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GRADUATE COLLEGE

THE EFFECTS OF MALATHION ON BEHAVIOR
IN THE EUROPEAN HOUSE CRICKET,
Acheta domesticus.

A DISSERTATION
SUBMITTED TO THE GRADUATE FACULTY
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degree of
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JAMES DAVID BIGGS
Norman, Oklahoma
1977

THE EFFECTS OF MALATHION ON BEHAVIOR
IN THE EUROPEAN HOUSE CRICKET,
Acheta domesticus.

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Abstract

The LD₅₀ range of malathion in Acheta domesticus was determined to be 3.6 - 4.8 micrograms per gram. Doses of malathion at or below the LD₅₀ were considered sublethal in this study. Malathion in sublethal doses was found to affect aggressive calling and courtship calling, locomotor activity, the response of females to a mixture of calling and courtship songs, and thermal preferendum of both sexes.

The effects of calling by males and on locomotor activity were attributed tentatively to a reduction of inhibition by mushroom body interneurons. All of the behavioral changes following malathion treatment could be biologically significant in natural populations.

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Introduction

Mass production and protection of foods and fibers requires the control of various insect pests, largely through the use of chemical pesticides. Economic considerations favor broad-spectrum pesticides, although unintentional effects of a pesticide for target and non-target species can be expected to increase as specificity decreases. Elucidation of many unintentional effects of insecticides, especially during the last 30 years, has added to the vast literature on economic poisons. Bittner (1960) noted that although this literature was vast, many questions regarding the actions of contact insecticides remained. The behavioral effects of insecticides are among these questions.

Studies of the effects of insecticides on animal behavior could be of value in several areas. They could indicate directions for further investigations on the biochemical lesions producing death in insects poisoned by organophosphates. Behavioral effects of insecticides might prove sufficiently disruptive to a pest species to obviate the necessity for lethal applications, thereby reducing both cost of application and environmental contamination. Studies of intoxicated animals could also aid in pure research on behavior, e.g., the use of DDT in localizing receptors in the labella of Sarcophaga bullata (Hodgson and

Smyth, 1955). Warner et al. (1966) feel that "...it is probable that ethology will come to play one of the dominant roles in studies of the problem of micro-chemical contamination of the environment." (p. 225).

Warner et al. (1966) considered behavioral parameters to be valuable indices of toxication for several reasons. Since behavior represents the integrated result of a diversity of physiological events, they considered it "more comprehensive" than individual physiological measurements. They also felt that the sensitivity of behavior patterns to changes in the "steady state" of the organism and the possibility of executing behavioral studies without detectors implanted in the test animals represented advantages. Similar considerations led to the choice of behavioral rather than electrophysiological methods for this study.

Materials and Methods

The present study involved six series of experiments, investigating respectively the toxicity of malathion to Acheta domesticus and the effects of this insecticide on aggressive calling, courtship calling, locomotor activity, thermal behavior, and response of females to the calling song. Adult Acheta domesticus were used in all experiments. Except for the experiments in which stridulation was investigated, males and females were used in equal numbers. The crickets were housed in a variety of aquaria or containers made of wood, screening, or synthetic resins. Egg cartons and pieces of wood, styrofoam and cardboard were placed in cricket cages to increase crawl space, which is necessary in rearing this species (Sparks, 1959). Temperatures of incubating eggs were maintained at $30 \pm 1.5^{\circ}\text{C}$. in an environmental chamber. Most crickets were kept in this chamber at all times, the remainder being kept in cages fitted with electric lights and chick brooder thermostats, as recommended by Sparks (1959). Rearing at room temperature is unsatisfactory because of high nymphal mortality (de Baillon, 1920), longer developmental times (de Baillon, 1920; Kemper, 1937; Sparks, 1959) and, at high temperatures, reduced sizes of adults (Sparks, 1959).

Various techniques were tried for providing water, the most satisfactory being through gauze wicks protruding from narrow-mouthed bottles of water. The bottles were placed on their sides on the cage floors and the wicks were rested on

pieces of aluminum foil. Crickets are omnivorous, "...exhibiting marked tendencies toward carnivory" (Gangwere, 1961). They are known to eat dead insects, cloth, paper, bread, sugar, bran, oats, fruits and meat (Gangwere, 1961). Sanford (1971) found Gaines MealTM dog food provides a satisfactory diet for this species, and this was used in this study. Since Sparks (1959) noted that finely ground food resulted in less waste, the dog food was ground to a powder in a blender and presented in jar lids.

Pans full of a mixture of sand, peat moss, sawdust and wood shavings were used for oviposition. The mixture was autoclaved before use and was frequently moistened with tap water until hatching was considered complete.

In order to select crickets to be used in an experiment, I shook one of the egg cartons or pieces of styrofoam from the floor of a cage over a container until all of the crickets had fallen off the object. I then reached blindly into the container and seized a single cricket, repeating the process until an adequate number of healthy adult crickets fulfilling the sex distribution needed for the experiment had been selected. A cricket was rejected only if it was immature, if enough crickets of its sex had already been chosen, or if it appeared behaviorally or anatomically to be in suboptimal health. These crickets ordinarily struggle vigorously when clasped in a closed hand. Crickets failing to struggle well were considered

behaviorally symptomatic of ill health and rejected. Anatomical reasons for rejection included absence of an entire antenna or cercus, absence or considerable fraying of an elytron or wing, deformity or break in the exoskeleton, or absence of tarsal segments.

Malathion of secondary analytical standard grade was obtained from Oklahoma State Department of Agriculture and was used in all experiments. Of a number of organic solvents tested on the crickets, corn oil, which had been used as a solvent for malathion and some other insecticides in insect studies (Harvey and Brown, 1951; Brown and Brown, 1956), proved most satisfactory.

Test solutions were applied topically to the ventral thorax instead of being injected, since Gerolt (1969) had shown that parathion, DDVP, dieldrin and DDT accumulate in the integument, reaching the site of action via the tracheal integument rather than via the hemolymph. In order to minimize effects of variable areas of contact between the cuticle and the malathion solution (Matsumura, 1963), the concentrations of the solutions were chosen to result in a dose of 1.0 microliter of solution per 0.1 gram cricket mass, delivered from pipettes of 0.02 ml capacity graduated in units of 1.0 microliter. Each of these pipettes was tested for trueness of bore by observation of the length of an air bubble passed up and down the bore in alcohol (Ungureanu and Ungureanu, 1959). Flow of solution during

dosing was governed by a pneumatic pipette filler. This technique provides rapid, accurate delivery after limited practice.

Crickets were held in glassine envelopes while masses were determined on balances. Since prior anesthesia had been shown to protect Anopheles larvae against DDT (White and Jones, 1968), all handling and experimental techniques were designed to avoid the necessity of anesthesia. In each experiment, animals to be malathion-treated were selected, placed individually in one-quart jars, transferred to glassine envelopes and weighed, and the masses recorded. The crickets were returned to their respective jars, dosed with malathion and left undisturbed for 60 minutes prior to testing. The time period was chosen in consideration of findings on the uptake of topically applied malathion (Matsumura, 1963), the temporal pattern of cholinesterase inhibition in cockroach ventral nerve cords following malathion treatment (O'Brien, 1956) and a sixty-minute latency in the effects of parathion on oxygen consumption in Blattella (Harvey and Brown, 1951). A series of control animals was treated with corn oil alone.

In order to extrapolate from the laboratory findings to predictions of effects of malathion on natural populations, one must observe not only the differences between the two series described above but also differences between the latter group and animals that have not been handled.

Therefore, in each behavioral experiment, a series of insects was transferred directly from cages to the experimental setting. In order to examine the extent to which corn oil treatment, as opposed to handling, affected the insects' behavior, it was necessary to test a series of insects treated as the corn oil controls, but sham-dosed with clean, dry pipette. Since isolation is known to increase aggression in male crickets (Alexander, 1961; Sanford, 1971) and since the malathion-dosed crickets were to be maintained in isolation for one hour prior to testing, it was necessary to test for effects of isolation in the aggressive calling experiment. For this, a series of crickets was isolated in jars without weighing or sham-dosing and their calling observed.

Experiments

Toxicity

Worden (1961) noted that the LD₅₀ may not be a reliable standard, since values obtained in different laboratories under similar conditions may vary considerably. Dowden and Bennett (1965) suggested the most significant contribution of the related LC₅₀ may be the use of this value as a starting point in studies of sublethal effects of toxins. Despite the recognized limitations of the use of this value, no better index of toxicity has been suggested.

An extensive investigation of the literature revealed no information on the toxicity of malathion to Acheta domesticus. American Cyanamid Company, original patent holders on the compound (U.S. patent 2,578,652), made a search through December, 1970 and also failed to find any information on the question (M.H. Woolford, jr., personal communication).

The 24-hour LD₅₀ of malathion for Acheta domesticus was thus determined. After weighing and dosing, crickets were left isolated in quart jars until evaluation. Ten males and ten females were tested at each dosage level, since Ciocco (1940) had pointed out the usually lower susceptibility of females of many insects and other animals to a number of poisons. The toxicity is plotted in Fig. 1. The line was fitted by eye (Finney, 1952). A cricket was considered dead if it was immobile and showed no visible motion of any leg when a jet of air from a rubber bulb was

blown through a dropper pipette onto one of the cerci or no twitching of the palps when a similar jet of air was blown onto them. These examinations were made with the unaided eye.

Aggressive Calling

In order to minimize the effects of ambient sound, aggressive calling experiments were conducted inside a small anechoic chamber described by Frings and Lollis (1971). This chamber was placed in an environmental chamber for temperature regulation. The number of bursts of aggressive calling produced by two male crickets of the same treatment group during the first fifteen minutes of confinement in a one-gallon aquarium placed inside the anechoic chamber was counted as the measure of aggressive behavior. A piece of styrofoam approximately 10 cm X 10 cm X 2 cm was placed on the floor of the aquarium during each test, after preliminary experiments had indicated more uniform results with this addition. A microphone connected to a tape recorder was suspended inside the aquarium. A timer which could be set to sound a bell at the end of the 15-minute interval was placed outside the chamber and produced an audible recording. Twenty replicates were run for each treatment.

Preliminary experiments had shown that excessively large numbers of calls were obtained when five or more males were placed together in the aquarium; that a single male placed in the apparatus did not call at all; that the

presence of females was accompanied by a mixture of song types from the males; and that, despite the anechoic chamber, ambient sound levels increased the number of aggressive calls per 15-minute period. The latter finding necessitated suspension of testing during periods of unusually high ambient sound.

Courtship Calling

The same chamber-aquarium apparatus used in the aggression study was used in investigating courtship calling. Crickets destined to be used in this study were prevented from mating prior to their use in experiments. They were purchased when immature and each female was removed before maturity and placed in a cage of females only, while the males were left with the remainder of the immatures.

Each test involved recording the calls produced in the fifteen-minute period immediately following the introduction of one male and one female into an aquarium. The females were all taken directly from the cage of virgins, whereas the males were variously treated as described under Materials and Methods. Ten replicates were run for each treatment. Since the courtship call of A. domesticus is a sustained series of notes of variable length rather than the discrete burst of aggressive calling, it seemed that a count of the number of bouts of courtship calling would be less representative of the vigor of courtship than a measure of the total time spent in calling. The duration of each male's

courtship calling was cumulated over the fifteen-minute period by stop-watch.

Locomotor Activity

Diverse devices have been used as actographs in studies of small animal movement. Nowosielski and Patton (1963) used balanced cages connected to marking pens so that movement of a cricket within the cage affected the tracing produced by the pen. Amos (1967) and Gage (1966) used photographic records of the successive positions of animals at regular intervals in studying activity; Phipps (1963) constructed a device in which blocking of an infrared beam focused on a phototransistor caused deflection of a marking pen; Schechter et al. (1963) studied locomotor activity of cockroaches by means of a capacity sensing device; and Bittner (1960) studied the movements of insecticide-poisoned bees by permitting the insects to move about on soot-covered glass plates. Contact of the insects' tarsi and wings displaced the delicate film of soot, producing clear tracks on a black background. Since each insect track on a soot-covered plate uncovers a finite area of clear glass underneath, it follows that one could calculate the area of glass uncovered by each track and add these areas together, the sum serving as an index of locomotor activity.

In this study, crickets were confined for 15 minutes in a shallow rectangular cage, the floor of which was a glass plate completely covered with soot on the side on which the

cricket walked. The plates were coated with soot by being held over a shallow metal dish of burning benzene. Each plate was coded by a number inscribed on the back and the completely sooted plates were run through a photo-electric device (Hayashi Denko Co., Ltd.; Automatic Area Meter, Type AAM-5) designed to measure the surface area of regularly- or irregularly-shaped objects. As an object passes through the machine, the opaque parts of the object block a slit-shaped beam of light focused on a photoelectric cell. The machine cumulates the opaque area passing across this beam. Translucent parts of an object are not measured.

After a plate had been walked on by a cricket for 15 minutes, it was again run through the area-measuring machine and the resultant area was subtracted from the area of the untracked sooted plate. The difference between the two area measurements was, then, the sum of the areas of the tracks made by the cricket.

Thermal Gradient

Preliminary experiments with a gradient apparatus designed for use with snakes were inconclusive, probably due to the lack of uniformity of temperature from one side of the gradient to the other at a given point along the length of the apparatus. A gradient device with hot and cold water piped into an outer container (Fig. 2) produced a fairly uniform and repeatable gradient. A polyvinyl chloride (PVC) pipe extended the length of the apparatus left of the

mid-bottom and another right of the mid-bottom. A series of holes was drilled in line down each pipe so that water escaping from the holes formed jets aimed along the midline of the water surface. The holes were spaced according to the recursive mathematical series 1,1,2,3,5,8....n, the unit of distance being 0.5 cm. The series began at opposite ends for the hot and cold water pipes. A length of 1.5 inch diameter, potable-water rated PVC pipe was sectioned lengthwise to form a trough. Leads from a Yellow Springs telethermometer, Model TD, were placed at the ends and at nine evenly spaced points along the bottom of the trough to delineate ten temperature zones. The ends of the trough were sealed and the top covered with wire screen.

Once a gradient was established, crickets were introduced into the cold end of the trough and allowed fifteen minutes to stabilize their positions in the gradient. Ten crickets of the same sex were tested simultaneously. The gradient apparatus was located in a photographic darkroom and all room lights were turned off during runs. At the end of the fifteen minute period, an electronic flash photograph of the apparatus was made from above to produce an instantaneous record of the positions of the crickets, and the temperature of each telethermometer lead was recorded.

Response to Calling and Courtship Songs

An arena with floor and sides of synthetic resin and an open top covered with wire screen was used as a runway

for female crickets introduced at one end. At the other end of the runway, a container of approximately 1,000 male and female crickets was placed. The population of crickets served as a source of calling and courtship songs. Preliminary observation of such populations had shown that the songs occur almost constantly and, so far as the human ear could determine, to the virtual exclusion of aggressive calls. The sound level of calling seemed constant to the human ear. The time required for a female to travel a linear distance of 100 centimeters to a point 25 centimeters from the container of calling crickets was measured. Twenty females were tested at each treatment. Since the arena was 50 cm. wide, there was an abundance of area in which a female could move about without moving toward the sound source.

Controls for visual and olfactory attraction were not necessary, since Regen's (1912) classical experiments had demonstrated that males that were not calling were not attractive to females and that females with the tympanal organs destroyed were not attracted to calling males.

Shuvalov and Popov (1973) used the rate at which female Gryllus campestris and G. bimaculatus approached a source of calling songs as a measure of the "strength of the reaction" of the females to those songs. Popov and Shuvalov (1974) found that variable changes of intensity dynamics with changing distance from the source made the fol-

lowing of an intensity gradient by female G. campestris
unlikely at distances greater than about two meters.

Results

Toxicity

Figure 1 shows the dosage response curve. Each point represents the probit of the percentage of crickets dying at the indicated dosage. The LD₅₀ range determined empirically is 3.6 micrograms to 4.8 micrograms per gram. At 24.2 micrograms per gram, 100% kill was obtained and at 0.24 micrograms per gram, no animals were killed. These values were not useful for plotting of the dosage-mortality curve (Finney, 1952) and were thus not considered.

Aggressive Calling

The mean numbers of calls per pair of male crickets at each treatment is presented in Table 1. The calls during each fifteen-minute period are shown both as a total and broken down into five-minute intervals. A chi-square test was calculated to determine the likelihood that the distribution of the means of all of the treatment groups above and below the grand mean could be expected by chance. The obtained value of $\chi^2 = 38.84$ well exceeds the value of 20.28 necessary for significance ($p = 0.995$, 7 df) (Hoel, 1966). Further examination of the malathion-treated groups alone was done by use of the chi-square statistic, using the number of 15-minute periods in which no aggressive calling occurred in corn oil-treated males as an expected frequency with which to compare the corresponding frequencies of the treated groups. The obtained chi-square value of 16.66 exceeds the value of 12.84 necessary for significance at the

0.005 level (3df) (Hoel, 1966). Thus, although there seems to be no clearly dose-related reduction of aggressive calling, malathion does significantly reduce this behavior.

Alexander (1961) noted that isolation of a few days increased aggressiveness in crickets. This study indicates that isolation for one hour increases aggressiveness, as measured by aggressive calling. An average of 208 bursts of aggressive calling per 15-minute period occurred in crickets that were isolated for one hour prior to testing, whereas 133 bursts per 15-minute period occurred in crickets that were tested directly after removal from a population cage. Both handling (See Materials and Methods section) and treatment with corn oil, in contrast, reduce aggressive calling.

In any treatment not involving malathion dosage, the average number of calls during the second and third five-minute segments of the fifteen minute recording period was decidedly higher than in the first five minutes. This contrast is not seen at any level of malathion treatment. Furthermore, in corn oil treated animals the number of five minute periods in which no calling was recorded was greater for the second and third five minute periods than for the first. In malathion treated animals, the number of first five minute periods in which no calling was heard was greater than the corresponding numbers of second and third five minute periods except at 3.6 microgram per gram. These trends seem clearly to indicate that malathion affects

either the rate at which dominance is established or the stability of the dominance relationship.

Courtship Calling

The courtship call of Acheta domesticus is a sustained series of notes. Total time spent in courtship calling was thus recorded instead of number of bursts, as was done in aggressive calling. Table 2 summarizes the findings. Each total represents the sum of the courtship calling times of ten males. The effects of malathion on courtship calling are quite different from the effects on aggressive calling and are dose-related over the range 2.4 micrograms to 4.8 micrograms per gram. At the lowest level of malathion treatment, 0.36 microgram per gram, the total time is approximately 0.2% above the corn oil total, which difference is negligible.

The trend is perhaps most clearly seen by comparing progressively lower dosages. Malathion at 4.8 micrograms per gram is highly stimulatory to courtship calling, and this stimulation persists at 3.6 micrograms per gram. At dosages of 2.4 microgram per gram and 0.48 microgram per gram, a depression in courtship calling seems to occur and at 0.36 microgram per gram, there is no effect. This evidence would seem to imply effects of malathion on, to take the simplest case, two aspects of the cricket's neural mechanisms, each having a different threshold. Statistical analysis by Rank-Sum Tests (Hoel, 1966), reveals that significant differences among malathion-treated animals

exist only in comparisons between 0.48 microgram per gram and 3.6 microgram per gram ($p = 0.053$). It should be noted that the comparison between corn oil-treated and 3.6 microgram per gram ($R = 85.5$) animals and that between 2.42 microgram per gram and 4.8 microgram per gram ($R = 87$) are significant at the 0.1 level (U.S. Department of Commerce, 1963).

Locomotor Activity

The results of the smoked-plate actographic experiments are summarized in Table 3. Hoel (1966) describes a use of the X^2 statistic for testing whether the locations of two distributions differ. This test was employed to examine whether the locations of the distributions of the track areas (See Materials and Methods) differed between males and females, since female crickets are, on the average, larger than males. The result ($X^2 = 8.4$, 18 df) shows that the probability that the distributions of the male and female track areas are in the same location is ≈ 0.975 .

Rank sum tests were run on the data, comparing each level of malathion dosage with the corn oil-treated controls. The sexes were treated separately.

Among females, both the 4.8 microgram per gram and 2.4 microgram per gram treatments showed significantly higher locomotor activity than the control group at the 0.01 level. The 3.6 microgram per gram group was significantly higher in track area than the control at the 0.053 level and 0.36 microgram per gram group was significantly higher at the

0.025 level.

Among males, the 4.8 microgram per gram group was higher than the corn oil controls ($p = 0.05$), as was the 2.4 microgram per gram group ($p = 0.005$). The males treated at 2.4 microgram per gram were also significantly more active than those at 0.36 microgram per gram ($p = 0.05$).

Thermal Gradient Behavior

The gradient apparatus described under Materials and Methods was capable of producing a reasonably repeatable range of temperatures, although the locations of specific temperatures along the gradient varied. Table 4 shows the temperatures below which half of the crickets had settled at the end of the fifteen-minute runs. At most treatments, males had higher preferenda than females, although in those treated with corn oil alone and those treated at 4.8 microgram per gram, medians for males and females were identical.

Comparison of corn oil controls with malathion treated animals shows a general trend of the latter to seek higher temperatures. In females, a clear difference is seen only at 4.8 microgram per gram. In males, the median preferred temperature at every malathion dosage was higher than that of corn oil-treated animals. Comparison of corn oil-treated animals with those that were sham-dosed with a dry pipette shows that corn oil itself produced a considerable drop in the median chosen temperature of males but not of females. Such differences are not entirely unexpected in a species with one sex strongly territorial and combative.

Although males and females treated with corn oil showed the same median preferred temperature, the effect of this treatment as seen by comparison with the direct control series (see Materials and Methods for definition of the control series' designations) was greater for males than females. Furthermore, the effects of malathion work in the direction of returning the crickets' temperature selections to those of crickets which have not been weighed, handled, dosed, or placed in a container for an hour before being tested.

Female Response to Calling and Courtship Songs

The ranges and median times required for female crickets to travel 100 cm toward the sound source are shown in Table 5. The longest median time was in the group that was placed directly in the arena after being removed from a rearing cage. The medians of the handled control series and the corn oil control series were identical. The median for the 3.6 microgram per gram group was slightly higher than the corn oil group, whereas the value for 2.4 microgram per gram was lower than the corn oil median. The median value for crickets treated at 4.8 microgram per gram was the lowest for any group tested, representing approximately one-half of the time for the other malathion-treated groups, the corn oil control series and the handled controls. Comparisons of various groups by rank-sum tests showed significance ($p = \ll 0.005$) of the reduction in time to run the arena at 2.4 and 4.8 microgram per gram but not at 3.6 microgram per gram. Females running down the sides of the arena often

made a sharp turn toward the center along a line that carried them directly toward the sound source when they were approximately 25-50 cm from it.

The shortest times at each treatment probably represent values near the minimum time in which a cricket can run the prescribed distance under the experimental conditions. The crickets arriving in these times seemed to follow straight paths which took them variously toward the center of the sound source or to one side of it. Crickets requiring the longest times spent as long as several minutes walking around the upper end of the arena, antennating and palpating the substrate before appearing to orient toward the sound source definitely.

Discussion and Conclusions

Perhaps because insecticides are evaluated in terms of mortalities in test populations, considerable importance attaches to the lethality of a studied dosage to the test population, whether lethality per se is being studied or not. Some work, e.g., Barlow (1968), Bittner (1960), Pesson et al. (1971), employs dosages which are termed "lethal", whereas other work investigates levels termed "sublethal" (Warner et al., 1966; Kennedy, 1947; Anderson, 1968; Anderson and Peterson, 1969; Cairns et al., 1967; Ogilvie and Anderson, 1965; Weiss, 1959; Macek, 1968). Macek (1968), noting that previous workers had considered as "sublethal" dosages which killed some animals, questioned whether the term "sublethal" properly applied.

The lethal-sublethal problem essentially resolves itself into two considerations. First, lethality is evaluated in terms of populations, in the statistical sense, rather than in terms of individuals. Exposures killing 50% or less of a population can as well be called "sublethal" as "lethal". Second, the time over which a toxin is permitted to act on a population prior to evaluation affects the judgement of the lethality of the dosage. In the present study, crickets surviving 24 hours after malathion exposure seemed to live as long as a week only if they had received lower doses of the insecticide.

The mechanisms by which organophosphates kill insects have not been satisfactorily elucidated. There seems to be

general agreement that toxicity in vertebrates is primarily related to cholinesterase inhibition, but, although acetylcholine has long been known to exist in insects (Gautrelet, 1938) and both that compound and cholinesterase have been isolated from the heads, brains, and other organs of various insects, including crickets (Corteggiani and Serfaty, 1939; Tobias et al., 1946; Roeder, 1948; Lewis and Fowler, 1956; Colhoun, 1958, 1959, 1963), there is no general agreement on the nature of the insect synaptic transmitter substance.

Acetylcholine increases in insects treated with organophosphates (Lewis and Fowler, 1956; Colhoun, 1958, 1959; Farnham, 1966), but also increases after treatment with DDT (Tobias et al., 1946). Colhoun (1963) suggested that insect peripheral nerves are cholinergic but that the transmitter substance at nerve endings is of a different nature. Farnham (1966) found the severity of poisoning in diazinon- and diazoxon-treated flies to be closely related to the extent of inhibition of cholinesterase in the thoracic ganglion. The more severe a fly's symptoms, the farther into the nervous system the inhibition was found to extend. Based on studies of the locomotor activity of bees fatally poisoned with parathion, Bittner (1960) had suggested that increasing severity of behavioral symptoms would probably prove to follow a progression of cholinesterase inhibition from peripheral sites in the nervous system inward.

McCann and Reece (1967) found evidence for a cholinergic

neuromuscular mechanism in insects and Boistel (1968), in examining criteria which he felt should be fulfilled in order to identify conclusively a cholinergic synapse, concluded that "...it would appear that these conditions are fulfilled in the case of ganglion of the cockroach nerve cord" (p. 10). Barker (1970), however, found that cholinesterase-reactivating drugs used as organophosphate antidotes in vertebrates did not reduce mortality in bees poisoned with parathion, carbaryl, or monocrotophos. Faeder et al. (1970) also found no effects on neuromuscular transmission in insects when several drugs affecting cholinergic transmission were applied to them. Naharishi (1971) concluded that the effects of "various organophosphates" on insect nerve and muscle are explicable in terms of cholinesterase inhibition. In the case of malathion, however, O'Brien (1956) has shown that cholinesterase inhibition as a cause of death in insects is suggested by in vitro findings but countermanded by in vivo evidence. After an early inhibition, cholinesterase activity rose until death.

O'Brien (1956) found that malathion inhibited succinoxidase, but not to an extent that could explain death. Kovalenok and Kazakova (1967) found succinic dehydrogenase inhibition in the neurophile of the thoracic ganglion in Dipterex poisoning, whereas the same enzyme showed increased activity in thoracic indirect flight muscle. Harvey and Brown (1951) found that TEPP immediately stimulated oxygen consumption, whereas parathion stimulated it only after a

60-minute latency. With both poisons, stimulation was followed by depression lasting until death. Brown and Brown (1956) found inhibition of cytochrome oxidase in coxal muscles after injection of corn oil solutions of malathion into the hemolymph of Periplaneta americana, but the inhibition was below 20%. O'Brien (1957) concluded that the insecticidal action of malathion is probably not related to carbohydrate metabolism, because insect enzymes were generally less inhibited than corresponding mamalian enzymes, although insects are more susceptible to the poison than mammals.

There remains, therefore, considerable doubt regarding mechanisms of toxicity of malathion. In the absence of this evidence, one cannot explain behavioral effects in terms of specific biochemical lesions, but the behavioral effects may suggest directions for further chemical investigations.

Insecticides in appropriate exposure levels may stimulate biological processes. Luckey (1968) found stimulation of growth in Acheta domesticus reared under suboptimal conditions when the young crickets were exposed to sublethal doses of several insecticides. Lowered fecundity and longevity (Adkisson and Wellso, 1962; Attiah and Boudreaux, 1964), increased fecundity and longevity (Havertz and Curtin, 1967; Knutson, 1955; Kuenen, 1958; Ouye and Knutson, 1957; Parry and Ford, 1971) and a general lack of effect on honey production by honeybees (Robinson, 1955; Wolfenbarger and Robinson, 1957; Morse, 1961) have also been reported follow-

ing insecticide exposure.

Rose (1944) felt that the first sign of DDT-induced excitation in a mosquito indicated fatal poisoning. Barlow (1968), however, found that Sitophilus granarius poisoned with what should have been a lethal dose might survive and overcome the reversal of their photonegativity caused by DDT. Kennedy (1947), Barrera et al. (1959) and Kuhlow (1959) have investigated the excitation of mosquitoes produced by levels of DDT exposure which did not kill the animals and Hodgson et al. (1955) and Smyth and Roys (1955) used DDT in studying insect chemoreception. Soliman and Cutkomp (1963) and Soliman (1966) found that parathion did not lower sucrose thresholds in Musca domestica vicina. Pyrethrins have also been shown to affect feeding behavior of Aedes aegypti (Lal et al., 1965) and Anopheles gambiae (Smith and Chabeda, 1969).

There seem to be few studies of the effects of organophosphate insecticides on insect behavior. Bittner (1960) found that the patterns of tracks formed when DDT-poisoned bees moved about on soot-covered glass plates were different from the patterns formed by parathion-poisoned bees. He reviewed earlier work on disruption of the dance communication system of bees by some other compounds. Pesson et al. (1971) found compound-specific patterns in actographic records of houseflies poisoned with parathion, dipterex, fenthion and some non-organophosphate poisons.

In the present study, the various behaviors investigated

do not seem to be affected in a common way. There is an unusual finding at the dosage level 0.36 microgram per gram, however. In the studies on aggressive calling, locomotor behavior and thermal gradient behavior, the values for this dosage seem unexpected. There is no obvious explanation for this observation.

The studies of aggressive calling and courtship calling by males may shed light on the effects of malathion on a specific structure in the cricket. One might attribute the reduction in aggressive calling to a generalized expression of intoxication, reflecting enzyme inhibitions which reduce muscular activity. The tendency toward increased courtship calling, however, would seem to be a contradiction of this interpretation, as would the increase in locomotor activity.

Huber (1955) suggested that the physiological priming mechanisms for courtship and aggression are different. Huber later (1960) reported that the mushroom bodies are involved in selection of song type and in song-release and that the central body and mesothoracic ganglion are also involved in song production, the latter containing all of the neurons and connections for the coordination of the 28 muscles involved in song production. Later (1962), Huber reported that cutting both connectives between the higher centers and the mesothoracic ganglion eliminates singing, although the ganglion remains intact. Huber's (1965) review of brain-controlled behavior in Orthoptera also pointed out that electrical shocks to the mushroom bodies

of crickets produce normal song rhythms, whereas stimulation of the central body produces only anomalous song. Finally Huber (1967) reported that removal of the dorsal parts of the mushroom bodies completely stops all stridulation and that focal stimulation of certain areas in the brain of male crickets, near the mushroom bodies, either inhibits singing or releases sound patterns very similar to normal song.

Vowles' (1955) study of the mushroom bodies in Hymenoptera showed that these bodies are composed of internuncial cells which are entirely contained within the mushroom bodies.

Mushroom body cells synapse with afferent fibers from the optic and antennal ganglia and with efferent fibers passing to the nerve cord and subesophageal ganglion.

Malathion could, then, affect both aggressive and courtship calling by producing some undefined lesion in the mushroom bodies. Interference with the handling of incoming impulses from the sensory apparatus of the head, with outgoing impulses to the subesophageal ganglion, or both, could produce behavioral effects. Taken in total, Huber's work demonstrates that normal song-type production does not occur in the absence of the mushroom bodies and that stimulation of various foci on the mushroom bodies leads to normal song-release, which happens with stimulation of no other structure. Males with lesions in the dorsal protocerebrum sometimes sing to the point of exhaustion. Malathion's effects on courtship and aggressive calling could be mediated through an effect of the insecticide on the mushroom bodies.

As Khalifa (1950) noted, the courtship display of Acheta domesticus is limited to song. The effects of malathion thus change the entire courtship display of this species.

Many questions regarding excitatory and inhibitory influences of the neuronal pathways of which mushroom body internuncials form parts remain to be answered. Further investigations involving malathion could play a part in elucidating "...das komplizierte Zusammenspiel von Sinnesorganen und mehreren zentralen Stellen bei der Kontrolle und Koordination des akustischen Verhaltens..." (Huber, 1960, p. 124). Huber (1960) noted that it was unclear from his work whether a single central body circuit changes its discharge rhythm in conformity with neural commands from the mushroom bodies or whether for each of the three song types there is a separate circuit. The essentially opposite findings on effects of malathion on courtship and aggressive calling would be compatible with either of these systems; these findings thus shed no light on this question.

In locomotor behavior, malathion stimulates at all levels, although that at 3.6 microgram per gram is not so great as would be expected from surrounding values. In the case of this behavior, Huber's findings may again clarify the circumstances. By 1960, Huber had concluded that the brain contains neurons of which the input controls the vigor and duration of locomotion. The neurons did not appear to be brought together in "centers", but were distributed over a large region of the protocerebrum. It was demonstrated

for the first time that the mushroom bodies contain inhibitory neurons for locomotion. Absence of both mushroom bodies was shown to increase walking and jumping activity (failure of inhibition) whereas absence of the central body decreased walking activity.

Huber found later (1962) that removal of the subesophageal ganglion causes a marked decrease in flight activity and locomotor activity. Further work (Huber, 1967) showed that removal of the dorsal parts of the mushroom bodies in crickets causes a "remarkable increase" in locomotor activity, which increase is not elicited by destruction of the sensory equipment of the head. The effect would appear, then to be due to the removal of inhibition originating in or near the mushroom bodies. Confirmation of this explanation was obtained when stimulation to the calyces and lateral neuropile reduced locomotor activity. Huber hypothesized that, since no locomotor-inhibitory center has been found outside the mushroom bodies, the subesophageal ganglion and central body must act together as locomotor exciters to the mechanisms in the thoracic ganglia and that both are inhibited by commands from the mushroom bodies.

The locomotor behavior of crickets dosed with malathion is suggestive of mushroom body involvement. At all dosages, median locomotor activity is greater than that of animals treated with corn oil. This increase may be attributable to the removal of mushroom body locomotor inhibition under

the influence of malathion. Evidence of effects of malathion on two song types, aggressive calling and courtship calling, and locomotor behavior together leave no alternative which is superior to the explanation of an effect on mushroom bodies.

Investigations of the control of locomotion in some insects closely related to crickets have demonstrated mechanisms that are not known in crickets. Pearson et al. (1973) have identified two types of central interneurons in the cockroach and have discussed the probable roles of certain cuticular sense organs in the control of locomotion. Odhiambo (1966) has implicated corpus allatum hormone in locomotor control in a species of locust. It is conceivable that similar mechanisms in crickets may act in concert with the mushroom bodies in the control of locomotion. Research on such possible mechanisms in crickets might further clarify modes of malathion toxicity.

Another aspect of this study, response of females to calling and courtship songs, involves, among other phenomena, locomotion. Granted that crickets treated with malathion are more active, is this increased activity expressed in uncoordinated movement, as is seen in the course of poisoning of insects by various insecticides (Bittner, 1960; Pearson et al., 1973) or is the increased activity accompanied by more rapid approach toward an attractive stimulus?

Malathion-treated animals showed a clear trend to travel toward the source of calling and courtship calls

more rapidly than insects treated otherwise. Orientation of the insects toward the sound source seems the only reasonable explanation of this phenomenon, since opportunities for locomotion in directions other than toward the source were ample.

The primary function of the cricket calling song is the attraction of females (Alexander, 1961). It was not possible to investigate this aspect of acoustic behavior directly, as was done for the aggressive and courtship songs, since males did not produce the calling song under circumstances conducive to such study. Furthermore, females were not found to be attracted to tape recordings of calls, possibly because of deficiencies in the transducing equipment. The use of actual crickets as a sound source might be unjustified if crickets were known to produce sexual attractant pheromones, but these are not known, nor do they seem likely in light of the acoustical attractant system known in these animals (Regen, 1912). A further possible objection is that females might be attracted via the olfactory mode to some non-pheromonal scent arising from the cage of crickets, and that it was, in fact, orientation by scent which was facilitated in malathion-treated animals. Again, olfactory attraction of this sort is not known in crickets. Moreover, investigations of the effects of organophosphate insecticides on chemical senses of insects have consistently produced negative results (Soliman et al., 1963; Soliman, 1966). It seems reasonable, therefore, to assume

that the females were orienting to the container of crickets as a source of attractive acoustical stimuli.

Since males spend more time in courtship calling when treated with malathion, at least at higher dosages, and since females show heightened responsiveness to the calling and courtship songs of males, it is possible that mating might be more frequent in natural populations exposed to this insecticide. If this were true, increased fecundity might follow, as has been reported in various insects treated with phosphamidon (Parry and Ford, 1971; Havertz and Curtin, 1967; Knutson, 1955). Although diversity of insects, pesticides, and experimental designs makes it unlikely that a common mechanism underlies the similar findings, it is interesting that the effects on the insect populations and, potentially, the environmental impact, are similar.

There seems to have been no report in the literature of an investigation of possible effects of any organophosphate insecticide on thermal gradient behavior of any animal. In Acheta domesticus temperature is known to exert pronounced effects on developmental times (de Baillon, 1920; Kemper, 1937; Sparks, 1959), nymphal mortality (de Baillon, 1920) and size of adults (Sparks, 1959).

The greatest effects of malathion on median selected temperature occur at different dosages for males and females. The effect in males was most pronounced at 4.8 micrograms per gram, the upper limit of LD₅₀ range, whereas in females the effect was maximum at 2.4 micrograms per gram. The

shift was also greater in females, a rise of 11°F. over corn oil treated controls, whereas males showed, at the greatest, only a 7.5°F. upward shift in median selected temperature. These findings were both unexpected in light of Ciocco's (1940) finding that female animals are generally less susceptible to toxins.

An upward shift in selected temperature could have several effects in malathion-intoxicated insects. Toxicity of malathion to Musca domestica varies directly with temperature (Negherbon, 1959). An increase in a cricket's body temperature could increase the effects of exposure, but, as White and Jones (1968) found, cold may provide protection against insecticide effects.

Different effects of insecticides at different exposure levels are not uncommon; any of them would be expected to kill insects at some dosage, yet as Luckey (1968) and others have shown, growth, longevity and fecundity may be greater in insects exposed to certain appropriate levels. Ogilvie and Anderson (1965) found that DDT in low doses caused a downward shift in the temperature selected by young Atlantic salmon, whereas higher doses produced an upward shift and Teeman and Matsumura (1973) reported that rats treated with high doses of acaricide, chlordimeform, showed initial hyperexcitation followed by tranquilization, whereas those treated with low doses showed tranquilization initially.

Work such as that of Ogilvie and Anderson (1965) and Teeman and Matsumura (1973) indicates that effects of an

insecticide on a behavioral parameter are not necessarily dose-related in a linear fashion. The complexity of physiological functions involved in a behavior pattern suggests that the expression of insecticide effects on that behavior might be equally complex.

There is undoubtedly work on the behavioral effects of insecticides which has gone unreported because of the difficulty of explaining the findings in terms of current fragmentary knowledge of sublethal pesticide effects. It is desirable that such work be reported for its potential benefit to our understanding of the interactions among organisms, their environments, and the pesticides which have become virtually omnipresent features of those environments. In many cases, the explanation of behavioral findings in terms of specific physiological events must await the clarification of mechanisms of toxicity of insecticides; many fundamental questions regarding the physiology of nervous systems must be answered as well.

It is apparent (Huber, 1955; Roeder, 1967) that the concept of spatially localized functional nerve centers must be abandoned in the case of cricket and mantis locomotor control, and Huber's (1960, 1965, 1967) reports of several loci within the mushroom bodies for production of certain song types contraindicate spatial localization of function in this system. Schürmann's (1974) studies of the mushroom bodies of Acheta domesticus provide anatomical evidence which supports the behavioral indications of complexity.

The potential for complexity of an insecticide effect on mushroom body-related behavior may be magnified by the emergence at some dosage of modifying effects which did not exist at lower dosages. Plots of dosage against response would not be meaningful in such a system, although effects could be pronounced. Investigations using electrophysiological techniques to study the effects of insecticides on the mushroom bodies are needed to test the tentative conclusions which can be drawn from behavior, as in the present study.

Biological changes secondary to physiological effects of insecticides may be of considerable economic and environmental importance. Raised thermal preferenda in both sexes following malathion exposure could alter the relationship of a species to man, increasing the tendency of insects to seek the warmth of homes and other heated buildings. Increased courtship calling by males and heightened responses of females to calling songs could contribute to increases in fecundity and concomitant increases in population density, which changes could also alter the pest status of a species such as Acheta domesticus.

Treatment	first 5 min.	second 5 min.	third 5 min.	15 min. total	5 min. mean	first 5 min.	second 5 min.	third 5 min.	15 min.
Direct	35	45	53	133	45	0	2	0	0
Handled	19	29	28	76	25	1	3	5	0
Corn Oil	8	21	19	48	16	5	7	13	3
Isolated	39	83	86	208	69	1	2	1	0
0.36 mcg/g	4	10	3	17	6	13	9	12	8
2.4 mcg/g	3	6	4	13	4	11	9	9	3
3.6 mcg/g	7	15	6	28	9	11	9	14	7
4.8 mcg/g	9	8	5	22	7	10	9	8	6

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Table 1: Mean numbers of calls per 15-minute period by pairs of male Acheta domesticus.

Calls per 15 minute period are shown as totals and as numbers of calls in the component 1st, 2nd and 3rd 5-minute periods. Columns 6-9 show the numbers of 5-minute periods and 15-minute totals in which no calling occurred.

Treatment	Total Calling Time
Direct	2107.6 sec.
Handled	629.0 sec.
Corn Oil	803.6 sec.
0.36 mcg/g	816.3 sec.
0.48 mcg/g	567.8 sec.
2.4 mcg/g	707.5 sec.
3.6 mcg/g	1464.1 sec.
4.8 mcg/g	1622.0 sec.

Table 2: Total time spent in courtship calling by ten male Acheta domesticus, each of which was confined with a single female for 15 minutes.

Treatment	Average Areas of Tracks (cm ²) male	female	Median
Direct	7.14	9.47	7.94
Handled	5.88	6.03	5.55
Corn Oil	2.83	2.99	2.12
0.36 mcg/g	4.40	9.46	3.59
2.4 mcg/g	8.33	13.41	10.05
3.6 mcg/g	4.89	6.73	4.91
4.8 mcg/g	8.01	6.11	8.64

Table 3: Smoked plate actograph data

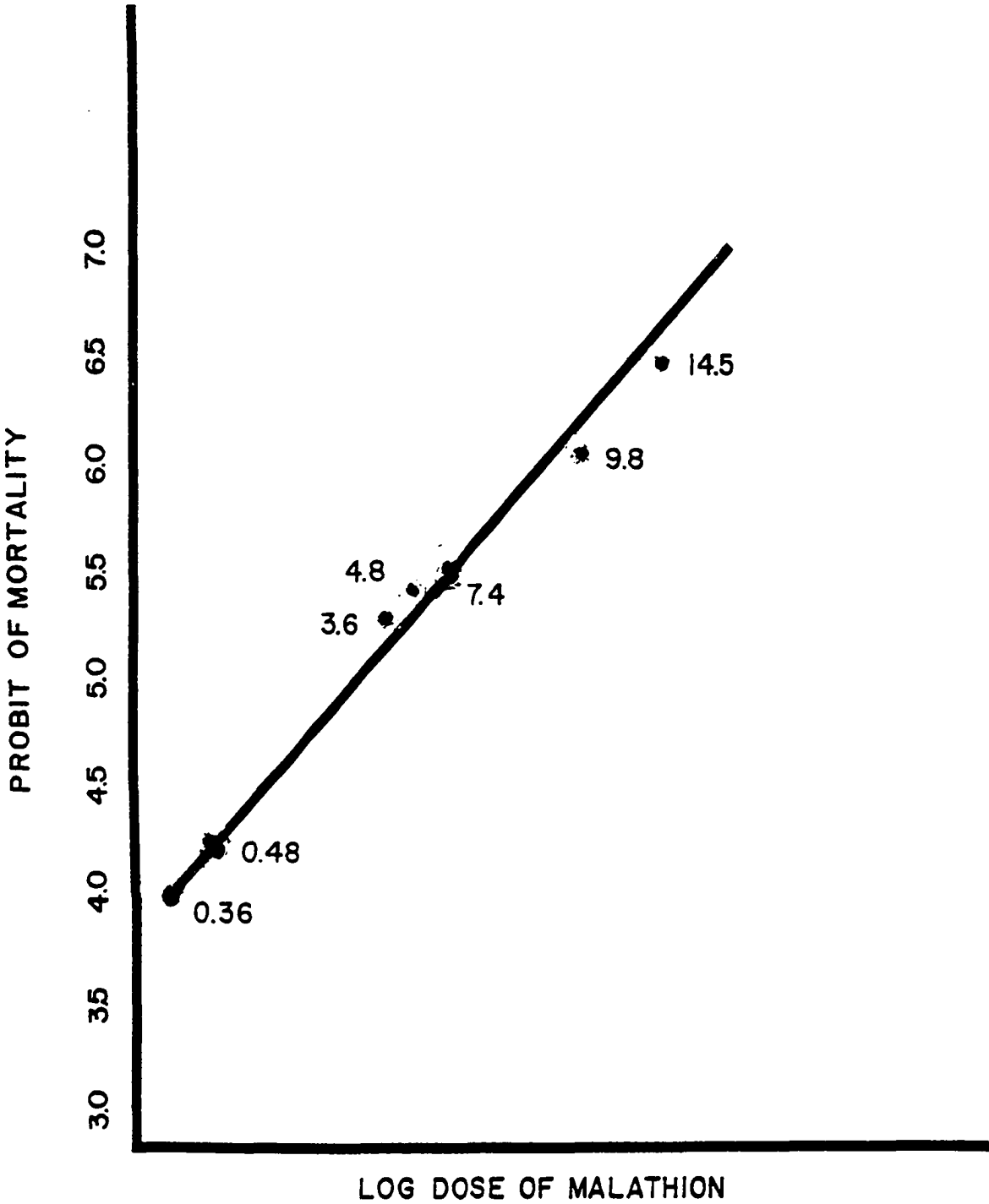
Treatment	Female		Male	
	Zone	Lower Temperature Boundary ($^{\circ}\text{F}$)	Zone	Lower Temperature Boundary ($^{\circ}\text{F}$)
Direct	8/9	61	6/7	71
Handled	8/9	62	2/3	92
Corn Oil	7/8	65	7/8	65
2.4 mcg/g	8/9	65	5/6	76
3.6 mcg/g	8/9	63	6/7	71
4.8 mcg/g	6/7	72.5	6/7	72.5

Table 4: Zone designation and lower temperature boundary delineating median preferred temperatures of Acheta domesticus in a thermal gradient.

Treatment	Range	Median
	Min:Sec.Sec X 10^{-1}	Min:Sec.Sec X 10^{-1}
Direct	0:5-9:25	3:15
Handled	0:8-6:55	1:12.5
Corn Oil	0:15-6:00	1:12.5
2.4 mcg/g	0:10-8:20	1:07.5
3.6 mcg/g	0:8-5:25	1:35
4.8 mcg/g	0:10-3:35	0:34.5

Table 5: Ranges and medians of time required by female Acheta domesticus to travel 100 cm. toward a source of calling and courtship songs.

Figure 1



TOXICITY OF MALATHION TO ACHETA DOMESTICUS

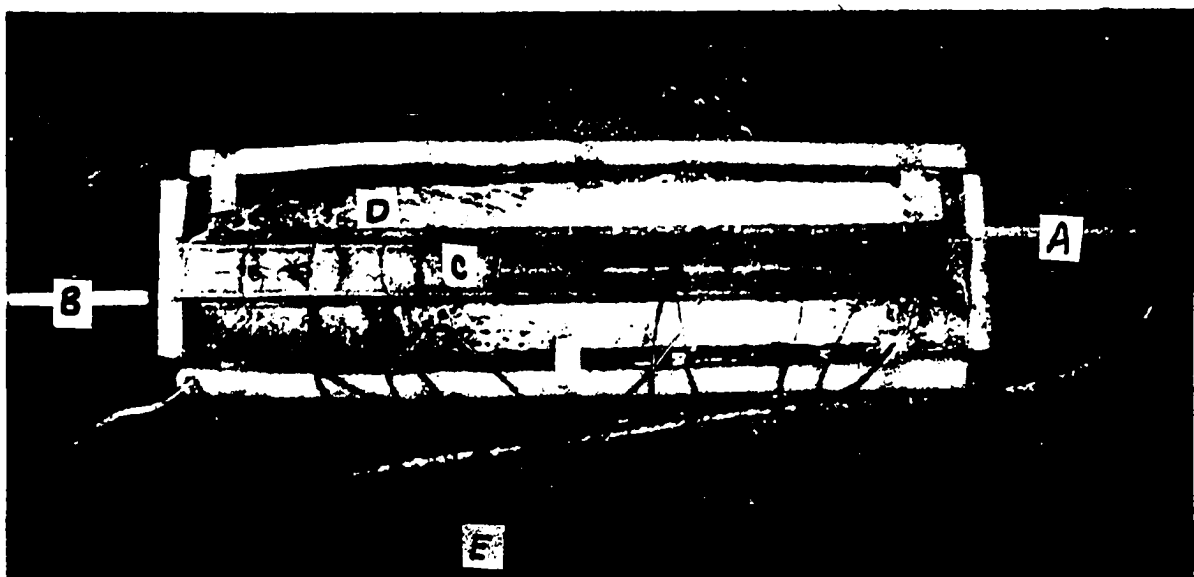


Figure 2

Thermal gradient apparatus. Hot water enters the outer trough (D) via holes in the pipe (A) which extends to the left; cold water enters the outer trough (D) via holes in the pipe (B) which extends to the right; crickets are in an inner water-free trough (C) which is tempered by the water flowing around it. Thermometer leads (E) are connected to a Yellow Springs telethermometer.

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