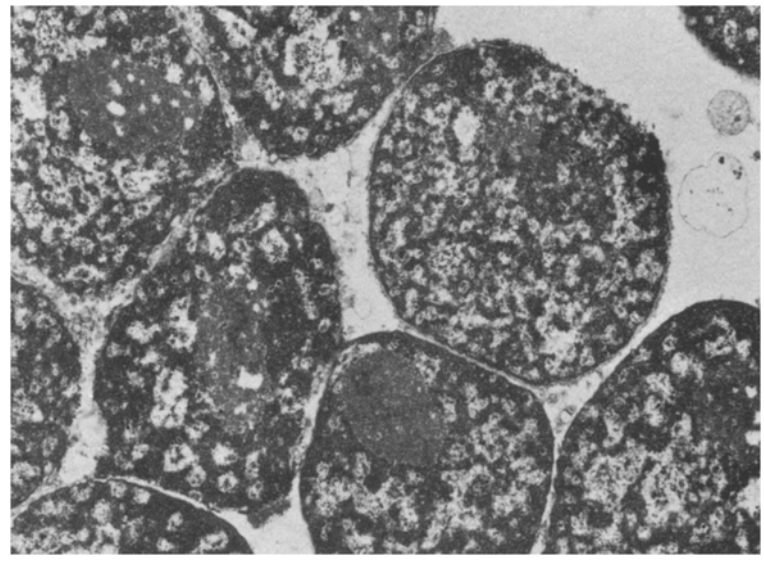

Fig. 3. A group of karyoplasts from layer 3 of treated lymphocytes. × 11,600.

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.

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Sectioning of polyacrylamide slab gels with a modified small animal stereotaxic instrument[1]

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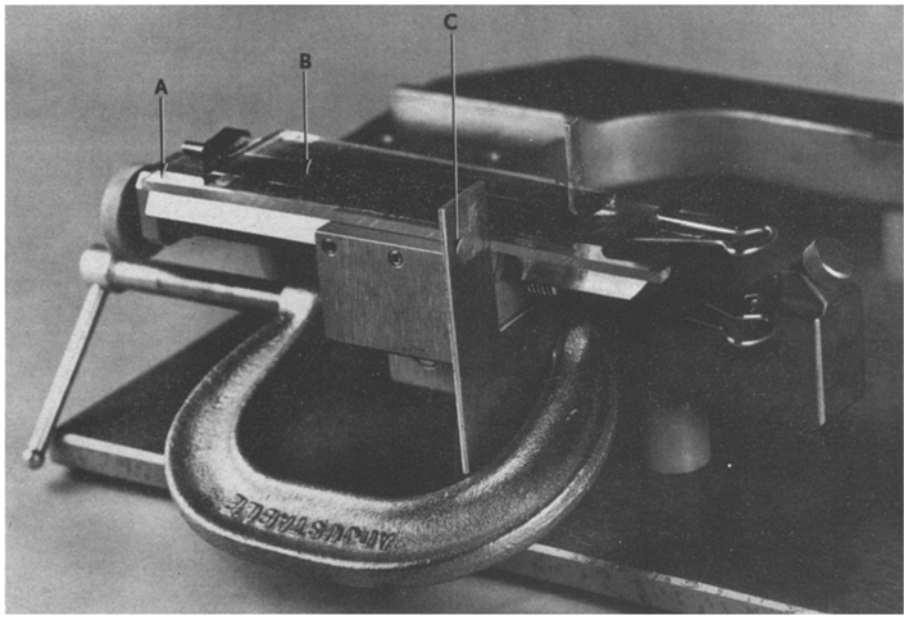
Summary. A simple and inexpensive modification of the Kopf model 900 small animal stereotaxic instrument allows it to be used temporarily as a precision polyacrylamide slab gel slicer.

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-



Overall view of stereotaxic apparatus modified for gel sectioning. A: Perspex gel support (fastened to moving stage with paper clips at each end). B: Slab gel in position on apparatus. C: Stationary cutting plate down which knife blade or wire is moved vertically to cut each slice.

amide. Following overnight precipitation of the BSA in 10% trichloroacetic acid (TCA) the gel was washed for several hours in 7.5% acetic acid and cut into 3 cm strips in preparation for sectioning. The strips were quickly blotted on one surface with a tissue and then placed on the perspex gel support to be sliced with a Weck surgical prep knife blade. Several series of 15 consecutive slices were made at thicknesses of 1 and 2 mm. The slices were incubated in 1 ml of Soluene-350 (Packard) for 5 h at 50 °C in scintillation vials and then assayed for radioactivity 48 h after the addition of 10 ml toluene-based scintillation fluor. The relative SD of the counts per min (cpm) for the 15 slice series were 5.2% for the 2 mm thick slices and 7.2% for the 1 mm thick series. We routinely use the apparatus to slice gradient slab gels (2.7% cross-linked with bisacrylamide) ranging in acrylamide concentration from 7.5% to 20% without difficulty. Gels composed of 5% acrylamide or less are sticky and difficult to slice with our apparatus at room temperature. Also, gels with acrylamide concentrations in the range from 15% to 25% are somewhat crumbly and cannot be sectioned with the precision reported for the more porous (10% acrylamide) gels. We have observed, however, that cross-linking with 10% N,N'diallyltartardiamide (DATD, frequently used because the gels can be dissolved in 30 min by 2% periodic acid at room temperature) reduces both stickiness and crumbling and improves the precision of sectioning at both low and high acrylamide concentrations.

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