A Simple Starch-Gel Electrophoretic Method to Investigate Esterase Inhibition by Common Organic Insecticides[1]

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Starch-gel electrophoretic methods to resolve components of complex protein mixtures are available to use in analyzing serum from many species, but most analyses are involved and time consuming. Also present starch-gel electrophoretic techniques (6, 7, 8, 9) used to separate blood proteins obtained from Tenebrio molitor (L.) larvae give poor separation of components. The objective of this work was to simplify and accelerate the conventional starch-gel electrophoretic technique and to adapt it to resolving components of insect blood protein for investigating the interaction between common organic insecticides and enzymes.

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Materials and Methods

Reagents and Equipment

Starch. Partially hydrolyzed potato starch is prepared specifically for starch-gel electrophoresis (Cannaught Medical Research Laboratories, Toronto, Canada).

Apparatus. The electrophoresis apparatus consisted of a large tray 7 1/4 in. wide with eight 1/2 in. x 3/4 in. x 6 in. Plexiglas trays or channels. The trays were supported with 2 in. x 2 in. x 7 1/4 in. electrode chambers in each side, and two removable end gates to permit direct contact between the gel and the buffer. A cheese cutter with piano wire was used to slice the gel to four equal 1/16 in. layers using the 3/16 in. x 13/16 in. x 8 1/2 in. slicing tray and the 1/16 in. x 3/4 in. x 9 1/2 in. Plexiglas pieces (Figure 1). A Spinco Duostat supplied power (Spinco Division, Beckman Instruments, Inc.). A Densicord densitometer with 595 mu filter (Photovolt Corp.) completed the apparatus used.

Blood protein. *T. molitor* larvae were reared as described by Kadoum *et al.* (4). The blood protein was prepared by excising the entire prothoracic legs of 50 *T. molitor* larvae and introducing a capillary tube to collect their blood. The 50 larvae were identical in age and weight in each of these test groups: control and larvae treated with sublethal dosages of endrin. Blood samples were obtained from endrin-treated larvae 6 and 24 hr after treatment.

Endrin treatment. Fifty or 150 ug of endrin per gram of three-month-old larvae was applied on the entire dorsal side of each larva.

Procedure

Starch gels were prepared according to Smithies (7, 8) except that 15 gm of hydrolyzed starch per 100 ml of 3 M urea solution in 0.038 M aluminum lactate buffer was mixed in a 500 ml Pyrex suction flask. The removable end gates were placed in their positions at the sides of the gel tray; then hot gel was poured into the gel tray, smoothed and covered with Saran Wrap. After one hour the Saran Wrap was lowered to the gel surface and the gel was placed in the refrigerator ready for use, or kept overnight to produce a stronger gel. Blood protein samples of 10 ul were added to the gels, as described by Marsh et al. (6).

Electrical contacts to the gels were placed directly into the 0.038 M (pH = 3.1) aluminum lactate buffer solution in the electrode compartments by removing the end gates and the gel was covered with Saran Wrap during electrophoresis. After electrophoresis the gel was cut horizontally into four equal 1/16 in. layers by the aid of the slicing tray and a piano wire attached to the cheese cutter. The two middle layers were stained in 0.5% solution of nigrosin in 0.038 M aluminum lactate buffer for three hours. Gel strips were finally destained for 16 hr in running tap water.

Results and Discussion

Preliminary experiments with acetate, borate, aluminum lactate, and tris-glycine buffer at varying pH indicated that the aluminum lactate buffer at 0.038 M and pH 3.1 most satisfactorily resolved protein components (Figure 2). On the other hand, very poor separation of blood components was obtained with the other buffers mentioned. Protein components migrated very slowly and heavy streaks from origin were obtained using buffer with pH higher than 3.1. Higher than 0.038 M concentrations of aluminum lactate buffer produced noticeable heat in the gel strips. Urea in starch gel did not affect blood protein components, which were identical whether starch gel was prepared with or without urea. However, urea in starch gel produced clearer gel with high resolving power. To establish suitable electrophoretic conditions, factors such as voltage and time were studied. Optimum conditions with the electrophoretic apparatus used and with 8 gels were found to be: 0.038 M lactate buffer (pH = 3.1), a constant current of 45 ma (approximately 180 volts). Under those conditions heat production was negligible, and protein components were spread over about 4 in. of the 6-in.-long gel in 2.5 hr.

The modified method was therefore employed to compare protein fractions in blood of endrin-treated and untreated T. molitor larvae. There was no significant difference between electropherograms obtained from treated and control larvae, and the patterns were

identical. Colvin et al. (2) reported that assays of acetylcholinesterase in brain-spinal cord homogenates of fish revealed no inhibition by endrin at a final concentration of 10 uM. However, limited inhibition (4-10%) was observed with succinate dehydrogenase when measurements were conducted at an endrin concentration of 0.5 uM in mitochondria preparation of fish liver, and 24-59% inhibition was obtained using 80 uM endrin.

Bunyan et al. (1) reported that serum electropherograms from pheasants showed no differences between control and heavily thimet-poisoned birds, while serum cholinesterase zymograms gave erratic results. Electropherograms from pheasant tissues were indiscrete bands on a continuous background compared with distinct electropherograms obtained in this study (Figure 2). Serum of T. molitor larvae yielded electropherograms with discrete bands migrating to the cathodes but no cathodic bands were obtained using aluminum lactate buffer with pH 3.1.

The technique described should resolve the components of complex protein mixtures equally well, particularly in analyses of serum proteins; this simple starch-gel electrophoretic technique should demonstrate inhibition of cholinesterase and other esterases by organophosphorus insecticides very well. Procedures described by Hunter and Burstone (3) and Karnovsky and Roots (5) could be used to obtain carboxylic esterase and cholinesterase zymograms.

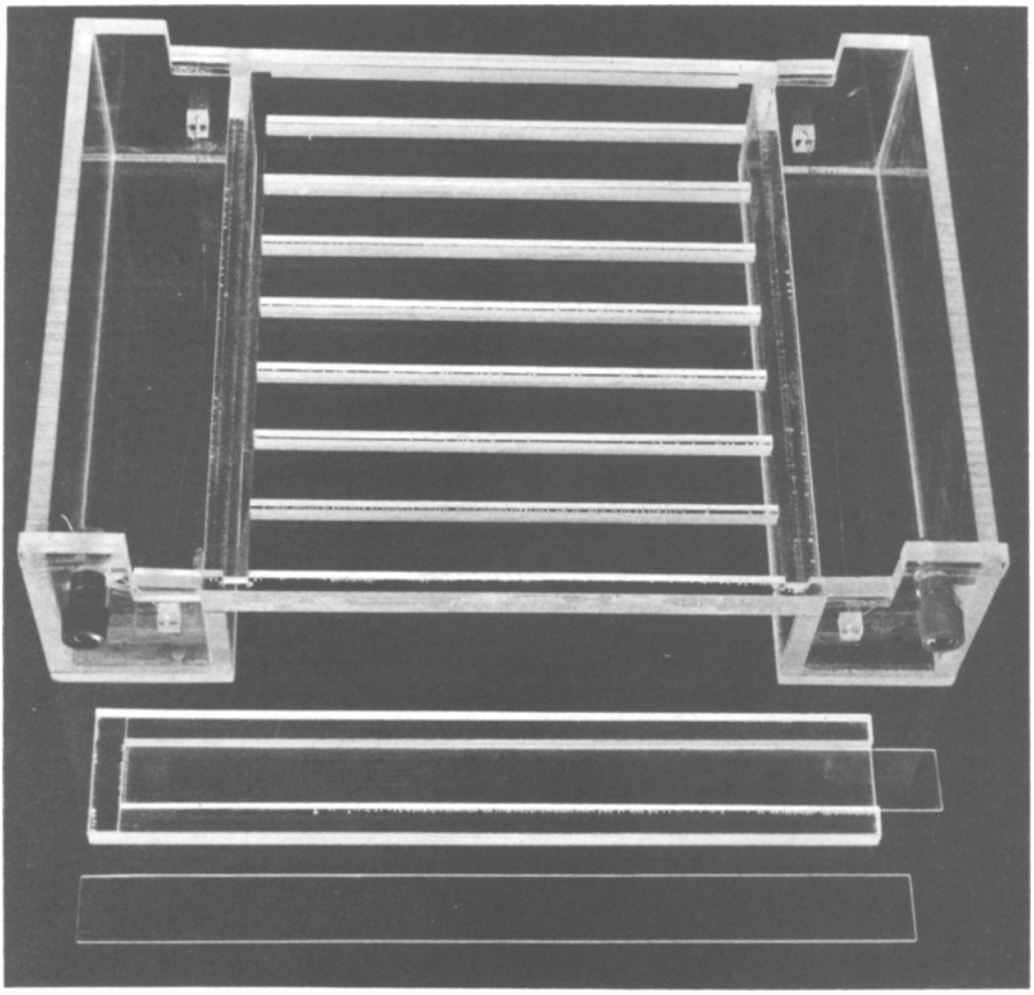

Figure 1. (Upper) Starch-gel apparatus, illustrating gel tray with end gates removed.

(Lower) Slicing tray with 1/16 in. thick Plexiglas pieces that help cut the gel.

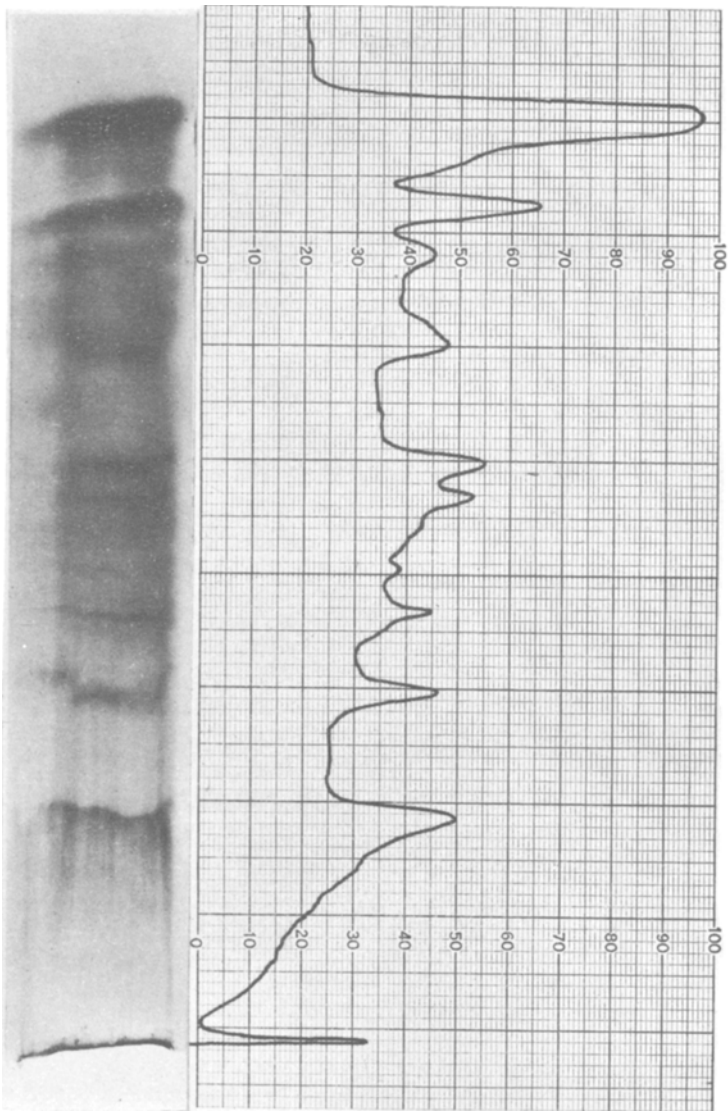

Figure 2. (Right) Densitometer scan of starch-gel pattern with control serum, using a 595 mu filter. (Length of electropherogram = 10 cm.)

(Left) Starch-gel pattern for blood protein isolated from control I. molitor larvae. Electrophoresis for 2.5 hr at 45 ma in 0.038 M aluminum lactate buffer (pH = 3.1).

References

1. P. J. BUNYAN and A. TAYLOR, J. Agr. Food Chem. 14, 132 (1966).
2. H. J. COLVIN and A. T. PHILLIPS, Bull. of Environ. Cont. & Toxic. 3, 106 (1968).
3. R. L. HUNTER, and M. S. BURSTONE, J. Histochem. Cytochem. 8, 58 (1960).
4. A. M. KADOUM, S. O. NELSON and L. E. STETSON, Ann. Entom. Soc. Amer. 60, 885 (1967).
5. M. J. KARNOVSKY, L. ROOTS, J. Histochem. Cytochem. 12, 219 (1964).
6. C. L. MARSH, C. R. TOLLIFF and L. C. PAYNE, Amer. J. Clin. Path. 41, 217 (1964).
7. O. SMITHIES, Biochem. J. 61, 629 (1955).
8. O. SMITHIES, Ibid. 71, 585 (1959)
9. O. SMITHIES, Adv. Protein Chem. 14, 113 (1959).