this regard, the patterns of behavior characteristic of hermit crabs which the glaucothoes and young crabs of *Birgus latro* show toward empty gastropod shells are of great interest and represent an excellent example of the retention of ancestral behavior during development.

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7. To keep the experimental age of the glaucothoes as constant as possible, it was necessary to work with them as they appeared, usually within 24 to 48 hours after the terminal molt. They were tested with whatever shell sets and experimental chambers were available at that time. The opportunistic nature of the program is reflected in the different sample sizes. The duration of the experiments was variable, usually about a week, but occasionally a few days to 2 weeks. The responses shown in Table 1 represent the situations at the conclusion of the experiments at which time none of the glaucothoes had metamorphosed to the first crab stage.
8. I thank Drs. D. F. Dorward and J. E. Nelson, both of Monash University, Australia, for commenting on this paper. Supported in part by NSF grants GB-1003 and GB-3651 and by the Eniwetok Marine Biological Laboratory. Contribution No. 297 from the Hawaii Institute of Marine Biology, University of Hawaii, Honolulu 96822.

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Density Gradient Centrifugation: Fixation of Bands by Photopolymerization of Acrylamide

Abstract. *A method for immobilization of proteins in a density gradient is described. This method eliminates the collection of drops or the use of a schlieren optical system, but visualizes the results of the Amido Schwarz stain. Although only sucrose gradients have been examined, the method possibly may be extended to other gradients.*

The widespread use of density gradient centrifugation has led us to develop a technique for immobilization of the separated materials *in situ*. The gel which results from the polymerization of the tube contents may then be treated in the same manner that gels of disc electrophoresis are treated. Banding, immobilization, and localization of some proteins are reported here.

An all-glass gradient-maker was used in pouring the gradients (*1*). The solutions used for preparation of the light and heavy components of the gradient are made by mixing aliquots of stock solutions B, D, and E, and water and sucrose. Stock solution B is 11.4 g tris buffer, 1.2 ml *N,N,N′,N′*-tetramethylethylenediamine (TEMED, practical grade, Matheson Coleman and Bell 8563), and 98 ml of distilled water; the *p*H is adjusted to 6.9 with 85 percent phosphoric acid. Solution D is 24 g of acrylamide, 5 g *N,N′*-methylenebisacrylamide (Bis, Eastman 8383), and distilled water to give final volume of 100 ml. Solution E is 8 mg of riboflavin in 100 ml of distilled water (*2*). Solutions B and D as well as the sucrose solutions are stored in the refrigerator; E is prepared fresh daily. The polymerizable solution consists of 1 part B, 2 parts D, 1 part E, and 4 parts water. The sucrose solutions are prepared in double strength; for example, 10 and 50 percent sucrose solutions are prepared for a final 5 to 25 percent sucrose gradient. Each double-strength sucrose solution is diluted with an equal volume of polymerizable solution. Three milliliters of each of these two resulting solutions are used for pouring the gradient into 5 by 1.5-cm cellulose nitrate tubes.

The sample in a volume of 0.05 ml is layered onto the surface of the poured gradient mixture with a microliter pipette. In this work a 50SW swinging bucket rotor was used in a Spinco model L preparative ultracentrifuge. The tubes were spun at 50,000 rev/min for the appropriate time.

When the run is completed, the tubes are removed from the buckets and overlayered with about 2 mm of water with a fine capillary pipette. The overlayering helps produce a sharp edge at the top of the tube contents after polymerization is completed. The tubes are photopolymerized by a fluorescent light in about 30 minutes. The gels are removed from the tube by shaking or by puncturing the bottom of the tube, whereby they slide out easily.

For total protein, the gels are stained in a 1 percent solution of Amido Schwartz dye (Naphthol Blue Black C, 1; No. 20470, Allied Chemical Corp.) in 7 percent acetic acid. This procedure stains the proteins and binds them to the gel. Excess dye may be removed by washing repeatedly in 15 percent acetic acid or by electrophoretic destaining in 15 percent acetic acid. The destained gels may be viewed and stored in 15 percent acetic acid. Figure 1 shows the results of a typical run. The conditions are noted in the legend.

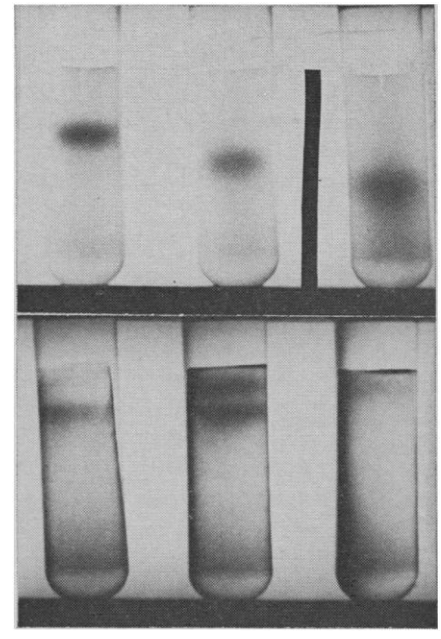

Fig. 1. (Top) Left to right, 40 μg of bovine serum albumin centrifuged for 19, 24, 30 hours in a 5 to 25 percent sucrose gradient at 50,000 rev/min. (Bottom) Left to right, 40 μg of lysozyme, 40 μg of lysozyme and 40 μg of alcohol dehydrogenase, and 40 μg of alcohol dehydrogenase centrifuged for 6 hours in a 5 to 10 percent sucrose gradient at 50,000 rev/min. The black bar indicates a length of 46 mm.

The results of density gradient centrifugation are usually analyzed by schlieren optics or by fractionation of the run by drop collecting. For work involving only localization of the sample, this immobilization technique may be advantageous. The technique described here may be of use in enzyme studies; the techniques for visualization of many enzymes by specific reagents on polyacrylamide gels are available (*3*).

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References and Notes

1. The gradient-maker was a gift from Dr. George D. Guthrie (Indiana University, Bloomington).
2. These solutions are double-strength preparations for those used in disc electrophore → J. Davis, *Ann. N.Y. Acad. Sci.* **121**, 404 (1964).
3. Disc Electrophoresis Information Center, 5635 Fisher Lane, Rockville, Md. 20852.
4. Supported by PHS research grant GM11860. We thank Dr. A. E. Brooks for his help during this work.
5. After this manuscript was accepted for publication, a similar method was brought to our attention [W. B. Jolley, H. W. Allen, O. M. Griffith, *Anal. Biochem.* **21**, 454 (1967)].

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