Fluorescent lamps were mounted above the plexiglass. One plexiglass sheet was lowered until it rested on the glass pane of the collecting box, while the other piece of plexiglass was raised to a height of 14" above the glass pane. In this way an illusion of depth in the form of a high and low "ceiling" was created. Fig. 2 is an illustration of the appearance of the apparatus from inside looking up. The mirror is used to eliminate the cue of interposition. The edges of the plexiglass are not visible. With this arrangement only two depth-perception cues are available to the flies: motion parallax and textural differences of the stimulus' surfaces.

Doane, W. W. Yale University, New Haven, connecticut. Separation and quantitation of α-amylases with disc electrophoresis.

An improved method was developed to analyze α-amylases in Drosophila separated by acrylamide gel disc electrophoresis. The procedure provides both quantitative and qualitative estimates of enzyme activity from single flies or individual tissues. (A previous method, DIS 40:97, was abandoned.)

The technique of Ornstein & Davis (1962) is employed with minor modifications: 1) omit $K_4Fe(CN)6$ from small pore gel, 2) use 1/2 the amount of N,N,N',N'-tetramethylethylenediamine, 3) substitute 0.47 M tris-phosphate buffer, pH 6.9, for tris-HCl in large pore gels, and 4) add 1 µl. 10% 3-dimethylaminopropionitrile (DMAPN) to each sample gel to insure polymerization. Supernatant from a single fly, homogenized in 10 to 15 µl. distilled water and centrifuged in a capillary tube, is put directly on top of spacer gel, mixed with 0.1 ml. large pore gel and layered-over with another 0.1 ml. of this gel. Electrophoresis is done at 4°C. with a constant current of 4 ma per gel tube for 50-60 minutes.

After electrophoresis, gels are removed from their tubes and placed on a corrugated rack in a moist chamber. A glass plate (3 1/4" x 4"), coated with a starch-acrylamide film, is placed over the gels, weighted (125 gm.), and left to incubate at 25°C. for 30 minutes. Plates are made by mixing 2 ml. small pore gel as usual but altering the pH to 7.4 and including 1.5% Connaught hydrolyzed starch, previously boiled 5 min. in the water used in making "small-pore solution #2". Ten µl. of 10% DMAPN solution is added and the mixture spread between two glass plates, one of which is coated on its periphery with dried albumin. Cover-glass chips are set in the albumin to separate the plates so the film forms with uniform thickness. (Film thickness, sample size and incubation time must be suitably adjusted for densitometric analysis.) Films are polymerized over a fluorescent bulb 15-20 min. and immersed in tris-HCl buffer, pH7.4 (optimum for melanogaster, Doane, unpubl.), where they may be stored under refrigeration 1-2 weeks or used immediately. Prior to use, plates are rinsed with water, the outsides dried, and the two separated from one another, leaving the starch-acrylamide film on the side edged with albumin. The latter plate is placed directly on electrophoresed gels to incubate, care being taken to avoid any additional moisture.

Following incubation, the starch-acrylamide plate is set in I-KI reagent 1-2 min. to stop the reaction and to render unhydrolyzed portions of the film blue. Not only do amylase bands stand out distinctly (ranging from lighter shades of blue to nearly colorless), but the imprint of the individual gels may be discerned along with their origins and leading edges (the latter caused by tracking dye). The stained plate is rinsed with water, then 7% acetic acid, and re-covered with the other glass plate removed prior to incubation.

The stained starch film, sandwiched between two plates, may be stored several months without deterioration by placing it, with a little 7% acetic acid, in a container in the dark and under refrigeration. It serves a double purpose: 1) it is used as a photographic negative for contact prints of amylase banding patterns, and 2) it is scanned densitometrically (Joyce-Loebl Microdensitometer) to determine relative activity in different bands (see research note, this issue). Amylases in the electrophoresed gels remain unharmed and may be cut out, eluted, and further analyzed.