Starch-Gel Electrophoresis of Liver Esterases Selectively Inhibited by Pesticides

by C. E. MENDOZA and G. V. HATINA
Food Division, Research Laboratories, Food and Drug Directorate, Ottawa 3, Ontario, Canada

Esterases have been gaining importance in pesticide residue detection and identification (1, 2, 3, 4, 5, 6, 7, 8, 9, 10). The activity of some esterases can be inhibited by nanogram amounts of pesticides and selectively inhibited by certain pesticides (6, 10). The use of liver esterases from avian and mammalian species has shown promise in TLC (thin-layer chromatographic) determination and confirmation of certain inhibitors (6). Liver esterases of different species have been reliably differentiated by starch-gel electrophoresis (Mendoza and Hatina, unpublished). The electrophoretic patterns of esterases were affected by pH and concentration of starch and buffer used in preparation of gels. This paper describes starch-gel electrophoresis of liver esterases selectively inhibited by pesticides.

Materials and Methods

Bridge buffer - Approximately 0.3 M boric acid buffer pHs 8.6 was prepared with NaOH.

Gel buffers - The tris buffer [tris (hydroxymethyl) aminomethane] of different molarities was made with HCl to the desired pH just before use.

Bulletin of Environmental Contamination & Toxicology,
Vol. 5, No. 2, 1970, published by Springer-Verlag New York, Inc.

Extraction of esterases - The esterases were extracted from fresh livers of male Rhesus (Rh) monkey, chicken, and turkey, steer, barrow, and wether by the method previously reported by Mendoza et al. (6). The aqueous 2000-g supernatant used in this study was generally used for TLC (6).

Preparation of the gel - In each gel, 26.25 g of hydrolysed starch (obtainable from Connaught Medical Research Laboratories, University of Toronto, Canada) per 250 ml of buffer was used. The procedure of Smithies (11) was generally followed.

Electrophoresis - The gel used was 8 x 108 x 186 mm. Three power supplies of equivalent performance (two with 150 mA and 500 v capacities, obtained from Gelman Instrument Co., Michigan; and one with 150 mA and 400 v capacities, Heathkit, Model PS-4) and platinum electrodes (1 mm in diameter) were used. The bridge buffer was used twice only and was replaced daily. The paper bridges (each with 5 layers of Whatman No. 1 filter paper) were replaced every third day.

The gel was prepared for sample application by cutting vertical slots approximately 8 mm long and 5 cm from the end of the gel. The strip wetted with approximately 10 µliters of enzyme solution was inserted into the slot. The paper strips were removed after 15-min electrophoresis under 160 v and before a 300-v power was applied. Electrophoresis was terminated when the brown borate line travelled 8 cm.

Staining procedure - The gel was incubated for 10 min in a solution of α-naphthyl acetate and Fast Blue RR (5). The gel was then flooded with cold water and was fixed in a solution of methanol: acetic acid: water (5:1:5).

To determine the effect of pesticides on the activity of different liver esterases, 450 µl of each enzyme solution was incubated with 50 µl of 0.2 M methyl parathion, diazinon, carbaryl, Dyrene, or tepa in methanol at 37°C for 30 min.

Results and Discussion

For convenience, the term band was used to mean either esterases or isozymes hydrolyzing α-naphthyl acetate. In this experiment, however, no attempt was made to characterize components of the bands.

Effect of temperature. Fig. 1 shows a zymogram typical of liver esterases incubated at 0, 24, 37, 48, 60, and 70°C, for 30 min. After incubation at 24-48°C, beef, pig, or chicken esterases showed activity comparable to those in an ice-bath, whereas sheep, turkey, and monkey esterases showed increased activity. At 60°C, pig, chicken, and turkey esterases showed only a slight decrease over the control. After 1 hr at 70°C, further inactivation of pig liver esterases and complete inactivation of chicken esterases were observed. Complete inactivation of beef, sheep, monkey, and turkey esterase was noted after 30 min at 70°C. The results obtained were comparable to the effect of heat treatment on amidase activity of the liver of wether (13).

Fig. 2 shows that zymograms of beef, pig, sheep, monkey, chicken, or turkey liver esterases incubated at 37°C for 30 min were comparable to those of esterases incubated for 60 min. The zymograms were on starch-gels made with 0.02-M tris HCl buffer at pH 7.5.

Effect of pesticides on liver esterases - Fig. 3 (A-C) shows zymograms of liver esterases incubated with pesticides at 37°C for 30 min. In this experiment, Dyrene heated with methanol was studied only with the pig and sheep esterases although recent data (Mendoza and Wales, unpublished), showed that it was inhibitory to other liver esterases.

Beef esterases (Fig. 3A). The major esterase bands showed resistance to inhibition by 0.02-M methyl parathion, diazinon, tepa, or freshly dissolved Dyrene in acetone-methanol, the exceptions being the fastest bands of esterases treated with methyl parathion, carbaryl, and diazinon. The slowest moving esterase bands were strongly inhibited by 0.02 M carbaryl. The fastest band was slightly inhibited by tepa and was not affected by Dyrene that was dissolved with 20% acetone in methanol. Slightly increased activity was observed with methanol-treated extracts (blanks).

Sheep esterases (Fig. 3A). Strikingly different results were obtained with sheep liver esterases incubated with 0.02-M methyl parathion, carbaryl, diazinon, heated Dyrene, and tepa. Methyl parathion and tepa did not affect esterases while carbaryl strongly inhibited the activity of intermediate bands and slightly inhibited the slowest moving band. Diazinon weakly inhibited the slow-moving bands but strongly inhibited the fastest-moving band. The heated Dyrene inactivated esterases almost completely.

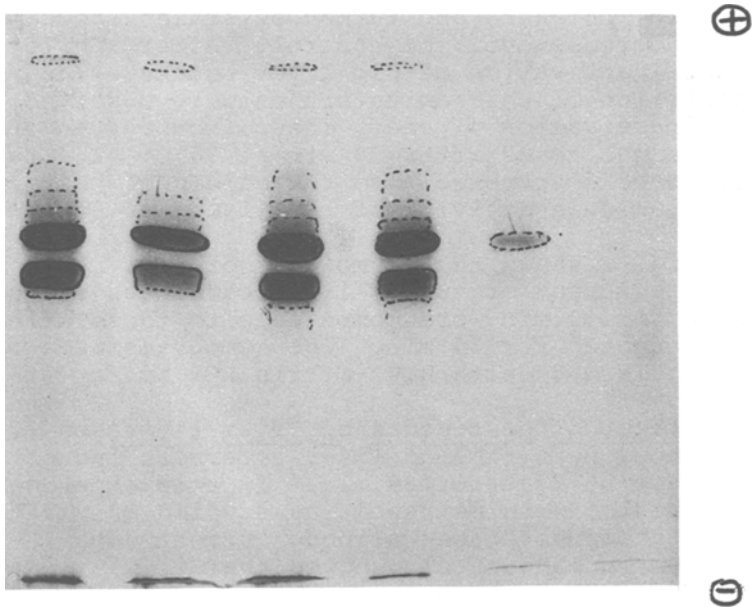

Fig. 1. A zymogram typical of liver esterases incubated for 30 min. Outlines of the bands are traced with india ink to improve the contrast; solid lines indicate dark bands and broken lines indicate weak or light bands. This zymogram is of beef esterases incubated at the following incubation temperatures: from left to right -- control (ice-bath), 24, 37, 48, 60, and 70°C. The gel was made with 0.02-M tris-HCl buffer at pH 7.5

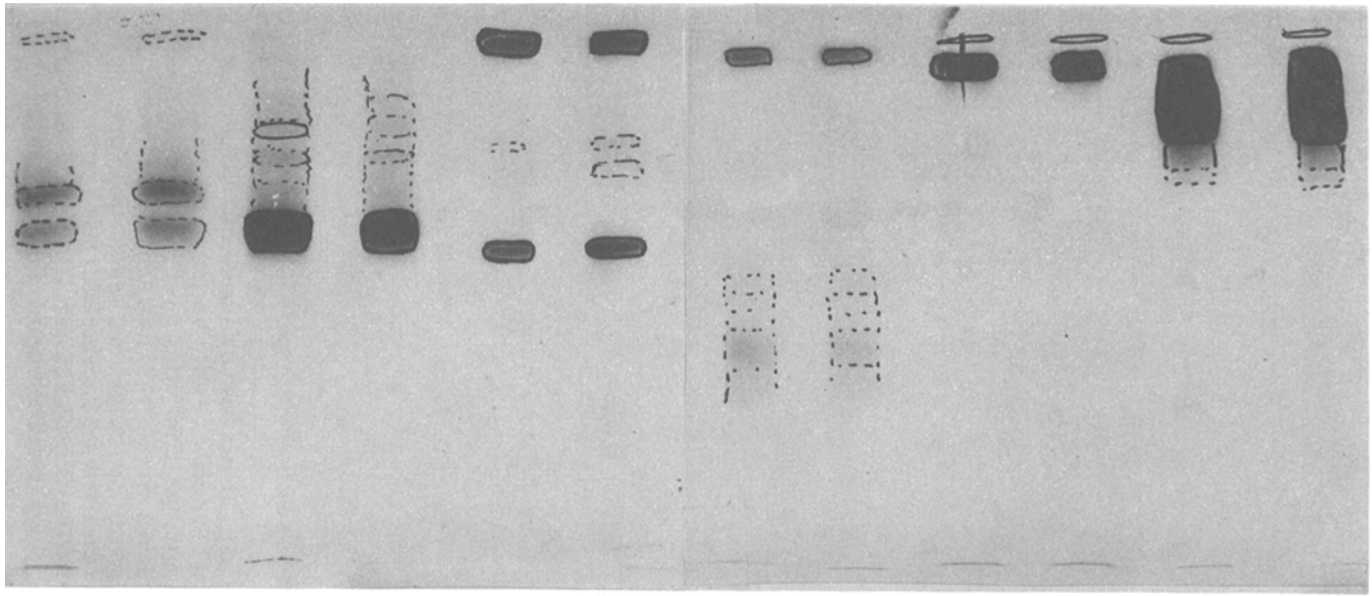

Fig. 2. Zymograms of A) beef, B) pig, C) sheep, D) Rh monkey, E) chicken, and F) turkey liver esterases. Outlines of the bands are traced with india ink to improve the contrast; solid lines indicate dark bands and broken lines indicate weak or light bands. Each pair of slots had zymograms of esterases incubated at 37°C for 30 (left) and 60 min (right).

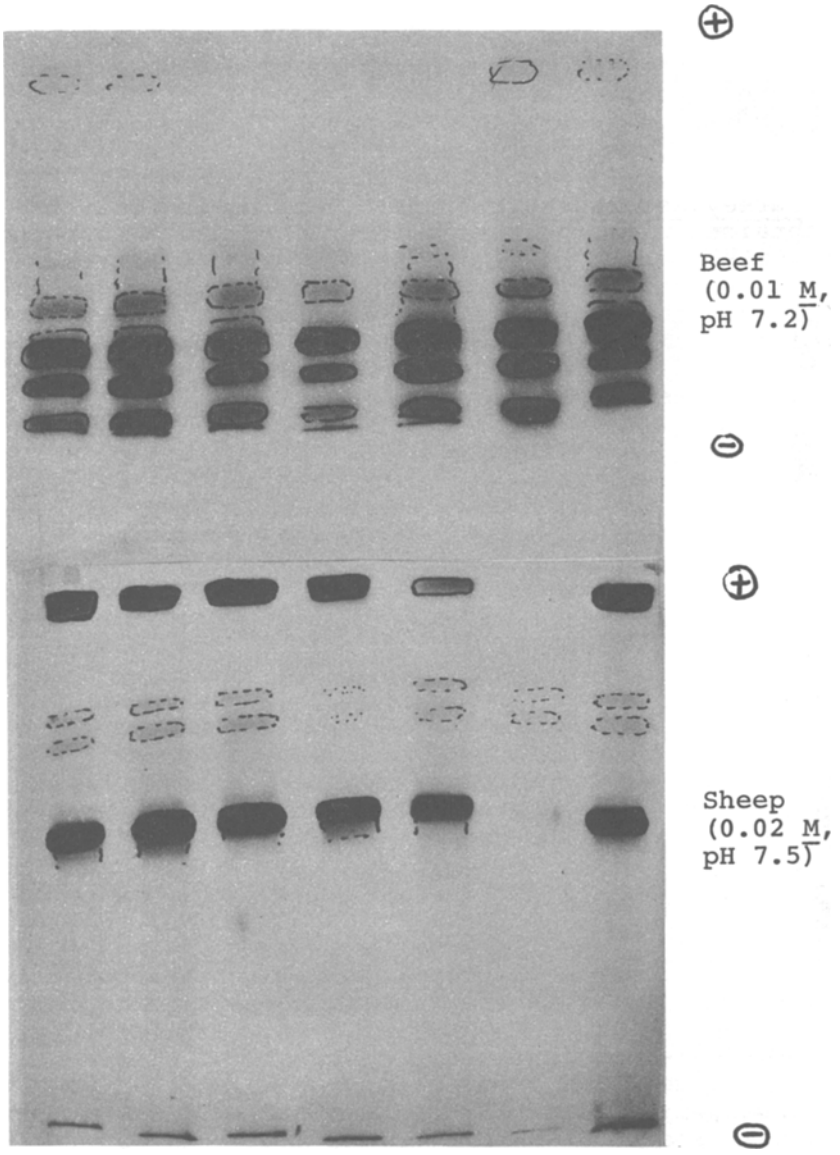

Fig. 3A. Zymograms of liver esterases incubated at 37°C for 30 min. Outlines of the bands are traced with india ink to improve the contrast; solid lines indicate dark bands and broken lines indicate weak or light bands. From left to right of each gel are zymograms of esterases treated with water, methanol, methyl parathion, carbaryl, diazinon, Dyrene, and tepa. Concentrations and pH of buffer used in preparation of gels are in parenthesis.

Chicken esterases (Fig. 3B). Chicken liver esterases were strongly inhibited by 0.02-M methyl parathion or diazinon, followed by 0.02-M carbaryl. Weak enzyme inhibition was observed with 0.02-M tepa or Dyrene dissolved in acetone-methanol. Chicken liver esterases incubated with methanol showed no increase in activity over the control.

Turkey esterases (Fig. 3B). Strong inhibition was obtained with the turkey liver esterases incubated with 0.02-M methyl parathion at 37°C for 30 min. Both 0.02-M carbaryl and 0.02-M diazinon selectively inactivated the fast-moving band. Dyrene, freshly dissolved in acetone-methanol, and tepa had negligible effect on this esterase. Methanol slightly activated turkey esterases.

Pig esterases (Fig. 3C). Strong inhibition was observed with pig liver esterases incubated with 0.02-M methyl parathion or diazinon. Carbaryl at 0.02-M had a slight inhibitory effect on the main bands. Freshly dissolved Dyrene in acetone-methanol and tepa did not inhibit esterases. However, when the methanol solution of Dyrene was heated, complete inactivation of esterases was observed.

Rh Monkey esterases (Fig. 3C). Diazinon at 0.02 M gave the strongest inhibition of monkey esterases incubated at 37°C, followed by 0.02-M methyl parathion or carbaryl. Dyrene, freshly dissolved in acetone-methanol, and tepa negligibly inhibited the esterase bands. Methanol had no effect on esterases.

Conclusion

The studies presented here indicate that selective inhibition of liver esterases or bands by pesticides can be evaluated rapidly by starch-gel electrophoresis. The liver esterase inhibition - starch-gel electrophoretic procedure described may be useful in screening pesticides with selective or specific toxicity to a particular species. A similar method has already been used to demonstrate the relation of esterase inhibition with acute organophosphorus poisoning in pheasants (14). The ability of Dyrene heated in methanol to strongly inhibit liver esterases is noteworthy and will be reported elsewhere.

Acknowledgement

We thank Dr. K.A. McCully, Miss P.J. Wales, and Miss M. van Walbeek for criticism of the manuscript.

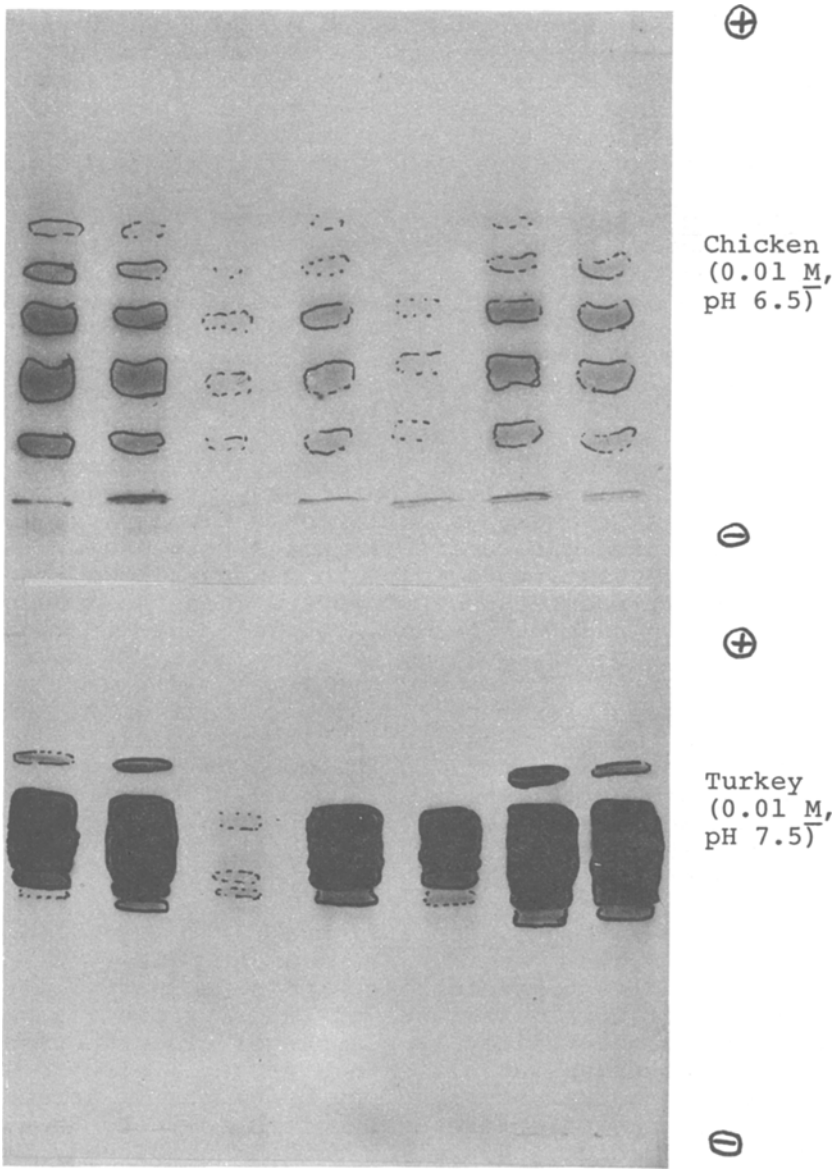

Fig. 3B. Zymograms of liver esterases incubated at 37°C for 30 min. Outlines of the bands are traced with india ink to improve the contrast; solid lines indicate dark bands and broken lines indicate weak or light bands. From left to right of each gel are zymograms of esterases treated with water, methanol, methyl parathion, carbaryl, diazinon, Dyrene, and tepa. Concentrations and pH of buffer used in preparation of gels are in parenthesis.

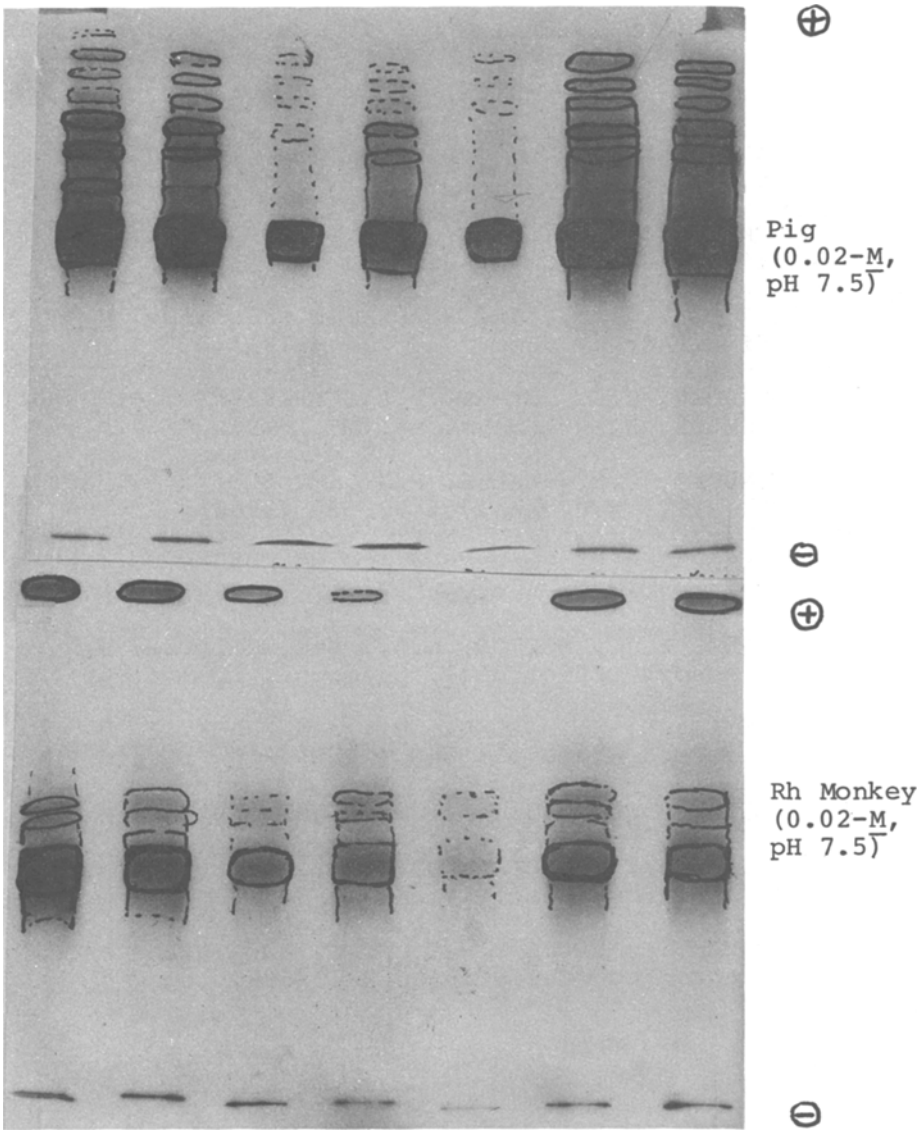

Fig. 3C. Zymograms of liver esterases incubated at 37°C for 30 min. Outlines of the bands are traced with india ink to improve the contrast; solid lines indicate dark bands and broken lines indicate weak or light bands. From left to right of each gel are zymograms of esterases treated with water, methanol, methyl parathion, carbaryl, diazinon, tepa, and Dyrene. Concentrations and pH of buffer used in preparation of gels are in parenthesis.

References

1. ACKERMAN, H. J. Chromatog. 36, 309 (1968).

2. BUNYAN, P.J., Analyst 89, 615 (1964).

3. FALLSCHEER, H.O., and COOK, J.W. J. Assoc. Offic. Agric. Chem. 39, 691 (1956).

4. MCKINLEY, W.P., and JOHAL, P.S. J. Assoc. Offic. Agric. Chem. 46, 840 (1963).

5. MCKINLEY, W.P., and READ, S.I. J. Assoc. Offic. Agric. Chem. 44, 726 (1961).

6. MENDOZA, C.E., GRANT, D.L., BRACELAND, B., and MCCULLY, K.A. Analyst 94, 805 (1969).

7. MENDOZA, C.E., WALES, P.J., MCLEOD, H.A., and MCKINLEY, W.P. Analyst 93, 34 (1968).

8. MENDOZA, C.E., WALES, P.J., MCLEOD, H.A., and MCKINLEY, W.P. Analyst 93, 173 (1968).

9. WALES, P.J., MCLEOD, H.A., and MCKINLEY, W.P. J. Assoc. Offic. Anal. Chem. 51, 1239 (1968).

10. WINTERLIN, W., WALKER, G., and FRANK, H. J. Agr. Food Chem. 16, 808 (1968).

11. SMITHIES, O. Biochem. J. 61, 629 (1955).

12. UICHIDA, T., DAUTERMAN, W.C., O'BRIEN, R.D. J. Agr. Food Chem. 12, 48 (1964).

13. BUNYAN, P.J., JENNINGS, D.M., and TAYLOR, A. J. Agr. Food Chem. 16, 332 (1968).