References and Notes

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4. The National Water Quality Network, established in 1957, is operated by the Public Health Service as a cooperative project with federal, state, and local agencies as well as industry. It now consists of 128 geographically dispersed, surface water sampling stations located on major rivers and the Great Lakes. Some 175 individual agencies participate in its operation and are herewith acknowledged as contributors to this report. Analytical work is accomplished in the field and in Network laboratories located in Cincinnati, Ohio, and includes organic, radiological, biological, microbiological, and physical analyses.
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7. Each aromatic fraction was dissolved in 0.2 ml CCl_4. Volumes ranging from 0.2 to 0.5 μl were injected into a Perkin-Elmer model 800, flame ionization, dual column, temperature controlled, gas chromatograph equipped with a 91 cm, 0.32-cm O.D. stainless steel column packed with 5-percent Dow-11 on 60- to 80-mesh Chromosorb W. Helium, the carrier gas, was fed at 60 ml/minute. Corroborating identifications of DDT were made by increasing the temperature between 125° to 200°C at 5°C per minute. Dieldrin was determined isothermally at 170°C.
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24 June 1963

Acetylcholine-like Activity in Sciatic Nerve

Abstract. *Extracts of sciatic nerve exhibit acetylcholine-like activity that is only partly attributable to acetylcholine. The extracts show relatively greater activity on the rectus abdominis muscle of the frog than on the ileum of the guinea pig. To prevent the action of the extract on the frog rectus abdominis muscle, a greater concentration of* d*-tubocurarine is required than is necessary to prevent the action of known acetylcholine.*

The different sensitivities of the ileum of the guinea pig and the rectus abdominis muscle of the frog to acetylcholine and its congeners have been exploited to show the presence of choline esters other than acetylcholine in tissue extracts (*1*). In measuring acetylcholine-like activity in acetone extracts of sciatic nerve prepared (*2*) from rabbits treated with physostigmine, we observed that a higher value was obtained in measurements on the frog muscle than that obtained on the guinea pig ileum (*3*). When assayed on the frog muscle, the sciatic nerve contained activity equivalent to 2.55 ± 0.43 μg of acetylcholine per gram of fresh weight; on the guinea pig ileum, 1.57 ± 0.41 μg acetylcholine per gram of fresh weight.

Examination of subcellular fractions (*4*) obtained by differential centrifugation of the sciatic nerve revealed another discrepancy. When assayed on the frog muscle, the acetylcholine-like activity was almost equally divided between the soluble fraction of the nerve, which contained 47.3 ± 4.6 percent of the activity, and the total particulate material. When assayed on the ileum, 79.3 ± 0.60 percent of the activity was in the soluble fraction.

These observations suggested that a choline ester other than acetylcholine may be present in the sciatic nerve extracts, since the frog muscle is sensitive to low concentrations of many choline esters, whereas the guinea pig ileum is especially sensitive to acetylcholine and relatively insensitive to other choline esters (*1*).

An alternative explanation—that the extracts contained material that either sensitized the frog muscle to acetylcholine or prevented the response of the ileum to acetylcholine—was ruled out by showing that on both muscles the effects of the extract (boiled or unboiled) and of known acetylcholine are additive.

The following evidence is consistent with the idea that the guinea pig ileum responded to acetylcholine (or a closely related compound) in the nerve extract. The slopes of the dose-response curve for the extract and for acetylcholine were identical.

The effects of equally active doses of the extract and of acetylcholine were prevented by atropine at the same dose, 6 μg/liter; neither pyrilamine maleate, 5 μg/liter, nor lysergic acid diethylamide, 50 μg/liter, which are, respectively, antagonists of histamine and 5-hydroxytryptamine, affected the activity of the extract or of acetylcholine. The atropine-treated ileum recovered its sensitivity concurrently with both the extract and acetylcholine. The action of the extract on the ileum, like that of aceytlcholine, was destroyed both by boiling for 10 minutes at *p*H 10 and by incubation with acetylcholinesterase prepared from bovine erythrocytes. Finally, when the extract was chromatographed on paper (*5*), all material with activity was eluted in the area, R_F 0.00 to 0.20, that included known acetylcholine (R_F 0.12).

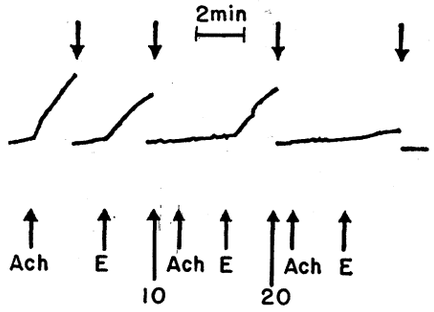

Fig. 1. The effect of *d*-tubocurarine on the action of 200 ng of acetylcholine (Ach) and an extract (E) equivalent to 90 mg of sciatic nerve on the frog rectus abdominis muscle. Numbers refer to μg of *d*-tubocurarine chloride pentahydrate, which was permitted to act for 10 minutes. The upper arrows indicate a change of bathing solution.

That the rectus abdominis muscle of the frog responds to substances other than acetylcholine in the sciatic nerve extract is evident from the following observations. The action of the extract was not prevented by a concentration of *d*-tubocurarine that prevented the action of acetylcholine (Fig. 1). Doubling the concentration of *d*-tubocurarine, however, did prevent the action of the extract (Fig. 1). The differential action of these two concentrations of *d*-tubocurarine was noted in six of seven experiments. Furthermore, only 66.5 ± 15.6 percent of the material acting on the frog muscle was recovered in the eluate of material with R_F 0.00 to 0.20; the lower dose of *d*-tubocurarine completely prevented the action of this eluate.

The extract of sciatic nerve was inactive on both preparations if the rabbits were not treated with physostigmine. It is likely that the extract contains, besides acetylcholine, another choline ester to which the frog rectus abdominis muscle is especially sensitive. Other than acetylcholine, the only known, naturally occurring choline ester with potent activity on this preparation is propionylcholine, but since this compound has an R_F value of 0.15 in the solvent system used in this work, it would be included in the examined eluate and hence cannot be the active material (*6*).

E. A. Carlini*
J. P. Green

Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut

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1. V. P. Whittaker, *Handb. Exptl. Pharmakol.* **15**, 1 (1963).
2. Rabbits were anesthetized with sodium pentobarbital, 30 mg/kg of body weight, and injected intraperitoneally with physostigmine, 1 mg/kg of body weight. After about 10 minutes, when the animals were salivating, they were killed by injecting 10 ml of air into the marginal ear vein. The sciatic nerve was cut on its emergence from the spinal cord, and its two main branches, the peroneal and tibial nerves, cut just before entering the foot. The nerves were homogenized in 4 ml of 0.3 *M* sucrose containing 14 mg of physostigmine per liter. The homogenate was extracted with 20 volumes of acetone; after centrifugation, the acetone was blown off with a stream of nitrogen. When standard solutions of acetylcholine were carried through the acetone extraction, recoveries of acetylcholine, as measured on the ileum of the guinea pig and the rectus abdominis muscle of the frog, ranged from 76 to 92 percent.
3. The muscle chamber contained 5 ml of solution. Each liter of the Tyrode's solution used in the experiments on the frog rectus abdominis contained 6.4 g NaCl, 0.3 g KCl, 0.17 g $CaCl_2$, 0.35 g $NaHCO_3$, 0.7 g glucose, and 0.01 g physostigmine. The Tyrode's solution used on experiments with the guinea pig ileum and the method for recording contractions have been described (*4*).
4. E. A. Carlini and J. P. Green, *Brit. J. Pharmacol.* **20**, 264 (1963).
5. Descending chromatography was performed on Whatman 3 MM paper in water-saturated *n*-butanol. Known acetylcholine was carried through the same procedure and all values are corrected for losses.
6. Supported by the U.S. Public Health Service (GM-K3-10313-01 and GM-K3-2459) and the American Heart Association.

* Postdoctoral fellow of the Rockefeller Foundation. Present address: Laboratórios Farmacologia e Bioquimica, Escola Paulista de Medicina, Caixa Postal 12,993, São Paulo, Brazil.

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Sex Attractant of Cabbage Looper, Trichoplusia ni (Hübner)

Abstract. *When virgin male cabbage loopers were exposed to filter-paper strips containing an extract from female abdomens, they exhibited a response lasting from 2 to 5 minutes. Gases of a component (or components) of these extracts emanating from a chromatography column attracted the males and then evoked the same response. The attractant is fairly volatile and has a relatively low molecular weight.*

A sex attractant has been extracted from female cabbage loopers, *Trichoplusia ni* (Hübner). The intensity and regularity of the response during the preliminary tests, corroborated by subsequent tests, demonstrated that a lure was present in cabbage looper females and that this lure could be extracted with methylene chloride.

Problems with insect resistance to insecticides and persistence of insecticide residues have stimulated investigators to seek new and different methods of insect control (*1*). The use of sex attractants is one of the methods receiving considerable attention. Readers are referred to reviews on sex attractants (*2*).

Cabbage loopers in laboratory cages were observed to mate between midnight and 4 A.M. (*3*). During this period male cabbage loopers appeared to be definitely attracted to females prior to mating. With this information and the methylene chloride extraction technique of Ouye and Butt (*4*), we undertook to determine whether an attractant for males was present in female cabbage loopers.

Pupae, obtained from larvae reared on a semisynthetic diet (*5*), were separated by sex and emerging male and female adults were kept apart for 4 days. Approximately 25 males were spray-marked with a water suspension of rhodamine B and combined with 50 virgin females in a 60- × 80- × 60-cm (24- × 32- × 24-in.) screened cage for observation. Females which appeared attractive to males were immediately collected and the terminal two or three segments or "tips" of the abdomen were extracted with methylene chloride. An extract of one female abdomen dispensed on a strip of filter paper exposed to virgin males in a separate cage caused receptive males first to move their antennae 90 degrees from a position along the anterior margin of the forewing to an elevated position in a wide V above the head. This response was followed at once by rapid vibrations of the wing, slight elevation of the abdomen, and eventual flight to the source of the stimulus. Stimulated males, while in hovering flight, "fingered" the treated paper with their antennae, and repeatedly tried to mate with the paper strip and nearby males. During attempts at mating, the tip of the male's abdomen was curved under, the brown tufts of hair near the tip of the abdomen were fanned out, and the genital claspers were clearly exposed. No response was obtained when filter paper with the solvent alone was exposed to virgin males.

The filter-paper strip that provoked the initial response was then placed in a cylindrical (8 cm in diameter × 23 cm long) ice-cream carton fashioned into a trap by inserting screen funnels into each end. Another trap containing a filter-paper strip impregnated with methylene chloride only was used as the untreated check. The traps were then placed at opposite ends of a screened cage (60 × 80 × 60 cm) containing 50 virgin males 4 days old. A 6-cm fan provided air circulation within the cage. Receptive males showed the characteristic pattern of behavior described. Within 15 minutes the carton containing the female extract trapped five males. None were caught in the check trap.

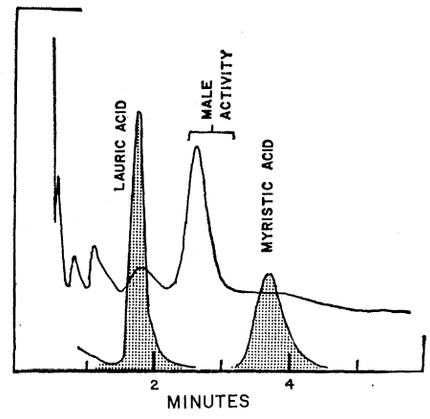

Fig. 1. Gas chromatograph of crude methylene chloride extract of one 7-day-old female cabbage looper and the methyl esters of lauric and myristic acids used as reference.

Extracts from 4-day-old females collected at random during the initial tests also attracted males. Extracts of virgin females 7 to 8 days old were used for subsequent studies of the response of individual males and for gas chromatography analysis of the crude extract.

In further tests male adults, which had been separated by sex as pupae, were individually tested for a response to the female extract in wide-mouth jars (14 × 5 cm). The mouth of a jar was fitted with an inverted copper-screen cone that supported the extract-treated strips of filter paper during testing and held cotton soaked with 5-percent sugar solution when tests were not being conducted. All jars were covered with a metal cap to keep the cotton damp during periods when tests were not being conducted. Adult males were held under continuous light and tested daily between 8 and 11 A.M. until death. All tests were conducted at room temperature (24° to 25°C). Thirty minutes before testing, the lid and the cotton were removed from each jar. A strip (½ × 1 cm) of filter paper containing extract equivalent to 1/20 of a female was then placed on the cone in each jar and the males were observed. Excitation of the antennae accompanied by continuous wing vibration was selected as the criterion of a positive response. A total of 134 virgin males were used in three separate tests.

Of the 134 males tested, 133 (99.2