Resumen por el autor, D. E. Minnich.

Un estudio cuantitativo de la sensibilidad tarsal a las
soluciones de sacarosa en la mariposa
Pyrameis atalanta L.

El autor ha demostrado previamente que los tarsos de la mariposa *Pyrameis atalanta* L. son sensitivos en contacto con la estimulación química. Una de las sustancias que la mariposa puede distinguir claramente con los tarsos es una solución IM de sacarosa. Si se ponen en contacto con esta solución, *Pyrameis* siempre responde, sin relación alguna con su condición nutritiva. El animal puede también responder si los tarsos tocan el agua destilada, pero solamente después de un periodo más o menos prolongado de inanición total. Además, la ingestión de agua inhibe inmediatamente la respuesta. Es posible, por consiguiente, mantener a *Pyrameis* en un estado de 100 por ciento de reacción a la solución IM de sacarosa, pero solamente en uno de 0 por ciento de respuesta al agua sola. Bajo estas condiciones, la concentración mínima de sacarosa necesaria para producir una respuesta, esto es, la concentración del umbral de respuesta a la sacarosa, varía directamente con la condición de la nutrición. Durante los periodos de inanición de sacarosa el umbral decrece gradualmente, y puede alcanzar niveles tales como M/3200, M/6400 y aún M/12800. Pero con la iniciación de un periodo de dieta a base de sacarosa el umbral se eleva súbitamente a un nivel generalmente de M/10, que se conserva próximamente constante mientras la dieta continúa. Cuando se compara con la de otros animales, la sensibilidad de *Pyrameis* a la sacarosa está muy desarrollada. Este hecho está sin duda relacionado con el hecho de que los azúcares forman el alimento principal de este insecto.

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A QUANTITATIVE STUDY OF TARSAL SENSITIVITY TO SOLUTIONS OF SACCHAROSE, IN THE RED ADMIRAL BUTTERFLY, PYRAMEIS ATALANTA LINN.

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ONE FIGURE

CONTENTS

Introduction.....	445
Methods.....	446
Results.....	448
Conclusions.....	456
Bibliography.....	457

INTRODUCTION

It is well known that many insects are extremely sensitive to distance chemical stimulation. The males of certain Lepidoptera, in particular, exhibit at the time of mating a degree of sensitivity probably unique in the animal kingdom. Thus Riley ('94, p. 39) describes an experiment in which a male of *Philosamia cynthia* Drury was successful in seeking out a female a mile and a half away, and Fabre ('79-'04), in his classic experiments upon several other species of Lepidoptera, reports equally remarkable results. But while we do know something of the degree of olfactory sensitivity possessed by insects, we know absolutely nothing concerning their acuity of taste or contact chemoreception. This is due to the fact that the only organs of taste heretofore described were located on the mouth parts or within the buccal cavity, where experimentation was virtually impossible.

Recently, however, I have shown (Minnich, '21) that the nymphalid butterflies, *Vanessa antiopa* Linn. and *Pyrameis atalanta* Linn., possess taste organs or contact chemo-receptors on their ambulatory tarsi. In a more detailed study of

Pyrameis alone (Minnich, '22) I have shown further some of the substances which can be differentiated through the tarsal organs. These substances consist of distilled water and several aqueous solutions, including a solution of 1M saccharose. In the course of this investigation two facts were discovered which have made possible a quantitative study of the tarsal sensitivity to saccharose solutions. First, contact of the four ambulatory tarsi with a 1M saccharose solution will always effect an extension of the proboscis, irrespective of the nutritional condition of the animal. From the time of its emergence from the pupa until its death, whether starved or abundantly fed, the butterfly will continue to respond with the utmost constancy to this stimulus. Second, contact of the tarsi with distilled water alone will also effect an extension of the proboscis, but only after a prolonged period of inanition with respect to water. Thus, a butterfly deprived of water for a sufficient number of days, generally four to seven, will finally become 100 per cent responsive to water. This response, however, ceases at once if the animal be allowed to drink water. By the mere administration of water, therefore, Pyrameis can be maintained in a state of 0 per cent responsiveness to water, but of 100 per cent responsiveness to 1M saccharose. Under these conditions, the minimal concentration of saccharose necessary to effect a response is readily determined. It is the purpose of the present paper to report the data obtained in this fashion.

METHODS

All butterflies were hatched in captivity. Upon hatching they were kept without food or water until they became 100 per cent responsive to water. They were then placed on a diet of distilled water which was administered morning and evening. Although some individuals appeared quite weak after the period of total inanition, they generally revived after their first access to water, and for some days the water diet was sufficient to maintain them in a normal condition. Sooner or later, however, the butterflies began to show signs of weakness which gradually increased until further trials became impossible. At this point 1M saccharose

was substituted for distilled water, and again the animals generally revived. The diet of saccharose was continued for three days, after which the animals were subjected to a second period of total inanition, followed by a second period of water diet, followed in turn by a second period of 1M saccharose diet. Those individuals which survived the entire experiment were thus carried through six nutritional periods: a first period of total inanition, a first period of water diet, a first period of 1M saccharose diet, a second period of total inanition, a second period of water diet, and a second period of 1M saccharose diet.

The method employed in making trials was the following. The animal was placed in a holder manipulated by hand, and was held with its four ambulatory tarsi in contact with a flat layer of absorbent cotton contained in a Syracuse watch-glass and saturated with the stimulating substance. Each trial with a given concentration of saccharose was immediately preceded by a trial with distilled water alone. If there was any evidence of response in the preliminary trial the saccharose test was abandoned, thereby avoiding any possibility of misinterpretation. No visible movement of the proboscis during one minute constituted a no response, while extensions of the proboscis during the same interval, whether partial or complete extensions, constituted a response. After each trial with saccharose the feet were carefully rinsed in distilled water and dried by contact with clean filter-paper.

Excepting the first three to five days after hatching and days of responsiveness to water alone, an attempt was made to determine the threshold concentration of saccharose for each animal daily. In general, this was accomplished by means of four determinations, although occasionally as many as six or seven were made. These determinations were made at least one hour apart. Between them, however, tests with other substances, viz., 1M saccharose, 2M NaCl, and M/10 quinine hydrochloride, were being made at minimal intervals of fifteen minutes. As far as I was able to observe, the trials with these various substances in nowise affected the threshold of response to saccharose.

In selecting the concentrations of saccharose for making determinations I followed no fixed, uniform procedure. Instead, I was guided by the immediate reaction of the individual together with its previous behavior. In general, the first day of experimentation was begun with a concentration of M/10 or M/100, while each day thereafter was begun with the concentration equal to or just below the threshold concentration of the previous day. If this concentration produced a response, lesser concentrations were tried; if it produced no response, greater concentrations were tried. This general procedure is brought out clearly in column IV of table 1, where the concentrations used on butterfly no. 13 are given in the order tried, with the reaction indicated in each case.

The quality of saccharose employed in making solutions was U. S. P. Stock solutions of M/10 and M/200 were made up from time to time during the experiments, while the other dilutions employed were prepared fresh daily from these stock solutions.

The present experiments were carried out at the same time and on the same animals as the experiments described in a previous paper (Minnich, '21). For a more detailed account of general methods than the one given here the reader is referred to that paper.

RESULTS

A total of seven butterflies was experimented upon. Three of these died quite early in the experiment, probably as a direct result of starvation. Of the remaining four, two survived to within a few days of the end of the experiment, while two others not only survived the entire experiment, but were still in vigorous condition several days later when they were killed and preserved for further study.

The most complete data were obtained on butterfly no. 13, and, since they are typical of the results obtained on the animals as a whole, they are presented in full in table 1. It will be noted that during the first three days after hatching no trials were made. The first determination was thus made on the 4th day when the threshold concentration was found to be M/200.

TABLE 1

Showing the responses of butterfly no. 13 to distilled water and to various concentrations of saccharose, under different nutritional conditions. Changes in nutritional conditions were always made in the evening after the trials of the day were completed. This is indicated by a repetition of the day number followed by (p.m.). Threshold concentrations are indicated in *italics*

I CONDITIONS OF NUTRITION	II DAY OF AGE	III RESPONSE TO DISTILLED WATER	IV RESPONSE TO SACCHAROSE SOLUTION
Given neither wa- ter nor food	1	Not tried	Not tried
	2	Not tried	Not tried
	3	Not tried	Not tried
	4	No response No response No response No response	M/10 Complete extension M/100 Complete extension <i>M/200 Complete extension</i> M/400 No response
	5	Complete extension Complete extension Partial extensions Partial extensions	Not tried Not tried Not tried Not tried
	6	Complete extension Complete extension Complete extension Complete extension	Not tried Not tried Not tried Not tried
	7	Complete extension Complete extension	Not tried Not tried
	7 (p.m.)		
	8	No response No response No response No response	M/200 Complete extension <i>M/400 Partial extensions</i> M/200 Complete extension M/400 No response
	9	No response No response No response No response No response	M/400 Complete extension <i>M/800 Partial extensions</i> M/1600 No response M/800 No response M/400 Partial extensions
	10	No response No response	<i>M/800 Complete extension</i> M/1600 No response
Offered distilled water morning and evening			

TABLE 1—*Continued*

I CONDITIONS OF NUTRITION	II DAY OF AGE	III RESPONSE TO DISTILLED WATER	IV RESPONSE TO SACCHAROSE SOLUTION
Offered distilled water morning and evening	10	No response No response	<i>M/800 Partial extensions</i> <i>M/400 Partial extensions</i>
	11	No response No response No response No response	<i>M/1600 Complete extension</i> <i>M/3200 Complete extension</i> <i>M/6400 No response</i> <i>M/3200 No response</i>
	11(p.m.)		
	12	Not tried	Not tried
Offered 1M sac- charose morn- ing and evening	13	No response No response No response No response	<i>M/200 No response</i> <i>M/100 No response</i> <i>M/50 No response</i> <i>M/10 Complete extension</i>
	14	No response No response No response No response	<i>M/50 No response</i> <i>M/10 Complete extension</i> <i>M/50 No response</i> <i>M/10 Complete extension</i>
	15	No response No response No response No response	<i>M/50 No response</i> <i>M/10 Complete extension</i> <i>M/50 No response</i> <i>M/10 Complete extension</i>
	15(p.m.)		
Given neither wa- ter nor food	16	No response No response No response No response	<i>M/50 Partial extension</i> <i>M/100 No response</i> <i>M/50 No response</i> <i>M/10 Complete extension</i>
	17	No response No response No response No response	<i>M/100 Complete extension</i> <i>M/200 No response</i> <i>M/100 Partial extensions</i> <i>M/200 No response</i>
	18	Complete extension Partial extensions No response No response	Not tried Not tried <i>M/200 No response</i> <i>M/100 No response</i>
	19	Complete extension Complete extension	Not tried Not tried

TABLE 1—Continued

I CONDITIONS OF NUTRITION	II DAY OF AGE	III RESPONSE TO DISTILLED WATER	IV RESPONSE TO SACCHAROSE SOLUTION
Given neither wa- ter nor food	19	Complete extension No response	Not tried <i>M/100 Complete extension</i>
	20	Complete extension Complete extension Complete extension Complete extension	Not tried Not tried Not tried Not tried
	(20(p.m.))		
	21	No response No response No response No response No response	M/1600 No response M/400 No response M/200 No response <i>M/100 Partial extensions</i> M/50 Partial extensions
Offered distilled water morning and evening	22	No response No response No response No response No response	M/200 No response M/100 Partial extensions M/50 Complete extension <i>M/200 Partial extensions</i> M/400 No response
	23	No response No response No response No response No response	M/400 No response M/200 No response M/100 No response M/50 Complete extension <i>M/100 Partial extensions</i>
	24	No response No response No response No response	<i>M/100 Complete extension</i> M/200 No response <i>M/100 Complete extension</i> M/200 No response
	25	No response No response No response No response No response No response	M/200 Complete extension <i>M/400 Complete extension</i> M/1600 No response M/800 No response <i>M/400 Partial extensions</i> M/800 No response
	26	No response No response No response	M/800 Complete extension M/3200 Partial extensions <i>M/6400 Complete extension</i>

TABLE 1—*Concluded*

I CONDITIONS OF NUTRITION	II DAY OF AGE	III RESPONSE TO DISTILLED WATER	IV RESPONSE TO SACCHAROSE SOLUTION
Offered distilled water morning and evening	26	No response No response No response	M/25,600 No response M/12,800 No response M/6400 No response
Offered 1M sac- charose morn- ing and evening	(26(p.m.))		
	27	No response No response No response No response No response	M/100 No response M/50 No response <i>M/10 Complete extension</i> M/50 No response <i>M/10 Complete extension</i>
	(28(a.m.))	Animal found dead	

The 5th, 6th, and 7th days, no trials were made with saccharose since the animal responded to water. During the 7th day the animal became so weak that further experimentation was impossible. Accordingly, trials were suspended, water was administered, and the butterfly was allowed to recuperate.

On the following morning, viz., the morning of the 8th day, the butterfly had entirely recovered, its responsiveness to water had disappeared, and trials with saccharose were resumed. As the period of water diet continued, the threshold of response to saccharose gradually fell from M/400 on the 8th day to M/3200 on the 11th day. At the close of the 11th day the animal again evinced signs of weakness, and it was necessary to change the diet to 1M saccharose.

Absence from the laboratory prevented any trials on the 12th day. When experimentation was renewed on the morning of the 13th day, however, the butterfly had completely recovered its normal vigor. But the threshold of response had risen from M/3200 to M/10, and at this level it steadily remained throughout the entire period of saccharose diet, viz., the 13th, 14th, and 15th days.

Following the first period of saccharose diet came the second period of total inanition, which lasted from the 16th to the 20th days, inclusive. During the first two days of this period, the

16th and 17th days, the threshold of response to saccharose again fell to $M/100$. On the 18th, 19th, and 20th days the responsiveness to water gradually reappeared and prevented any conclusive determinations with saccharose.

At the close of the trials on the 20th day the second period of water diet was begun, and on the following morning the responsiveness to water had completely disappeared once more. The threshold concentration of saccharose on this the 21st day was still $M/100$. The water diet was continued until the close of the 26th day, the threshold concentration of saccharose gradually falling meantime until it reached $M/6400$. In the last two trials of the 26th day the butterfly had become extremely weak—a fact which may account for the negative results obtained.

The physical condition of the animal on the evening of the 26th day necessitated the administration of saccharose, and accordingly the second period of saccharose diet was begun. On the morning of the 27th day the butterfly appeared greatly revived. But tests with saccharose showed that the threshold of response had again risen to $M/10$, precisely as it had done at the beginning of the first period of saccharose diet. On the evening of the 27th day the animal seemed quite as vigorous as usual, but on the morning of the 28th day it was found dead.

In figure 1 the threshold concentrations of saccharose for butterfly no. 13 are represented graphically. A glance at the curve shows that during periods of total inanition followed by periods of water diet, i.e., during saccharose inanition, the threshold of response gradually fell, while immediately a period of saccharose diet was begun the threshold abruptly rose. Two facts are thus brought out clearly. First, the minimal effective concentration of saccharose for the tarsi varies directly with the nutritional condition of the animal. During periods of saccharose diet the threshold remained at $M/10$; during periods of complete inanition and water diet, i.e., saccharose inanition, the threshold gradually fell to $M/3200$ or $M/6400$. Second, the minimal effective concentration after prolonged inanition with respect to saccharose may be extremely low. At the close of the first period it was $M/3200$; at the close of the second, $M/6400$.

In table 2 I have summarized the results obtained on all seven butterflies. While the results there presented exhibit rather wide individual differences, they show the same general features which are brought out in the behavior of butterfly no. 13. Thus the threshold of response in all animals fluctuated directly with the nutritional condition. Furthermore, the minimal effective concentration after prolonged inanition with respect to saccharose was very low in general. The extremely low thresholds, M/3200, M/6400, and M/12,800, which were observed in four of the seven animals, are particularly noteworthy.

TABLE 2

Showing the threshold concentrations of saccharose solution, under varying nutritional conditions, in seven butterflies. An asterisk signifies the death of the butterfly within twenty hours after the determination indicated

ANIMAL NUMBER	FIRST PERIOD OF TOTAL INANITION	FIRST PERIOD OF WATER DIET	FIRST PERIOD OF SACCHAROSE DIET	SECOND PERIOD OF TOTAL INANITION	SECOND PERIOD OF WATER DIET	SECOND PERIOD OF SACCHAROSE DIET
11	Not tried	M/100	M/10	M/50	M/12,800	M/10
12	Not tried	M/200	M/10	M/50	M/400*	
13	M/200	M/3200	M/10	M/100	M/6400	M/10*
22	Not tried	Not tried	M/100	M/400	M/3200	M/100
23	Not tried	M/100*				
24	Not tried	M/12,800*				
25	Not tried	M/400*				

This responsiveness to very dilute solutions of saccharose affords further evidence of a point previously made by me, viz., that the nature of tarsal stimulation is not osmotic, but chemical (cf. Minnich '21, p. 198). Throughout the present experiments all animals were tested four times daily with several solutions other than those mentioned in this paper. Among these were 1M saccharose and 2M NaCl. On the days when the four butterflies mentioned in the foregoing paragraph were responding to such dilutions of saccharose as M/3200, M/6400, and M/12,800, three of the four gave no response whatever to 2M NaCl. Yet the NaCl solution was vastly the more effective osmotically. It might be objected that the salt solution produced such a powerful osmotic effect as to inhibit response, whereas a more

Fig. 1 Graphic representation of threshold concentrations of saccharose during various nutritional periods in butterfly no. 13. In order to bring out the significant features of the curve in the available space, the logarithm of the concentration has been plotted instead of the concentration. Portions of the curve shown in broken lines indicate periods of sensitiveness to water alone, during which determinations with saccharose were impossible.

dilute solution would have produced a response. But this objection is at once ruled out by the fact that a 1M solution of saccharose always yielded a 100 per cent response. The tarsal sense organs, therefore, appear to be quite unaffected by osmotic pressure and must be regarded as specific chemoreceptors.

The tarsal sensitivity of the red admiral to saccharose is quite remarkable when compared with the sensitivity of other animals to the same substance. Among the marine invertebrates saccharose appears to have little or no stimulating power apart from the osmotic effect of high concentrations. The same holds true for the lower aquatic vertebrates. According to the table given by Parker ('12, p. 228), *Amphioxus*, *Ammocoetes*, *Mustelus*, and *Amiurus* are entirely unresponsive to saccharose. Moreover, the weakest solution which the human tongue can detect is but M/50. But as we have seen in two of the seven butterflies tested, nos. 11 and 24, the tarsi were able to discriminate an M/12,800 solution. In other words, the tarsi of these two butterflies were 256 times more sensitive to saccharose than the human tongue.

The sensitivity of *Pyrameis* to saccharose is thus a highly specialized one. The reason for this is not far to seek. *Pyrameis* feeds on nectar, exuding sap, and juices of ripe and decaying fruit. In all of these substances sugars are found. In the laboratory I have kept the butterfly alive and in good condition for thirty days on 1M saccharose solution. Sugars thus appear to be the most important food of this insect, and the highly developed sensitivity to saccharose is doubtless directly correlated with this fact. Like the organs of olfaction, therefore, the organs of taste may be very highly developed in certain lepidopterous forms.

CONCLUSIONS

1. In *Pyrameis* the threshold of response to saccharose solutions varies directly with the nutritional condition of the individual. During periods of total inanition followed by periods of water diet, i.e., during saccharose inanition, it gradually falls. But with the initiation of a period of saccharose diet, it rises abruptly to a level which remains approximately constant throughout the remainder of the period.

2. After prolonged inanition with respect to saccharose the threshold concentration may fall as low as M/3200, M/6400, or even M/12,800 in some individuals. The tarsal sensitivity of *Pyrameis* to saccharose may thus be as much as 256 times that of the human tongue.

3. The highly developed sensitivity to saccharose is doubtless correlated with the fact that sugars form the chief food of this insect.

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