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.

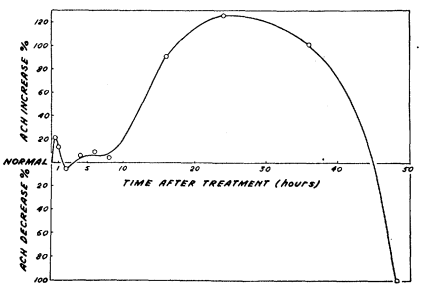

Fig. 1. Total acetylcholine content of thoracic nerve cord of roaches treated topically with 5 μg of tetraethyl pyrophosphate per roach.

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References and Notes

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6. W. Feldberg, *J. Physiol. (London)* 103, 367 (1945).

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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-

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Table 1. Comparison of performance in approach and avoidance situations for each cat following intramuscular injection of reserpine. All figures are percentages.

Responses	Cat							
	A	B	E	F	G	H	K	L
Avoidance								
Correct	29	47	67	71	29	82	53	31
Escape	10	25	4	18	10	8	8	21
No response	61	28	29	11	61	10	39	48
Pattern								
Correct	76	100	83	93	63	82	81	83
Error	9	0	0	7	12	0	4	3
Food only*	0	0	0	0	12	11	13	0
No response	15	0	17	0	13	7	2	14

* The cat approached and ate visible food but refused to approach patterns.

tioned stimulus was used as the index, it was found that reserpine affected the conditioned avoidance response to a visual cue much more severely than it affected the same response to an auditory cue. Friedman two-way analysis of variance (*3*) showed that this visual-auditory differential is significant at better than the 0.01 level. An extreme illustration of the differential is shown in Fig. 1. Since these responses were learned by two subgroups in counterbalanced order, it is possible to demonstrate that this differential susceptibility is not related to order of acquisition. It is related to the difficulty of learning the two responses, for avoidance to tone was acquired in fewer trials regardless of the order of learning. With sufficiently large doses of reserpine it was possible to block the avoidance responses to both stimuli, but examination of the time course of effects shows that the visually cued response is affected earlier and usually recovers later than the response to the auditory stimulus.

At times, when presentation of the conditioned stimulus did not elicit the conditioned avoidance response, a cat that had been injected with reserpine could be seen to cringe, growl, and sometimes attempt to escape from the box, on occasion even climbing to the ceiling of the compartment. These observations can be interpreted as evidence that the sensory mechanisms necessary for perception of the stimuli are functional and that the motor capacity of the animals to make the conditioned response is unimpaired. The adequate performance of a conditioned avoidance response to one stimulus but not to another, although the latter was followed by arousal and apparently affective reactions, suggests that the reserpine has not blocked what we might term the "anxiety-evoking potential" of the conditioned stimulus. The dosage levels used throughout these experiments were appreciably lower than those generally reported in current psychopharmacological investigations of reserpine, ranging from 7.5 to 65 μg/kg for intramuscular administration and from 25 to 300 μg total dose for central administration. Clear behavioral effects were demonstrated with doses as low as 7.5 μg/kg (intramuscular administration). The failure of Weiskrantz and Wilson (*4*) to find an analogous split between their approach and avoidance situations may be attributable to their excessively high doses (0.75 mg/kg) of reserpine, which resulted in a total behavioral depression.

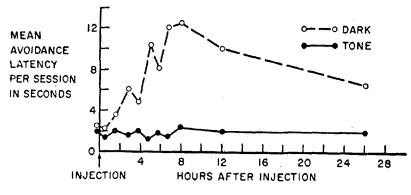

Fig. 1. Mean latency in seconds for avoidance responses to two different conditioned stimuli in one cat following central injection of 200 μg of reserpine (total dose).

Examination of the effects of reserpine on conditioned avoidance responses and on pattern discrimination for food shows a definite difference. Table 1 shows results based on 730 trials in shock avoidance and on 355 trials in pattern discrimination after intramuscular injection of reserpine (*5*). The two distributions of percentages of correct trials differ at slightly better than the 0.01 level, according to the sign test (*3*). This difference is not related to ease of acquisition in terms of trials to criterion. All cats showed such a split in behavior regardless of whether they learned pattern discrimination more or less quickly than they learned avoidance. These data suggest that reserpine affects behavior acquired under motivation from punishment more readily than it affects behavior reinforced by reward. It is true that the responses differ along other dimensions than that of approach versus avoidance but the data of Grastyán *et al.* (*6*) suggest that this may represent a basic physiological dichotomy (*7*).

The retention, under reserpine, of a visually mediated approach response simultaneously with the blocking of a visually cued avoidance response further demonstrates that the visual pathways remain functional during the period in which the animal is affected by reserpine.

It is felt that the data cannot be reconciled with the interpretation that reserpine at these doses blocks the conditioned avoidance response by interference with sensory perception, with motivation to perform, or with motor coordination. Therefore we propose that this selective action of reserpine may be attributed to an interference with the specific conditioned association between the stimulus and a directed evasion response—that is, interference with learned associations (*8*).

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References and Notes

1. Detailed reports on these studies are in preparation.
2. We wish to express our thanks to Dr. Jurg Schneider of Ciba Pharmaceutical Products Inc. for supplying us with reserpine (Serpasil).
3. S. Siegel, *Nonparametric Statistics for the Behavioral Sciences*, (McGraw-Hill, New York, 1956).
4. L. Weiskrantz and W. A. Wilson, Jr., *Ann. N.Y. Acad. Sci.* 61, 36 (1955).
5. Comparable data from central injections are not given here because of their sporadic nature, which is due to the fact that the placebo material in which reserpine is dissolved tends to destroy appetite when it is centrally injected. We were not aware of this phenomenon until we injected the placebo alone.
6. E. Grastyán *et al.*, *Kisérletes Orvostudomány* 9, 88 (1957).
7. Experiments are in progress to clarify whether or not it is the motivational dimension which is crucial to this distinction.
8. The research reported here was carried out with the support of the National Science Foundation (grant No. G-3354).

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Colorimetric Assay for Dipicolinic Acid in Bacterial Spores

Dipicolinic acid (pyridine 2,6-dicarboxylic acid) is a major component of bacterial spores (*1, 2*) and is unique in that it has been found only in such spores. It is synthesized during sporulation. The spores release dipicolinic acid during germination (*1*), or upon hydrolysis (*2*) or heating (*3*). Methods of analysis so far published (*2, 4*) are based upon ultraviolet absorption of the compound after acid digestion of the spores, isolation of the dipicolinic acid by ether extraction, paper chromatography of the extract, and elution of the acid-bearing spots. Though accurate, this method is extremely time-consuming and laborious. This report (*5*) describes a more convenient colorimetric method that