The above-described procedure has also been successfully employed on a smaller scale using, most frequently, a 500-ml measuring cylinder of conventional type as the chromatographic vessel. Such a system was found to provide a convenient means of performing quick checks on the identity and state of purity of corticosteroids and related substances.

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Microanatomical Study of DDT-moribund *Anopheles quadrimaculatus* Say

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Histological studies following administration of DDT have been made principally on the roach (*Periplaneta americana* L. [*1, 2*]), on the honeybee (*Apis mellifera* L.[*1*]), and on the housefly (*Musca domestica* L. [*3*]). For the most part these studies were made on one stage only and did not include examinations of all the tissues of these insects.

Since the action of DDT still remains obscure, it seemed advisable to make intensive histological studies of *all* the tissues of an insect from representative periods during its life span. The common malaria mosquito of the Southeastern United States, *Anopheles quadrimaculatus* Say, was selected for these studies.

Fourth-instar larvae, pupae, and adults of both sexes were exposed topically to massive doses of pure *p,p'*-DDT, and when moribund (about 2–8 hr) were studied by examination of dissected living specimens and by examination of sectioned fixed specimens. All test animals were acutely poisoned and showed every sign of extreme intoxication. Twenty-five test animals from each stadium were used with equivalent numbers of controls.

Dissections were made in insect Ringer's and were either examined while fresh under the microscope with and without phase contrast, or were examined after fixation with alcohol or formalin in whole mounts prepared of the various tissues.

Complete transverse, sagittal, and frontal serial sections at 6–15 μ were made from alcoholic–formalin-, Bouin-, or Carnoy-fixed material. Sections stained with Delafield's and Haidenhain's iron hematoxylin and counterstained with eosin were examined at 100 and at 1000 diameters.[1]

Every effort was made to study all the representative tissues of these insects and to determine by comparison with controls whether visible changes in the structure and staining reactions had occurred. Detailed cytological, histological, and histochemical studies of normal *A. quadrimaculatus* are in preparation for publication at a later date.

Tissues examined included: (1) hypodermis; (2) tracheae; (3) thoracic molting glands in larvae; (4) micro- and macro-oenocytes; (5) imaginal discs of undifferentiated and differentiated thoracic and abdominal muscles; (6) imaginal discs of eyes, antennae, legs, wings, and halteres; (7) anal papillae of larvae; (8) neurocytes and neuropile of frontal, supraesophageal, thoracic, and abdominal ganglia; (9) neurosecretory cells in supraesophageal ganglion; (10) corpora allata–cardiaca complex; (11) immature, differentiating, and mature gonads and associated structures (mucus gland, atrium, spermatheca of female and accessory glands of male); (12) dorsal vessel; (13) alary muscles; (14) micro- and macro-pericardial cells; (15) thoracic ventral nephrocytes in larvae; (16) ventral diaphragm of adult; (17) internal and external fat body; (18) buccal cavity; (19) salivary glands; (20) esophagus; (21) gastric caecae of larvae; (22) dorsal and ventral diverticula of adult (23) midgut; (24) Malpighian tubules; (25) hind-gut; and (26) rectal papillae of adult.

No microanatomical changes in cell or nuclear structure (e.g., cytoplasmic vacuoles, hypertrophy, atrophy, surface irregularities, karyorrhexis) or abnormal reactions to hematoxylin and eosin (e.g., overstaining, diffuse staining, blotching, or failure to stain) were encountered in any of the tissues of any of the stages that were examined. These findings indicate that DDT does not operate to produce visible structural damage to cells during representative periods of the life span of *A. quadrimaculatus*.

The present observations confirm and extend those of Richards and Cutkomp (*2*), who found absence of definite pathology in DDT-poisoned roaches. No comparison can be made between the present observations and those of Chang (*1*), who reported breakdown of Golgi bodies in the ganglia of DDT-poisoned roaches and honeybees, since the special methods required for their demonstration were not used in the present studies.

Absence of definite pathology in the central nervous system of the mosquito and the roach following DDT administration is not peculiar to insects, for similar findings have been reported by Globus (*4*) in monkeys, dogs, cats, and rats acutely poisoned with DDT.

A few investigators have reported cellular changes in insects (*1, 3*) and in vertebrates following DDT

[1] Grateful acknowledgment is made to Ralph D. Lillie and James H. Peers, Laboratory of Pathology and Pharmacology, for extending me the courtesy of their laboratory facilities. I am indebted to Joseph Woodard of their laboratory for having cut and stained most of the sections.

administered in different ways, but these changes are generally considered to be nonspecific (*2, 4*) and do not adequately account either for the symptoms or for the death of the animals (*2, 4, 5*).

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A Rapid Screening Test for the Determination of the Approximate Cholinesterase Activity of Human Blood

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Organic phosphate insecticides are coming into extensive use. The chief physiological action of these highly toxic compounds is their inhibition of the cholinesterase (ChE) activity in man, as well as in insects. Compounds having a similar action are considered as possible war gases. Among the first symptoms to appear as a result of overexposure to these compounds are nausea, vomiting, diarrhea, and headache. In very severe poisoning weakness and generalized muscular fasciculations also appear. Since these symptoms are nonspecific, it is important to know whether or not they are caused by ChE-inhibiting agents. The most practical method of detecting the true cause of the symptoms is the determination of the ChE level of the blood, which reflects the activity of the enzyme in all parts of the body.

The methods commonly used to determine blood ChE activity require apparatus found only in clinical or chemical laboratories. For screening purposes a simpler and more generally applicable test is desired, by which overexposure may be detected so that appropriate treatment could be administered with minimum delay.

Although plasma ChE is inhibited more readily than red cell ChE (*1–3*), in cases of actual human poisoning by organic phosphate insecticides both plasma and red cell ChE levels were significantly reduced (*2–4*). It seemed, therefore, that the whole-blood ChE level would serve equally well as an index of overexposure. Based on this premise, a rapid screening test that can be carried out in the field without the use of specialized equipment has been developed and is the subject of this report.

This method is essentially a visual colorimetric method in which the change in pH (ΔpH) resulting from the liberation of acetic acid from a ChE substrate (acetylcholine iodide) is estimated by the change in color of an indicator, brom thymol blue (BTB). The color of the solution at the end of 20 min will determine the approximate ChE activity. Only one drop of fingertip blood is required.

The only apparatus and materials required are a blood-diluting pipette (WBC dilution, 1:20), a dropping pipette with bulb attached, a 2- and a 1-ml rubber-stoppered serum bottle containing acetylcholine iodide[1] (6 mg) and brom thymol blue[2] (0.5 mg), respectively, and two 1-ml bottles each containing 1 ml of sterile distilled water (pH 6.8–7.0). The reagents can be added to the bottles by dissolving them separately in alcohol (300 mg substrate/25 ml and 25 mg indicator/10 ml) and adding 0.5 ml of the substrate solution to the 2-ml bottle and 0.2 ml of the indicator solution to the 1-ml bottle. After stoppering the bottles, the alcohol is removed under reduced pressure at room temperature by inserting a hypodermic needle through the rubber stopper. Before using, 1 ml of distilled water is added to each of the reagent bottles by means of the dropping pipette, and the bottles are shaken. One of the bottles that had contained distilled water can then be used as the test bottle. Acetylcholine iodide was used as the substrate because it is nonhygroscopic and stable (*5*). All these materials are easily incorporated into a small kit that can be carried in the pocket.

Because this method is based on a change in pH, the finger from which the blood sample is to be taken, as well as the lancet, must be uncontaminated with acid or alkali. After being washed with water and sterilized with alcohol, both the fingertip and the lancet must be wiped dry. After pricking the finger, blood is drawn up to the 0.5 mark on the pipette, and then the outside of the pipette is wiped with a clean piece of gauze. The blood sample is diluted with BTB solution up to the 11 mark. The blood-BTB solution is then expelled into the empty bottle, and any remaining blood in the pipette is washed into the bottle by drawing the blood-BTB solution up and down several times. A second volume of BTB is drawn up to the 11 mark and added to the blood-BTB in the test bottle. Two volumes of the substrate solution, each measured to the 11 mark, are then added to the blood-BTB solution. The test bottle is stoppered and shaken. After noting the color of the solution (it should be green) and the time, the test bottle is immediately placed in the axilla next to the skin so that the test will be carried out at constant (body) temperature. At the end of exactly 20 min the color of the solution is again observed, preferably in daylight or fluorescent light, by holding a piece of white paper about 1 in. below the bottle held in a horizontal position and looking *down* through the side of the bottle.

[1] Hoffmann-LaRoche.
[2] Harleco, water-soluble.