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THE ODOROUS ATTRACTANT OF THE AMERICAN COCKROACH, PERIPLANETA AMERICANA (L.)

I. QUANTITATIVE ASPECTS OF THE RESPONSE TO THE ATTRACTANT*

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INTRODUCTION

Effective use of attractants for insect control depends upon basic information concerning the behavior of insects towards the attractants. As yet no adequate quantitative studies on the response of insects to attractants of autogenous origin have been made. The possibility of making such studies was provided by the observations of Roth and Willis (5), who found that female American cockroaches emit a specific odorous attractant for the male and that this substance adheres to paper or other substances with which the females come in contact.[1] They also showed that the response of the male to paper impregnated with the attractant is wholly similar to its response to the female. The present paper deals with the quantitative aspects of the response of the roach to the attractant and is essentially a study of olfaction and adaptation. The results obtained provide a background for the development of a method of assay dealt with in the subsequent paper (6).

Materials and Methods

Care and Marking of Cockroaches.—Male *P. americana* were kept in greased glass battery jars 8 inches high by 6 inches in diameter, with water tubes and food dishes so placed as to afford a maximum continuous area of open floor space in the jars. The insectary was dimly lit except during tests, when it was fully lighted. The temperature was 28 ± 1°C. during tests, the relative humidity 50 per cent. Ventilation was forced.

Conditions for testing require that the roaches be kept in a way that permits their free response and that each roach be marked conspicuously for instant recognition

* This work was conducted under the Army Quartermaster Research and Development Program to establish methods for controlling insects of economic and medical importance.

[1] We gratefully acknowledge the furtherance of this work by Dr. Louis M. Roth in supplying us with roaches and with papers suitable for extraction.

by the experimenter. For marking, they were anesthetized with CO_2 and painted with a variety of materials transferred with a thin, tapered glass rod. Usually 10 roaches were put in each jar, and, being marked either on the thorax or the wings, they could be readily identified by the use of five different colors. Success in painting depended on keeping the roaches at rest until the paint dried thoroughly. We have observed that roaches are startled when a current of air is blown upon them, but, after the first impulse to flee, they hug the jar quite closely and quietly. We have thus been able to use brief anesthesia for painting and to dry the paint by placing the jars immediately in a stream of warm air. Even though the roaches would ordinarily be violent on recovering from anesthesia because of the toxic and irritating effects of the paint, in the stream of air they remain quiet and are intoxicated to a minimum degree since the fumes are rapidly blown away. Even so, as judged by their lack of sexual excitability, the roaches do not recover fully for 2 or 3 days. All tests were made in the afternoon in the insectary.

Preparation of the Sex Attractant.—Solutions of the attractant were obtained by extracting with petroleum ether at room temperature papers on which virgin females had been kept for about a week. Approximately 2000 square inches of paper which had lined the sides of 20 jars containing 30 to 40 females each were extracted at one time, and the decanted supernatant solution used to extract two additional lots of paper. The extracts so obtained were concentrated to one-twentieth the original volume by vacuum distillation at 22°C. (preparation P-3-C). The distillates were collected in a freezing bath but had no value as attractants. The concentrates were kept in a refrigerator and constituted the bases from which dilution series were made. Petroleum ether extracts were also prepared with the aid of a Soxhlet apparatus, pooled, and concentrated. One such, preparation 68-5, after partial fractionation by the countercurrent procedure, was used in several of the tests.[2]

Technique of the Test.—In making a test, a 15 mm. disc of No. 2 Whatman filter paper was hooked on to the end of a stiff monel metal wire suspended from the center of a bar which was placed across the mouth of the jar containing the roaches. The wire was of such a length that the disc of paper could be centered $1\frac{1}{2}$ inches above the open area of the floor of the jar. The paper was flooded in the horizontal position with 0.04 ml. of a solution of the attractant in petroleum ether and, when dry, presented to the roaches. It was kept in the jar for 1 minute and then transferred to another jar, and so in sequence until all the jars of roaches had been tested. Test sequences were made at 5 or 15 minute intervals.

In the present paper we are not concerned with the constitution or weight of the material used, but solely with its activity as measured by the response it stimulated.

RESULTS

General Response of Roaches to Attractant.—In confirmation of Roth and Willis (5), we have observed that the approach of the American cockroach to the mating act is direct, lacking in courtship, but accompanied by characteristic motions. The effect of the receptive female, or of paper impregnated with attractant, upon the male is to arouse its interest, as manifested first by sudden alertness, then movement of the antennae, then active search for the source of odor, and then more or less vigorous fluttering of the wings, usually accompanied by protrusion of the abdomen. Repeated tests with our extracts consistently showed that this order of activity corresponds with the influence of increasing concentrations of attractant. Except for copulation, wing flutter is the surest visible sexual response and when it occurs in the presence of the attractant it is a certain indication of the recognition of the specific odor by the male.

[2] We are grateful to Dr. J. D. Loconti of these Laboratories for making this preparation available to us.

We have found that the isolated roach is not as suitable for studying the response to this olfactory stimulant as are roaches in groups, perhaps because of their gregarious habits. This may be readily observed by first testing roaches in groups, then isolating the highly reactive ones and testing them again, and finally regrouping and retesting. For example, with a group of 20 roaches which had been selected for the consistency of their reaction, the response during a period of 11 days following isolation was negligible but recovered immediately when the roaches were regrouped. Similarly, roaches which upon emergence as adults were isolated and immediately tested were almost wholly unresponsive to the stimulus of impregnated paper and remained so during a test period of 4 weeks, whereas roaches of comparable age, which were grouped, reacted strongly from the 6th day on.

Roaches react with different intensity to the attractant at different times. A roach may be highly sensitive for a few days or weeks or months and then become quiescent gradually or suddenly. A few seldom or never react. During a test, fluttering may be intense and persistent, or intense and irregular, or swiftly transient, or recurrent, or hesitant, or lackadaisical; quite commonly near the threshold of response inchoate movements of copulatory activity will be made. In all cases, however, we have used wing lift as the single criterion of response, and have ignored, in the present study, its persistence, intensity, and recurrence.

Effect of Concentration of Attractant on Per Cent of Roaches Responding.—When different concentrations of the attractant are presented to *P. americana* on different days, the percentage response is proportional to the log of the concentration throughout a wide range (Fig. 1). When the entire range of concentrations is tested, an S-shaped curve results. However, irregularities were observed in the extreme ranges and certain evidence suggested that they were due to impurities in the extracts. Therefore, we have postponed consideration of the extremes of the curve until such time as purified extracts are obtained, and have restricted the present study to the range of linear response.

Effect of Ascending and Descending Concentrations of Attractant on Response.—When different concentrations in a *descending* series are presented at intervals of 5 or 15 minutes, the response is similarly proportional to the log of the concentration and the curves approximate those of the random series shown in Fig. 1. The response to an *ascending* series of concentrations within the same range as used in the descending scale is also proportional to the log of the concentration, but the slope of such a curve is not as steep as the slope of the curve of the descending series, especially with the 5 minute interval (Fig. 2). An examination of the charts shows further that the ascending and descending curves cross as a result of the difference in slopes. It is apparent, therefore, that the threshhold of response, which is the concentration of attractant required to elicit a given percentage of response, is higher for the ascending series than that for the descending at the upper levels of response, but that it is lower at the lower levels.

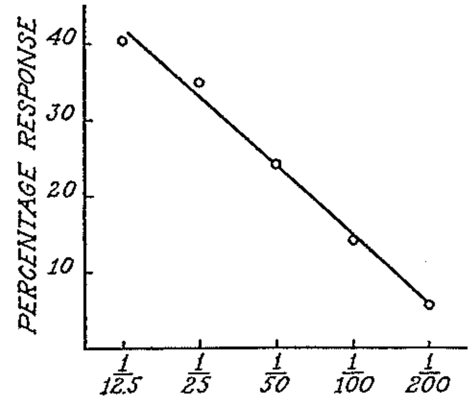

FIG. 1. Effect of concentration of attractant on per cent of roaches responding.

Effect of Repeated Stimulation with a Constant Concentration of Attractant on Response.—If, instead of serial concentrations, the same concentration is repeatedly used to stimulate the roaches for the same length of time and at the same interval, the percentage response will not be the same throughout, but, in a test of five rounds, will almost invariably be lowest in the last round. This holds true at all levels tested (Table I). It therefore became necessary to find out what effect further extensive and intensive testing would have on the response of the roaches and to see whether the apparent adaptation was haphazard or conformed to a characteristic pattern. We accordingly subjected 40 roaches to 30 consecutive rounds of stimulation with a concentration of 1:25 of preparation 68-5 for periods of 1 minute at 5 minute intervals. The initial response to this concentration of attractant was about 40 per cent. The succeeding responses, which are shown plotted in Fig. 3 (*a*), indicate that there is not a continuous decline from round to round, but rather a cyclic alternation of

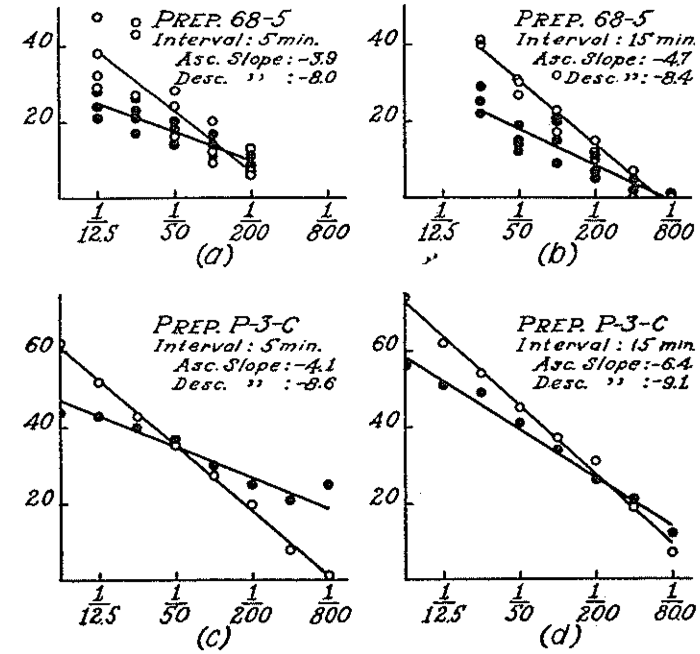

FIG. 2. Effect of ascending and descending concentrations of attractant on per cent of roaches responding. ○, descending series; ●, ascending series. The ordinates represent percentage response; abscissae, concentrations of attractant. The slopes are based on abscissa values of 1, 3, 5, etc., in place of the values of the concentrations shown.

TABLE I

Effect of Repeated Stimulation with Different Concentrations on Response

No. of round	Percentage response to different concentrations of attractant*															
	1/3.13	1/3.13	1/6.25	1/6.25	1/6.25	1/12.5	1/25	1/25	1/25	1/25	1/25	1/25	1/25	1/50	1/100	1/200
1	59	47	39	36	31	40	35	31	30	31	33	39	35	24	14	8
2	54	42	29	34	36	50	31	26	23	30	31	34	39	22	15	9
3	44	46	26	26	31	38	32	31	24	23	28	29	36	16	13	11
4	37	35	22	28	26	32	25	32	26	24	25	29	32	16	11	8
5	38	32	17	26	24	27	21	25	22	23	19	19	31	13	9	2

* Based on total of 140 cockroaches per test. Interval, 5 minutes. Tests made with preparation 68-5.

excitement and rest. However, the line of regression suggests that there may be, in fact, a progressive decline in the percentage of roaches responding. The decline becomes more significant when the roaches are stimulated by higher

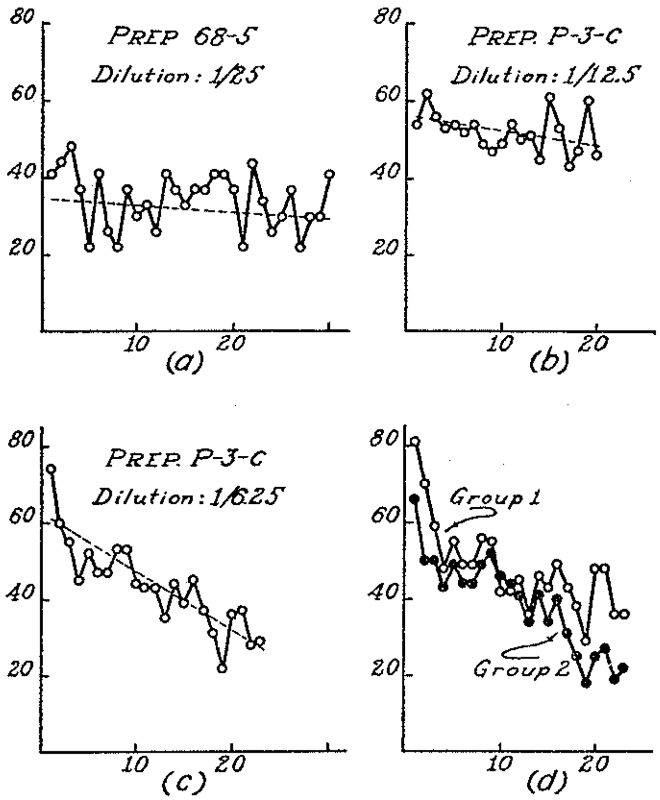

FIG. 3. The cyclic response of roaches to repeated stimulation with different concentrations of attractant. The ordinates represent percentage response; the abscissae, number of rounds. The dotted lines represent lines of regression. Interval between rounds in test (*a*), 5 minutes; in tests (*b*) and (*c*), 15 minutes. Interval between tests (*a*) and (*b*), 75 days; between tests (*b*) and (*c*), 5 days.

and higher concentrations of the attractant, even when the interval is 15 minutes instead of 5 (Fig. 3 (*b*) and (*c*)). In these tests 140 roaches were used. The greater decline at higher concentrations of attractant is undoubtedly due to the higher percentage of roaches activated by the stronger concentrations,

leaving a smaller reserve of rested roaches to respond to the subsequent doses. These additional experiments show again a cycle of response alternating with rest.

The rhythmic pattern of Fig. 3 represents, of course, the composite response resulting from the influences of the different cycles of the individual roaches comprising the group. Examples of types of cycle exhibited by six individual roaches selected from the data of the tests shown in Fig. 3 are shown in Fig. 4.

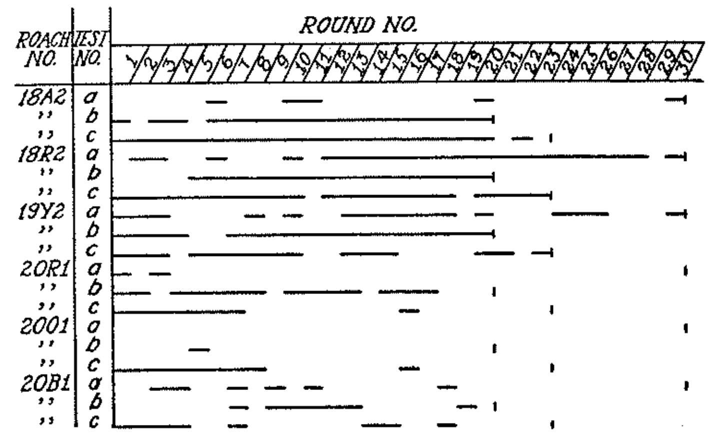

FIG. 4. Responses of six individual roaches to repeated stimulation with different concentrations of attractant (see Fig. 3, *a*, *b*, and *c*). Horizontal line represents positive response in round indicated. Vertical line represents end of test.

Reproducibility of Cyclic Response in Different Groups of Roaches.—It appeared of further interest to find out whether any two groups of roaches under simultaneous test would show the same pattern of cyclic response. Information on this would be of considerable importance to the development of any assay method in which two groups of roaches might be involved. To examine this question, the data on which the curve of Fig. 3 (*c*) is based were divided in random fashion so as to provide sets of data for two groups of roaches of 70 each, instead of the original single group of 140 roaches. The effect of this, shown in Fig. 3 (*d*), was to produce two very similar curves with the peaks and troughs showing a remarkably synchronous relationship. This finding was amply confirmed by additional experiments. Further, by dividing the roaches into two groups of 70 each at the beginning of the experiment and testing simultaneously with separate doses of the attractant in each round, it was shown that the findings were not due to errors of dosage. Variations in temperature, humidity, and other variables under our control also were ruled out as sources of error. It appeared safe to conclude, therefore, that the findings were not the result of a chance distribution of responses. It is to be expected, however, that with smaller and smaller groups of roaches this close reproducibility would not be observed. For purposes of assay, groups at least as small as 70 appear satisfactory.

DISCUSSION

Although the duration of the olfactory stimulus we employed was much greater than the comparatively instantaneous stimuli used in the classic experiments of Adrian (1) in the demonstration of adaptation, or than the duration of the stimulus in the recent experiments of Dethier (2) in showing adaptation of the gustatory sense, there can be no doubt of the conformity of our results to the criteria of adaptation of nervous mechanisms. The phenomenon of adaptation is presumably an aspect of fatigue. An explanation of its action, suggested by Adrian, is that repeated subliminal stimulations fail to attain an effective potential within or upon the receptors because of "leakage." The idea of leakage does not, however, seem to account for the disproportionately feeble response to a relatively large dose following adaptation. We have seen that a greater degree of adaptation occurs with minimal than with maximal effective doses. This is evident from the fact that the slopes of the titration curves of the descending series were similar to those of the random series, while the slopes of the ascending series were much less steep. It would seem strange if weak concentrations of a stimulant should affect the cell so as to produce more "leakage" than stronger concentrations might be thought to do.

We believe it likely, rather, that the effect of a stimulus on the sensory mechanism is to set up, through enzymatic means, a series of reactions in which the enzyme is recovered when the series is completed. In such a way the sensory mechanism is kept free to respond to a repeated stimulus. However, when subliminal doses are given, they fail to catalyze the complete series of reactions and the enzyme is left in a transitional state, incapable of responding to further quantities of the stimulant. Bound to the stimulant, it thus constitutes a block to further reaction, and with its accumulation in the transitional state the amount of reactive enzyme available to the stimulus is decreased. Consequently a larger dose of stimulant must be released to activate the remaining quantity of enzyme. If this concept is valid, it is easy to see why adaptation should occur more readily in the subliminal range of stimulation.

The above theory of the mechanism of adaptation thus differs from the idea of leakage of products or potentials, and rather views the products as being bound in an unreactive transitional complex. We look upon the idea of enzymatic mediation of the sensory mechanism favorably, *blockage* of sensation entailing an inhibition of enzymatic components. This is contrary to Kistiakowsky's theory (4) in which *stimulation* of sensation entails an inhibition of enzymatic components.

Elsberg and his colleagues (3) deduced from their work in man that the brain was the site of adaptation to olfactants; Dethier, from his work on the

gustatory response of the blowfly to sugar solutions concluded that adaptation occurred predominantly in the central nervous system. Our results with the cockroach can be interpreted equally well on the basis of any assumed site of adaptation. The behavior of the roach is such that a chain of events, beginning, in their outward manifestations, with the first questing movement of the antennae and ending in the copulatory act, may be readily observed. The sequence of cessation of responses under the influence of adaptation is exactly the reverse of the order of their appearance; that is, the copulatory act of abdominal protrusion and wing flutter are the first to fail to appear; then locomotion becomes more fitful and eventually stops; then the antennae and the palpi decrease their movement; and finally there is an actual withdrawal of the roach from the sphere of activity.

Since our observations have consistently shown that the order of the chain of events exhibited by the roaches when stimulated by the attractant depends on increasing intensity of stimulation, then decreasing the effective intensity of stimulation, as by adaptation, should bring about a failure of the responses in the reverse order, and this has proved to be the case. It might be deduced from these results that adaptation occurs in different regions of the central nervous system controlling these different responses, or perhaps in only one locus with over-all control. On the other hand, there would seem to be no reason for excluding the possibility that adaptation may take place anywhere in the conducting system.

SUMMARY

1. Procedures have been developed for studying quantitatively the response of the male American cockroach to the odorous attractant of the female.

2. The percentage of male roaches responding to extracts of the attractant of the female has been found to vary with the log of the concentration of the attractant throughout a wide range.

3. Adaptation to the olfactory stimulus has been demonstrated.

4. A theory of adaptation is offered.

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