

SENSORY STIMULATION OF THE OYSTER, OSTREA VIRGINICA, BY CHEMICALS

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INTRODUCTION

Although the adult oyster is immobile it is, like a great many other organisms, highly sensitive to environmental changes, both chemical and physical. The obvious reaction of the oyster to stimuli is a quick closing of the valves, brought about by contraction of the adductor muscle. This may be observed following mechanical or chemical irritation or sudden decrease in the intensity of the light; the so-called shadow reaction. It is not the purpose of this paper to give a complete account of the oyster's sensitivity to all environmental factors, but it is of interest that the only reaction, ordinarily to be observed, to stimuli of various kinds is the shell closure. Because of its immobility and its consequently limited number of possible reactions the oyster is a favorable object for experiments on sensitivity.

The sensitivity of invertebrates and lower vertebrates to a wide variety of chemical substances has been studied by numerous investigators. It is unnecessary to make a complete survey of such works here, but certain of them should be mentioned to indicate the scope of the chemical senses. Amphioxus (Parker, 1908) has been found to respond to solutions of salts, acids, alkalies, alkaloids, alcohol, ether, chloroform, turpentine, and several essential oils, but not to cane sugar of a concentration as high as 2 molar. Kribs (1910) gave the threshold concentrations of a number of substances in the stimulation of the Oligochaet, Aelosoma, as follows: Mineral acids, N/3,000; organic acids, N/2,000; hydrates, N/1,500; carbonates, N/1,200; chlorides, M/80; bromides, M/50; ferric sulphate, M/10,000; copper sulphate, M/80,000; zinc sulphate, M/80,000. He stated that increase in temperature or exposure to bright light lowered the threshold.

Crozier (1915) studied by means of reaction times and threshold concentrations the responses of *Holothuria surinamensis* to salts, acids, carbohydrates, and alkaloïds. Similar observations have been made by Olmstead (1917) on *Synaptula*; Hecht (1918) on *Ascidia*; Crozier and Arey (1919) on *Chromodoris*; Arey and Crozier (1919) on *Chiton*; Agersborg (1922) on *Nudibranchs*; Copeland (1923) on *Palaemonetes*; Copeland and Wieman (1924) on *Nereis*; and various others. A recent review of the significant findings of these workers is that of Parker and Crozier (1929).

MATERIAL AND METHODS

The experiments described in the following pages were made at the biological laboratory of the United States Bureau of Fisheries at Beaufort, N. C. Large local oysters (*Ostrea virginica*) were employed.

By sawing through the posterior portion of the right valve of a specimen, it was possible to remove the severed piece of shell without injury to the underlying tissues. The mantle, with its border of 2 rows of tentacles, 2 to 5 millimeters long, was in this manner exposed. The method by which a chemical solution was brought into sudden contact with the tentacles has already been described (Hopkins, 1932). By means of an arrangement of constant levels a stream of water flowed through a small tube across a few tentacles. By making a quick turn of a 3-way stopcock the stream of water was suddenly replaced by the chemical solution to be tested. The total time from turning of the stopcock until the retraction of the tentacles was measured with a stopwatch. The time required for the solution to go from the stopcock to the tentacles was measured by use of a colored solution. This value was then subtracted from the total time measured, giving the actual latent period, or the time required for the reaction to take place after the solution reaches the tentacles.

The test solutions were made up in sea water so as to eliminate any possible effect of distilled water. When stimulated by a chemical solution the tentacles of the oyster retract sharply, so that the latent period may readily be measured. The reaction to distilled water, or to sea water diluted with equal parts of distilled water, or to sea water concentrated to half its original volume, is quite different. In these cases the tentacles retract very slowly, and it is almost impossible to determine the time of beginning of the movement. This is in marked contrast to the quick jerk of the tentacles when stimulated by an irritating solution and is clear evidence that the latent periods determined in the experiments were not due to differences in osmotic pressure.

EXPERIMENTAL OBSERVATIONS

No attempt has been made to list all substances which may stimulate the oyster. The purpose of the experiments was to find out how the reaction time varies with concentration in enough types of compounds to indicate whether the same principles obtain in all cases. One substance was tested which in man is an adequate stimulus for the sense of smell, namely, cumarin. This has an odor similar to that of vanilla and at fairly high dilutions, at least, seems to have no taste as distinct from odor.

As representatives of substances commonly identified with the sense of taste the following compounds were employed: Several inorganic salts; quinine sulphate; cane sugar; hydrochloric acid (though these results were not totally satisfactory because of the difficulties concerned with acid in sea water); and alkalies, namely,

sodium hydroxide and potassium hydroxide. The alkalies are often thought to be stimuli for the common chemical sense rather than for taste.

The results are presented graphically and each point represents the average value of from 10 to 20 latent period determinations. Five minutes were allowed to elapse between succeeding tests to prevent possible fatigue of the sensory endings. A single complete series, involving tests for enough concentrations to produce a relatively complete curve, was made within two or three successive days and under conditions as nearly constant as possible. The temperature of the water during a series varied over approximately 3° C. and was in general close to 20° C. It is not known at present how great is the error due to temperature fluctuations, but the probable error in any group of tests with a single concentration did not exceed ±0.05 second, except in certain cases which will be mentioned later.

CUMARIN

In general the latent period values were not more than about five seconds, for above this the reaction was so weak as to be indistinguishable from the frequent slight movements of the tentacles. However, in the case of cumarin the reaction was sharp and clear up to about 14 seconds, although the probable error increased to about ±0.3 second. In Figure 1 the results of a series with one specimen are presented. It is unnecessary to give more for, aside from a slight individual variation, the other series agree perfectly with this one. Concentrations from 0.0004 to 0.011 per cent were tested, and the points as shown in the figure fall with reasonable accuracy into a hyperbolic curve. Crozier (1918, 1918a) obtained similar curves in studies on the stimulation of the earthworm by acids and alkalies. The relationship between concentration (C) and latent period (T) is what would be expected for a simple monomolecular chemical reaction; namely, $C \times T = K$.

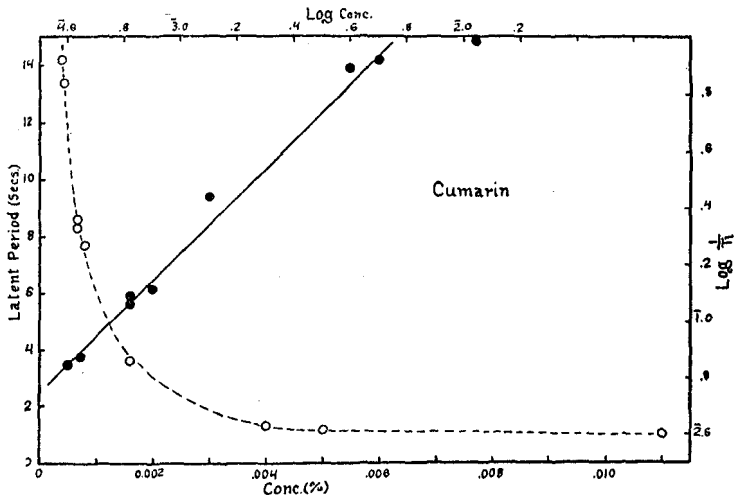


FIGURE 1.—Graph showing average latent period values (open circles) as ordinates for different concentrations (abscissae) of cumarin, and same data (solid points) plotted as log of reciprocal of latent period and logarithmic concentration

On the same graph the data are given logarithmically, as logarithmic concentration (abscissæ) and logarithmic 1/latent period (ordinates). The points fall close to a straight line with the exception of that referring to the highest concentration. Whether the latent period at high concentrations actually reaches a limit and changes the form of the logarithmic curve or whether the error is too high to permit accurate determination is not quite clear. The latter possibility, however, is very likely, for at high concentrations the latent period is extremely short and the reaction involves not only the tentacles but also the adjacent portion of the mantle.

POTASSIUM CHLORIDE

Latent period studies were made with a number of inorganic salts, but at this place data need be given for only KCl, NaCl, and MgCl₂.

A typical record is that shown in Figure 2 for potassium chloride. The latent

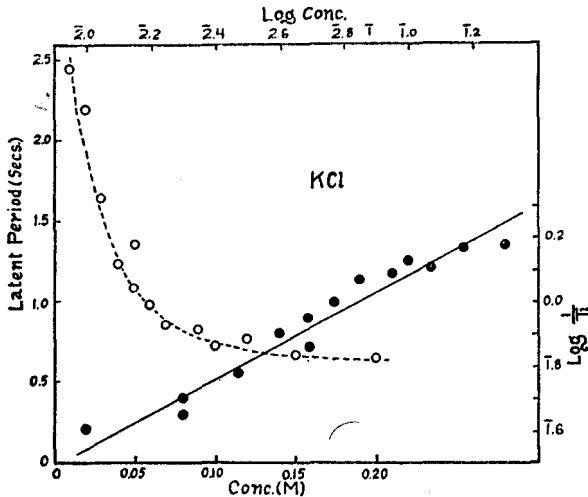


FIGURE 2.—Average latent period values (open circles) for solutions of potassium chloride of different concentrations; and the same data expressed logarithmically

period values all fell within 2.5 seconds, but the curve is nevertheless very similar to that just described. It was possible to measure the reaction time for concentrations as low as 0.01 M., but solutions of 0.2 M. or higher caused such violent contractions of the whole mantle that the difficulties of experimentation were great.

The logarithmic curve (fig. 2) is similar to that shown in Figure 1, except that the angle made with the horizontal axis is less. Typical of nearly all of the records for salts is an angle of less than 45° for the logarithmic curve. It is assumed that this is due to some factor, such as diffusion, which causes the latent

periods to be longer than expected. That is, the relationship, $C \times T = K$, must be modified to $C \times (T - X) = K$. The significance of the factor, X, will be discussed later.

SODIUM CHLORIDE

In Figures 3 and 4 the results of two series of tests with solutions of NaCl are given. The series were made with different specimens at different times, and the latent period values of series 1 are consistently higher than those of series 2. This difference probably was not due to temperature for that was approximately the same in both cases. It may have been due to difference in light intensity, for according to Kribs (1910) bright light sensitizes the chemical receptors of Aelosoma. It was possible to measure the reaction time at concentrations only as low as 0.1 M., which is very high as compared to the threshold for KCl. It is well known, however, that the potassium ion is very highly stimulating to many organisms (Hopkins, 1932).

In Figure 4 the two curves are presented in logarithmic form. The curves are drawn through the points as parallel lines, though this need not necessarily be the case. The angle of the curves is less than 45°, as in the case of KCl, and all other salts.

MAGNESIUM CHLORIDE

It has been a source of confusion in this work that in certain experiments the latent period seemed to change almost suddenly from short to long. For example, during a series of tests with one concentration of a salt, the latent period for a number of tests would be consistently about one second. Then, for no obvious reason, the reaction would follow a latent period of perhaps 1.8 seconds. At other times it might shift from long to short; or two separate and distinct reactions at the two levels might be observed. When the tests were first made with MgCl₂ this was found to occur

and it was thought to be peculiar to this salt. However, it has been found to be possible with other salts as well. In Figures 5 and 6 the results of one series of tests with

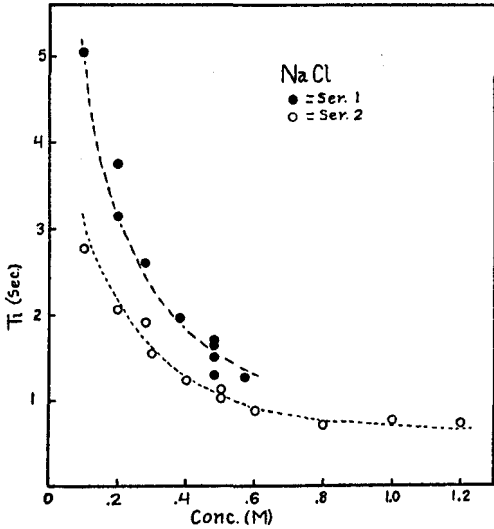


FIGURE 3.—Average latent period values (ordinates) of the reaction of the oyster to different concentrations (abscissae) of sodium chloride. Two series of experiments made under different conditions are given. (See fig. 4)

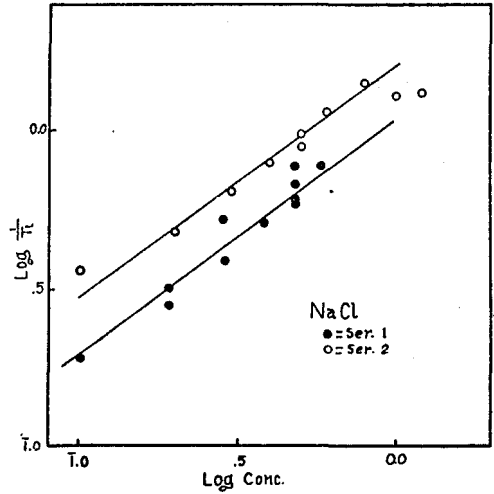


FIGURE 4.—Data given in Figure 3 plotted as logarithmic reciprocal of latent period and logarithmic concentration

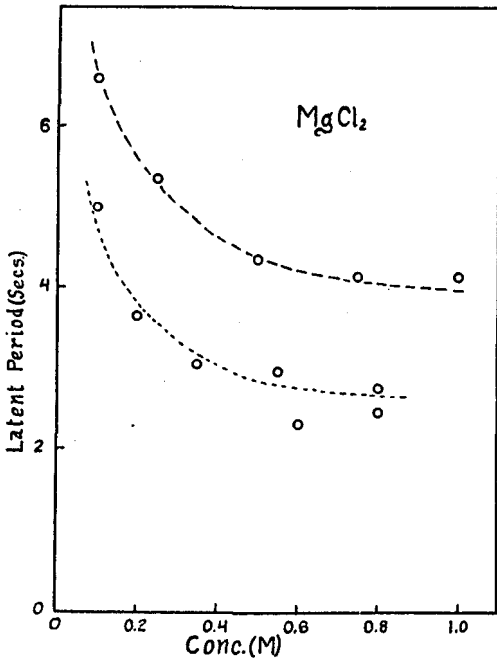


FIGURE 5.—Curve showing two different latent period levels in the response of a single specimen to magnesium chloride. The points represent averages. (See fig. 6)

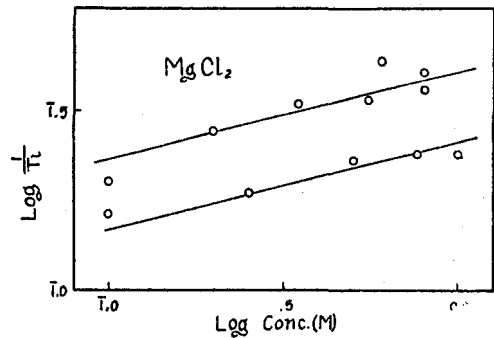


FIGURE 6.—Logarithmic presentation of data given in Figure 5 showing relation between logarithmic concentration and logarithmic reciprocal of latent period

MgCl₂ are given. The points fall clearly into two approximately parallel curves about one second apart.

The two curves are mathematically comparable. Further experiments are in progress for the purpose of finding the significance of this behavior.

QUININE SULPHATE

The reactions of the tentacles to quinine are in every way comparable to those to the salts just described. The curve (fig. 7) is a typical hyperbola and the points, logarithmically expressed, fall around a straight line which has an angle, however,

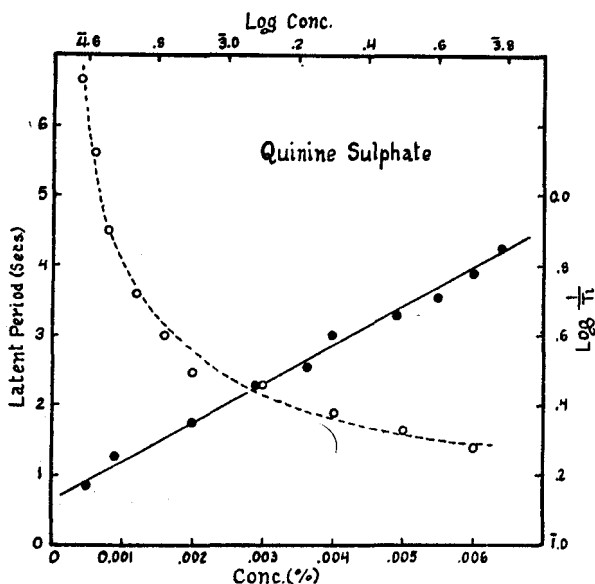


FIGURE 7.—Average latent period values of the response of the oyster to quinine. On the same graph (solid points) the data are expressed logarithmically

up of a significant concentration of the test chemical in the medium. For this reason it is impractical to use artificial sea water entirely. Experiments might be made in which the specimens are immersed in natural sea water and only the solution of alkali in carbonate-free water. This procedure, however, would probably not be satisfactory, for in order to bring about stimulation it is necessary for the test solution to come into contact with the tentacles which are already immersed in sea water, and reaction between the molecules of alkali and carbonate would take place close to the sensory surfaces. In such a case precipitation compounds would be formed.

In these experiments such possible sources of error were recognized, but a few series of tests were made without attempting to eliminate the difficulties. Nevertheless, the results appear to be of considerable significance. Stock molar solutions of KOH and NaOH were made in sea water. The precipitate of magnesium and calcium carbonate was allowed to settle and the solution decanted and filtered. Immediately preceding a series of tests the desired concentration was made from the stock solution and sea water. Again a precipitate was formed and removed by decanting and filtration. When the test solutions came into contact with the tentacles there was probably further precipitation. However, in all except possibly the most dilute solutions the hydroxyl-ion concentration was sufficient to remove all of the carbonates from the test solutions. The concentrations of alkali as stated in terms of mols of alkali originally dissolved, while not strictly correct, are relatively correct with relation to one another.

This will appear more clearly when Figures 8 and 9 are examined. One series of tests each for NaOH and KOH are given in the graphs. Three such series were

less than 45° . A distinct and measurable tentacular reaction was observed as a response to concentrations as low as 0.0004 per cent. According to Hecht (1918) the taste threshold in man for quinine sulphate is 0.00004 M. Since the oyster is sensitive to 0.0004 per cent, or about 0.0000046 M., the latter is about eight times as sensitive to this substance as man.

ALKALIES

The difficulty of working with alkalis in sea water is great unless artificial, carbonate-free sea water is employed. In experiments of the present kind it is necessary for the specimens to be immersed in a considerable quantity of running water in order to insure against building

made, but the others are entirely comparable and need not be given. In Figure 8 the curves representing the latent period values for each concentration are entirely different for the two alkalis. While the latent periods for KOH fell into a curve typically like those described in the preceding pages, the results obtained with NaOH fall distinctly into two such curves (A and B, fig. 8) which cross one another at a concentration of about 0.07 M.

In measuring the latent period of the tentacular reaction the time up to the first visible sharp reaction is used. It was observed in the early experiments with NaOH (fig. 8) that the latent period for concentrations of 0.1 M. or above were very short, and at the next lower concentrations much longer than expected. There appeared to be a sudden break in the curve.

When tests with various concentrations were repeated it was found that one series would give a long reaction time and another series with the same solution would give a short latent period. The points appeared to fall along the lines as shown in the figure. It is probable that the reactions in both latent period groups occurred in all cases, but that at some times those of one group would be sharp enough to be detected while at other times the clear reactions would fall into the second group. For some time during the tests it was thought that the error of the method was simply very large, but the latent period values persisted in falling along the lines shown.

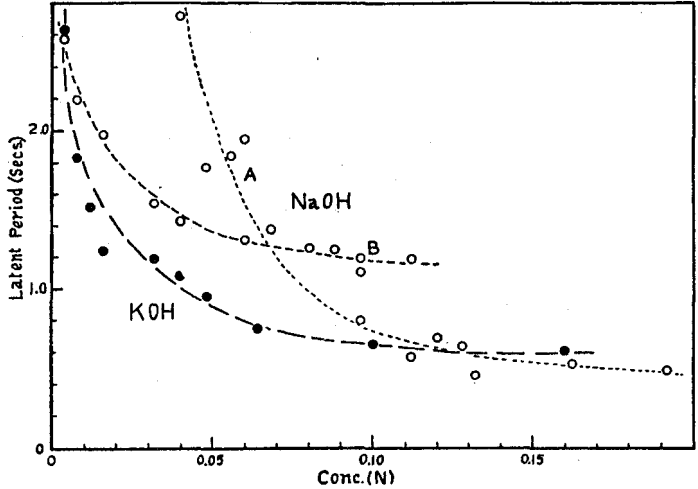


FIGURE 8.—Average latent period values for different concentrations of KOH (solid points) and NaOH (open circles). (See fig. 9)

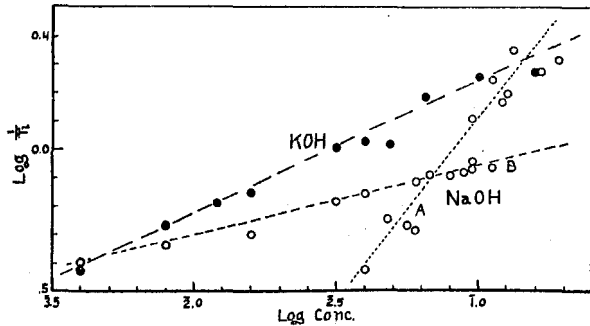


FIGURE 9.—Latent period data given in Figure 8 expressed logarithmically. (See text)

are required to satisfy the values obtained with NaOH. The difference is probably of some significance for all of the tests were made upon a single specimen within the period of a few days.

In the description of the experiments with salts it was pointed out that the logarithmic curves made an angle of something less than 45° with the horizontal. In Figure 9 it appears that the angle made by the KOH curve is only about 20°,

The records obtained with KOH, on the other hand, were of a decidedly different sort (fig. 8). So far as could be determined the latent period curve was single. The points fall into a curve similar to those of the salts. By plotting the results logarithmically (fig. 9), the dual nature of the results with NaOH is made even clearer. While the points representing the reaction to KOH may be considered to fall into a single straight line, two such lines

are required to satisfy the values obtained with NaOH. The difference is probably of some significance for all of the tests were made upon a single specimen within the period of a few days.

while that of one NaOH curve (B) is even less, and of the other (A) is approximately 45° . If it be assumed, as seems reasonable, that the small angle of the curves is due to the time required for the stimulating substance to penetrate a layer of mucus on the tentacles, a possible explanation of these curves may be obtained. It has been pointed out that the potassium salts are much more efficient than sodium salts in the process of stimulation.

While the threshold for KCl is 0.01 M., that for NaCl is 0.10 M., or 10 times as high. It may be that the two ions in solutions of KCl or KOH stimulate separately, and that the observed latent period is that due to the ion which initiates the reaction first. Then, if it be assumed that the curve for KOH (figs. 8 and 9) is a record of the stimulation by potassium ions, it is probable that the NaOH curve B represents the response to sodium ions, and curve A, OH ions. If this is the case, it would appear that the OH ions penetrate the layer of mucus much more rapidly than the ions of Na or K. Consequently the concentration of OH ions \times the latent period is a constant, for the logarithmic curve has the angle of 45° . However, the time required for penetration of the mucus by Na and K ions must be subtracted from the latent period in order for this relationship to obtain.

CANE SUGAR

Parker (1908) stated that it appears that cane sugar does not stimulate the sensory receptors of *Amphioxus*. Similar results were obtained with the oyster. Only when a very high concentration of sugar was employed did the tentacles retract, and then the reaction was similar to that due to diluted or concentrated sea water. The effect obviously was due to osmosis by extraction of water. Crozier and Arey (1919) obtained similar results with *Chromodoris*, but found (Arey and Crozier (1919)) *Chiton* to be sensitive to 0.5 M. sucrose and lactose.

Negative evidence such as this is, however, of doubtful significance. The reaction of the oyster to the solutions employed is negative. The retraction of the tentacles or mantle appears to be of a protective nature and results in the withdrawal of these organs from the source of irritation. It is not impossible that substances such as sugar may stimulate the receptors positively and that the reaction as to irritation does not take place.

DISCUSSION

A source of constant difficulty in the experiments described in the foregoing pages is the occasional sudden change in the latent period values from a low to a high level, or vice versa. One example was given (figs. 5 and 6) in which two distinct curves were obtained. It was thought possible that frequent stimulation might result in fatigue of the receptors or in sensitization like that observed by Irwin (1918) to follow successive stimulation with a strong potassium salt. It seemed also possible that changes in light intensity or temperature might cause variations in the threshold (Kribs, 1910). However, no evidence has been obtained that any of these suggestions is the case. There appear to be definite levels at which the response to any concentration of a salt takes place. It has frequently happened that two separate and distinct responses may be observed, and measured, following stimulation. At other times either the lower or the higher reaction time may be measured. It is as if both such reactions always occur and that in some cases one is distinct and the other too weak to be observed.

The question arises as to whether the reactions are due to stimulation of different sensory endings the thresholds of which are different. Or possibly different receptors are stimulated separately; one, for example, by the anions, another by cations, and still another by undissociated molecules. This is a problem which may be solved, and further experiments are under way for this purpose.

What appears possible is that there is a measurable refractory period of the tentacular muscle fibers and that following initial stimulation contractions follow at regular intervals. Stimulation results first in retraction of the tentacles involved and then, after a considerably longer latent period, the adductor muscle contracts, closing the valves. In Figures 10 and 11 the results of a series of tests are given to show the relationship existing between the initial response of the tentacles and the following reaction of the adductor muscle. It is not practicable to employ the response of the

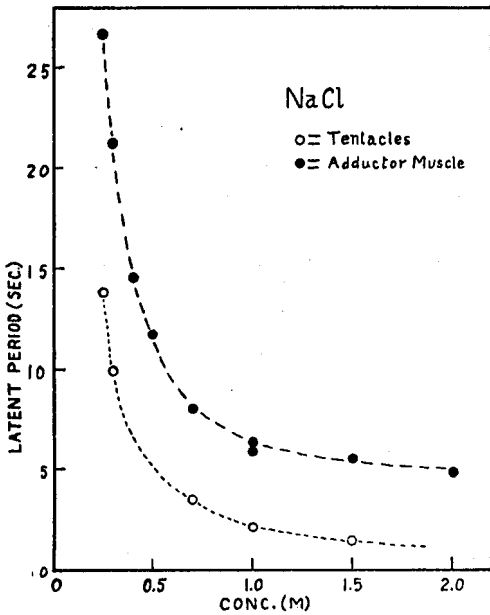


FIGURE 10.—Average latent period values for the tentacular (open circles) and adductor muscle (solid points) reaction to a series of concentrations of sodium chloride. (See also fig. 11)

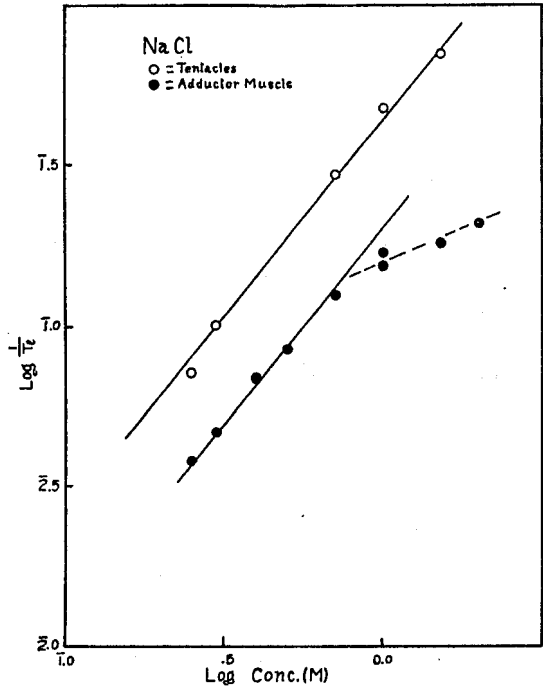


FIGURE 11.—Logarithmic expression of data given in Figure 10 for responses of tentacles and adductor muscle to sodium chloride

adductor muscle in this work, for after the oyster has been stimulated a few times the shells close and remain so for a considerable period.

Certain experiments with mixtures of substances are of interest with regard to this phase of the subject. Cumarin (0.0009 per cent) produced a reaction following 8.4 seconds; and the latent period for quinine (0.0007 per cent) was 9.2 seconds. A mixture containing both 0.0009 per cent cumarin and 0.0007 per cent quinine gave a latent period value exactly the same as that of cumarin alone, 8.4 seconds; that is, the latent period measured was that of the substance (cumarin) in the mixture which alone would produce the shorter reaction time. In another experiment the latent period for cumarin (0.0013 per cent) was 6.05 seconds, and that for quinine (0.001 per cent) was 5.85 seconds. The solution consisting of both 0.0013 per cent cumarin and 0.001 per cent quinine gave a latent period, within a small error, the same as that

of quinine alone. The two substances stimulate entirely separately. Both reactions probably took place, but only the shorter one could be measured.

It is to be expected that, if the two substances act on the same sensory ending, the effect of such a mixture would be additive and therefore cause reaction following a latent period shorter than that of either alone. Such is the case when mixtures of such salts as KCl and NaCl are employed. Two solutions were made up as follows: KCl, 0.1 M.; and NaCl, 0.5 M. The latent periods of the reaction to these and to mixtures of the two solutions were as follows:

	Seconds		Seconds
NaCl.....	3.69	2 parts KCl } 1 part NaCl } KCl.....	3.52
1 part KCl.. }	3.64		
2 parts NaCl }			
1 part KCl.. }	3.59		
1 part NaCl }		3.50	

The effect of the two salts in the above case was additive. It is possible to keep the latent period constant by replacing a part of the KCl with the proper amount of NaCl. However, this is not true in the case of mixtures of quinine and cumarin, or of cumarin and NaCl.

Crozier (1916) demonstrated the physiological antagonism between the salts of certain univalent and bivalent cations in the stimulation of the frog's foot. Tests with mixtures of NaCl and MgCl₂ on stimulation of the oyster failed to demonstrate any antagonism. However, the high proportion of these salts in sea water would suffice to prevent any considerable effect of such antagonism. These tests, nevertheless, are of some interest for the results seem to throw some light upon the question of changing latent periods.

In Table 1 data are presented to show the results of an attempt to find the latent period due to mixtures of NaCl and MgCl₂ each of which had approximately the same effect. It will be observed that most of the values are between 2.45 and 2.65 seconds, but that certain of them are about one second higher. On one day the experiments with NaCl were made and the latent period was of the high level. On the next day the latent period for MgCl₂ was about the same. On the third day a mixture was employed and the latent period was a full second lower. It appeared as if the effect of the mixture had been increased, but a series of tests with NaCl gave low values also. During the following two days the latent periods of the reaction to solutions of a single salt or to mixtures remained likewise low. Two days later, however, the reaction time to MgCl₂ was again high, but that to a mixture was low.

TABLE 1.—*Latent periods of reaction to mixtures of NaCl (0.5 M) and MgCl₂ (0.5 M)*

Date	Time	Salts	Average latent periods	Temperature	Latent period level	Date	Time	Salts	Average latent periods	Temperature	Latent period level
Feb. 1...	10-11 a. m.	NaCl.....	3.07	°C.	High.	Feb. 6...	11-12 m.	{NaCl, 25 per cent. MgCl ₂ , 75 per cent.}	2.43	°C.	Low.
Feb. 2...	2-3 p. m.	MgCl ₂	3.65	10.3	Do.	Feb. 7...	4-5 p. m.	NaCl.....	2.45	10.5	Do.
Feb. 3...	10-11 a. m.	{NaCl, 50 per cent. MgCl ₂ , 50 per cent.}	2.69	9.5	Low.	Feb. 7...	10-11 a. m.	MgCl ₂	3.61	12.5	High.
Feb. 4...	3-4 p. m.	NaCl.....	2.58	11.5	Do.	Feb. 7...	3-4 p. m.	{NaCl, 10 per cent. MgCl ₂ , 90 per cent.}	2.40	12.8	Low.
Feb. 4...	11-12 m.	MgCl ₂	2.80	12.3	Do.						
Feb. 4...	3-4 p. m.	{NaCl, 50 per cent. MgCl ₂ , 50 per cent.}	2.59	13.0	Do.						

The table shows well the confusion given rise to by such fluctuations. Obviously temperature was not responsible for such behavior. It is clear that there are in this case two different latent period levels which appear. The reason for the change from one to the other is puzzling.

The existence of two latent period levels of a different nature was shown in the tests with NaOH. (Figs. 8 and 9.) In this case two typical curves crossed each other, and it was suggested that one curve represented the response to Na ions and the other that to OH ions. Crozier made studies of the sensory response of earthworms to acids (1918a) and to alkalies (1918b). His method was to measure the time required for withdrawal of the worm from the test solution. Certain of his logarithmic curves (1918b, fig. 3) show sharp breaks suggesting the existence of two intersecting curves. His method was not adapted to show whether each curve was continuous in both directions beyond the intersection. The response studied—locomotion for a certain distance—represents not a simple response but all of the neuromuscular activity involved in locomotion.

Crozier found it necessary to subtract from the reaction time a figure representing "the mechanical resistance to, or disadvantage of, its method of progression," in order for the effect, $\left(\frac{1}{R. T.}\right)$, to be directly proportional to the concentration. After subtraction of this factor the logarithmic curves make an angle of 45° with the y-axis. In the present experiments, also, it was found that a constant must be subtracted from the latent period values in order for the equation, $C \times T = K$, to obtain. While in the case of the complicated locomotor activity of the worm it is conceivable that the "mechanical disadvantage" might account for this factor, it appears improbable that such an explanation would suffice for the comparatively simple retraction of the delicate tentacles of the oyster. More likely appears the hypothesis that the layer of mucus constantly present serves to impede the penetration of the molecules of stimulating chemical. Further experiments are under way to clear up this point.

SUMMARY

(1) The tentacles on the mantle of the oyster react to chemical irritation by retracting sharply. They are sensitive to odorous compounds, such as cumarin, and to salts, acids, alkalies, and quinine, but show no clear reaction to cane sugar.

(2) Graphs plotted to show the relationship between concentration and latent period indicate that the effect, considered to be represented by the reciprocal of the latent period, is directly proportional to the concentration. However, in order for this to be the case it is necessary to subtract a constant from the latent period values. This constant is supposed to represent the impedance due to mucus covering the receptors.

(3) In certain cases it was found that the latent period fluctuated between two levels. For a series of concentrations the values obtained would fall into two well-separated and distinct curves, mathematically comparable. This does not appear to be due to fatigue or to variations in temperature.

(4) The latent period values for solutions of sodium hydroxide in sea water appear to be most clearly represented by two intersecting curves. Expressed logarithmically one of these makes an angle of less than 45° with the horizontal and may indicate the response to sodium cations; the angle of the other is approximately 45° and may

represent the effect of OH ions. Similarly plotted, the results for KOH fall into a single curve making an angle of less than 45°. If this interpretation is correct, the concentration of OH ions is directly proportional to the effect, but for the effect of ions of Na and K a factor must be introduced, as in the case of the salts, for this relationship to hold.

(5) The response of the adductor muscle to stimulation of the tentacles bears a relationship to concentration similar to that of the tentacular reaction, but the reaction time is longer.

(6) When mixtures of cumarin and quinine are employed the latent period of the reaction observed is the same as that of the substance which, alone, would produce reaction after the shorter latent period. The two substances act independently, one neither enhancing nor inhibiting the effect of the other.

(7) Mixtures of two salts, such as NaCl and KCl or NaCl and MgCl₂, are additive in effect and presumably act on the same endings in the same manner.

BIBLIOGRAPHY

AGERSBERG, H. P. KJERSCHOW.

1922. Some observations on qualitative chemical and physical stimulation in Nudibranchiate mollusks with special reference to the rôle of the "rhinopores." *Journal of Experimental Zoology*, vol. 36, No. 4, November, 1922, pp. 423-444, 1 fig. Philadelphia.

AREY, LESLIE B., and W. J. CROZIER.

1919. The sensory response of Chiton. *Journal of Experimental Zoology*, vol. 29, No. 2, October, 1919, pp. 157-260, 14 figs. Philadelphia.

COPELAND, MANTON.

1923. The chemical sense of *Palæmonetes vulgaris*. *Anatomical Record*, vol. 24, 1923, pp. 394-407.

COPELAND, MANTON, and H. L. WIEMAN.

1924. The chemical sense and feeding behavior of *Nereis virens*. *Biological Bulletin of the Marine Biological Laboratory*, Vol. XLVII, pp. 231-238, 1 fig. Woods Hole, Mass.

CROZIER, W. J.

1915. The sensory reactions of *Holothuria surinamensis* Ludwig. *Zoologischer Jahrbucher, Abteilung für Allgemeine Zoologie und Physiologie der Tiere*, Bd. XXXV, Nr. 3, 1915, pp. 233-297. Jena.

1916. Regarding the existence of a "common chemical sense" in vertebrates. *Journal of Comparative Neurology*, vol. 26, 1916, pp. 1-8.

1918. On sensory activation by alkalis. *American Journal of Physiology*, vol. 45, 1918, pp. 315-322. Boston.

- 1918a. Sensory activation by acids. *American Journal of Physiology*, vol. 45, 1918, pp. 323-341. Boston.

CROZIER, W. J., and LESLIE B. AREY.

1919. Sensory reactions of *Chromodoris zebra*. *Journal of Experimental Zoology*, vol. 29, No. 2, October, 1919, pp. 261-310, 8 figs. Philadelphia.

HECHT, SELIG.

1918. The physiology of *Ascidia atra* Lesueur. II. Sensory physiology. *Journal of Experimental Zoology*, vol. 25, No. 1, February, 1918, pp. 261-299, 2 figs. Philadelphia.

HOPKINS, A. E.

1932. Chemical stimulation by salts in the oyster, *Ostrea virginica*. *Journal of Experimental Zoology*. In press.

IRWIN, M.

1918. The nature of sensory stimulation by salts. *American Journal of Physiology*, vol. 47, 1918, pp. 265-277. Boston.

KRIBS, H. G.

1910. The reactions of Aelosoma to chemical stimuli. *Journal of Experimental Zoology*, Vol. VIII, No. 1, January, 1910, pp. 43-74, 2 figs. Philadelphia.

OLMSTEAD, J. M. P.

1917. The comparative physiology of *Synaptula hydriformis* (Lesueur). *Journal of Experimental Zoology*, vol. 24, No. 2, November, 1917, pp. 333-379, 2 figs. Philadelphia.

PARKER, G. H.

1908. The sensory reactions of *Amphioxus*. *Proceedings, American Academy of Arts and Sciences*, Vol. XLIII, No. 16, pp. 413-455.

PARKER, GEORGE H., and W. J. CROZIER.

1929. The chemical sense. *In* *The Foundations of Experimental Psychology*, edited by Carl Murchison, x+907 pp., Chapter 8, pp. 350-391, 24 figs. Clark University Press, Worcester, Mass.

