The morphology of cytoplasts and karyoplasts produced by cytochalasin D[1]

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Summary. The denucleation of L cells and human lymphocytes in suspension was carried out by incubation with cytochalasin D 25 and 50 µg/ml and with ultracentrifugation over a discontinuous Ficoll gradient. Ultrastructural examination of the separate layers confirmed the presence of the anucleate cytoplasm (cytoplasts) with intact membrane and the separated nuclei (karyoplasts).

One of the effects of cytochalasin B (CB), a metabolite of the fungus *Helminthosporium dematiodeum*, is that of denucleation of cells growing in a monolayer culture[2,3]. This action of the cytochalasin has been utilized to produce separate nuclei (karyoplasts) and anucleate cytoplasm (cytoplasts) from cells grown on glass slides[4–6]. These cytoplasts and karyoplasts are viable alone for up to 36 h[4,5] but if reconstituted before this time longer survival is obtained[6]. The problem with the above methods is that they are limited to cells which grow on glass or plastic and cannot be used with cells in suspension. More recently cells in suspension have been denucleated with cytochalasin B when centrifuged at high speeds over a discontinuous Ficoll gradient[7]. This method was tried with L cells but better denucleation was obtained with cytochalasin D (CD) at a concentration of 50 µg/ml and the ultrastructural morphology of the cytoplasts and karyoplasts was studied.

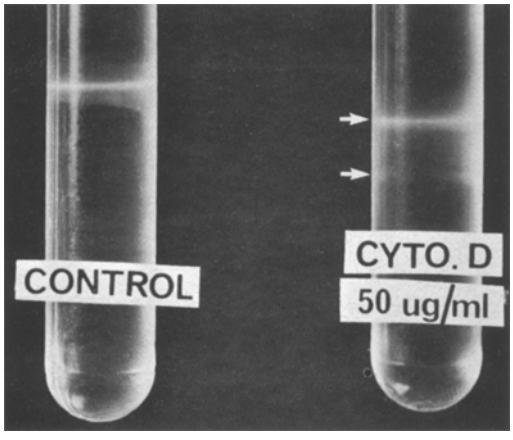

Fig. 1. Result of ultracentrifugation of a suspension of L cells with and without incubation with cytochalasin D 50 µg/ml. Note 2 clear layers in the Ficoll in the treated specimen (arrows).

Materials and methods. The method of Walker and Kaul[7] was used with the following changes. CD at a concentration of 25 and 50 µg/ml was used instead of CB. A control without cytochalasin but with 0.5% dimethylsulphoxide (DMSO) was also prepared. The cell suspension consisted of approximately 20×10^6 L cells which had been grown in a medium of RPMI 1640 with 20% fetal calf serum. A suspension of lymphocytes was also processed. The cells were then centrifuged for 30 min at 35,000 rpm in an IEC B60 ultracentrifuge with an IEC 488 rotor head. Material for transmission electron microscopy (TEM) was fixed in ice-cold buffered glutaraldehyde 3% with postfixation in buffered 2% osmium tetroxide. The specimens were then dehydrated in increasing concentrations of alcohol, cleaned in propylene oxide and embedded in epoxy resin.

Results and discussion. The cellular material of both L cells and human lymphocytes in the tubes with the CD following ultracentrifugation was found to have separated into 3 layers (figure 1). The 1st layer contained mostly cytoplasts but some whole cells and an occasional karyoplast were present. The 2nd layer consisted mainly of karyoplasts but with some whole cells and a few cytoplasts. The 3rd layer at the bottom of the tube was scanty and consisted mainly of cellular debris with some karyoplasts. By the Trypan blue dye exclusion test only an occasional cytoplast was found to be damaged. The control showed either a single layer

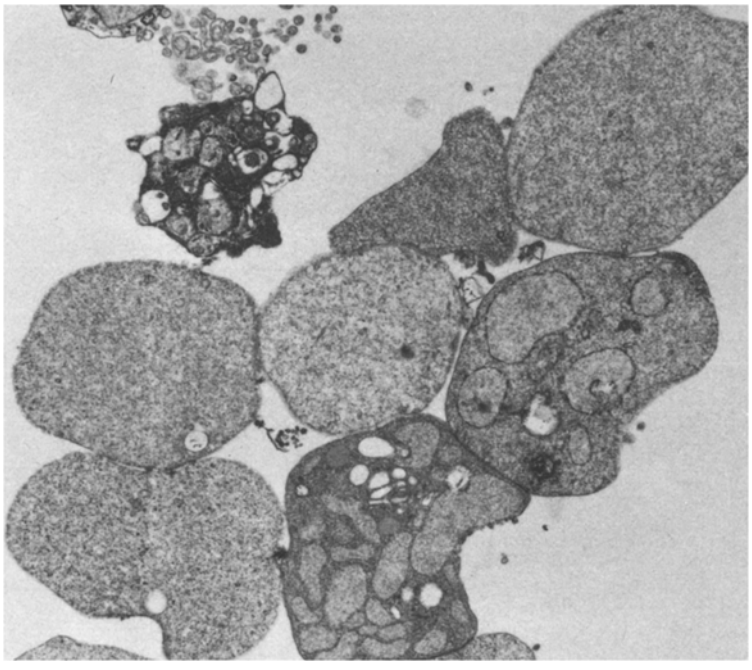

Fig. 2. A group of cytoplasts from layer 1 of the treated L cells. × 11,100.

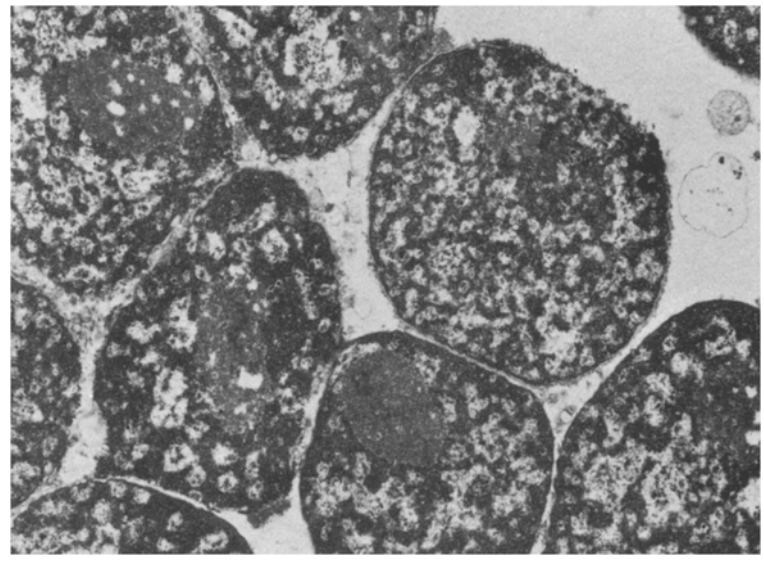

Fig. 3. A group of karyoplasts from layer 3 of treated lymphocytes. × 11,600.

although occasionally 2 ill-defined layers could be made out. No denucleation occurred in the control specimen. TEM studies of the 1st layer confirmed the impression gained by light microscopy. The majority of the cytoplasts contained mostly ribosomes but some contained dilated endoplasmic reticulum (ER) (figure 2). The cytoplasmic membranes of the cytoplasts were intact. The karyoplasts from the 3rd layer showed some nuclear edema. There was usually a very small rim of cytoplasm around the nucleus. Nucleoli were preserved (figure 3).

Our ultrastructural findings confirm the separation, by the action of cytochalasin D of cells into anucleate cytoplasm (cytoplasts) and bare nuclei (karyoplasts). By TEM the cellular fragments did not appear disorganized and previous work[4-6] also attests to their viability. The action of CD in our hands with L cells was superior to that of CB. This method should prove useful in providing large numbers of cytoplasts and karyoplasts which can be used in various recombination experiments.

1 This work was supported by a grant from the National Cancer Institute of Canada.
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Sectioning of polyacrylamide slab gels with a modified small animal stereotaxic instrument[1]

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Summary. A simple and inexpensive modification of the Kopf model 900 small animal stereotaxic instrument allows it to be used temporarily as a precision polyacrylamide slab gel slicer.

Radioactive polyacrylamide slab gels are commonly sectioned either at low temperature (usually while thawing) with an array of parallel razor blades[3] or at room temperature by a commercial electric gel slicer which many laboratories do not possess. The razor blade manifold does not work well at room temperature for the thicker gels (1–3 mm) which are likely to be used for the resolution of radioactive samples, and the slicing of frozen gels is inconvenient and somewhat tricky (they tend to shatter). The graduated moving stage of a small animal stereotaxic apparatus (Kopf model 900) has provided an extremely simple and inexpensive solution to the previously encountered problems of sectioning slab gels for the counting of trapped radioactivity. Other neurobiologists may wish to employ these stereotaxic devices similarly as temporary slab gel slicers.

The modified instrument with a slab gel in position is shown in the figure. The electrode carrier apparatus and other accessories are first removed from the basic stereotaxic frame. Next, a 16 cm × 3.6 cm × 0.3 cm perspex gel support, slotted on one side to accomodate the slightly raised millimeter vernier scale on the moving stage, is attached to the stage with a paper clip at each end. Finally, a cutting metal plate (7 cm high × 6.3 cm × 0.2 cm thick, with a 3.8 cm × 4 cm cutout as shown in the figure) is clamped against the front face of the stage support block. Cuts are made by drawing a knife blade or very fine wire down across this plate through the gel.

The general usefulness of the device and the precision of sectioning were evaluated by slicing gels uniformly labeled with radioactive bovine serum albumin (BSA, methylated to low specific activity with [^{14}C]-formaldehyde by the method of Rice and Means[4]). 0.5 µCi of the BSA solution was uniformly incorporated during casting into a 3 mm × 10 cm × 20 cm slab gel polymerized in a Bio-Rad model 220 electrophoresis cell using the separation gel formulation of Laemmli[5]. The acrylamide concentration was 10%, and the gel was 2.7% cross-linked with bisacryl-