PHOTOCHEMISTRY OF VISUAL PURPLE.

II. The Effect of Temperature on the Bleaching of Visual Purple by Light.

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I.

1. The determination of the influence of temperature on the velocity of a photochemical reaction is always of importance. This is particularly true when the mechanism of the reaction is unknown, as in the present instance of the bleaching of visual purple.

The usefulness of determining the temperature coefficient lies in this. Photochemical reactions have low temperature coefficients,—near unity (Sheppard, p. 304), though exceptions are known. Ordinary chemical reactions, however, have temperature coefficients which are much higher, between 2 and 3 or even more. The order of magnitude of the temperature coefficient may therefore give us some notion of the degree of complexity of a photosensitive reaction. A value of Q_{10} near unity may be taken to indicate that the reaction probably consists of a simple, direct photochemical transformation. On the other hand, a high value for Q_{10} shows most probably that the photochemical reaction proper is only one in a series of reactions which makes up the photosensitive process. The associated chemical reactions, because of their high temperature coefficients, raise the temperature coefficient of the reaction as a whole more nearly to their own level.

An example of just such a condition is the high temperature coefficient (Q_{10} = 2.5) of the photosensory process in *Mya*. On analysis (Hecht, 1918–19, *a*; 1919–20, *b*) a series of at least three reactions has been shown to exist, of which the photochemical proper is only the first. This has a temperature coefficient of 1.06 when investigated independently; the values for the temperature coefficients of the other reactions are 2.5 (μ = 19,680) and 13.5 (μ = 48,500) respectively. An analysis of a similar hypothetical condition has been made by Osterhout (1917) which shows the relation of the total temperature coefficient of a process to the temperature coefficients of the individual reactions composing it.

2. In the first paper of this series (Hecht, 1920–21, *c*) it was shown that visual purple solution, so prepared as to give an irreversible photochemical reaction, is bleached by light according to the kinetics of a monomolecular reaction. This in itself would seem to indicate that the reaction as a whole is a simple photochemical transformation.

Experiments with intermittent as compared with constant illumination supported this conclusion. Within the limits of experimental error it was not possible to demonstrate the presence of any initial induction period or of an after effect in the course of the reaction. The presence of either or both of these irregularities frequently bespeaks a complexity in the make-up of the reaction (Mellor, 1904, p. 116).

The evidence therefore indicates that the bleaching of visual purple is a comparatively simple process. This is further confirmed by the following experiments on the effect of temperature.

II.

Visual purple solutions are prepared from the dark-adapted retinas of frogs by the technique previously described.[1] The changes produced by light are followed colorimetrically by comparison with concentration standards. These are made by combining bleached and unbleached visual purple in varying proportions so as to give a series changing by 10 per cent steps. The experimental solutions and the standards are manipulated in capillary tubes arranged to give equal depths of solution. For the composition and preparation of the standards and for the methods generally, the reader is referred to the previous article in these studies (Hecht, 1920–21, *c*) where full details will be found.

[1] In making solutions of visual purple, pure bile salts must be used. I am indebted to Dr. S. Morgulis for furnishing me with a supply of these salts purified by himself.

In order to control the temperature of the exposed solution the capillary tube containing it is kept in a water bath. This is a cylindrical glass vessel, 12 cm. in diameter, and of 900 cc. capacity. The temperature of the bath is maintained constant by the addition of water at a higher or a lower temperature from a nearby reservoir. In this way the temperature is easily kept within 1°C. Such a bath is accurate enough for our purposes, as will presently be evident.

The exposure tube of visual purple is immersed, attached to the rotating apparatus, and kept in the dark for 5 minutes for its contents to reach the temperature of the bath. This is more than enough when we recall that a thermometer having a larger diameter than the capillary tube comes into thermal equilibrium in much less time. After 5 minutes the shutter is opened, and the light allowed to act on the solution, which is rotated in the usual manner. After the proper exposure, the light is shut off, and the concentration of the exposed solution determined. As in the previously reported work, readings are made to the nearest 5 per cent. The tube is then returned to the bath, allowed 5 minutes in the dark to come to the temperature of the bath, and again exposed for an interval. The concentration is again determined, and the process repeated until the solution is fully bleached.

The source of illumination is a 250 watt, concentrated-filament, stereopticon Mazda lamp run on the ordinary lighting circuit. The intensity used throughout these experiments on temperature effects is 50 meter candles. At this intensity bleaching is complete in half an hour.

III.

A number of preliminary experiments showed definitely that the effect of temperature on the velocity of the bleaching process is very small indeed. In this I can confirm the roughly quantitative, early work of Kühne (1879). For example, in a series of three experiments performed, one at 7°C., another at 23°C., and a third at 36°C., the velocity constants for the bleaching process were 0.038, 0.041, and 0.039 respectively. This gives a value for the temperature coefficient as $Q_{10} = 1.00$. The same value for Q_{10} was found for a set of experiments in which the velocity of decomposition was determined at five temperatures covering the same interval as the previous three. However, in two other series of experiments performed at three temperatures as before, I obtained a value for $Q_{10} = 1.15$.

These four sets of preliminary experiments were carried out by the use of a single tube of visual purple for each temperature. Individual experimental errors may thus possibly account for the differences between the first two and the last two sets of experiments. Therefore, in order to be thoroughly certain of the results, I performed two sets of experiments in which three tubes of visual purple

TABLE I.

Temperature and Bleaching of Visual Purple.

Temperature.	$k=\frac{1}{t}\log\frac{a}{a-x}$	
	Series 1.	Series 2.
°C.		
5.2	0.039	0.031
20.0	0.035	0.031
36.1	0.036	0.033

were bleached simultaneously at each temperature. The velocity constants for the two series are given in Table I together with the temperatures. Each figure is the average of three experiments which agreed among themselves in a way similar to the figures previously quoted for one preliminary experiment.

IV.

1. From these results it will be seen that the change in the velocity constant over a range of 30 degrees is practically *nil*. Graphically this is demonstrated by Fig. 1, which presents the details of the second series of Table I. The rectangles are the averages of the experimental findings at the different temperatures, whereas the curve is the isotherm of a monomolecular reaction

$$k = \frac{1}{t}\log\frac{a}{a-x}$$

in which the velocity constant has the average value of $k = 0.032$. It is apparent that the points at all temperatures fit the single curve as well as can be expected.

It may therefore be concluded that the temperature coefficient for 10°C. for the bleaching of visual purple by light is practically 1.00. This corroborates our idea that the bleaching reaction is a simple photochemical transformation, consisting most probably of a single reaction.

2. There is an additional point to be made with regard to the magnitude of the temperature coefficient. In any photochemical

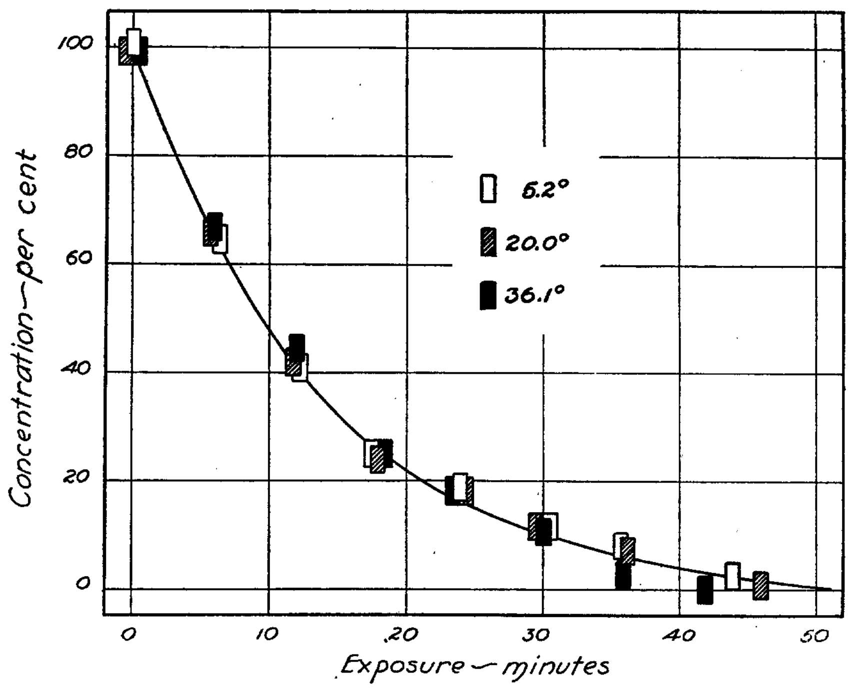

FIG. 1. Temperature and the velocity of photochemical decomposition of visual purple. The curve is a monomolecular isotherm; the rectangles are the experimental results of one set of experiments.

reaction there is always the possibility that the reaction proper takes place with a practically instantaneous velocity at the walls of the vessel, and that the kinetics of the reaction really represent the diffusion velocity of the photosensitive substance to the place of reaction.

The temperature coefficient of diffusion velocities, though lower than those of ordinary chemical reactions, is usually higher than 1.00,

—generally near 1.3 (Höber, 1914, p. 708). The fact that the temperature coefficient of visual purple bleaching is 1.00, combined with the other evidence already at hand, would therefore indicate that the kinetics of the reaction represent a real chemical occurrence rather than a process of diffusion.

SUMMARY.

The temperature coefficient of the bleaching of visual purple by light is 1.00 over a range of 30 degrees. This indicates that the monomolecular course of the reaction represents a real chemical process, as opposed to a possible diffusion process, and that the reaction is probably simple in nature.

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