Since the New York soil contained only living organisms of *B. radicicola* known to be capable of inoculating alfalfa, the inoculation of alfalfa by the organism isolated from the New York soil was to be expected.

It seems fair to conclude that *B. radicicola* grows but sparingly and shows no especial characteristics upon synthetic agar made in accordance with the formula reported by Grieg-Smith, which seems to be no more selective than the synthetic agar we have employed for many years in the Washington laboratories, and is perhaps less selective than the congo-red agar described by one of us.[3] Further development of technique or of culture media will be required before we may hope to secure reliable data regarding the relative distribution and quantitative function of *B. radicicola* in the soil.

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SOME EFFECTS OF SUNLIGHT ON THE STARFISH

Starfish have been much studied for their reactions to light. Their general reactions and behavior have been well described by Preyer, von Uexkull, Jennings and others, and there is general agreement in the results recorded by these writers. Details of behavior of the different parts affected by light are for the most part meager or omitted.

The general reactions of *Asterias forbesii* are essentially like those described for other starfish and there is no reason to suppose that its reactions are essentially different in detail so far as it is possible to observe them. It has been previously shown by the writer[1] that certain parts of the animal are sensitive to light. It has further been found that there is a definite time reaction between the moment when the light strikes the sensitive parts and the moment when they show a definite visible response, and the general reaction which follows, provided the light has sufficient intensity.

Individuals without the pigment or "eye" spots react as definitely to light as do those with the pigment spots intact. This was also found to be true for *Echinaster* (Cowles). The upper surface, the sides of the rays, the ventral surface and the tube feet are sensitive to light, since they show a direct response to it. The dermal branchia also show response to light stimuli. The behavior of dermal branchia is of peculiar interest, since their retraction must influence the extent of the aerating surface of the animal. The sudden illumination of a ray or a spot on it causes a retraction of the parts illuminated. If the area is large there is a bending of the ray ventralward no matter what the direction of the source of light. Following this primary reflex, there arise movements which lead eventually to the general response or behavior. Three stages are recognizable. These are: the initial or direct effect of light; the local direct response of the parts affected, and lastly the general effect and reactions in response to the influence of the preceding changes. It is apparently through these interactions that the external stimulus is finally transformed into reaction and behavior through the vortex of metabolic changes in protoplasm.

Loeb has maintained that "reactions are caused by a chemical effect of light" and that "the velocity or the character of the chemical reactions in the photosensitive elements of both sides of the body is different," and hence "the muscles or the contractile elements on one side of the organism are in a higher state of tension than their antagonists." One wishes for more direct evidence and, if such is possible, direct proof that light does influence the chemical processes of normal metabolism, than the above assumptions afford. While it is generally assumed that light does cause chemical changes in organisms and these must influence the reactions of the organisms, there is a significant absence of direct experimental proof.

[3] Kellerman, Karl F., "The Relation of Crown-gall to Legume Inoculation," U. S. Department of Agriculture, Bureau of Plant Industry, Circular 76, p. 4, 1911.

[1] Science, N. S., Vol. 35, p. 119.

Jennings sought an explanation of behavior based on physiological grounds and concluded that since the organism may react differently under apparently similar conditions, reactions are due to differences in physiological states. He cites instances in which the physiological conditions, such as hunger, for example, are known to modify reactions.

Mast (1911, page 369) admits that the "belief that light in some way influences the activity of organisms by chemical changes which it causes in them" is founded on hypothetical assumptions. Any direct evidence either in agreement with or opposed to these views, although it may need further verification, would be of importance.

It must be remembered that little is positively known concerning the character of chemical changes in metabolic processes. It is true, however, that of the various physiological states, or conditions which might effect them, the maintenance of the neutral or slightly alkaline condition in an organism is of the greatest importance, and this condition is not easily changed. Any change in this state it should be possible to detect provided a proper means be found. It is assumed that the organization of protoplasm involves and demands physical-chemical relations and changes of a progressive kind, with some range of disturbance possible without causing complete disorganization or breaking down of the chain of changes. These changes must be maintained within the limits of the conditions which make possible their continued recurrence. This has aptly been likened to a "vortex."

The natural result of a stimulus breaking in upon these regular changes may be to stop some, accelerate others, divert others into combinations different from those which would normally occur. That the stimulus (light) would cause a chemical change which would be the cause of the reaction is limiting the possibilities. From the viewpoint of the physiological processes it becomes a matter of importance to discover the nature of these disturbances. As previously stated, an acid or alkaline condition is of primary significance, the right condition being maintained through the interaction of certain basic and acid substances present. If it is not possible to detect these conditions directly it might still be possible to discover variations in the amount of elimination of products or alteration in their character. Accordingly, an attempt was made to discover any possible difference in these conditions.

To test for differences in respiration in the starfish two methods were used. In one series of experiments an indicator for carbon dioxide was introduced into the given amount of sea water with the specimen to be tested. Parallel experiments, one in the shade and one in the sunlight and one control, were compared. In a second series specimens were exposed in the shade and the sunlight in equal amounts of tested sea water, the sea water then after equal intervals of time being again tested.

Having made use of neutral red in class observation on the reaction of protoplasm and vacuoles in *Paramœcia,* this was tried in the starfish. Furthermore, neutral red might also show differences in *intra vitam* staining in light and shade. Dilute solutions of neutral red were made in sea water which is normally slightly alkaline in reaction, from 1:10,000 to 1:60,000. A more dilute solution was used in some cases. Given amounts, 200 c.c. to 400 c.c. of the same solution were placed in each of three large clean finger-bowls. One of these was kept for control. Two starfish equal in weight and as nearly alike as it is possible to select, which were found to react normally to light were placed one in each of the other two vessels. One of these vessels was then placed in the sunlight and the other in the shade. Both vessels were placed in a shallow aquarium of fresh sea water in order to maintain equality of temperature 18° centigrade. At intervals of two or five minutes a careful comparison was made to note possible changes in activity and degree of staining shown by each specimen. In practically every experiment at the end of five minutes, solutions and specimens showed distinct differences. In the vessel in the shade the solution showed a characteristic acid reaction, while at the same time

the one in the sunlight showed a very distinctly less amount of change, but when compared with the control it gave evidence of change. The specimen in the shade was usually more distinctly stained by the neutral red than the specimen in the sunlight, and the solution in the shade was apparently clear after the lapse of fifteen to thirty minutes, while that in the sunlight still distinctly showed the stain in solution. As might be expected in some of the experiments, the differences were more distinct than in others. It is taken that the acid reaction is due to the elimination of carbon dioxide.

A toxic effect was also evident in the experiments in the sunlight due probably to the action of the basic elements of the dye. What this is still remains to be determined. It is apparently due to effect of sunlight on protoplasm influencing metabolism in such a manner that the injurious changes occur; or it may be the effect of sunlight on the interaction of the basic dye and protoplasm or its metabolic products. A similar effect is seen in experiments with *Paramœcia*. In the sunlight there is a greater concentration of the hydroxyl ions which would give an alkaline reaction. The outcome is that hydrolysis takes place which interferes with the normal processes and produces injury to the protoplasm. In the shade the hydrogen ions have a greater concentration with the more acid reaction.

As a check upon these results a second set of experiments was made in which the reaction of the sea water was tested in which the specimens were placed without the presence of the indicator. In this series equal quantities of sea water, after being tested with the most accurate apparatus, were placed with carefully selected individuals in clean glass vessels and arranged, as in the former series, in the sun and in the shade. In this series it was possible to use the same specimen for the test at different times after exposure for equal intervals of time in the sun and in the shade. The results agreed as closely as could be expected with those in the former series.

In testing the sea water in each case an N/10 solution of hydrochloric acid and an N/10 solution of sodium hydroxide, and phenolphtalein were used. It was found in a series of ten parallel experiments that at equal intervals of time after the lapse of about five minutes from the beginning of each experiment up to fifteen minutes, the sea water from the vessels in the sunlight showed less acid reaction than that taken from those in the shade. In four cases the sea water with the specimens in the sunlight remained slightly alkaline, but less so than the normal sea water; four showed a slightly acid reaction, the two remaining were neutral. Of the parallel series in the shade at the same intervals of time, seven showed an acid reaction, two were neutral and one was very slightly alkaline. Normal sea water is alkaline. It thus appears that the metabolic processes of protoplasm under these different conditions of illumination differ to a degree sufficient to affect the sea water through differences in elimination of the products of metabolism. It is to be remembered that ten or fifteen minutes is usually sufficient for continuous sunshine to cause a starfish to take up a characteristic fixed position with respect to the light in as protected a place as possible.

These experiments show that sunlight modifies the normal physiological changes taking place in protoplasm, checking some of the processes and probably accelerating others. It appears that the acid and alkaline relations are affected probably through a disturbance in the relations of the hydrogen and the hydroxyl ions. The starfish with one half of its upper surface in the light and one half in the shade moves from the light into the shade because of this interference with its normal physiological activities.

These experiments were performed in the Biological Laboratory of the Brooklyn Institute of Arts and Sciences, Cold Spring Harbor, Long Island, July and August, and I am under obligations to Dr. C. B. Davenport, the director of the laboratory, for the privileges and opportunities so kindly extended.

Hansford MacCurdy

Alma College,
October 3, 1912