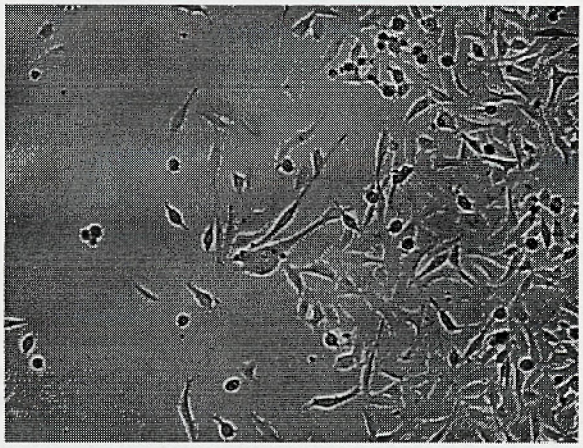

Figure 2. The same cells under 0.5% agarose (Sigma, type VII, cat# A-4018) before C2 ceramide (100ng/mL) has taken effect *

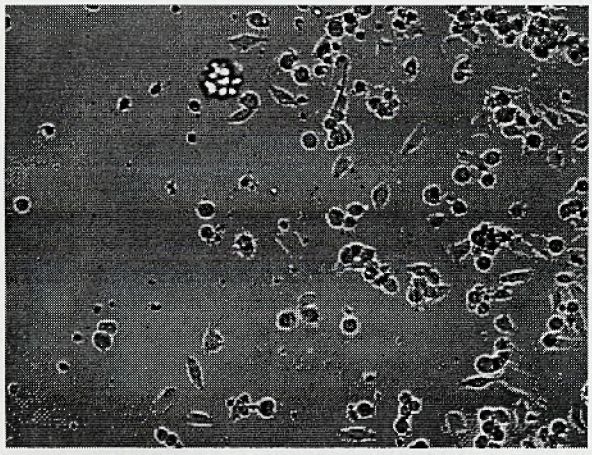

Figure 3. The same cells under 0.5% agarose after 1.5 hours treatment withC2 ceramide.

Block Trimming On The Microtome For Serial Sections

Rough trim the excess plastic from the specimen face with a razor blade, keeping all the tissue edges. Put the specimen chuck into the microtome arm, and put in a dry glass knife. Shave off enough plastic from the front (face) in very small increments (start with 1 µm slices, decrease to 0.5 µm, then 0.1 µm until you get a mirror smooth face). If you must keep all the sample, then start with a very thin shaving just to get the face smooth. You should be able to tell when you are into the tissue by looking at the thin shavings. Mark the top edge of the one that will become the top of your trapezoid--with a magic marker on the chuck. Be sure to keep track of the plane of your sample because the clearance angle of the knife will necessitate that you return the specimen to the original position in which you trimmed the face. Rotate the chuck/specimen 90 degrees (*e.g.*, clockwise). The edges that will become the bottom and top of your trapezoid are now vertical instead of parallel to the earth. Turn the knife holder about 30 degrees away from center (*e.g.*, right) and approach the block carefully. Trim the left side of the block which will become the bottom of your trapezoid; this will make a smooth facet that will start with the face and go toward the microtome slanting outward at about 30 degrees. Leave the specimen in this position and turn the knife to the other side (*e.g.*, left). Trim this side like the first; it will become the facet over the top of your trapezoid. The top and bottom should now be parallel. If they are not, you can make minute adjustments by slightly rotating the block. The purpose of this exercise it to make the scratches caused by rough trimming be parallel, not perpendicular as happens when trimming with a razor blade, to the top and bottom edges of the face.

If the face is too wide, you can trim it, either on the microtome or with a razor blade. I usually just chop the sides with a razor blade to save time; I make a trapezoid with the top edge slightly shorter than the bottom edge. However, folks with unsteady hands can make the same trapezoid by turning the block back to its original position with the magic marker at the top, then slightly rotating it right or left about 2 or 3 degrees. Trim this side with the knife still at 30 degrees away from center. Then rotate the block 2 or 3 degrees in the other direction and turn the knife 30 degrees to the opposite side. Trim the other side.

Before sectioning, turn the block back 2-3 degrees to be in the original position it was when you faced it with the mark at the top. Use a fresh knife, or even better if you have one, use a diamond. When aligning, be sure to have both the bottom edge of your block parallel to the edge of your knife as well as the plane of your block face parallel to the plane of the knife edge. To do the former, you may have to rotate your specimen very slightly. To do the latter, you may have to change the angle of your specimen in the microtome arm. These tiny adjustments may be necessary because the diamond may not be mounted in its holder exactly like the glass one you used for trimming.

Pick up the sections on slotted grids with a support film.

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A Method for the Easy Collection of Serial Sections:

Make up 0.2% sodium lauryl sulphate (a detergent) in distilled water. Grids are dipped in this just prior to collecting sections, then drained on filter paper before introducing under the water in the knife boat and bringing up under the sections. The sections can be manipulated along filmed slots with an eyelash before the residual film of water dries. You can play this game under the binocular microscope for some minutes while watching the water evaporate. Its a bit like watching paint dry!

Sometimes, raising the water meniscus (i.e., to convex) is useful when trying to position sections prior to collection.

Too much carry-over of detergent can cause the sections to run together and bunch up, this is a surface tension effect of the detergent.

The main problem with this practice is that this can only be done after cutting a batch of sections. If sectioning is to continue, then it is advisable to flush the boat with fresh water, otherwise the block face will probably "wet" when resuming cutting. I normally pipette out the water and replace it three times - that works.

After sectioning, we keep the detergent and re-use it. It goes cloudy sometimes, but all it needs is to be filtered through standard filter papers.

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