# Inhibition of Growth and Esterase Activity in Mouse Fibroblast Cell Cultures by Several Organophosphorus Insecticides

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The presence of esterases was demonstrated in sonically disrupted cell suspensions (sonicates) of mouse fibroblast L-929 cells by their ability to hydrolyze choline and phenyl esters. The presence of more than one esterase in L-cells was indicated by the fact that organophosphorus insecticides gave widely differing $pI_{50}$ values when PhAc and BuCh substrates were compared. The esterases were much less sensitive to the organophosphorus compounds tested than esterases from insect or whole animal mammalian sources and more closely resembled mammalian pseudocholinesterases (EC 3.1.1.7) than true or acetylcholinesterases (EC 3.1.1.8). Bioassays of the toxicity of the organophosphorus insecticides used on cultures of L-cells gave relative toxicities of: phosphamidon > Bidrin > SD 11319 > Azodrin, in contrast with their toxicity to mice where the order was SD 11319 > Azodrin > Bidrin > phosphamidon. There appeared to be no correlation between cell growth inhibition by the insecticides and esterase inhibition.

Before an insecticide can be registered for use on food crops, information on the behavior of the chemical in plants and animals must be accumulated to ascertain the potential hazards associated with that use. In the past, systems for studying the toxic effects of a chemical have been essentially limited to whole animals or subcellular particles, such as liver microsomes. It would be valuable to have available a system in which one could investigate the effects of a chemical on intact cells without the complex interactions which apply in the whole animal. Furthermore, great savings in time are made possible by the use of tissue culture techniques.

Lewis and Richards (1945) found that DDT resulted in no appreciable differences in cytology, mitosis, or migration of chick embryo and intestine cells when compared to control cells. Solarino *et al.* (1966) noted only minute changes in cellular morphology after treatment of fowl embryo plantaris tendon fibroblast cells with DDT, but felt they were important because subsequent changes in an intact animal treated with DDT may stem from these effects. Chung *et al.* (1967, 1968) found that DDT caused a decrease in total HeLa cell population, although total cell protein was not changed. Changes noted in RNA and DNA were not directly related to DDT or dieldrin concentrations.

Gabliks and coworkers (Gabliks and Friedman, 1965; Gabliks, 1965a; Gabliks *et al.*, 1967), using Chang liver, mouse liver, HeLa and L-929 cells, evaluated the acute and chronic toxicity of a number of insecticides representing a number of chemical classes. The dinitrophenol insecticides were the most toxic, and all insecticides were significantly more toxic during chronic than during acute cytotoxicity tests. Furthermore, HeLa cells treated with insecticides were more susceptible than controls to polio virus and diphtheria toxin (Gabliks, 1965b), indicating that treatment with an insecticide alters the physiology of the cell. This was further substantiated by the fact that some insecticides apparently act against vaccinia virus in Chang liver cells (Gabliks, 1967).

Wilson and Walker (1966) suggested that malathion acted to increase the death rate of chick embryo cells or affected the time spent by cells between divisions. Wilson *et al.* (1968) found malaoxon strongly inhibiting the activity of chick embryo cell cholinesterase at concentrations where it did not appreciably affect cell growth. However, growth studies (Wilson and Stinnett, 1969) indicated that malathion was more toxic than malaoxon to the growth of two cell types.

Since the organophosphorus insecticides function as inhibitors of cholinesterases, an investigation of organophosphates in tissue culture would necessarily involve an investigation of the esterases of that system. Engel (1961) discovered cholinesterase activity in the cytoplasm and perinuclear area of chick embryo skeletal muscle cells. Long-term cultures of mammalian brain cells (Geiger and Stone, 1962) showed localized intracellular nonspecific esterases and acetylcholinesterase. Jones *et al.* (1956) found the esterase enzyme in primary explants of chick embryo intestines to be predominately the specific acetylcholinesterase. Induction of acetylcholinesterase synthesis by addition of substrate has been demonstrated in chick embryo leg muscle cells (Goodwin and Sizer, 1963). Substrate specificity studies in the presence of added acetylcholine suggested the presence of an acetylcholinesterase and a nonspecific esterase in chick embryo lung (Burkhalter, 1963; Burkhalter *et al.*, 1957).

In the present study, the effects of several organophosphorus insecticides on the growth of mouse fibroblast L-929 cells have been evaluated. Because the mode of action of these compounds *in vivo* is known to be inhibition of acetylcholinesterase, an attempt has been made to characterize the esterases of L-929 cells and to evaluate the effects of the organophosphorus insecticides on them.

It would be valuable if a correlation between cell growth inhibition and esterase inhibition could be demonstrated. In a recent study, Litterst *et al.* (1969) were unsuccessful in demonstrating a correlation between the effects of several insecticides on cell growth and their effects on DNA, RNA, and protein synthesis in HeLa cell cultures.

## METHODS AND MATERIALS

**Tissue Cultures.** Mouse fibroblast L-929 cell cultures (Earle, 1943) were maintained under sterile conditions as *in vitro* suspension cultures in 89% modified Eagle's Essential Medium (Eagle, 1955) 10% horse serum, 1% glutamine, and 100 units of penicillin and 0.1 mg of streptomycin per 100 ml of medium (all components obtained from Microbiological

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Associates, Inc., Bethesda, Md.). The cells were grown in 200-ml volumes in 500-ml Erlenmeyer flasks inside a controlled environment incubator at 33° C, 80–90% relative humidity, and a gas phase of 6% $CO_2$ in air. Nutrient medium was replenished every other day by centrifuging the cultures at 275 G for 25 min, decanting the depleted supernatant medium, and resuspending the cell pellet in freshly prepared growth medium.

Cellular concentration in the cultures was determined by diluting the cells into white blood cell pipettes with 5% 1 to 300 trypsin (Nutritional Biochemical Corp., Cleveland, Ohio) in physiological saline. The cells were transferred to hemocytometers where quadruplicate counts were performed microscopically at 100X.

**Substrates and Inhibitors.** The following chemicals used as substrates for L-cell esterases were obtained commercially: phenyl acetate (PhAc), phenyl propionate (PhPr), phenyl butyrate (PhBu), acetylcholine chloride (ACh), propionylcholine iodide (PrCh), butyrylcholine iodide (BuCh), acetyl-β-methylcholine chloride (MeCh), and benzoylcholine chloride (BzCh). 3-Hydroxy-*N*,*N*-dimethyl-*cis*-crotonamide, dimethyl phosphate (Bidrin), 3-hydroxy-*N*-methyl-*cis*-crotonamide, dimethyl phosphate (Azodrin), and 3-hydroxy-*cis*-crotonamide, dimethyl phosphate (SD 11319) were supplied by Shell Development Co., Modesto, Calif. 2-Chloro-*N*,*N*-diethyl-3-hydroxycrotonamide, dimethyl phosphate (phosphamidon) was from CIBA Agrochemical Co., Vero Beach, Fla. Eserine sulfate was obtained from Calbiochem, Los Angeles, Calif.

**Evaluation of Organophosphorus Insecticide Toxicity to L-Cells.** Cells from a stock donor L-cell culture were removed, after determining the concentration, to set up new control and experimental cultures in freshly prepared nutrient medium, each new culture having a cell concentration of 350,000–450,000 cells per ml. Test volumes of aqueous solutions of Bidrin or phosphamidon were added via sterile pipettes to the test cultures and equal volumes of sterile, distilled water were similarly added to the control cultures. All cultures were gauze-stoppered and placed on stirrers inside the incubator for 1 hr to allow mono-dispersal of the cells. At this point, 0-hr cell concentrations were determined. All cultures were replaced in the incubator and allowed to grow for a total period of 72 hr with sampling for cell concentration quantitation, and bacteriological agar inoculation at 24, 48, and 72 hr. At each point of investigation, comparisons of populations of control and test cultures relative to 0-hr populations were used to assay toxicity.

Another technique (Perlman, 1968; Smith *et al.*, 1963) was used in the bioassay of Bidrin, phosphamidon, Azodrin and SD 11319 toxicity to L-cells. From donor L-cell cultures, six new cultures were prepared, each containing 350,000–450,000 cells per ml. Each insecticide, in a constant volume of distilled water, was added to five of the cultures from a glass syringe through a Millipore Swinex filter, pore size 0.22 μ (Millipore Filter Corp., Bedford, Mass.). The final concentrations were 1 to 15 $\times 10^{-3} M$ in the five test cultures. An equal volume of distilled water was similarly added to the control flask. All flasks were placed in the incubator for 1 hr. Zero-hr cell concentrations were then determined on each culture. The cultures were incubated for 24 hr, when cell concentrations were again determined. Comparisons of each test culture with the control culture on the basis of relative population increases were made, and inhibition of cellular proliferation by the varying concentrations of insecticides was calculated. $ID_{50}$ (concentration of the insecticide required to limit cellular proliferation to 50% of that observed in the unsupplemented control culture) was determined by plotting the data on log-probit paper.

**Esterase Activity Quantitation.** An appropriate volume of donor culture was centrifuged at 275 G for 25 min in order to isolate the desired number of cells as a cell pellet. The supernatant medium was decanted and the cells were resuspended in physiological saline with subsequent recentrifugation. This washing procedure was performed twice in order to remove any medium components from the exterior of the cells. The cells were finally resuspended in a hypertonic bicarbonate buffer (0.37*M* sodium bicarbonate and 0.164*M* sodium chloride). In some experiments, the cells were added in this state to Warburg flasks for esterase analysis. In most of the experiments, the cells resuspended in the bicarbonate buffer were sonified by a Branson Sonifier (Branson Sonic Power, Danbury, Conn.) utilizing a micro tip. The resultant sonically disrupted cell suspension (sonicate) was then added to Warburg flasks for esterase analysis. Protein determinations of both whole cell preparations and sonicates were by the method of Lowry *et al.* (1951) as modified by Oyama and Eagle (1956), using bovine serum albumin (Nutritional Biochemical Corp.) as a standard.

Acid released from ester hydrolysis was determined manometrically by measuring $CO_2$ released from the bicarbonate buffer at 37° C. Seven-milliliter total volume, single sidearm Warburg flasks were used, with the substrate in 0.4 ml of buffer in the side arm and enzyme from the L-cells or horse serum in 1.6 ml of buffer in the main compartment. The flasks were equilibrated for 15 min at 37° C prior to initiating the reaction by mixing the enzyme and substrate. Manometer readings were taken at 5 min intervals for 60 min. When the rate of $CO_2$ evolution decreased within this period, only the initial linear portion was used in deriving the $b_{60}$ values (microliters of $CO_2$ evolved per 60 min).

The optimum substrate concentration for L-cell esterases was evaluated with choline and phenyl esters. Esters which were not water soluble were emulsified in the bicarbonate buffer with Triton X-100 (Rohm and Haas Co., Philadelphia, Pa.). When thus employed, the final amount of Triton X-100 in each Warburg flask was 1 mg.

The cell concentration was also evaluated in order to obtain optimum L-cell esterase activity. Sonicates of $32 \times 10^6$ cells (34 mg of protein), $64 \times 10^6$ cells (68 mg of protein), and $128 \times 10^6$ cells (136 mg of protein) per Warburg flask were used with the choline ester substrates. For all whole cell esterase measurements, $32 \times 10^6$ L-cells (34 mg of protein) were used. Horse serum hydrolysis of choline substrates was studied using 0.44 ml of horse serum (34 mg of protein).

**Esterase Inhibition.** Solutions of Bidrin, Azodrin, SD 11319, and eserine sulfate in bicarbonate buffer were added to the main compartments of the Warburg flasks and incubated with the whole cells, cell sonicate, or horse serum for 15 min at 37° C before mixing with the substrate. Comparisons between uninhibited control esterase activity and inhibited esterase activity allowed for the calculation of $pI_{50}$ (negative logarithm of the molar inhibitor concentration necessary for 50% inhibition of the esterase) for each inhibitor.

For studies of inhibition of esterase hydrolysis of choline esters, BuCh was used as the substrate in $10^{-1} M$ final concentration in each Warburg flask. For studies of inhibition of esterase hydrolysis of phenyl esters, PhAc was used as the substrate in $10^{-2} M$ final concentration in each Warburg flask.

## RESULTS

**Toxicity of Organophosphorus Insecticides to L-Cells.** L-cells grew normally, relative to the control cells, in the presence of $10^{-3}M$ Bidrin in cultures whose population kinetics were assessed at 24, 48, and 72 hr of growth (Table I), although higher concentrations of Bidrin demonstrated progressively greater inhibition of growth. Phosphamidon was more potent as seen by the fact that $10^{-3}M$ resulted in 27% inhibition at 24 hr as compared to the controls. Bacteriological media plates inoculated from all cultures were negative for microbial growth.

Twenty-four hr data on L-cell growth inhibition by phosphamidon, Bidrin, Azodrin, and SD 11319 are shown in Table II. The results are expressed as the negative logarithm of the $ID_{50}$ to allow ready comparison with $pI_{50}$ data.

**Characterization of L-Cell Esterases.** HYDROLYSIS OF SUBSTRATES BY WHOLE L-CELLS. Attempts to hydrolyze choline and phenyl ester substrates by whole L-cells in bicarbonate buffer were unsuccessful. No $CO_2$ was produced when ACh, BuCh, PrCh, PhAc, PhPr, or PhBu substrates were incubated for 60 min with 32, 64, or 128 $\times$ $10^6$ cells under standard Warburg conditions.

**Selection of Cell Quantities for Esterase Studies.** L-cells which were disrupted by sonication hydrolyzed both choline and phenyl esters. With activity expressed as $\mu$moles $CO_2$ produced by $10^{-1}M$ ACh hydrolysis per 100 mg protein per hr, the activity for a sonicate of 16 $\times$ $10^6$ cells was 19; 21 for 32 $\times$ $10^6$ cells; 23 for 64 $\times$ $10^6$ cells; and 13 for 128 $\times$ $10^6$ L-cells. Using BuCh ($10^{-1}M$) as the substrate, the activity for a sonicate of 16 $\times$ $10^6$ L-cells was 21; 20 for 32 $\times$ $10^6$ cells; 21 for 64 $\times$ $10^6$ cells; and 14 for 128 $\times$ $10^6$ cells. Accordingly, 32 $\times$ $10^6$ L-cells were used as the source of esterase in all subsequent experiments. Sonicates of this amount of cells consistently contained approximately 34 mg protein.

**Substrate Specificity of L-Cell Esterases.** Hydrolysis of choline and phenyl esters was not inhibited by excess substrate (Figures 1 and 2). Relative rates of hydrolysis for the choline esters were BuCh > ACh > MeCh > PrCh (Fig. 1). BzCh was not hydrolyzed at substrate concentrations between $10^{-4}M$ and $10^{-1}M$. Relative rates of hydrolysis for the phenyl esters were PhAc > PhPr > PhBu (Fig. 2). Phenyl ester hydrolysis proceeded at a much greater rate than choline ester hydrolysis.

**Hydrolysis of Choline Esters by Horse Serum.** In order to approximate the amount of protein in 32 $\times$ $10^6$ L-cells, 34 mg horse serum protein was used per Warburg flask. The horse serum esterases and L-cells esterases exhibited similar substrate specificities. However, the absolute rate of hydrolysis of any choline ester substrate was approximately 10 times faster than that seen with L-cell esterases. Horse serum also hydrolyzed BzCh. Also, under stated conditions the $K_m$ for horse serum cholinesterase was approximately 1.7 $\times$ $10^{-1}M$, while the $K_m$ for L-cell cholinesterase was approximately 7.5 $\times$ $10^{-3}M$, with ACh as the substrate.

**Inhibition of BuCh and PhAc Hydrolysis by Insecticides.** Eserine sulfate and Bidrin inhibited both BuCh and PhAc hydrolysis by L-cell esterases at relatively high levels. However, the degree of inhibition with Azodrin and SD 11319 varied with the substrate investigated. Azodrin and SD 11319 inhibited PhAc hydrolysis, but Azodrin and SD 11319 concentrations as high as $1M$ did not inhibit BuCh hydrolysis. The $pI_{50}$ values for each inhibitor with BuCh and PhAc are listed in Table II. Eserine sulfate and Bidrin more severely inhibited the enzymatic hydrolysis of PhAc than BuCh. The degree of inhibition of PhAc hydrolysis resulting from eserine sulfate, Bidrin, and Azodrin showed little variance. SD 11319 was the most potent inhibitor of PhAc hydrolysis while eserine sulfate was the most potent inhibitor of BuCh hydrolysis.

**Table I. Effect of Bidrin and Phosphamidon on L-Cell Culture Growth**

| Insecticide Concentration in Culture | Relative Cell Number[a] and Per Cent Inhibition[b] at Indicated Time | | | | | |
|---|---|---|---|---|---|---|
| | 24 hr | | 48 hr | | 72 hr | |
| | Bidrin | | | | | |
| 0 (Control) | 2.0 | (0) | 3.0 | (0) | 3.5 | (0) |
| $1 \times 10^{-3}M$ | 2.0 | (0) | 3.0 | (0) | 3.1 | (11) |
| $5 \times 10^{-3}M$ | 0.78 | (61) | ...[c] | (100) | ... | (100) |
| $1 \times 10^{-2}M$ | ...[c] | (100) | ... | (100) | ... | (100) |
| | Phosphamidon | | | | | |
| 0 (Control) | 2.6 | (0) | 4.5 | (0) | 5.0 | (0) |
| $1 \times 10^{-3}M$ | 1.9 | (27) | 1.6 | (65) | 1.4 | (72) |
| $1 \times 10^{-2}M$ | ...[c] | (100) | ... | (100) | ... | (100) |

[a] Relative cell number = culture cell concentration at experimental time indicated divided by culture cell concentration at 0 hr. [b] Per cent inhibition = 100 minus experimental relative cell number divided by control relative cell number times 100 (given in parentheses). [c] Cells completely lacking.

**Table II. Toxicity to L-Cells and Inhibition of L-Cell Esterases Resulting from Organophosphorus Insecticides**

| Insecticide | $pID_{50}$[a] | $pI_{50}$[b] BuCh ($10^{-1}M$) | $pI_{50}$[b] PhAc ($10^{-2}M$) |
|---|---|---|---|
| Eserine | ... | 2.6 | 3.7 |
| Bidrin | 2.4 | 1.8 | 3.1 |
| Azodrin | 2.0 | ...[c] | 3.3 |
| SD 11319 | 2.1 | ...[c] | 4.7 |
| Phosphamidon | 2.9 | ... | ... |

[a] Negative logarithm of the molar concentration of insecticide required to limit cellular proliferation to 50% of that observed in the unsupplemented control culture. [b] Negative logarithm of the molar concentration of insecticide necessary for 50% inhibition of the esterase enzyme using the indicated substrate. Mean of six replicates. [c] No inhibition observed at insecticide concentrations through $1M$.

## DISCUSSION

L-cell growth was found to be inhibited by the organophosphorus insecticides, phosphamidon, and Bidrin. Bioassays of toxicity of phosphamidon, Bidrin, Azodrin and SD 11319 upon L-cells covered an $ID_{50}$ range between 1.18 $\times$ $10^{-3}M$ and 9.2 $\times$ $10^{-3}M$ ($pID_{50}$ between 2.0 and 2.9) (Table II). Relative toxicities of these insecticides to L-cells were phosphamidon > Bidrin > SD 11319 > Azodrin. Relative toxicities of these insecticides to mice were SD 11319 > Azodrin > Bidrin > phosphamidon (Menzer and Casida, 1965; Clemons and Menzer, 1968). There is no reason to expect that the toxicity relationships in the two systems would be the same. The L-929 cell strain is a genetically abnormal line which does not have a nervous system and in which the enzymes normally associated with mammalian liver microsomes would likely not be present. L-cell toxicity probably depends on a complex of metabolic reactions, whereas mouse toxicity surely depends on acetylcholinesterase inhibition in some key part of the nervous system balanced aganist detoxication mechanisms found in other parts of the animal. Furthermore, when organophosphorus insecticide toxicity to L-cells is measured by bioassay ($pID_{50}$) and compared with inhibition of cellular esterases ($pID_{50}$), there seems to be little correlation between the findings

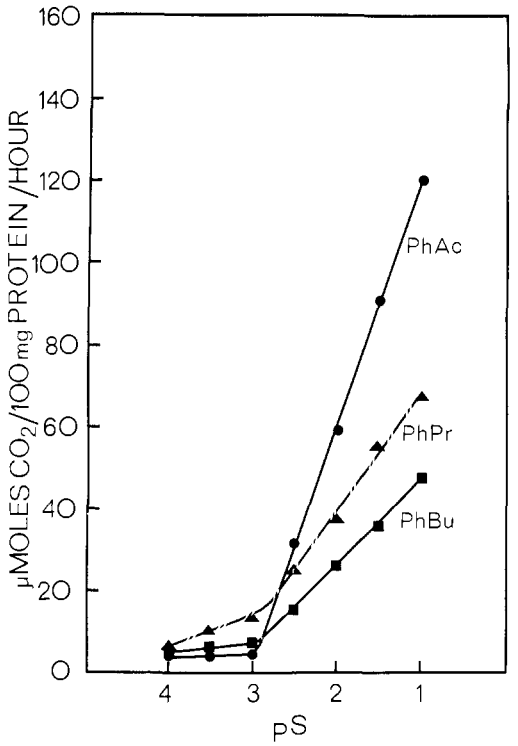


**Figure 1. Activity-substrate concentration curves for hydrolysis of choline esters by L-cell sonicate esterases (pS = negative logarithm of molar substrate concentration)**

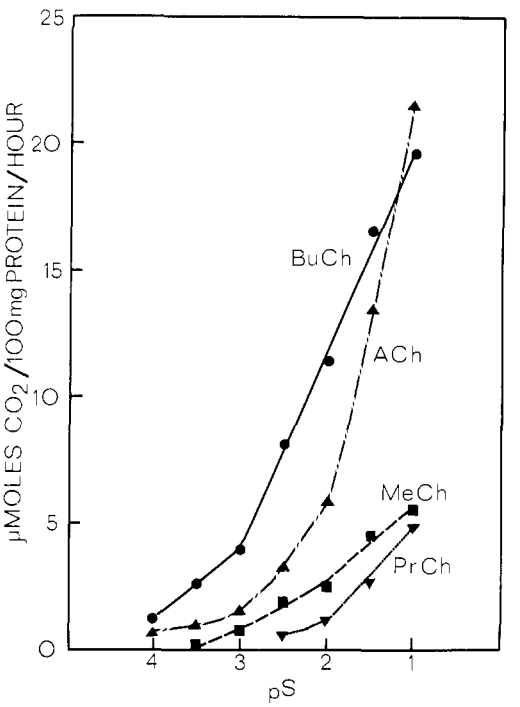


**Figure 2. Activity-substrate concentration curves for hydrolysis of phenyl esters by L-cell sonicate esterases (pS = negative logarithm of molar substrate concentration)**

(Table II). Similar results were obtained by Wilson *et al.* (1968), who noted that malaoxon strongly inhibited cholinesterase activity of chick embryo muscle cells at concentrations which did not appreciably affect growth of the cells.

One or more esterases have been shown to be present in sonicates of L-929 mouse fibroblast cells. These esterases may be differentiated from those occurring in the horse serum of the culture growth medium as follows. (a) Horse serum esterases would exist as relatively large intact protein molecules and would therefore necessarily be in the exterior cell medium. The process of washing the whole cells with saline should have removed any such proteins. However, if the horse serum esterases were associated with the exterior of the cellular surfaces and were not washed off, some substrate hydrolysis should have been noted when whole L-cells were used as the esterase source. Whole cells were assayed with the various substrates repeatedly but no substrate hydrolysis was noted. Esterase activity resulted only when L-cells were sonicated. (b) The $K_m$ values for the enzymes from the two sources were widely different when ACh was used as the substrate. Choline substrates were generally hydrolyzed at least 10 times faster by horse serum esterases than by L-cell esterases. (c) Horse serum esterases hydrolyzed BzCh ($10^{-1}M$) at a rate of 13 μmoles $CO_2$/100 mg of protein hr, while L-cell sonicates did not hydrolyze BzCh at all (concentrations tested were $10^{-4}M$ through $10^{-1}M$).

Enzymatic hydrolysis of choline esters by L-cell sonicates was not inhibited by excess substrate. This is characteristic of a pseudocholinesterase type of enzyme and not of true acetylcholinesterase (Augustinsson and Nachmansohn, 1949). Another such indication is the fact that BuCh, not ACh, was hydrolyzed fastest of all the choline esters by L-cell esterases. Relative rates of hydrolysis of substrates ($10^{-1}M$) by cultured chick lung cell esterases were tributyrin > PrCh > ACh > BuCh > MeCh. BzCh was not hydrolyzed (Burkhalter *et al.*, 1957). These results agree with those of the present study in that the same choline esters were hydrolyzed, BzCh was not hydrolyzed, and the highest rate of hydrolysis was seen with the noncholine phenyl ester, PhAc.

Poulsen and Aldridge (1964) have noted that the degree of inhibition of a single enzyme by an organophosphorus compound should be the same, regardless of the substrate being used. If organophosphorus inhibition of an enzyme which hydrolyzes two separate substrates is not approximately the same, then two or more enzymes are hydrolyzing the substrates. Main (1969) has analyzed the kinetics of enzyme inhibition by diisopropylphosphorofluoridate and amiton to gather evidence for the existence of multiple cholinesterases in serum and erythrocytes. In the present study, the $pI_{50}$ for eserine sulfate-inhibition of L-cell esterase hydrolysis of BuCh (Table II) was 2.6, but the $pI_{50}$ for eserine sulfate-inhibition of L-cell esterase hydrolysis of PhAc was 3.7. Similarly, $pI_{50}$'s for Bidrin-inhibition of L-cell esterase-hydrolysis of BuCh and PhAc were 1.8 and 3.1, respectively. Bidrin and eserine sulfate inhibited both BuCh and PhAc hydrolysis; Azodrin and SD 11319 inhibited PhAc hydrolysis, but did not inhibit BuCh hydrolysis. When these data are viewed in the light of the studies noted above (Poulsen and Aldridge, 1964; Main, 1969), it would appear that the hydrolysis of the substrates was effected by more than one esterase. These data, plus the fact that L-cell sonicates hydrolyze both choline and phenyl esters, indicate that L-cell esterases more closely resemble mammalian pseudocholinesterases than "true" or acetylcholinesterase. They differ from most mammalian pseudocholinesterases in not hydrolyzing BzCh.

It has been established that L-cells possess esterases which are susceptible to inhibition by organophosphorus insecticides, although they are much less sensitive to the compounds used than esterases from either insect or mammalian sources

(Clemons and Menzer, 1968; Menzer and Casida, 1965). Furthermore, there appears to be no correlation between cell growth inhibition and esterase inhibition.

## LITERATURE CITED


Augustinsson, K. B., Nachmansohn, D., *Science* **110,** 98 (1949).
Burkhalter, A., *Nature* (London) **199,** 598 (1963).
Burkhalter, A., Jones, M., Featherstone, R. M., *Proc. Soc. Exp. Biol. Med.* **96,** 747 (1957).
Chung, R. A., Huang, I-Lo, Brown, R. W., J. AGR. FOOD CHEM. **15,** 497 (1967).
Chung, R. A., Lin, Yi-Do, Brown, R. W., J. AGR. FOOD CHEM. **16,** 298 (1968).
Clemons, G. P., Menzer, R. E., J. AGR. FOOD CHEM. **16,** 312 (1968).
Eagle, H., *Science* **122,** 501 (1955).
Earle, W. R., *J. Nat. Cancer Inst.* **4,** 165 (1943).
Engel, W. K., *J. Histochem. Cytochem.* **9,** 66 (1961).
Gabliks, J., *Proc. Soc. Exp. Biol. Med.* **120,** 168 (1965a).
Gabliks, J., *Proc. Soc. Exp. Biol. Med.* **120,** 172 (1965b).
Gabliks, J., *Arch. Environ. Health* **14,** 698 (1967).
Gabliks, J., Bantug-Jurilla, M., Friedman, L., *Proc. Soc. Exp. Biol. Med.* **125,** 1002 (1967).
Gabliks, J., Friedman, L., *Proc. Soc. Exp. Biol. Med.* **120,** 163 (1965).
Geiger, R. S., Stone, W. G., *Federation Proc.* **21,** 366 (1962).
Goodwin, B. C., Sizer, I. W., *Federation Proc.* **22,** 383 (1963).
Jones, M., Featherstone, R. M., Bonting, S. L., *J. Pharmacol. Exp. Ther.* **116,** 114 (1956).
Lewis, W. H., Richards, A. G., *Science* **102,** 330 (1945).
Litterst, C. L., Lichtenstein, E. P., Kajiwara, Kazuto, J. AGR. FOOD CHEM. **17,** 1199 (1969).
Lowry, A., Rosebrough, N. J. Farr, A. L., Randall, R. J., *J. Biol. Chem.* **193,** 265 (1951).
Main, A. R., *J. Biol. Chem.* **244,** 829 (1969).
Menzer, R. E., Casida, J. E., J. AGR. FOOD CHEM. **13,** 102 (1965).
Oyama, V. I., Eagle, H., *Proc. Soc. Exp. Biol. Med.* **91,** 305 (1956).
Perlman, D., *Science* **160,** 42 (1968).
Poulsen, E., Aldridge, W. N., *Biochem. J.* **90,** 182 (1964).
Smith, C. G., Grady, J. E., Northam, J. I., *Cancer Chemother. Rep.* **30,** 9 (1963).
Solarino, G., Fumarola, D., Giordano, D., *Med. Pharmacol. Exp.* **15,** 351 (1966).
Wilson, B. W., Stinnett, H. O., Hooker, W. P., Asmundson, R. V., *J. Cell Biol.* **39,** 143a (1968).
Wilson, B. W., Stinnett, H. O., *Proc. Soc. Exp. Biol. Med.* **130,** 30 (1969).
Wilson, B. W., Walker, N. E., *Proc. Soc. Exp. Biol. Med.* **121,** 1260 (1966).



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