

THE EFFECT OF EXPOSURE PERIOD AND TEMPERATURE ON THE PHOTSENSORY PROCESS IN CIONA.

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I.

Mya and *Ciona* are two animals which are sensitive to light, and which respond to it by a vigorous retraction of the siphons. As a result of previous work¹ it is known that the receptor mechanism underlying this sensitivity to light is composed of two associated processes. The first of these takes place during the necessary exposure to light. It follows the Bunsen-Roscoe law, and is practically uninfluenced by temperature. This primary process is therefore photochemical in nature.

The characteristic siphon response of the animals does not take place immediately after the necessary exposure to light, but only after a definite latent period. The second peripheral process occurs during this latent period, and occupies practically all of it. Only an insignificant portion of it is concerned with the conduction of the stimulus. This secondary, latent period process in *Mya* has a temperature characteristic of $\mu = 19,680$, and is uninfluenced by light. It is therefore probably an ordinary "dark" chemical reaction.

The relation between the initial photochemical reaction and the secondary latent period reaction is such for *Mya* that the velocity of the latent period process is directly proportional to the concentration of the products resulting from the photochemical reaction. From

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¹ For a summary of much of this work see Hecht (1925).

this connection it has been assumed that the relation between the two reactions is in the nature of a catalysis. However, further information as to the possible chemical nature and interrelation of these two processes has not been available. The purpose of the present paper is to present data on the photic sensitivity of *Ciona* which make possible certain suppositions as to the nature of the two processes and the mode of their interaction.

II.

If the exposure of *Ciona* to light is maintained until the animal responds by a siphon retraction, the reaction time of the animal is of minimum duration. Exposure for shorter intervals, down to a certain point, results in no change in the reaction time. Exposure below this critical value (sensitization period) causes the resulting reaction time to increase. This change in total reaction time is obviously an increase in the latent period. Experiments were made to determine the relation between the length of exposure and the duration of the latent period.

To study this accurately, a carefully constructed shutter was used to control the exposure. The shutter consists essentially of a metal plate moving horizontally between the animal and a fixed opening through which shines the source of light. A variable opening in the moving plate determines the duration of the exposure. In principle this shutter therefore resembles the one described previously (Hecht, 1919-20), except that the horizontal movement of the plate is obtained by a falling weight instead of a spring. The significant point in a shutter of this type is to know accurately the speed of the moving plate. This is accomplished here by a simple device. As the plate moves, its forward end makes momentary electrical contact with five fixed points along its path. These contacts are recorded on a kymograph by a signal magnet, simultaneously with the record of an electrically driven tuning fork of 50 vibrations per second. Knowing the distance between the contacts and the time taken for the plate to go from one to another, it is simple to construct a graphic record of the movement of the plate. From this record the exposure is ascertained by reading off the time required for the opening in the plate to move past the stationary opening through which the light

passes to the animal. With each set of experiments ten kymograph records were made, and their average used as a measure of the exposures.

An experiment consists in measuring the reaction time of *Ciona* to a given intensity as the result of different exposures. A given ani-

TABLE I.

Relation between Exposure Period and Latent Period. Illumination Intensity Is 10,000 Meter Candles.

Series.	Exposure.	Reaction time.	Latent period.	Reciprocal of latent period.
	<i>sec.</i>	<i>sec.</i>	<i>sec.</i>	
IX, Mar. 28, 1925. 10 animals.	0.16	3.31	3.15	0.318
	0.29	2.44	2.15	0.465
	0.39	2.08	1.69	0.592
	0.49	1.91	1.42	0.704
	0.58	1.84	1.26	0.794
X, Apr. 5, 1925. 5 animals.	0.16	3.28	3.12	0.321
	0.29	2.48	2.19	0.457
	0.39	2.24	1.85	0.541
	0.49	2.08	1.59	0.629
	0.58	2.02	1.44	0.694
XIIIa, Apr. 14, 1925. 7 ani- mals.	0.16	3.37	3.21	0.312
	0.29	2.51	2.22	0.451
	0.40	2.21	1.81	0.553
	0.51	2.06	1.55	0.645
	0.60	2.00	1.40	0.714

mal, after thorough dark adaptation,² is exposed for a definite time to a certain intensity and the reaction time measured with a stop-

² A curious fact has come to light in this connection. After prolonged adaptation, say for overnight, the *first* reaction time of *Ciona* to a given intensity is always definitely *longer* than those which follow and which remain constant for long periods. For example, the first, second, and third measurements made at hourly intervals with three animals (Series IX) were respectively 3.6, 3.3, and 3.3; 4.2, 3.2, and 3.3; and 3.7, 2.9, and 2.9. About 10 hours dark adaptation is required to produce this effect. It represents an increase in the latent period, because the same effect is produced when very short and constant exposures are used. The phenomenon was not investigated further, but the precaution was always observed of taking an initial reading with an animal before an experiment was begun.

watch. It is then placed in the dark for an hour, after which its reaction time to the same intensity but to a different exposure is again measured. At hourly intervals the process is repeated until five or six exposures are tested. The measurements are then repeated in the reverse order, with the same intervals in the dark. The observations are made in the dark with the aid of a dim ruby lamp. To

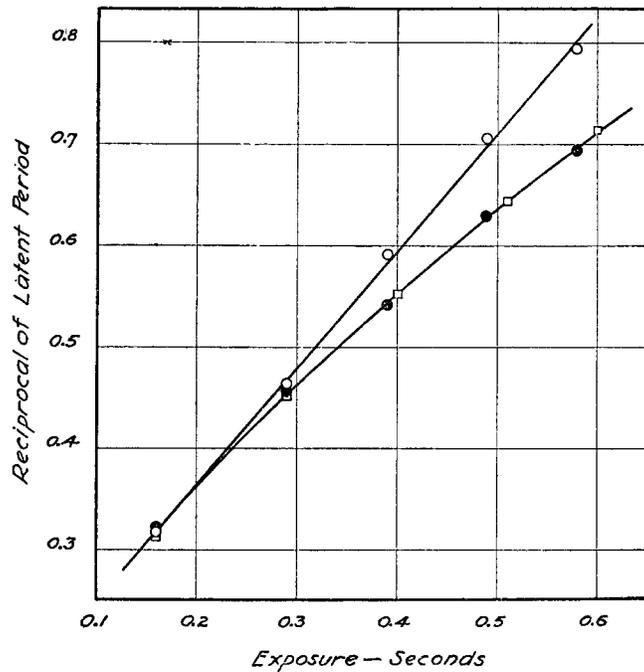


FIG. 1. Relation between the duration of the exposure to light and the reciprocal of the latent period. The connection between the two is practically linear, being exactly so for Series IX (white circles), and very nearly so for Series X (black circles) and XIIIa (squares).

this red light *Ciona* is absolutely insensitive, as tested by many independent experiments.

Three series of experiments were made, one with 10 animals, one with 7 animals, and one with 5 animals. The results, as Table I shows, are uniform in demonstrating that the reaction time shortens as the exposure lengthens. The fourth column in Table I gives the

values of the latent period corresponding to each exposure, and is the difference between reaction time and exposure period. It is clear that the latent period varies inversely with the exposure.

In order to interpret this relation, let it be assumed that in order to produce a stimulus,—and hence a response,—a definite amount of material must be formed as a result of the latent period process. The reciprocal of the time occupied by the process will therefore serve as a measure of the velocity of the reaction. These reciprocals are given in the fourth column of Table I, from which it follows that the velocity of the latent period process varies directly with the exposure. Fig. 1 shows that this relation is very nearly a linear one, being exactly linear in Series IX, and very nearly so in the other two series.

The exposure periods measure the time during which the light acts on the sensitive material in the sense cell. Since these exposures are very short intervals of time, the resulting photochemical effect may be considered, within the experimental error, as directly proportional to the exposure. Fig. 1 then shows that the velocity of the latent period process is directly proportional to the concentration of substances formed by the photochemical action of the light during the exposure.

So direct a relationship may obviously be interpreted in a variety of ways. A simple explanation is as follows. L is an indifferent (neutral) substance in the sense cell, and under proper conditions is converted into an active (charged) substance T , the accumulation of which in a definite quantity sets off chemically (electrically) the stimulus to the attached nerve. The proper conditions for this reaction $L \rightarrow T$ to proceed are furnished by the presence of photolytic products of the primary photochemical reaction. These photolytes may merely catalyze the reaction $L \rightarrow T$; or one of them, say a substance B , may actually combine with L so that the reaction will be $B + L \rightarrow T$. Either idea accounts for the relevant data. The velocity of catalyzed reactions is very often proportional to the concentration of catalyst. Similarly, if the substance L is present in excess, the velocity of $B + L \rightarrow T$ will be proportional to the concentration of B . The catalysis relation is simple and goes well with a completely reversible photochemical reaction. The direct chemical relation is compatible with a pseudoreversible photochemical reaction. The two ideas are

not mutually exclusive since catalytic phenomena may be chemical in nature.

The suggested relation between the two reactions is essentially that they form a coupled photochemical reaction. This carries with it the implication, often found with such processes, that the secondary "dark" reaction involves much more chemical change, both in number of molecules and in quantity of energy, than would be expected from the relatively small amount of light energy used by the primary photochemical reaction (*cf.* Bodenstein, 1913). A scheme of this kind is valuable in suggesting a plausible picture to account for the relatively great sensitivity of such photosensory processes as those present here, and more particularly in the retina.

III.

It has been assumed that the latent period process is a chemical reaction. Experiments to test the influence of temperature on the latent period confirm this assumption. They show that the order of magnitude of the temperature effect is clearly that associated with chemical reactions.

The experiments are very similar to those described in the last section, and consist in exposing *Ciona* to light for a fixed period shorter than the sensitization period, and measuring the resulting reaction time at different temperatures. The exposure is made with the shutter already described and is calibrated in like manner. The animals, in individual dishes, are kept in the dark in a large thermostat, the temperature of which is controlled by the addition of hot or cold water. An animal is kept at a given temperature at least half an hour before its reaction time is measured, and at least 1 hour in the dark is allowed between readings. One reading is made at each temperature.

Two series were run: one with 9 animals, and one with 5 animals. The data secured are given in Table II. The latent period is the difference between the measured reaction time and the measured exposure, since the exposure is well below the sensitization period (*cf.* Table I). The data show clearly that the latent period varies inversely with the temperature.

Since the exposure is fixed in duration, the only influence which

temperature could have on it is an increase in the velocity of the photochemical reaction, and a consequent increase in the concentration of photolytes present at the end of the period. This effect, however, is excluded first by the general fact that photochemical reactions are practically uninfluenced by temperature changes, and second by the specific findings with $M\gamma a$, where the temperature coefficient of the

TABLE II.

Relation between Temperature and Latent Period. Exposure Is 0.62 Seconds. Illumination Intensity Is 10,000 Meter Candles.

Series.	Temperature.	Reaction time.	Latent period.
	°C.	sec.	sec.
XIII, Apr. 12, 1925. 9 animals.	5.5	5.38	4.76
	7.5	3.87	3.25
	9.2	3.39	2.77
	10.9	2.95	2.33
	12.7	2.52	1.90
	13.7	2.38	1.76
	15.8	2.04	1.42
	18.0	1.72	1.10
	18.3	1.70	1.08
	20.7	1.52	0.90
XIV, Apr. 19, 1925. 5 animals.	7.3	6.12	5.50
	10.3	3.96	3.34
	11.7	3.63	3.01
	12.9	3.10	2.48
	15.5	2.60	1.98
	15.6	2.66	2.04
	17.5	2.23	1.61
	18.5	2.12	1.50
	18.9	2.06	1.44
	20.4	1.90	1.28

exposure period is 1.06 (Hecht, 1919-20). The effect of temperature in the present instance is therefore an influence on the latent period reaction alone.

The exact relation between this reaction and temperature is given by the Arrhenius equation

$$\ln \frac{k_2}{k_1} = \frac{\mu}{R} \left(\frac{1}{T_1} - \frac{1}{T_2} \right)$$

in which k_1 and k_2 are the velocity constants at the two absolute temperatures T_1 and T_2 , R is the gas constant, and μ the *temperature characteristic* (Crozier, 1924) which is constant for a given chemical reaction. The velocity constants k_1 and k_2 are unknown, but since only their ratio is necessary, we may substitute for them the reciprocals of the time required to produce the same chemical change. In the present instance the same amount of work is indicated by a retraction

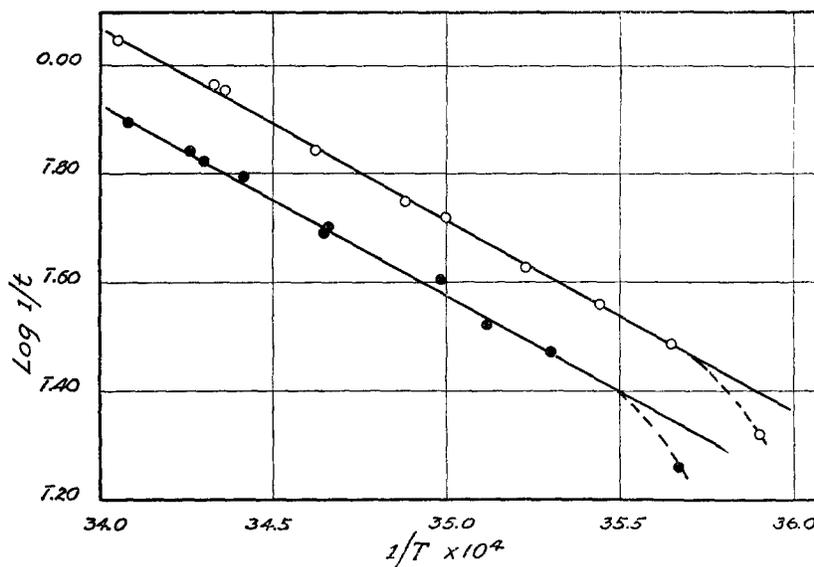


FIG. 2. Temperature and latent period. The points are computed from the experimental data, whereas the two straight lines give the Arrhenius equation when $\mu = 16,200$. The white circles are Series XIV, and the black circles are Series XIII.

at the end of the latent period. Therefore the ratio of the reciprocals of the latent period at different temperatures serve to give the value of μ .

The computation may be carried out graphically by plotting $\log \frac{1}{t}$ against $\frac{1}{T}$, where t is the latent period and T the absolute temperature. The data from the two series of experiments are given in Fig. 2. The two straight lines drawn so as best to express the

data give the values of the Arrhenius equation when $\mu = 16,200$. It is apparent that the two independent series give identical slopes, and therefore identical values of μ . The constancy of μ and its general order of magnitude indicate that the latent period for *Ciona* is a chemical reaction of fairly simple nature. This corroborates the similar findings with the latent period for *Mya*, where $\mu = 19,680$ (Hecht, 1918-19) and confirms the general similarity of the photochemical system in the 2 animals.

IV.

The term μ in the Arrhenius equation represents theoretically the energy required to transform a given species of molecule from an inactive to an active state. Rice (1923) has developed this idea to mean that since many apparently diverse chemical reactions yield the same value of μ , this energy of activation applies to a common molecule, for example, the hydrogen ion or the hydroxyl ion which is concerned as catalyst. Subsequently Crozier (1924, 1924-25) has shown that, theory aside, a great many diverse protoplasmic activities fall together into a comparatively small number of classes in each of which the processes possess an almost identical value of μ . This fact he has used to support the idea that a given value of μ indicates a common protoplasmic catalyst; and a skillful use of many data has enabled him to make suggestive identifications of some of the catalysts.

It is therefore not without significance that the numerical value of μ which the latent period yields is common to a large group of biological activities which are very probably oxidations. More particularly, it falls within that group of oxidations which seem to be associated with iron as catalyst, and for which the value of μ lies between 16,100 and 16,200 with striking precision (Crozier, 1924-25).

The fact that the latent period of *Ciona* is perhaps an oxidation reaction catalyzed by the presence of iron tempts one to present the aptness with which such an arrangement corresponds with the hypothetical scheme which has already been suggested for the chemical mechanism underlying the photosensory properties of *Ciona* and *Mya*, and to a certain extent of the retina (Hecht, 1925). The hypothesis is briefly this. A photosensitive substance is changed by the action

of light into one or more photolytes which are also perhaps the precursors from which the sensitive substance is regenerated. These photolytes, as they are formed, act as a catalyst for a secondary reaction, the products of which then set off the associated nerve ending in the form of a "stimulus." The mechanism is therefore that of a coupled photochemical reaction, of which the secondary is all ready to go, but must wait for an essential component (in this case a catalyst) until the products of the primary photochemical reaction are formed by the light.

According to the value of μ , the secondary (latent period) reaction for *Ciona* may be an oxidation catalyzed by iron. It is a noteworthy fact that iron in a variety of forms and compounds is very photosensitive; and it is possible that the initial reaction in the sensitivity of *Ciona* to light is the photochemical conversion of an iron compound into a catalytically active form. The secondary oxidation reaction can then proceed under its influence. Such photochemical reactions are known; a simple one is the photocatalytic decomposition of hydrogen peroxide in the presence of potassium ferrocyanide (Kistiakowsky, 1900; Winther, 1920). It is the iron compound which is acted upon by the light, because if it is first exposed to light and then added to the hydrogen peroxide in the dark, the usual liberation of oxygen takes place. In fact, by making the liberated oxygen do work (*e.g.* like closing an electrical circuit) it is possible to reproduce most of the essentials of such a sensory system as is found in *Ciona* and *Mya*. For example, a short exposure period causes a "response" after a latent period in the dark, and the length of this latent period may be changed by varying the exposure.

It would be of no advantage either to press such an analogy, or to attempt a more definite chemical statement of the sensory system in *Ciona*. It may, however, be worth adding, that though a sense cell is, and must needs be, a complex structure because of the mere fact that it is a living entity, its function as sense cell may still be carried out by a comparatively simple mechanism not much more involved than the one here outlined for the photic sensitivity of *Ciona*.

SUMMARY.

1. Experiments are presented which show that the latent period in the photosensory response of *Ciona* is inversely proportional to the duration of the exposure period to light. From this it is found that the velocity of the chemical reaction which determines the latent period is directly proportional to the concentration of photochemical products formed during the exposure period. This is interpreted as showing that the two processes form a coupled photochemical reaction, of which the secondary reaction proceeds only in the presence of products from the primary reaction. This coupling may be a catalysis or a direct chemical relation.

2. Further experiments show that the relation between temperature and the latent period is accurately described by the Arrhenius equation in which $\mu = 16,200$. The precise numerical value of μ tentatively identifies the latent period process as an oxidation reaction which is catalyzed by iron.

3. The photocatalytic properties of certain iron compounds are used as a model for the coupled photochemical reaction suggested for the photosensory mechanism of *Ciona* and *Mya*.

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