References and Notes

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.

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17 June 1963

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.

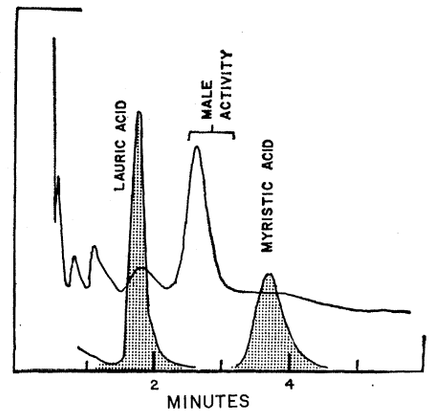

Fig. 1. Gas chromatograph of crude methylene chloride extract of one 7-day-old female cabbage looper and the methyl esters of lauric and myristic acids used as reference.

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

percent) responded. Receptive adults responded within 2 to 3 minutes after the strips were placed on the cones. The duration of response, while the moths were continuously exposed to the strips, ranged from 2 to 5 minutes. The ability of the male to detect the attractant is lost after exposure and regained only after a "rest" of several hours. The major portion of virgin males initially responded to the presence of the extract when they were 3–4 days old. Initial responses for males whose ages in days were 1–2, 2–3, 3–4, 4–5, 5–6, 6–7, 7–8, 8–9, and 9–10 were 5, 20, 29, 26, 10, 6, 2, 1, and 1 percent, respectively. Over 95 percent of all virgin males responded at least once by the time they were 7 days old. A mating response was obtained from one male 17 to 18 days old. Records obtained from frequency-response studies indicated that 80 percent of the virgin males responded from three to eight times to the presence of the extract. The number of males responding 0, 1–2, 3–4, 5–6, 7–8, 9–10, 11–12, and 13–14 times were 1, 10, 23, 32, 47, 14, 6, and 1, respectively. One male responded for 13 consecutive days. Consecutive responses for 2, 3, 4, 5, 6, and 7 days were recorded 68, 36, 15, 17, 9, and 4 times, respectively. The first response of virgin males occurred 1 day after emergence (5 percent) and the maximum response (77 percent) occurred at 8 days. Mortality of males at 8 days was only 8 percent. Thus, in order to insure optimum detection of the attracting substance for a specific period, males at least 7 to 8 days old should be used for bioassay.

The methylene chloride extract of the abdominal tips from 7-day-old virgin females was analyzed by gas-liquid chromatography with a column (2.5 mm × 185 cm), containing 5-percent neopentylglycol succinate on 80- to 100-mesh, acid-washed Chromosorb W, operated at 190°C with a flow of 53 ml of argon through the column per minute. The effluent gases of a very prominent component (or components) having a retention time of 2.6 minutes strongly attracted virgin males and elicited mating responses. The attractant gases emerged approximately midway between the methyl esters of lauric and myristic acid which were used as convenient reference compounds and had retention times of 1.8 and 3.7 minutes, respectively (Fig. 1). The position of the attractant between the two reference compounds indicates that it is fairly volatile and has a relatively low molecular weight. Detectable but lesser quantities of the attractant were found in dead and newly emerged females. No peak was found at the retention time of the attractant component (or components) on chromatographs of methylene chloride extracts prepared from male cabbage looper moth abdominal tips (*6*).

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6. We thank Mrs. S. Robinson for preparation of the photograph. This research was done in cooperation with the Texas Agricultural Experiment Station.

25 June 1963

Low Dose Radiation of the Developing Brain

Abstract. *X-irradiation administered in single doses of 10 to 40 r has a widespread effect on the developing rat brain. It first diminishes the formation of cytoplasmic basophilic material in the nerve cells and inhibits their growth. Single doses of 20 to 40 r cause permanent alterations of individual nerve cells, and interfere with their organization into neuronal assemblies, such as layers of the cerebral cortex.*

Little work has been done on the pathological effects of single low doses of ionizing radiations on the morphogenesis and cytogenesis of the developing mammalian brain, but related studies (*1*) suggest that low doses of x-radiation may alter neural development. A threshold dose for initiating eye malformations during early neurulation in rats is 25 r and, in mice genetically disposed to show them, certain spinal anomalies are increased when as little as 25 r are given during the formation of the body axis. A threshold dose for killing certain classes of primitive differentiating neural cells in early development in rats and mice is 30 to 40 r. The appearance of four cases of exencephalia in a large series of mice exposed to 5 or 15 r on one of the first 2 days of pregnancy has been attributed to the radiation. In other experiments (*2*), small doses of radiation repeated daily during the fetal life of rats did not lead to cytoarchitectural changes in the adult cerebral cortex when less than 60 r per day were given on five successive days.

We recently began experiments to determine the pathological effects on the developing nervous system of x-irradiation administered in single doses of 10 to 50 r (*3*, *4*). The brains of irradiated rats were studied grossly and histologically, and were compared with matching littermate or cousin controls.

Irradiation with 30 r disturbed the development of the cerebral cortex when given on the 16th, 18th, or 22nd day of pregnancy, or on the day after birth. This disturbance was expressed in several ways depending on which day the radiation was administered. When 30 r was administered on the day after birth, a lag in development of the neurons was seen within 72 hours, particularly of those in the outer half of the cortex, layers 2, 3, 4, and 5. The lag was characterized in the dorsal neocortex by shorter and thinner primary apical dendrites; incomplete delineation and differentiation of layer 4, which is normally under way at this time; and diminished formation and distribution of cytoplasmic basophilic (*5*, *6*) material in the nerve cells, particularly in the more distal parts of the

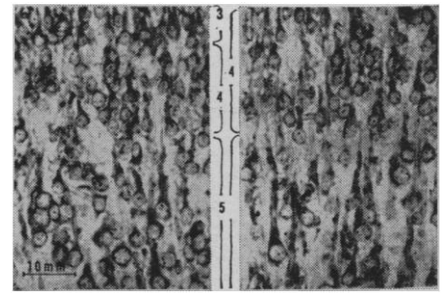

Fig. 1. Diminished growth of neurons in the dorsal cortex (left) of a 4-day-old rat irradiated 72 hours earlier with 30 r, compared with that of a normal litter mate (right). Layer 4 is sparse and cells in layer 5 have smaller, less basophilic dendrites than normal. (× 500, cresyl violet, Luxol fast blue)