The Effects of Chronic Doses of an Organophosphorus Inhibitor on Cholinesterase Activity in Boll Weevils

Though absolute proof has not been established, the consensus of most workers is that cholinesterase (ChE) inhibition is the primary biochemical lesion leading to the death of insects poisoned by organophosphorus (OP) insecticides[1,2]. Effects of OP poisoning on mammalian ChE have been reported in some detail, but information on chronic effects in insects is limited. As part of a general investigation of systemic, OP insecticides in cotton plants, we examined changes in the ChE activity of adult boll weevils (*Anthonomus grandis* Boheman) that received sustained doses of Bidrin (3-hydroxy-*N*,*N*-dimethyl-*cis*-crotonamide dimethyl phosphate) in their diet.

Insecticide-susceptible weevils were reared and tested under continuous light at 27 °C. During tests, adults of mixed sexes were fed pellets of treated or untreated synthetic diet[3] that were changed daily and prepared fresh at least every 3 days. Only the pure, *cis* isomer of Bidrin was used for all treatments. A complete toxicological study of the fate of Bidrin in boll weevils was reported[4].

A colorimetric procedure[5] was used to measure the ChE activity[6] in whole-body homogenates of adult weevils. Treated insects were homogenized in buffered substrate at 0 °C to minimize reaction of residual anticholinesterase with uninhibited ChE. Each day sufficient insects were collected to permit triplicate analyses and all tests were repeated at least once. In each analysis, the ChE activity of treated insects was compared directly with that of insects of the same age maintained concurrently on untreated diet under identical conditions. All tests were initiated with adult weevils that were 5 to 7 days old.

Insects fed a chronic, sublethal dose (1 ppm) of Bidrin evinced no symptoms typical of OP poisoning. A diet containing 4 ppm Bidrin killed 50% of the population after 6.5 days and caused symptoms typical of OP poisoning, including hyperactivity, poor equilibrium, excessive excretion, and eventual prostration.

In weevils fed chronic sublethal doses of Bidrin, ChE inhibition was cumulative until maximum depression (44.5% I) at 4 days after tests were begun. Throughout the remainder of the test period, however, ChE activity in treated insects tended to recover at a nearly constant rate to 89% of normal after 21 days. In insects removed from treated food after 6 days and held on untreated food thereafter, ChE activity recovered at only a slightly faster rate than in treated insects (Figure 1). When fed a chronic lethal dose, maximum ChE inhibition (56.5%) in survivors occurred 24 h after tests were begun, but on succeeding days ChE activity recovered steadily to 84% of normal after 11 days (Figure 2). Again, enzyme activity of insects transferred from treated to untreated food recovered at about the same rate as that of treated insects. After transfer, however, mortality diminished strikingly to only 2% after 24 h and fewer on succeeding days.

These results suggest a secondary lesion was incurred, during OP poisoning of weevils, that complemented ChE inhibition in eliciting the mortal response. On the other hand, it was also possible that measurement of total ChE in whole-body homogenates did not provide a reliable estimate of toxic action and that only a small fraction of the enzyme, perhaps at a vital site, was directly associated with death.

Of considerable interest was the physiological mechanism that stopped cumulative inhibition and allowed recovery of ChE activity in spite of continued treatment. That the enzyme recovered at nearly the same rate in

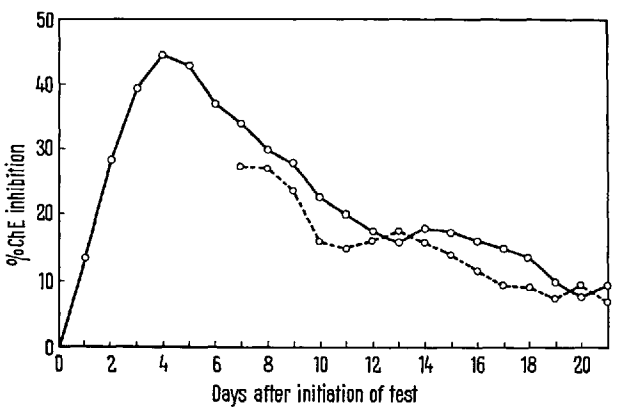

Fig. 1. % ChE inhibition among boll weevils held on chronic sublethal diets (1 ppm Bidrin). Each point is an average of 6 analyses with 90 treated insects compared with an equal number of analyses of untreated insects. Broken line represents data from insects transferred from treated to untreated food on 6th day.

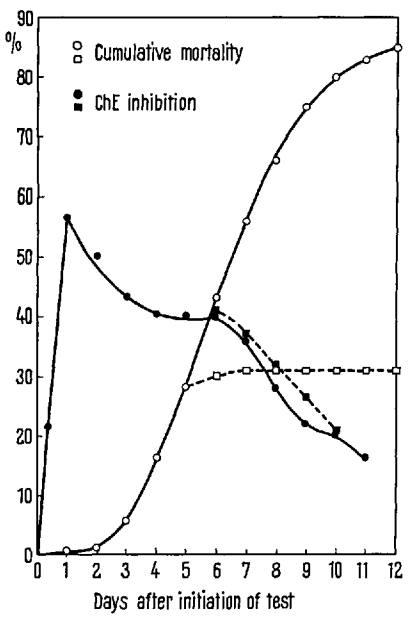

Fig. 2. % cumulative mortality (○) and % ChE inhibition (●) among boll weevils held on chronic lethal diets (4 ppm Bidrin). Broken lines represent data from insects transferred from treated to untreated food on 5th day (■ ChE activity, □ mortality). The number of analyses was the same as indicated for Figure 1.

[1] R. D. O'BRIEN, *Toxic Phosphorus Esters* (Academic Press, New York and London 1960).
[2] D. F. HEATH, *Organophosphorus Poisons* (Pergamon Press, New York-Oxford-London-Paris 1961).
[3] E. S. VANDERZANT and T. B. DAVICH, J. econ. Ent. *54*, 923 (1961).
[4] D. L. BULL and D. A. LINDQUIST, J. agric. Food Chem. *12*, 310 (1964).
[5] D. R. SIMPSON, D. L. BULL, and D. A. LINDQUIST, Annals ent. Soc. Am. *57*, 367 (1964).
[6] Appropriate studies have indicated the ChE activity in whole-body homogenates of boll weevils is due to one enzyme that resembles 'true' cholinesterase (acetylcholinesterase).

insects transferred from treated to untreated diet as in insects continued on chronic treatments suggested that, after a time, an enhanced degradative system was destroying the toxicant before it reached the site of action and the inhibited enzyme was being replaced at a characteristic rate. However, experimental evidence did not support this conclusion. In daily comparisons of weevils that were fed sublethal treated diet or untreated diet, we have found (1) that both groups degraded non-toxic, topical doses of C^{14}-labeled Bidrin at equivalent rates, (2) that weevils held on treated diet were always more susceptible to topical and oral doses of Bidrin than those from untreated diet, and (3) that the in vitro inhibition of ChE by OP insecticides was always significantly greater in insects from treated diet.

Detailed investigations of these preliminary findings are in progress and will be reported later.

Zusammenfassung. Bei Imagines von *Anthonomus grandis* Boheman die kontinuierlich mit nahezu letaler und letaler Dosis phosphororganischer Insektizide gefüttert wurden, konnte eine befristete zunehmende Hemmung der Cholinesteraseaktivität beobachtet werden. Trotzdem die Behandlung nicht unterbrochen wurde, gewann das Enzym die fast normale Wirksamkeit wieder zurück.

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Quantitative Distribution of Glucose-6-Phosphate Dehydrogenase and Isocitric Dehydrogenase in the Human Nephron[1]

The broad purpose of our investigations is to determine quantitatively the activity of enzymes in the different anatomical units of the human kidney in health and disease. The ultramicrotechniques developed by Lowry[2] for analysis of brain tissue were adapted for analysis of renal tissue[3]. Immediately on removal from the body, the biopsy specimens of 4 healthy kidneys, weighing a few milligrams, were frozen rapidly in liquid nitrogen. Serial sections of 16 μ thickness were cut with a microtome in a cryostat at 20°C. Alternate sections of frozen-dried tissue were stained by means of the periodic acid-Schiff technique, counterstained with hematoxylin and studied under a microscope. When the various parts of the kidney had been identified in the stained sections, they were cut out of the adjacent unstained section with microscalpels (at 40–100× magnification). The dissected specimens were weighed on a quartz fibre fish-pole balance, useful range 5–50 mμg. Both dissection and weighing were done in a room maintained at low humidity (< 40%) and constant temperature (18–20°C). In addition, homogenates from lyophylised tissue were prepared. Specimens were assayed to determine glucose-6-phosphate dehydrogenase (G-6-PDH) and isocritic dehydrogenase (ICDH) with the kinetic data given elsewhere[4].

Distribution of glucose-6-phosphate dehydrogenase and isocitric dehydrogenase in the human kidney expressed in MKH units

Structure	G-6-PDH	ICDH
Homogenate	0.248 (0.118–0.502)	4.17 (2.22–6.79)
Glomerulum	1.539 (0.567–2.410)	2.26 (1.05–6.29)
Proximal tubule	0.929 (0.698–3.070)	17.26 (9.93–29.70)
Distal tubule	1.432 (1.045–1.590)	41.51 (26.2–63.5)
Collecting duct	–	37.5 (21.6–53.4)
Arteriole	1.419 (0.685–2.220)	1.58 (0.15–3.00)
Capsule	0.480	5.27 (4.44–6.71)

Means, and in brackets the least and the most active measurements, are given. 129 identified tissue pieces were used for the analysis of G-6-PDH and 179 for ICDH. Collecting ducts, arteriolar and capsular tissue were not available from all 4 biopsies.

To our knowledge no quantitative enzymatic data for the human nephron have been reported so far for G-6-PDH and ICDH. It seemed therefore important to make available typical results as given in the Table. Activity is expressed in moles of substrate split per kg tissue per h at 37°C (MKH). Accuracy and reproducibility of the method employed here, variation in enzyme activity within a species and individual variations from nephron to nephron have been discussed elsewhere[5].

The topography of enzyme activity in the human nephron in health has shown in general lower activity in the glomerulum than in the other parts for many enzymes, with the exception of acid phosphatase[6]. Glomerular G-6-PDH activity is very high and surpasses the distal tubular one. One might assume therefore an especially active pentose-monophosphate shunt in this structure. Activity of arteriolar tissue is similar to the glomerular activity for both enzymes. The results for ICDH parallel in magnitude those for malic dehydrogenase, as would be expected from another enzyme of the Krebs cycle[4].

Zusammenfassung. In den einzelnen Abschnitten des menschlichen Nephrons und in Nierenhomogenaten wurden quantitativ die Glucose-6-phosphat-dehydrogenase- und die Isozitronensäuredehydrogenase-Aktivität gemessen.

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Medizinische Poliklinik der Universität Basel (Switzerland), January 29, 1965.

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