Reports

Tetraethyl Pyrophosphate and Acetylcholine in Periplaneta americana

The known effect of organophosphate insecticides is to inhibit cholinesterase (*1*) and to increase the acetylcholine content of the nervous tissue of insects (*2*). As yet no evidence has been obtained for other factors related to the increase of acetylcholine, although treatment of roaches with physostigmine points to a correlation between nervous activity and acetylcholine (*3*). Accordingly, male roaches of *Periplaneta americana* L. were treated topically with a lethal dose (5 μg per roach) of tetraethyl pyrophosphate, and the acetylcholine content of thoracic cords was determined on the rectus abdominis muscle of the frog. The methods of dissection and extraction of acetylcholine from roach cords have been described (*4, 5*); they avoided *in vitro* syntheses of acetylcholine. Correction for sensitizing agents was made (*6*), and values for acetylcholine refer to acetylcholine chloride. Electrophysiological observations were also carried out on the gross nerve activity of the ventral cord. The acetylcholine content of thoracic cords between 1 and 48 hours is shown in Fig. 1. The level of acetylcholine in normal roach cords was 79 ± 6 μg/g (*5*).

It may readily be seen that two distinct peaks of acetylcholine were found for the 48-hour period. The first, occurring at ½ hour, was coincident with a period of intense nervous activity. The second peak, strikingly greater than the initial one, was found at 24 hours. At this time the ventral cord was electrically dead. Furthermore, from 24 hours onward the roaches showed definite signs of necrosis, as shown by a darkening and discoloration of thoracic tissues, and at 48 hours, when the acetylcholine content had fallen to zero, it was almost impossible to remove the nerve cords. It is concluded that the rise of acetylcholine in roach thoracic ganglia is related initially to hyperactivity and later on to an effect on nervous tissue distinct from hyperactivity. In insects of small size for which only a single peak is obtained, it is possible that the two characteristics are inseparable.

Studies of the mode of action of tetraethyl pyrophosphate have produced evidence that the blood of treated roaches contained acetylcholine as determined by rectus abdominis muscle of frog, *Venus* heart assay, alkaline hydrolysis, and hydrolysis by cholinesterase. Normal blood contained no acetylcholine, and blood extracts for assay were prepared similar to that for nerve tissue to avoid sensitizing agents. The amount was higher at 2 hours than at 8 hours after treatment. The blood results suggest a possible release by the central nervous system, for at 2 hours the level of acetylcholine in the thoracic cord had decreased (Fig. 1). However, the amount in the blood was not sufficient to warrant such an assumption, and, should acetylcholine be released into the blood by the central nervous system, the strikingly higher amount found in the thoracic cord at 24 hours cannot be in a freely diffusible form.

In contrast, the blood of DDT-treated roaches contained no acetylcholine, nor was there an abnormal amount of acetylcholine in thoracic ganglia at prostration. Blood from roaches treated with either tetraethyl pyrophosphate or DDT induced nerve volleys in the isolated nervous system of the roach. Since this result was not due to acetylcholine, there is, perhaps, a common factor in the blood of roaches poisoned by DDT and by tetraethyl pyrophosphate. As shown in the blood physiology, there is a specific difference in the mode of action of the two insecticides.

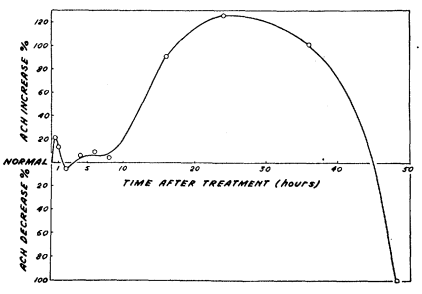

Fig. 1. Total acetylcholine content of thoracic nerve cord of roaches treated topically with 5 μg of tetraethyl pyrophosphate per roach.

E. H. Colhoun
Science Service Laboratory, Department of Agriculture, London, Ontario

References and Notes

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Differential Effects of Reserpine on Conditioned Responses in Cats

In order to study the differential effects of drug administration on a number of conditioned responses in the same animal, eight cats were trained in three tasks: (i) to traverse an elevated runway for visible food; (ii) to discriminate between a circle and a square for food in a Yerkes box; and (iii) to avoid shock in a hurdle box within 5 seconds of presentation of either an auditory (1200 cy/sec tone) or a visual (darkened compartment) stimulus. All cats were trained to 100 percent accuracy in avoidance and to 95 percent accuracy on three successive days in pattern discrimination. A cannula was then implanted into one lateral ventricle of the brain to permit central injection of solutions without anesthesia or other disturbance of the animal.

By use of the behavioral indices, the effects of central and peripheral injections of various substances were compared, and the interactions of these substances were studied. Materials studied included reserpine, serotonin, iproniazid, sodium pentobarbital, epinephrine, norepinephrine, acetylcholine, adrenocorticotrophic hormone, atropine, methamphetamine, potassium, and calcium (*1*). It is the purpose of this report to describe the differential effects of injections of reserpine (*2*) on the conditioned responses.

When either the latency of the conditioned avoidance response or the failure of the animal to respond to the condi-