Resumen por el autor, Dwight E. Minnich

La sensibilidad química de los tarsos de la mariposa *Pyrameis atalanta* Linn.

En la mariposa *Pyrameis atalanta* Linn. cada uno de los cuatro tarsos ambulatorios posee quimorreceptores de contacto cuya estimulación apropiada produce una respuesta en la forma de una extensión de la proboscis. Esta respuesta sin embargo varía algo con la naturaleza química del estímulo y con la condición de la nutrición del animal. Un estudio intenso de estas variaciones ha hecho posible establecer con certeza algunas de las sustancias que la mariposa puede distinguir mediante sus tarsos. De los cuatro estímulos probados, tres de ellos, a saber, el agua destilada, 1 M de sacarosa y 2 M de NaCl son claramente distinguidos por la mariposa. El cuarto, M/10 de cloruro de quinina es claramente distinguido del agua destilada y de la solución 2M NaCl y probablemente también de la 1M de sacarosa. Puesto que los órganos tarsales son quimorreceptores de contacto que sirven para distinguir el agua y el alimento normal tal como la sacarosa, pueden propiamente considerarse como órganos del gusto.

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THE CHEMICAL SENSITIVITY OF THE TARSI OF THE RED ADMIRAL BUTTERFLY, PYRAMEIS ATALANTA LINN.

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THREE FIGURES

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INTRODUCTION

In a previous paper (Minnich, '21) I showed that the tarsi of the butterfly, *Pyrameis atalanta* Linn., are sensitive to contact with certain substances in solution, and hence must possess contact chemoreceptors. In the same paper I also presented some preliminary data indicating what kinds of substances the animals were thus enabled to discriminate. It is the purpose of the present paper to give a further account of the qualitative range of sensitivity of these tarsal organs.

In the present work I have employed the same general methods as before, with two exceptions. First, instead of using both captured specimens and those hatched in the laboratory, only butterflies hatched in the laboratory have been employed. The complete adult history of every specimen was thus a known and controlled one. Second, a few animals have been studied, under varying nutritional conditions, over a long period of time, rather than a number of animals, in the same nutritional condition, for a short period of time. Indeed, the data to be presented have been obtained from but eight specimens, and chiefly from four of these. These four butterflies, however, were kept under very close observation for 19, 27, 29, and 30 days, respectively.

GENERAL METHODS

The experiments were carried out chiefly during the month of July. The laboratory was a basement room of northwest exposure, but direct sunlight was kept out by white-cloth shades at the west windows. Ventilation was accomplished entirely from the interior of the building, so that it was possible to avoid sudden and pronounced fluctuations of temperature. During the entire period of experimentation the extremes of temperature registered in the laboratory were 21°C. and 25.9°C. For the great majority of days, however, the temperature ranged from 22° to 24°, the variation for the day being less than 1°. Ventilation from the inside of the building also insured a more uniform condition of humidity than outside ventilation would have allowed. Every precaution was thus taken to maintain as great constancy of the general laboratory environment as possible.

The specimens employed were all exceptionally large and perfect. After hatching, they were housed in large cages (0.9m. x 0.4m. x 0.4m.) consisting of a light wooden framework covered with mosquito bar. Excepting periods of starvation, which were practiced from time to time during experiments, the animals remained in excellent condition. In one or two instances, near the end of an experiment, one leg of a specimen became more or less stiff and functionless as far as locomotion was concerned. Also the wings, in addition to being clipped slightly for purposes of identification, became somewhat frayed and considerably rubbed in their outer portions, due to the effects of the holder in repeated trials. But as far as I was able to observe, these slight mutilations in nowise affected the reactions which were being studied. In general, the hairy proximal portions of the wings and the rest of the body remained in almost perfect condition—much more so than is the case with animals in a state of nature. Thus butterflies caught in the field frequently have lost portions of the labial palpi, but of the eight animals in my experiments not one suffered such a mutilation.

Even in their most vigorous states, however, the butterflies flew but little. It was not that they were unable to fly, but that they merely did not. Neither did they creep much, usually

remaining at or near the place where they were released upon completion of a trial. This inactivity was doubtless due in a large measure to the effect of the spring clothes-pin holder on the wings in repeated trials, to continuous confinement, and to the constancy of the environmental conditions, viz., temperature, humidity, and light.

As in my previous work, the results of the present experiments have been obtained entirely through a study of the conditions which effect an extension of the proboscis. I have described this response in considerable detail (Minnich, '21, p. 178), so that it will be sufficient merely to mention the essential features here. In the unstimulated animal, the proboscis remains compactly coiled against the head, but upon appropriate stimulation it is extended and begins to probe the substrate. Not infrequently, however, a given stimulus fails to elicit a complete extension, producing only a partial extension followed by a subsequent recoil. Indeed, the partial extension may be so slight that the compact coil of the proboscis merely exhibits a jerk or two with no further sign of activity. Between such slight reactions and complete extension, all gradations may be observed.

It is clear that all extensions of the proboscis, whatever their degree, represent responses. But it is equally clear that these responses differ in intensity. To measure such differences is difficult. Nevertheless, I have endeavored to approximate a measurement by weighting all responses in which the proboscis was uncoiled less than one-half at 0.5, and all in which it was uncoiled one-half or over at 1. In figure 1, no. 1, the proboscis is shown as it normally appears in the unstimulated animal. In the same figure, nos. 2 and 3 illustrate responses which would be weighted at 0.5, while nos. 4 and 5 show responses which would be weighted at 1. As a matter of fact, the case illustrated in no. 4 is virtually never encountered, for the proboscis is rarely extended one-half or more of its length without being completely extended. This scheme for measuring the intensity of the proboscis response, therefore, virtually amounts to weighting small partial extensions at 0.5 and complete extensions at 1. I shall employ this scheme in all comparative statements, in order that

evaluations of groups of responses may be compared as well as the mere numbers of responses.

Fig. 1 Diagrams showing the proboscis in the unextended condition and in various stages of extension: 1, unextended; 2 and 3, partial extensions which would be weighted at 0.5; 4, partial extension which would be weighted at 1; 5, complete extension, which would be weighted at 1.

In the present experiments every trial was immediately preceded by a preliminary trial held under the same conditions. The purpose of the preliminary trial was fourfold: 1) to overcome

the death feint, although this reaction is virtually negligible in *Pyrameis*; 2) to eliminate the immediate effects of mechanical manipulation incident to the trial; 3) to accustom the animal to the grip of the holder on the wings, and, 4) to make certain that there was no sign of proboscis extension in the absence of the chemical stimulus to be applied. The last-mentioned point is of particular importance, for extensions of the proboscis were occasionally observed when no external chemical stimulus was apparent. Responses of this sort were most frequently observed in individuals which, through prolonged starvation, had become extremely sensitive. Most of these responses were of slight magnitude, although some consisted of complete extensions. The following examples will illustrate the cases in point.

In all trials butterflies were manipulated by means of a spring clothes-pin in which the wings were firmly held. The most simple method of placing an animal in the holder was often by direct use of the hands. This involved grasping the wings, and later the thorax and wing bases with the fingers. On grasping the wings with the fingers, as the specimen was pulled off the wall of the cage where it had been resting, there was occasionally a slight jerk of the proboscis coil. Again, while the butterfly was being held by the fingers in one position or another, further incomplete or, in rare instances, complete extensions of the proboscis were noted. Because of these occasional responses, direct contact between the hands and the body of the animal was avoided whenever possible. But even when the holder was applied directly to the wings, there were one or two instances of response. The stimuli effecting these responses cannot be postulated with certainty. I am inclined to believe that distance and contact chemical stimuli from the hands were chiefly responsible although it is possible that in conditions of extreme sensitivity mechanical stimuli may also exert some influence.

In several instances a butterfly which had remained undisturbed in its cage fifteen minutes or more was found exhibiting either a partial or complete extension of the proboscis. The previous trial having been completed fifteen to twenty minutes before, it was impossible to interpret such a case as a persisting

response. Internal stimuli due to prolonged inanition, or external chemical stimuli of an adventitious sort, too dilute for my detection, may perhaps explain these responses, though a certain statement concerning them is not possible.

Without multiplying examples, the above are sufficient to illustrate the behavior in question. One of the chief purposes of the preliminary trial was to detect responses of this sort and thus avoid an occasional misinterpretation. If there was but one barely visible jerk of the proboscis coil as the animal was pulled off the wall of the cage, and no further sign of extension during the preliminary trial, experimentation was continued. If, however, there was any more significant movement of the proboscis during the preliminary procedure, experimentation was discontinued and not resumed for at least fifteen minutes. Usually there was no evidence of response in the second preliminary, but in case there was, experimentation was again discontinued for a minimum period of fifteen minutes.

The maximum duration of all trials, preliminary and final, unless otherwise stated, was one minute. Failure to observe any visible movement of the proboscis during this period constituted a 'no response.' If the proboscis was partially extended early in a trial, the trial was continued, to ascertain whether complete extension would result. If the proboscis was completely extended, the trial was immediately terminated, and the animal returned to its cage. Trials thus lasted one minute or less, depending upon the response.

The chemical stimuli employed consisted of distilled water, and three aqueous solutions, viz., 1M saccharose, 2M NaCl, and M/10 quinine hydrochloride. The saccharose and quinine hydrochloride were USP quality; the sodium chloride, CP quality. The solutes being non-volatile, the distance stimulus afforded by each of the solutions was identical with that afforded by distilled water, viz., water vapor. In other words, the four stimuli used could be distinguished, if distinguished at all, only through direct contact.

With the exception of quinine hydrochloride, the stimuli selected were substances frequently encountered by *Pyrameis* in

its natural environment. The importance of water in this connection is too obvious to require comment. As for saccharose, it is one of the chief organic compounds present in the substances on which this species feeds, viz., fruit juices, exuding sap, and nectar. Thus apple juice is very attractive to these animals, and, if available, may constitute one of their chief foods in the autumn. In orchards where fallen apples litter the ground, I have observed great numbers of the butterflies feeding, just prior to hibernation. According to Browne ('99, pp. 9 and 10) the flesh of the average ripe apple contains 4 per cent of saccharose, this being, with the exception of water (84 per cent) and invert sugar (8 per cent), the only substance constituting more than 1 per cent of the total composition. Sodium chloride was selected as a common inorganic salt encountered by butterflies in surface waters. It is well known that many species of lepidoptera congregate about drying pools of water, and pools which have been contaminated with urine or manure seem particularly attractive. In such situations, NaCl is present in considerable quantity.

Saccharose and sodium chloride, in addition to the fact that they are frequently encountered by Pyrameis in its natural habitat, happen also to be substances which afford adequate stimuli for two of the four primary taste sensations in man, viz., sweet and salt. Quinine hydrochloride was chosen for experimentation, because it affords the adequate stimulus for a third of the human taste sensations, viz., bitter. With Pütter ('11, p. 608) I agree that there is not the slightest reason to suppose that a substance which affects the human taste organs in a certain way will affect the taste organs of a lower animal in the same way. It was not to ascertain whether Pyrameis could distinguish bitter that quinine hydrochloride was chosen, but rather to discover whether this salt, which produces such a bitter and disagreeable sensation when applied to the human tongue, would produce or fail to produce a reaction in the butterfly.

EXPERIMENTS WITH LOCAL STIMULATION OF INDIVIDUAL TARSI

Three butterflies were kept for five days after hatching without access to food or water. At the close of this period, they were subjected to a series of trials in which various chemical stimuli were applied locally to individual tarsi. The stimuli were applied on small cotton swabs ca. 1 cm. long and 1 to 2 mm. in diameter, the swab consisting of a bit of absorbent cotton wound on the end of a dissecting needle. Care was exercised to keep the cotton chemically clean while being handled. As a further precaution, swabs used to test the effect of dry cotton alone were heated prior to each experiment, in order to drive off any excess moisture.

The butterfly to be tested was placed in a holder (fig. 2) with the four ambulatory feet resting on a small platform of wire screen. In this position, the specimen was closely observed for one minute, this constituting the preliminary trial. In no case was any sign of response observed. Immediately following the preliminary trial, a dry cotton swab was applied to the ventral surface of the distal end of the ambulatory tarsus which it was desired to test. Except for a very few partial extensions this also failed to elicit any response. The dry swab was then replaced by a swab saturated with distilled water, and to this the animal almost invariably responded. Upon completion of the trial with distilled water, the specimen was returned to its cage for a minimum interval of fifteen minutes, after which another tarsus was tested in the same manner. This procedure was continued until each of the four ambulatory tarsi had been tested twice.

On completing the trials described above, the butterfly was placed on absorbent cotton saturated with distilled water, whereupon the proboscis was immediately extended. In this situation—presumably drinking in water continuously—it was allowed to remain as long as it would. After two or three minutes, however, the proboscis was recoiled, and the animal either crept away or was removed. Some minutes later, it was replaced on the wet cotton, and thus given a second opportunity to drink, but in no case was there any response.

After access to water, the butterfly was allowed to rest for a minimum interval of fifteen minutes, whereupon trials were resumed. The specimen was again placed in the holder with its four feet upon the screen platform and carefully observed for one minute. As before, however, there was never any indication of response during this preliminary trial. Next, the dry cotton

Fig. 2 Photograph showing holder employed in experiments on stimulation of individual tarsi. Note the appearance of the proboscis, *P*, in the unstimulated animal.

swab was applied to the tarsus, but this now failed to elicit even a single partial extension of the proboscis. The dry swab was followed by a swab saturated with distilled water. Prior to the administration of water, this had produced a very high per cent of response. It now failed to produce a response in nearly every trial. An additional stimulus was, therefore, employed, viz., a swab saturated with a 1M saccharose solution. To this stimulus

there was but one failure to respond. On completion of the trial with the sugar solution, the tarsus which had been tested was carefully rinsed by immersing in distilled water, and the butterfly was returned to its cage. Fifteen minutes later another tarsus was tested in the same manner, and so on, until each of the ambulatory tarsi had again been tested twice.

The data obtained from the above experiment are presented in table 1. It will be noted that specimens, both before and after access to water, exhibited essentially the same behavior toward the tactile stimuli afforded by the metal of the wire screen and the cotton of the dry swab, viz., failed to respond. It is true that in the case of the starved animals there were three trials in which partial extensions of the proboscis were produced by contact with dry cotton. Possible explanations of these responses will be discussed later. In comparison with the other stimuli employed, however, the response to dry cotton was virtually zero. With the cotton soaked in distilled water, the situation was very different. Before the animal was allowed access to water, this form of stimulus was 87.5 per cent efficient in producing a response, whereas after access to water, it was but 4.2 per cent efficient. Yet after this virtual disappearance of the response to water, a 1M saccharose solution was still 91.7 per cent efficient in evoking a response.

As noted above, a dry cotton swab applied to the tarsus occasionally elicited a slight response. Precisely what was the effective stimulus afforded by the cotton? Two possibilities may be suggested: first, the mere contact (pressure) of the cotton; second, the hygroscopic water present on the cotton fibers. The fact that the few slight responses observed were in starved animals and that these responses disappeared after access to water lends support to the latter suggestion. A final statement, however, as to the effective stimulus in these responses is not possible, at least not from the present data.

Returning to a consideration of table 1, the following facts are brought out clearly by the data there presented. First, contact (pressure) stimuli alone, such as those afforded by wire screen or dry cotton, when applied to the tarsi have little or no effect in

TABLE 1
Showing the effects of different stimuli on individual tarsi

A BUTTERFLIES 5 DAYS OLD WHICH HAD RECEIVED NEITHER FOOD NOR WATER												B SAME BUTTERFLIES AS IN A, AFTER ADMINISTRATION OF DISTILLED WATER											
ANIMAL NUMBER (1)	(2) No swab in contact with tarsi			(3) Dry swab in contact with one tarsus			(4) Distilled water swab in contact with one tarsus			(5) No swab in contact with tarsi			(6) Dry swab, in contact with one tarsus			(7) Distilled water swab in contact with one tarsus			(8) 1 M saccharose swab in contact with one tarsus				
	No response	Partial extensions	Complete extension	No response	Partial extensions	Complete extension	No response	Partial extensions	Complete extension	No response	Partial extensions	Complete extension	No response	Partial extensions	Complete extension	No response	Partial extensions	Complete extension	No response	Partial extensions	Complete extension		
32	8	0	0	8	0	0	0	1	7	8	0	0	0	0	0	8	0	0	0	1	7		
33	8	0	0	6	2	0	1	2	5	8	0	0	8	0	0	7	1	0	1	1	6		
34	8	0	0	7	1	0	0	1	7	8	0	0	8	0	0	7	1	0	0	0	8		
Totals.....	24	0	0	21	3	0	1	4	19	24	0	0	24	0	0	22	2	0	1	2	21		
Total weighted response....	0			1.5				21		0			0			1			22				
Per cent of effectiveness ¹	0			6.3				87.5		0			0			4.2			91.7				

¹ The per cent of effectiveness is calculated by dividing the total weight of responses obtained, as defined on page 109, by the total weight which would have been obtained had there been a complete extension of the proboscis in every trial. Thus, in column 3, three responses are listed. Since each represents only a small partial extension, their total weight is $3 \times .5$ or 1.5. The total weight, had there been a complete extension in every trial, would have been 24. $1.5 \div 24 = 6.3$ per cent.

producing an extension of the proboscis. Second, the same stimuli plus certain chemical stimuli will effect an extension of the proboscis. *Pyrameis* must, therefore, discriminate through its tarsi the presence of the chemical stimuli. Third, under certain conditions, distilled water is one chemical stimulus which is very efficient in evoking a response. Fourth, one condition which determines the responsiveness to water is the degree of inanition with respect to water. Satiety with respect to water inhibits the responsiveness thereto. This response may, therefore, be controlled, approaching 100 per cent or 0 per cent, according as the animal is or is not allowed access to water. Finally, although *Pyrameis* fails to respond to water in a condition of satiety with respect to the same, it, nevertheless, continues to respond vigorously to a 1M saccharose solution. The butterfly must, therefore, be able through its tarsi to distinguish sharply between water and an aqueous solution of a non-volatile substance such as saccharose.

EXPERIMENTS WITH SIMULTANEOUS STIMULATION OF ALL AMBULATORY TARSI

Because of the mechanical difficulties involved in stimulating individual tarsi, a more satisfactory method of experimentation is to allow all the ambulatory tarsi to come in contact with the stimulus at the same time. Experiments using this method were, therefore, more generally employed. In these experiments the wings of the butterfly were held in a spring clothes-pin, which was manipulated by the hand. Upon removal from the cage, the butterfly was first subjected to a preliminary trial of thirty seconds, in which the feet rested on clean filter-paper. This trial was carried out within 3 to 5 cm. of the place where the subsequent trial with a given chemical stimulus was to be made, so that the environment in the two trials was practically identical. During the trial, the butterfly was gently lifted and let down again at intervals of ten seconds, in order that it might become thoroughly accustomed to this sort of manipulation.

If there was no significant response during the preliminary trial, as was usually the case, the butterfly was lifted from the

filter-paper, across the few intervening centimeters, and set down with the feet in contact with a thin layer of cotton, contained in a Syracuse watch-glass and saturated with the solution to be tested. If the animal failed to respond promptly, various slight manipulations were employed to make certain that the ventral surface of every tarsus was afforded ample contact with the stimulus. Thus, if there was no response during the first few seconds of the trial, a slight, even pressure was given the holder, thereby causing the distal portions of one or more tarsi to press more firmly against the cotton and thus become immersed in the solution. If still there was no evidence of response after twenty to thirty seconds, the holder was turned slightly, forcing the butterfly to shift the position of some of its legs. And if both the above measures proved ineffective, toward the end of the trial the animal was occasionally lifted gently and let down again.

At the close of each trial the butterfly was placed over a watch-glass containing distilled water, and the tarsi were thoroughly rinsed. This was necessary in order to prevent contamination of the stimulating substance in one trial by adhering material from previous trials. If the proboscis remained extended after the trial, care was exercised to prevent the animals from drinking at this time. After the legs had been well rinsed, the butterfly was placed on clean filter-paper for a moment to absorb the excess moisture, and was then returned to its cage.

In the above manner, butterflies were tested with distilled water and solutions of 1M saccharose, 2M sodium chloride, and M/10 quinine hydrochloride, four trials being made daily with each of these substances. The order in which the four stimuli were employed was varied from time to time and a minimum rest period of fifteen minutes was allowed between consecutive trials.

The responses of each butterfly were studied under three nutritional conditions: first, a condition in which the animal was receiving neither food nor water; second, a condition in which it was receiving water only, and, third, a condition in which it was receiving both food and water. The general plan was the following. Immediately upon hatching, the specimen was placed

in a cage where it was kept without food or water. After three days, trials were begun with all four substances and were continued until the butterfly gave 100 per cent response to distilled water; that is, complete extension of the proboscis in each of the four trials of the day. In a few cases the experiment was continued for a day or so beyond this point before making any change in the nutritional conditions. In general, however, this was not possible because of the growing weakness of the specimen and the danger of its death.

When the experiment had reached the stage described, at the conclusion of the trials for the day the butterfly was placed on absorbent cotton saturated with distilled water and allowed to drink all it would. On the following morning it usually appeared quite revived. Before resuming trials, however, the animal was again given an opportunity to drink. If it failed to respond to the water, I several times forced an extension of the proboscis by touching one of the tarsi with sugar solution. Thus while the animal could not be compelled to drink, it could be compelled to bring the proboscis in contact with water. In this manner it was offered water in the morning, one hour before trials were begun, and in the evening, immediately after trials were concluded.

The butterfly was continued on this water diet until it again became so weak that further trials were impossible, whereupon experimentation was discontinued for the remainder of the day, and 1M saccharose was administered. Of this solution the animal always imbibed freely, and it was not until the abdomen was greatly distended that it ceased to feed. On the following morning it would again appear quite restored, and trials were resumed. The saccharose diet was continued for 3 to 4 days, administrations being made twice daily as in the case of water.

Following the period of saccharose diet, the butterfly was again kept without water or food until the response to water rose to 100 per cent. Then followed a period of water diet, and when this became insufficient, the sugar solution was again administered. In other words, the three nutritional states described above were repeated.

In continuing periods of starvation as long as possible, the risk of death became very great, and of eight specimens employed, six died in the course of the experiment. The longevity of these individuals was 8, 8, 9, 10, 19, and 27 days, respectively. In all these cases death was most probably due either directly or indirectly to the effects of starvation. In several of the shorter-lived specimens, I might have saved them had I appreciated the gravity of their condition soon enough, for animals in a state of complete collapse may sometimes be resuscitated in a surprisingly short time by the administration of 1M saccharose. Two specimens, however, survived the entire experiment, and several days after their last trials appeared vigorous in every way. They were killed for subsequent morphological study, having been under observation for twenty-nine and thirty days, respectively. Of the eight animals, I shall present the data from the four longest-lived only. The data from the four shorter-lived individuals are less complete and show nothing not shown by the others.

The observations on the four butterflies which survived longest are presented graphically in figure 3. The data for each animal numbered, respectively, 11, 12, 13, and 22, are presented in the form of four curves, each of which represents the responsiveness to a single substance. In these curves, the total weight of daily response, as defined on page 59, is plotted against age in days, the nutritional state for each day being indicated. An examination of the four curves for any one animal shows, with one exception, viz., no. 22, that no two coincide. In other words, there were differences of response to the different stimuli. It should be borne in mind at the outset of this discussion that the fact that a butterfly responds identically to two substances does not necessarily mean that it fails to distinguish them. This may merely indicate a positive response to both substances. On the other hand, differences of response do not necessarily show discrimination, unless they are differences of pronounced nature and regular occurrence. Are the differences noted above such as to indicate discrimination of the various stimuli or not? In order to answer this question, let us examine and compare the responses with respect to: first, relation to nutritional condition; second,

intensity, as indicated by the total amount of response produced, and, third, time aspects, viz., latent period and period of execution.

Let us take first the response to distilled water. This response was characterized by its close relationship to the nutritional condition of the animal. As shown above, in the experiments on stimulation of individual tarsi, table 1, the responsiveness to water could be increased or decreased by preventing or allowing access to water. The same results were obtained when all four tarsi were stimulated simultaneously. The curves of response to water in figure 3 show in every instance that with continued starvation the response finally rose to 100 per cent, while directly after water was administered it dropped to 0 per cent, where it generally remained as long as water or an aqueous solution was accessible. In other words, the response to water depended directly on the nutritional state of the animal. In this respect it was absolutely unique, for in no other response was any intelligible relationship to nutritional condition evident. The response to distilled water is thus sharply differentiated at the outset from the other responses studied. Consequently, we may omit it from further consideration, confining our attention solely to a comparison of the responses to the three solutions.

A survey of the curves of response to 1M saccharose (fig. 3) shows that each of the four butterflies responded to this solution in every trial, irrespective of nutritional condition. My notes show that during periods of inanition the response often became more persistent, lasting for some minutes after the trial had been completed. But even during periods when 1M saccharose was being fed twice a day, there was never a single failure to respond. Thus, a butterfly which had ceased to feed and had crept away, if replaced on the cotton soaked with the solution, immediately responded anew. And, if after ceasing to feed, the specimen remained on the cotton without creeping away, mere seizure of the wings or other slight agitation was usually sufficient to induce a fresh response. The response to 1M saccharose was thus entirely independent of the varying nutritional conditions of the animal.

Not only did the specimens always respond to 1M saccharose, but the extension of the proboscis was in every case a complete one. The total weighted response of all four animals under all conditions was, therefore, 100 per cent. Furthermore, the responses were rapidly executed. As shown in table 2, the average time required for this solution to produce a complete extension of the proboscis was between 1.7 and 3.4 seconds. The response to 1M saccharose was thus characterized by absolute constancy under all nutritional conditions, by maximum intensity, and by rapidity of execution. These facts all indicate that this stimulus must be a very powerful one. Indeed, it was by far the most effective of the stimuli tested.

TABLE 2

Showing the average time in seconds required by different stimuli to effect a complete extension of the proboscis

ANIMAL NUMBER	1 M SACCHAROSE		2 M SODIUM CHLORIDE		DISTILLED WATER		M/10 QUININE HYDROCHLORIDE	
	Number of trials	Average time	Number of trials	Average time	Number of trials	Average time	Number of trials	Average time
11	80	3.4	35	12.7	19	12.5	76	25.4
12	60	3.2	37	5.4	13	16.7	29	43.7
13	90	1.7	30	4.2	16	4.5	86	10.9
22	68	1.8	52	1.7	10	4.3	68	6.1

Unlike 1M saccharose, the responsiveness to 2M NaCl was characterized by extreme variability. One has only to examine the curves in figure 3 to become convinced of this fact. Take, for example, specimen 11. During the first three days of its life, no trials were made. On the 4th, 5th, and 6th days, the responses to this solution were 0. On the 7th, 8th, and 9th days, there was a variable number of responses for each day. On the 10th day the number of responses again fell to 0. On the 11th day it rose to 100 per cent, where it remained until the 16th day. The 16th, 17th, and 19th days (no observations were made on the 18th) the responsiveness was a little less than 100 per cent. On the 20th day it dropped to 0, only to rise to 100 per cent the 21st day, and again fall to 0 the following day, where it remained for the last two days of experimentation. The responses of this

specimen were thus extremely variable, and specimens 12, 13, and 22 showed essentially similar conditions. A careful study of each of these cases fails to show any apparent relationship between the nutritional condition of the animal and its response to the stimulus under consideration. Doubtless this response is very definitely determined, but the determining conditions are not evident from the present data. For the present, therefore, the outstanding characteristic of the response to 2M NaCl is its variability. It may be added that this variability is very great not only from one specimen to another, but also from time to time in the same specimen.

The variability of response to 2M NaCl means, of course, that the total amount of response to this substance was much less than to 1M saccharose. Considering the weighted responses of all four specimens collectively, the sodium chloride produced but 51.6 per cent response, as compared with 100 per cent for the sugar solution. On comparing the curves of the two responses, we find some days when specimens responded indistinguishably to both stimuli. But we also find days when there was a clear-cut difference of response. With every specimen there were periods, ranging from two to seven days, during which the animal gave 100 per cent response to 1M saccharose solution, and yet failed to evince even the slightest indication of response to 2M NaCl. The only plausible explanation of these facts is that the animal discriminated clearly between the stimuli. The fact that it responded at times to both is in nowise incompatible with this interpretation, while the fact that at other times it responded 100 per cent to one and 0 per cent to the other can hardly be explained in any other way. Clearly, therefore, the tarsi enable *Pyrameis* to distinguish a 1M saccharose solution from a 2M sodium-chloride solution.

There remains for consideration the response to quinine hydrochloride. In certain specimens, for example, no. 12 and to a slight extent no. 11, the curve of response to this substance (fig. 3) also shows some variation from day to day, though much less than with sodium chloride. No. 22, however, responded with a complete extension of the proboscis in every trial, and no. 13

11

12

13

22

Fig. 3 Curves of response to various solutions and distilled water. ----- 1M saccharose. Except where indicated by asterisk, the ordinate length represents the total weighted responses. Asterisks indicate cases where for some reason, usually the weakness of the animal, it was not possible to obtain a response. The ordinate length represents the per cent of response based on the number of trials actually made.

3;M/10 quinine hydrochloride; -----2M sodium chloride; ——— distilled water.
e, as defined on page 59, obtained from four trials made in the course of a single day. The
s impossible to complete the four trials of the day. In these instances the ordinate length

did the same excepting the last day of the experiment, when its responses suddenly dropped to 0. The total weighted response of all four specimens to the quinine solution was 84.7 per cent, as opposed to 100 per cent for 1M saccharose and 51.6 per cent for 2M NaCl. The amount of response produced by quinine was, therefore, intermediate between the other two substances.

A comparison of the curves for the quinine solution with those for sodium chloride (fig. 3) shows in every animal that there was a great diversity in the two responses. At times, the amount of response to each stimulus was the same; at other times, it was totally different. Here again, therefore, we must conclude that the two stimuli were differentiated.

A comparison of the saccharose and quinine curves yields no such conclusive evidence as the case above. For, while the two curves exhibit a rather wide divergence in animal no. 12, they very closely approximate one another in the other three animals. In this instance, however, there were distinctive differences of another sort. The rapidity with which the proboscis was extended to 1M saccharose has already been pointed out. As a comparison of the figures in table 2 will show, the response to quinine was very much slower. Thus, in animal no. 11 the average time required by quinine was seven to eight times that required by saccharose; in no. 12, thirteen to fourteen times; in no. 13, six to seven times, and in no. 22, three to four times. With 1M saccharose the extension of the proboscis began very shortly after the application of the stimulus and was rapidly completed. This was not the case with quinine. As a rule, the application of this stimulus was followed by a latent period lasting from a few seconds up to as many as sixty seconds, during which there was no sign of response. Then followed a period of reaction, beginning with slight relaxations of the proboscis which more or less gradually increased until extension was complete. The period of reaction also lasted from a few seconds up to fifteen or twenty seconds or even longer.¹

¹ The long latent period together with the long period of extension necessitated the prolongation of a number of trials with the quinine solution from the usual duration of one minute to two minutes.

The average response to M/10 quinine hydrochloride thus differed strikingly from that to 1M saccharose. That this difference, together with the slight differences of distribution noted, indicates a discrimination of the two stimuli, seems to me not only possible, but very probable. A final statement, however, cannot be made with certainty.

From the evidence presented, it is quite clear that the tarsal organs of *Pyrameis* are chemoreceptors of a rather wide range of sensitivity. Through them the butterfly is able to differentiate such solutions as 1M saccharose and 2M NaCl from distilled water and from one another. It is also able to differentiate M/10 quinine hydrochloride from distilled water, from 2M NaCl, and probably from 1M saccharose. The appropriate stimulation of these organs leads to an extension of the proboscis, the initial act in food taking. The tarsal organs are thus organs of chemical sense, concerned in the discrimination of food substances, and may be properly considered as organs of taste.

GENERAL SUMMARY AND CONCLUSIONS

1. In *Pyrameis atalanta* Linn. each of the four ambulatory tarsi possesses contact chemoreceptors.
2. The appropriate stimulation of these receptors produces a response in the form of an extension of the proboscis.
3. The manifestation of this response varies somewhat, depending upon the chemical nature of the stimulus and the nutritional condition of the animal.
4. An intensive study of these differences of response shows that *Pyrameis* is able to distinguish the following substances from one another through its tarsal organs: distilled water, 1M saccharose, and 2M NaCl. It is also able to distinguish M/10 quinine hydrochloride from distilled water, from 2M NaCl, and probably from 1M saccharose.
5. The efficiency of distilled water in evoking the proboscis response is directly dependent upon the nutritional condition of the animal.

6. The responsiveness to 1M saccharose, 2M NaCl, and M/10 quinine hydrochloride shows no apparent relationship to the nutritional condition of the animal.

7. According to the scheme of measurement employed in the present paper, the total response of all animals together was 100 per cent to 1M saccharose, 84.7 per cent to M/10 quinine hydrochloride, and 51.6 per cent to 2M NaCl.

8. Since the organs of the tarsi are contact chemoreceptors, and since they are concerned with the discrimination of substances to be taken as food, they may be appropriately termed organs of taste.

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