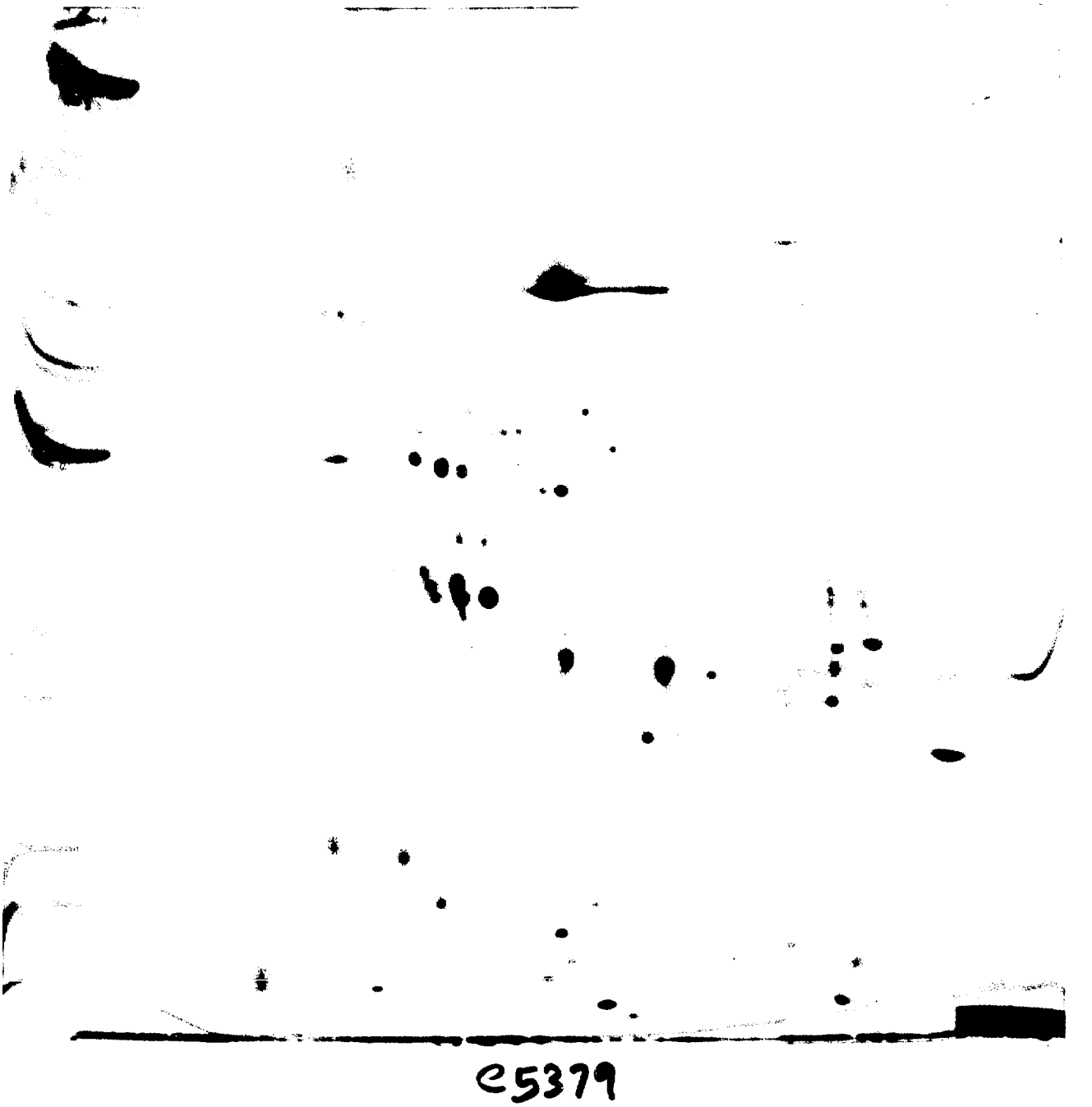


Figure 13 - 2-D pattern of human saliva

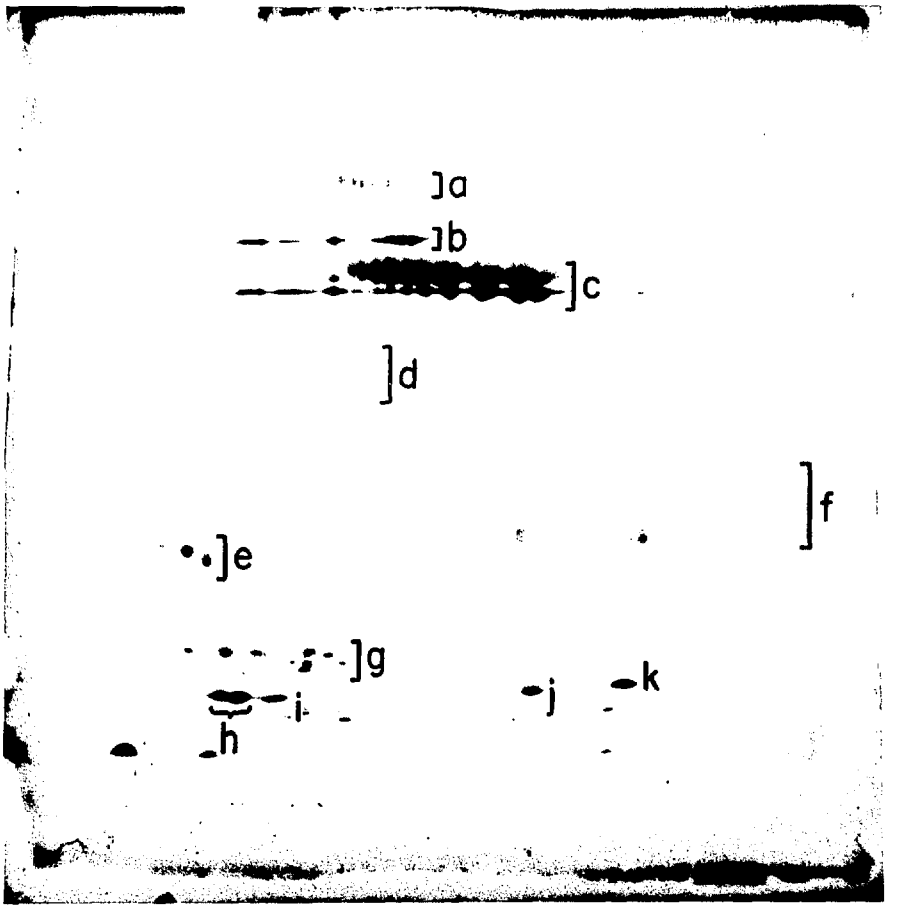


Figure 14 - 2-D pattern of human semen
a. Normal sample
b. Vasectomized sample