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IV. *On the Action of Light on Diastase, and its Biological Significance.*

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In the course of a series of investigations into the chemistry and physiology of foliage leaves, made some years ago by Messrs. Brown and Morris,* among other discoveries of interest, those observers ascertained that the quantity of diastase that the leaves of many different species of plants can be shown to contain, varies considerably during a day of twenty-four hours. It is at its maximum in leaves gathered in the early morning, and at its minimum after a period of exposure to light, as in leaves gathered at 5 P.M. Those observers put forward two hypotheses to explain this phenomenon. The first is that during the day, particularly when the sun is shining, the diastase is used up gradually in dissolving a portion of the starch as it is formed in the chloroplasts, such portion being converted into sugar to meet the immediate needs of the cell protoplasm. This theory involves the acceptance of the view that the starch of the chloroplasts is the final product of the assimilation of the CO_2 absorbed and that its manufacture is altogether an "upgrade" process. Their experiments, however, led them to contest this assumption, and to put forward in contradistinction to it the theory that starch is, in the chloroplasts as elsewhere, always a reserve product, and that its appearance indicates that more carbohydrate is being formed than can be at once either used by the protoplasm or removed from the cell, the surplus being temporarily deposited by the protoplasm as starch. On this view the needs of the cell do not call for the conversion of starch into sugar, but are supplied from the sugar, which all botanists agree is antecedent to starch. They therefore reject this hypothesis, in the light of the experiments referred to, which are detailed in their memoir.

Their second hypothesis, which they regard as a more probable one, is that the formation or secretion of diastase is irregular, being a starvation phenomenon, none of the enzyme being formed until the needs of the cell demand the transformation of

* "A Contribution to the Chemistry and Physiology of Foliage Leaves," 'Journ. Chem. Soc.,' May, 1893.

the stored starch, either for immediate consumption in the cell, or for removal to a more permanent reservoir.

There is a good deal to be said for this view; indeed the authors give some cogent evidence in support of their hypothesis. If we admit, however, that it is probably sound, it is insufficient to explain all the facts, for it gives no reason for the actual diminution of the diastase which can be observed after a subsequent period of illumination.

From the observations of MARSHALL WARD* and other observers who have studied the effect of light upon the living substance of many micro-organisms, it seems at least possible that the light to which the leaves are exposed during the daytime may have a destructive effect upon the enzyme, as it has upon the life of the bacteria and other lowly forms that have been investigated. This hypothesis need not, of course, vitiate the conclusions of BROWN and MORRIS, that diastase is secreted under the conditions they suggest; it rather supplements than contravenes them.

Some time after the appearance of BROWN and MORRIS's memoir, the author undertook a series of experiments to test the validity of the supposition just put forward. These have now extended over a period of three years, and have led to certain conclusions as to the effect of light in general, and of the different regions of the spectrum in particular, upon the secretion and destruction of diastase, which, perhaps, throw some light upon other problems connected with the nutrition of the vegetable organism.

Method of Experiment.

In carrying out these investigations various preparations of diastase were used. They comprised extract of malt, extract of foliage leaves (usually those of *Phaseolus vulgaris*), and diluted human saliva. To prepare the malt diastase, some ground malt was usually steeped in water for several hours and the mixture filtered. Alcohol was added to the filtrate till it contained about 30 per cent., when a flocculent precipitate fell. This was separated by filtration and dissolved in a feebly antiseptic solution, containing ·2 per cent. of potassic cyanide. In a few experiments the malt extract was used without the precipitation by alcohol, but the presence of sugar and colouring matter involved difficulties in the subsequent treatment. Experiments showed that 30 per cent. of alcohol caused the greater part of the diastase to be thrown down. The solution, however, always contained a little proteid matter.

The extract of the Phaseolus leaves was prepared by grinding the leaves, either fresh gathered, or after drying them in an air bath at 35° C., into a paste in a mortar and extracting them for several hours in water, the same proportion of KCN being added as an antiseptic. They were then filtered and the extract used without

* "On the Action of Light on Bacteria," 'Phil. Trans.,' vol. 185, B, pp. 961–986, 1895. (Other papers are quoted in Professor WARD's Memoir.)

further treatment. It was usually faintly tinged with green and contained a little proteid matter. When examined by the spectroscope it gave an absorption spectrum which will be described in a subsequent section of this paper. In a few cases the leaves before being dried were killed by exposure to the vapour of chloroform.

To prepare the salivary diastase, freshly-secreted saliva was diluted by the addition of about an equal bulk of water and the mucin was precipitated by a trace of acetic acid. It was then filtered and carefully neutralised by either dilute ammonia or crystals of potassium bicarbonate. The same antiseptic was added as in the other cases, ·2 per cent. being the quantity always present. The solution so prepared was faintly opalescent and contained a trace of proteid matter.

The diastasic extracts so prepared were found to keep extremely well, many preparations being quite unchanged after six months. The antiseptic used was found to be quite without effect upon the enzyme.

The extracts were exposed in different media to the action of the light for several hours; simultaneously further quantities were placed in exactly similar conditions to the exposed ones, except that they were protected in various ways from any illumination. Each experiment was thus carefully controlled, and every precaution was taken that the further results might be strictly comparable.

The source of illumination was in some cases sunlight, either that of a bright sun, or of the light reflected from a blue sky or light clouds. In some cases a dimmer light was employed, such as is reflected from the denser clouds of a wintry sky. A large number of observations were made by the use of the electric arc. Through the kindness of Mr. Barker, the Manager of the Electric Light Works, at Cambridge, the author had access to a naked arc lamp, of about 2000 candle power, which was made to play upon the extracts at a distance of about two feet.

During the exposures the temperature which was reached was ascertained by the use of a maximum thermometer. When the electric light was used, this rarely exceeded 27° or 28° C. When sunlight was employed and glass vessels used for the extracts, the illuminated and shielded vessels were exposed side by side in a beaker of water, so that the temperature of both should be the same.

The effect of an exposure for the usual duration of the illumination to a temperature of 27° C. was ascertained separately, and found to be but slight. It was taken into account, where necessary, in comparing the results of different experiments.

In some of the experiments, glass vessels were used to contain the extracts during illumination. These were, at first, tubes, but as the shape of the tube seemed likely to introduce an error, these were abandoned in favour of small flat-sided glass bottles, made to contain about 20 cub. centims. of fluid. The thickness of the liquid in these was about 5 millims.

As glass, however, has been shown by many observers* to be opaque to the ultra-violet rays, when these rays were under observation ebonite cells were employed, the

* Ward, *op. cit.*

faces of which were covered by quartz plates, cemented to the ebonite by gold size. Some comparative experiments on the effect of glass and quartz as the covering of the cells, were detailed by the author at the meeting of the British Association at Oxford in 1894.*

In a few experiments the diastasic solutions were mixed with agar-agar, at a temperature at which the agar-agar was on the point of gelatinisation, and the mixture poured over a flat glass dish, on which it almost immediately formed a film a few millimetres in thickness. The uncovered side was then illuminated.

After the exposure, equal volumes of the exposed and unexposed extracts were mixed with a preparation of starch and digested either at the laboratory temperature, or in an incubator at about 38° C. When the hydrolysis was fairly advanced, both digestions were boiled simultaneously and the products of hydrolysis ascertained by titration with FEHLING'S fluid. In the earlier experiments a weak starch paste (1 per cent.) was employed, but this was afterwards abandoned in favour of a 1 per cent. solution of soluble starch prepared by LINTNER'S method. Some dry potato starch was mixed with hydrochloric acid of 12 per cent. strength, and left in contact with the acid for some days. It was then strained off and dialysed in running water till all traces of the acid had disappeared. The starch was then readily soluble in boiling water, giving a solution which was but slightly opalescent and which filtered rapidly. The solution gave but a faint trace of cuprous oxide when boiled with FEHLING'S fluid, and became pure blue in colour when a little iodine was added to it.

The results of such a digestion, when expressed in terms of the weight of cupric oxide found on combustion, have been ascertained by KJELDAHL† to be proportional to the quantity of enzyme present, if the starch transformation is not allowed to fall below a cupric-reducing power of K 25–30 for the mixed products of hydrolysis.

The titration of the result of each experiment was performed by making the volume up to 100 cub. centims. with water, and boiling it with an excess of FEHLING'S fluid for fifteen minutes. It was then rapidly filtered, and the filter washed several times with boiling water. The filter paper with the cuprous oxide was then dried and burned in a platinum crucible till the oxide was all converted to the cupric condition, and the weight ascertained to the fourth place of decimals. With the comparatively small quantities of oxide present, this method was found to give accurate results.

In all cases where the extract contained sugar, as when fresh malt or leaf extract was used, a separate determination was made of the reduction caused by the volume of the extract, which was deducted from the results in the digestions under examination.

* 'Annals of Botany,' September, 1894.

† KJELDAHL, 'Résumé du Compte Rendu du Laboratoire de Carlsberg,' 1, 1879.

The Effect Produced by Exposure to all the Rays of the Spectrum.

As glass has been shown to be opaque to the ultra-violet rays of the electric light, it was necessary to expose the extract to the illumination without its interference. The first experiments were performed by mixing a diastasic solution with a little agar-agar, and supporting a film so prepared upon a shallow glass dish.

A quantity of malt was ground and extracted for several hours with a ·2 per cent. solution of potassium cyanide. It was then filtered and yielded a yellowish solution. Alcohol was added till about 30 per cent. was present, when a white precipitate fell. This was rapidly filtered off, drained, and dissolved in the same antiseptic liquid. A quantity of agar-agar solution, containing 3 per cent. of the jelly, was prepared, and just before it was cool enough to gelatinise, a quantity of the diastasic solution was stirred into it. Half of it was then poured into each of two Petri dishes, forming a film of about 3 millims. thickness, and the two allowed to cool. One of these was exposed for 10 to 12 hours to the naked light of the electric arc at a distance of about 2 feet. The lamp was of about 2000 candle-power. After removal, the agar-agar, which had become partially dried, was swelled up again by the addition of sufficient water. The two films, labelled L and D, were then detached from their supports and mixed carefully with 25 cub. centims. of 1 per cent. starch paste, care being taken to make the incorporation of the jelly with the paste as complete as possible. They were then digested in an incubator at 38° C. for 20 hours, the progress of hydrolysis being tested from time to time by adding iodine to a drop or two upon a porcelain slab. The exposed film was much less active than the non-exposed one. When the iodine showed the digestion to be well advanced, the two vessels were removed and simultaneously heated to 100° C., and the sugar titrated as described above.

The resulting weights of cupric oxide were D ·105 grm., L ·028 grm., showing a destruction of diastase in the proportion of about 78 per cent.

Other experiments with agar-agar were less satisfactory, owing to the difficulties of manipulation, and, instead of continuing the use of this medium, it was found better to expose the extracts to illumination in quartz-fronted cells. The first experiment under these conditions was performed with some of the same extract used in the one detailed above, when it was mixed with the agar-agar. The extract was placed in an ebonite cell, the face of which was covered by a quartz plate, about 3 millims. in thickness, which was cemented on by gold size. The cell was placed at the same distance from the lamp as the agar film had been, and remained there for 10 to 12 hours.

5 cub. centims. of this exposed extract was then added to 24 cub. centims. of 1 per cent. starch paste, and a similar quantity of the extract that had not been illuminated was added to a further 24 cub. centims. of the same. They were labelled L and D, and digested for $2\frac{1}{2}$ hours in an incubator at 38° C. The action

was more rapid than when the agar films were used, but the same difference in power was speedily apparent. On titration the weights of cupric oxide yielded were D ·012 grm., L ·005 grm., or about 58 per cent. of the diastase had been destroyed.

These results were repeatedly confirmed, using extracts of various strengths, and varying the times of exposure and the conditions of the subsequent digestions. Under the conditions of exposure quoted, however, the experiments agreed in showing a considerable destruction of the diastase of malt when exposed to the total rays of the spectrum.

Further experiments were then undertaken, using saliva instead of malt extract. It would be tedious to give details of all; a typical one may therefore be selected.

Two cells, with quartz fronts, were taken, and the outer face of one covered over with blackened paper, impervious to light. In each, 15 cub. centims. of saliva, prepared as described above, were exposed to the electric arc for 12 to 14 hours. After removal, 5 cub. centims. of each were mixed with 20 cub. centims. of the same starch paste and digested in the incubator at 40° C. On titration the weights of cupric oxide were D ·0658 grm., L ·0328 grm., so that a little more than half the ptyalin had been destroyed.

A similar result was arrived at with the diastase that can be extracted from foliage leaves. Some leaves of *Phaseolus vulgaris* were made into a pulp in a mortar and extracted with ·2 per cent. potassic cyanide solution. The solution when filtered was pale yellowish-green, but its spectrum showed no absorption bands, except a very faint narrow one at 680 to 690 $\mu\mu$. It cut off the red up to 720 $\mu\mu$, and the blue beyond 480 $\mu\mu$. Exposed for $9\frac{1}{2}$ hours to the electric arc, digested side by side with an unexposed control, and the digestion titrated, the weights of cupric oxide were L ·17 grm., D ·185 grm., a loss of diastase amounting to about 8 per cent.

The effect of the total rays of the spectrum, when allowed to act upon diastase, whether of animal or vegetable origin, is, therefore, destructive, the destruction varying however in amount under different conditions of exposure. The destruction further is progressive, the impairment of diastasic power continuing after removal from the light. An experiment bearing on this point is subjoined :—

Some solution of malt diastase, prepared as in the experiments already described, was exposed to the electric arc in a quartz-fronted cell for 10 to 11 hours, while a control was kept in darkness. 5 cub. centims. of each were then mixed with 25 cub. centims. of 1 per cent. starch paste and digested as usual. On titration the amounts of cupric oxide yielded were D ·085 grm., L ·036 grm., showing a destruction of 42 per cent. of the enzyme. The remainder of each was then set aside for six weeks, when another digestion experiment, in all respects the same as the first, was conducted with them. The final weights of cupric oxide were D ·089 grm., L ·001 grm., showing that the destructive action had gone on until almost all the diastase had disappeared.

The Effect of Light on Extracts Contained in Glass Vessels.

A series of experiments was next undertaken with the view of ascertaining in what part of the spectrum the deleterious rays are situated. The experiments of MARSHALL WARD on the influence of light on bacteria have shown that, so far as the latter organisms are concerned, the destructive effect was confined to the blue, violet, and ultra-violet rays, all the red end and the infra-red rays being harmless to them. As already pointed out, glass has been shown to be opaque to the ultra-violet rays, or at least to the ultra-violet of the spectrum of the electric arc. By the use of glass vessels, therefore, the effect of the rays of the visible spectrum and of the infra-red can be examined.

Experiments made with the electric arc, using a solution of precipitated malt diastase, gave a striking result. Two of these, which differed in the conditions of exposure, are detailed below.

20 cub. centims. of the diastasic solution were exposed in a glass cell to a lamp of about 500 candle power for 3 hours. 10 cub. centims. of this and 10 cub. centims. of an unexposed quantity of the same extract were then digested for 4 hours with 25 cub. centims. of starch paste. After titration the weights of cupric oxide were L ·0325 grm., D ·029 grm., or an increase of diastase amounting to 12 per cent.

In the second experiment the exposure was made for 20 hours to the larger arc light, of about 2000 candle power. 12 cub. centims. of the exposed and 12 cub. centims. of the unexposed solution were digested, each with 25 cub. centims. of 1 per cent. starch paste, at 39° C. for $3\frac{1}{4}$ hours. After titration the weights of cupric oxide were L ·04 grm., D ·03 grm., an increase of diastase amounting to 33 per cent.

As this investigation was undertaken at the outset to ascertain the effect of sunlight on the diastase of the leaf, some experiments may be recorded here in which varying intensities of sunlight were studied instead of the light of the electric arc. Several of these gave instructive results.

Three tubes containing a solution of malt diastase were taken; one, A, was placed in sunshine in the laboratory window; one, B, in diffused light in another window to which the sun had not access; and a third, C, was kept in darkness, the temperatures being kept equal by standing them in beakers of water. After 24 hours, during which there had been about 4 hours bright sunlight, they were examined in the usual way. After titration the weights of cupric oxide were A ·0845 grm., B ·0875 grm., C ·0845 grm. The exposure to bright sun had been without effect, while the effect of the diffused light had been to very slightly increase the diastase.

Another experiment, carried out under the same conditions, except that no observation was made on diffused light, showed that after an exposure of two days, during which there was a moderate amount of sunshine, the effect of the light was again slightly to increase the diastase.

The next experiment was continued for 10 days, putrefaction being guarded against by ·2 per cent. potassic cyanide. Two tubes of malt diastase solution were placed side by side in a beaker of water in the laboratory window, one being covered over with black paper. During this time there was a fair amount of sunshine. After 10 days exposure 15 cub. centims. of each were digested with 45 cub. centims. of 1 per cent. starch paste for 45 minutes at 38° C. After titration the weights of cupric oxide yielded were D ·23 grm., L ·09 grm. The 15 cub. centims. malt extract used contained sugar which, when titrated, gave ·08 grm. of cupric oxide. Deducting this, the values for the diastase in D and L were ·15 grm. and ·01 grm. respectively, a destruction in the latter case of 93 per cent.

Other experiments with malt extract gave a considerable destruction of the diastase after exposures lasting from 2 to 5 days.

Experiments with saliva, prepared as in other cases, gave similar results to those obtained with malt diastase.

Two tubes, A and B, were exposed in a beaker of water in the laboratory window for 9 days, B being covered with black paper. 17 cub. centims. of each were then digested with 40 cub. centims. 1 per cent. starch paste at the laboratory temperature for 1 hour. At the end of that time the sugar in each was titrated, the results being, in weights of cupric oxide, A ·08 grm., B ·132 grm., a destruction of the enzyme amounting to 39 per cent.

A further experiment, exposing the solution to diffused light, gave an explanation of the discrepancies hitherto observed.

Two tubes of diluted saliva, prepared as already described, were placed in a beaker of water in the laboratory window, A being exposed to the light, and B covered with a black paper screen. There was a fair amount of sunshine during part of each day for 3 days. The extracts were then examined in the usual way, and for the whole 3 days there was a gradual increase in the diastase. The tubes were then allowed to stand in the window for 8 weeks longer, from November 6th to January 2nd, and they were again examined. The result of the titration of the digestions was then, in weights of cupric oxide, A ·034 grm. B ·0634 grm., a destruction of enzyme amounting to 46 per cent.

The result of this series of experiments thus shows that the action of light on diastase is twofold. When the whole spectrum is employed the effect is the destruction of the enzyme; when only the visible and infra-red portions are used, there is first an increase of diastase, which on longer exposure is destroyed. The destructive rays are mainly those of the ultra-violet, but they are not altogether confined to that region, certain of the visible rays having the same property, but possessing a much feebler power. Certain other rays, whose situation the experiments so far have not accurately determined, have, on the other hand, the power of

increasing the amount of diastase in the solution. These may be suspected from the experiments already quoted to be situated at the red end of the spectrum.

An experiment may be quoted here which shows in a striking manner the difference between the two regions of the spectrum. Two cells were taken, one of which (Q), was faced with a quartz plate, the other (G) with a glass one. Each contained 10 cub. centims. of diluted saliva, and a third 10 cub. centims. was reserved in darkness as a standard (D). The two cells were exposed for about 12 hours to the electric arc. 2 cub. centims. of each were then digested for 45 minutes with 25 cub. centims. of 1 per cent. starch paste, boiled and titrated as before. The weights of cupric oxide resulting were: D (standard) ·0695 grm., Q ·05 grm., G ·074 grm. The diastase in Q was diminished therefore by 28 per cent., while that in G was increased by about 6·5 per cent.

Observations tending to localise definitely these different effects will be detailed later.

Effect of Light upon the Diastase in the Living Leaf.

It seemed obvious that of the two effects described, both must play a considerable part in the metabolism of the plant, for though the destruction of diastase appears to be the ultimate effect of the illumination, it is probably preceded by the greater production of the enzyme under the influence of the less refrangible rays, the latter phenomenon being shown to be the first observable under such conditions as allow both to be demonstrated, and to be in magnitude of considerable importance.

The destruction of diastase being, however, the result of illumination by the entire spectrum, the action of the latter upon the living leaf was the next subject of enquiry. The experiments were made upon what is known as the half-leaf plan, and the plant selected was the common scarlet runner, *Phaseolus vulgaris*, that plant having been shown by Brown and Morris to contain a good deal of diastase in its leaves. Leaves were gathered in the early morning, about 5 A.M., and were supported on a frame with their stalks in a test-tube containing water. The leaf of *Phaseolus* is ternate and each leaflet is of considerable size. Half of each of the three leaflets was covered by a piece of blackened paper up to the mid-rib, and the frame was then placed in the sunshine in the open air. The exposures lasted for several hours, and the position of the frame was adjusted repeatedly to face the sun as the day advanced. At the end of the exposure the leaves were killed by exposure to chloroform vapour and dried in the hot-air oven at 35–40° C. The mid-rib and main veins were then cut away, and equal weights of the dried leaf blades on the shaded and unshaded sides of the mid-rib taken for experiment. These were powdered and made into a paste in a mortar and mixed with equal volumes of a 1 per cent. solution of soluble starch, prepared as already described, for the estimation of their relative diastasic powers.

In the first experiment made in this manner the leaf was exposed to a bright sun

for 8 hours. The blades without the mid-ribs and main veins were used for the digestions, and the latter were conducted at 40° C. for 22 hours, 60 cub. centims. of the starch solution being used to each weighed portion of the leaf. Equal weights of the shaded and unshaded sides of the blade were tested side by side. After digestion and subsequent titration the weights of cupric oxide were :—

Shaded, ·0618 grm. ; unshaded, ·0498 grm. ; so that a destruction of diastase amounting to rather more than 19 per cent. had taken place.

In a subsequent experiment diffused light was employed instead of direct sunshine. The leaf, mounted and shaded as before, was exposed towards the north to the light from blue sky and white clouds for 8 hours, and then removed and killed with chloroform vapour.

After digestion and titration the weights of cupric oxide were :—

Shaded, ·0538 grm. ; exposed ·0458 grm. ; indicating a destruction of diastase amounting to nearly 15 per cent.

The destruction is seen, on comparing these two experiments, to vary with the intensity of the light.

An experiment was made to test the power of the electric arc upon the living leaf. A vigorous leaf was mounted on the frame as before and exposed for 9 hours to the electric lamp. The temperature reached during the exposure was taken by a maximum thermometer placed in a test-tube of water which was fastened on to the frame by the side of the leaf, and was found never to exceed 37·5° C.

The subsequent treatment was exactly the same as in the experiments conducted in sunlight and detailed above. The final weights of cupric oxide were :—

Shaded, ·0498 grm. ; exposed, ·0448 grm. ; or a diminution of about 10 per cent.

Comparing the destructive effect of the electric light and that of the bright sun, as shown in the experiments just quoted, it appears that the latter is nearly twice as powerful as the former.

Protection of the Diastase in the Living Leaf.

The destruction of diastase, though considerable in the living leaf, is much less than in the extract of malt and in saliva. Either the diastase of the leaf is more resistant than that of the other two extracts, or there is some constituent of the cell contents of the parenchyma that has the power of screening the enzyme from the deleterious rays. Attention is, from this point of view, at once drawn to the chlorophyll as possibly serving this purpose. Besides chlorophyll, however, the light is likely to be interfered with by the protoplasm through which it must pass, and by the proteids contained in the latter and in the cell sap.

As, moreover, the malt extracts and the saliva used contained small amounts of proteids, it became necessary to examine carefully to see whether the action of the light upon the enzyme was indirect, not destroying it, but hindering the manifesta-

tion of its activity by interfering with the proteids, with which in some manner, and to some extent, the enzymes are believed to be associated.

There remains still another possible interference, in the acid character of the cell sap of the leaf parenchyma. This is, however, scarcely likely to aid in the destruction of the ferment by light, as it has been found that the presence of a weak acid solution is favourable to the development of almost all ferments and to the activity of many. It was from this reason difficult to submit the influence of this faint acidity to experiment.

An investigation was first made as to the influence of varying amounts of proteid in the illuminated extracts. A solution of egg albumin (white of egg diluted with an equal volume of water), was used throughout this series of experiments. 10 cub. centims. of saliva, prepared as before, were placed in a quartz cell and five drops of the albumin solution added to it. In another similar cell, 10 cub. centims. of the saliva were placed, without any addition of albumin. In a paper-covered bottle were placed a third 10 cub. centims. of the saliva, with five drops of the albumin solution added. They were all exposed to the electric arc for 14 hours. On removal five drops of the albumin were added to the contents of the second quartz cell. Calling these three A, B, and C: A contained diastase illuminated in the presence of considerable albumin, B contained diastase illuminated in its absence, and C served as a control, to give the diastasic power of the saliva when not interfered with by light at all. They were all digested with weak starch paste as in other cases, and after titration, the weights of cupric oxide were: C (standard), ·1116 grm., A, ·1044 grm., and B, ·0943 grm. The extract, without proteid, had hence lost 15·5 per cent. of its diastase, while that which contained it during exposure had only lost 6·5 per cent.

A second experiment was conducted on the same lines, varying the porportion of albumin to saliva. 15 cub. centims. of the latter were taken, and 6 drops of the albumin solution. The cells A, B, and C were prepared as before, and exposed for the same time. The digestion was carried out with twice the relative volume of starch paste, and was prolonged for about three times the period of the first experiment. After titration the weights of cupric oxide were C (standard) ·2093 grm., A (with proteid during exposure) ·1708 grm., B ·0818. In this case the extract without proteid lost 60 per cent. of its diastase, while that which contained it during illumination lost only 18 per cent.

Further experiments, which it would be tedious to narrate in detail, confirmed these results; they all show that the destructive action of the light that has been established is not brought about by action on the proteid, for that a diastasic solution containing a very appreciable quantity of the latter is less and sometimes considerably less injured than one nearly free from it.

That the protection of the diastase in this series of experiments, is to be attributed to the presence of the proteid is demonstrated in the following one, which shows the

degree of protection to be in some way proportional to the amount of proteid present.

The extract used was the same saliva as that with which the last experiment was conducted. To 10 cub. centims. in cell A 2 drops of albumin solution were added; to 10 cub. centims. in cell B 1 drop only, and to a third 10 cub. centims. in cell C 2 drops as in A. They were then exposed to the arc light for 9 hours, during which the temperature rose to 29° C. A and B were faced by quartz plates, C with an impervious paper screen.

On removal, 1 drop of the albumin solution was added to B. 5 cub. centims. of each were then added to 60 cub. centims. starch paste (1 per cent.) and they were digested for 37 minutes at 39° C., after which they were boiled, and the products of the hydrolysis estimated by means of FEHLING'S solution, as in other cases. The weights of cupric oxide were C (standard) ·2651 grm., A ·1852 grm., B ·1648 grm. The diastase in B had been reduced 38 per cent., and that in A only 30 per cent., so that the double quantity of proteid in the latter had preserved 8 per cent. of diastase from destruction.

It is evident, however, that some other factors must enter into the causes of the differences observed, as the amount of protection afforded is not in exact proportion to the amount of proteid added. No more definite conclusion can be drawn at present than that proteids have the property of protecting diastase from the destructive rays of the spectrum.

There still remains the question as to the possible protective properties of chlorophyll. The investigation of this body is extremely difficult, as it is insoluble in all watery and neutral solutions, and is changed at once by acids and alkalies. The solvents usually employed for its extraction are alcohol or benzol, and it is very doubtful if these dissolve it unaltered.

Some solution of chlorophyll was prepared by extracting fresh leaves with alcohol, and diluted to such a strength that the absorption band in the green was just visible in the spectroscope. Double cells were constructed consisting of two ebonite rings separated by a quartz plate, and faced by another similar piece of quartz. In the inner cell was placed the diastasic extract, and in the outer one the chlorophyll solution. Two controls were prepared, one with alcohol in the outer cell, and the other with no screen at all. They were exposed to the arc-lamp for 14 hours, and the contents of them separately made to act on starch paste as in other cases. The result of the subsequent titration was to show that while there was a considerable destruction of diastase in the unscreened cell, there was little, if any, in those screened by either alcohol alone or alcohol containing chlorophyll.

A similar experiment made with a solution of chlorophyll in benzol had the same result.

The somewhat unexpected result of finding these solvents opaque to the deleterious rays was confirmed by further experiments. One of these will suffice here. 10 cub.

centims. of salivary diastase solution were placed in a quartz-fronted cell, and screened by alcohol in the outer cell; 10 cub. centims. were placed in a similar cell without a screen, and 10 cub. centims. in a paper-covered cell. These were labelled A, B, and C. They were exposed to the electric arc for 9 hours, the temperature rising to 31° C. 5 cub. centims. of each were then made to act on 25 cub. centims. starch solution for 1 hour 40 minutes. After titration, the weights of cupric oxide were, A, ·0398 grm., B, ·0218 grm., C (standard) ·0438. There was a destruction in B of 50 per cent., but in A of only 9 per cent., so that the alcohol was a very effective screen. Further experiments and observations with the spectroscope led to the conclusion that this reagent is like glass in its opacity to the ultra-violet rays. The protection it affords is not perfect, which is probably attributable to the fact that it allows all the blue and violet rays to pass, for, as we have seen, these are most likely deleterious, though to a less extent than those of the ultra-violet region.

The impossibility at present of preparing an extract of chlorophyll which can be used as a satisfactory screen to a diastasic solution during illumination, renders the proof of its possible protective power very difficult. Various considerations, however, that will be discussed more fully later, point very strongly to such a conclusion.

The Fate of the Rays of Light Absorbed.

In studying the mutual relations of light and diastase it becomes of interest to ascertain what is the fate of the deleterious rays themselves. That they destroy the enzyme is fairly evident. Do they pass on unaltered, or are they reflected, or absorbed and converted into some other form of energy? Some experiments bearing on this point may now be narrated, though a discussion of the matter had better be deferred till the results of the examination of the other parts of the spectrum have been detailed.

The first result of interest in this connection is that the deleterious rays do not pass through a diastasic solution, while the latter is more or less transparent to the beneficial ones. As this is a point of considerable importance, several experiments bearing on it may be quoted.

10 cub. centims. of diluted saliva were placed in the back compartment of a double quartz cell (A), 10 cub. centims. of the same in the front compartment (B), and 10 cub. centims. in a paper-covered cell (C). The three were then exposed to the electric arc for 9 hours, during which a maximum temperature of 31° C. was indicated by the thermometer. 5 cub. centims. of each were then digested with 25 cub. centims. of 1 per cent. starch solution for 1 hour 40 minutes, and then the digestions were titrated as usual.

The weights of cupric oxide found were, A, ·0413 grm., B, ·0218 grm., C, ·0438 grm. Comparing A and C, it is evident that the destruction of diastase indicated in B was almost totally averted in the case of A.

In another experiment both boiled and unboiled extracts were used as screens. Cells were arranged as under :—

A, 15 cub. centims. extract in outer quartz cell.
B, 15 „ „ inner „ behind A.
C, 15 „ boiled and filtered extract in outer quartz cell.
D, 15 „ extract in inner quartz cell behind C.
E, 15 „ „ paper-covered cell, to serve as a standard.

They were exposed to the arc lamp for 9 hours. Then 5 cub. centims. of each were digested with 20 cub. centims. of 1 per cent. solution of soluble starch, as before, at a temperature of 38° C.

The weight of cupric oxide given by the standard E was ·0603 grm. B, screened by the unboiled extract, gave ·0863 grm.; D, screened by the extract that had been boiled and filtered, gave ·0808 grm. There was no action naturally in C.

The extract in A not only cut off all the deleterious rays, but allowed the beneficial ones to pass. Comparing B with the standard E, the amount of diastase increased 43 per cent.; comparing also D with the standard, the diastase increased by 34 per cent. The diastase, therefore, whether boiled or unboiled, is opaque to the deleterious rays, while it is only partially so to the beneficial ones.

A further experiment on the screening powers of the boiled extract may be quoted. Cells were arranged as under :—

10 cub. centims. of diluted saliva were placed in the inner compartment and screened by boiled and filtered saliva (A). 10 cub. centims. were placed in a similar cell and screened by water (B); 10 cub. centims. were placed in a paper-covered cell (C). All were illuminated by the arc lamp for fourteen hours. 5 cub. centims. of each were then digested with 20 cub. centims. starch solution (1 per cent.). After titration the weights of cupric oxide were, A, ·1108 grm., B, ·0058 grm., C (standard) ·0888 grm.

The deleterious rays passed freely through the water, destroying 93 per cent. of the diastase. They failed to pass through the boiled extract, while the beneficial rays increased the diastase 24 per cent.

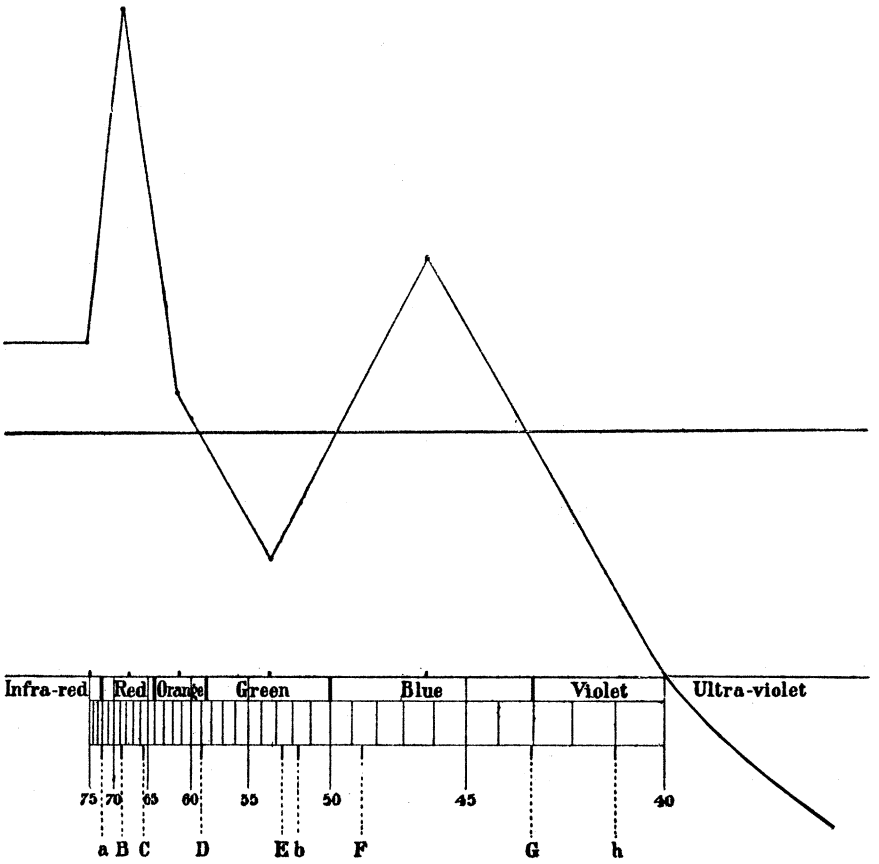
The deleterious rays seem from these experiments to be absorbed by the diastase, and the power of the latter to absorb them is not closely connected with its fermentative activity. Whatever be the change brought about by boiling in the molecule of the enzyme, it is not of such a nature as utterly to decompose it. When changed by the high temperature, it is still capable of intercepting these rays. To this point reference will be made later.

Localisation of the Beneficial Rays.

In investigating the action of the beneficial rays the method adopted was to eliminate the action of the ultra-violet ones by using glass vessels in which to expose

the diastasic solutions, and screening them by other vessels containing various coloured fluids. By the use of appropriate screens the spectrum was divided into seven regions, which may be roughly called the infra-red, red, orange, green, blue, violet, and ultra-violet. Fig. 1. shows the regions of the visible spectrum delimited as will be described; the red includes rays of wave-length 710–645 $\mu\mu$; orange 645–585 $\mu\mu$; green 590–500 $\mu\mu$; blue 500–430 $\mu\mu$; and violet those beyond 430 $\mu\mu$.

Fig. 1.



Curve showing the action of the rays of different wave-length upon the production and destruction of diastase. The ordinates are drawn over the centres of the bands examined, which are indicated upon the spectrum below the curve. The curve above the base-line indicates production, that below it destruction, of diastase.

The screens used were nearly those used by LANDOLT* in his investigations on Dispersion.

They may be described under the colours which they allowed to pass. The red

* LANDOLT, "Methode zur Bestimmung der Rotationsdispersion mit Hülfe von Strahlenfiltern," 'Ber. d. Deut. Chem. Gesell.,' 1894, p. 2872.

screen was composed of superposed solutions of hexamethyl-pararosanilin (crystal violet), ·03 grm. per litre, and potassic monochromate 10 per cent.

The orange screen was made by superposing solutions of nickel sulphate 30 per cent., potassic monochromate 10 per cent., and potassic permanganate about ·2 per cent. The green one consisted of superposed solutions of cupric chloride 60 per cent., and potassic monochromate 10 per cent.; the blue one of a solution of ammonio-cupric sulphate, diluted till it allowed the measured band quoted to pass.

The infra-red rays were examined by using a screen of a solution of iodine in carbon bisulphide.

In every experiment made the rays passing the several screens were carefully ascertained by a spectroscope provided with a wave-length scale.

Many experiments were made with each of the bands so isolated, with the exception of the violet one. It would be tedious to narrate these in detail and the results may therefore be summarised. The accompanying table will show at a glance the percentage increase or diminution in the diastase after an exposure of ten and a half hours duration to the electric arc. As the solutions received with the particular band under investigation the infra-red rays, the ascertained effect of the latter has been deducted in computing the results.

Band examined.	Cupric oxide in grms. reduced by solution exposed to band, after correction for effect of infra-red rays.	Cupric oxide in grms. reduced by unexposed solution.	Increase or diminution of amount of CuO reduced. Diminution indicated by — sign.	Increase or diminution of diastase per cent.	Mean increase or diminution.
Red 720–640 $\mu\mu$	·0557	·0354	+·0203	+57·4	
	·0565	·0393	+·0172	+43·7	
	·0921	·0568	+·0353	+62·1	
	·0518	·0343	+·0175	+51·0	+53·5
Orange 640–585 $\mu\mu$	·0338	·0323	+·0015	+ 4·6	
	·0296	·0282	+·0014	+ 4·9	+ 4·75
Green 585–500 $\mu\mu$	·0285	·0333	—·0048	—14·4	
	·0107	·0129	—·0022	—17·0	—15·7
Blue 500–430 $\mu\mu$	·0282	·0242	+·0040	+16·5	
	·0711	·0568	+·0143	+25·1	+20·8
	Cupric oxide reduced by solution exposed to infra-red rays.	Cupric oxide reduced by unexposed solution.	Gain.	Increase of diastase per cent.	Mean.
Infra-red	·0337	·0323	+·0016	+ 5·0	
	·0323	·0282	+·0047	+16·6	+10·8

The diastase used for these experiments was saliva prepared as already described, it being found difficult to prepare the other extracts quite free from colour. The results must be received with a certain amount of caution, as with such different screens it was not at all easy to get the intensity of the light the same in each case, though as much care as possible was taken. Slight differences in intensity in any one case were found, however, not to have a very marked effect during the time of the exposure.

As no screen satisfactorily isolated the violet rays or those beyond wave-length 430 $\mu\mu$, the effect of these can only be a matter of deduction. When experiments were made with the whole visible spectrum and the infra-red, the ultra-violet being excluded by either glass or alcohol, as already detailed, the ultimate effect of the light was deleterious to the enzyme. The infra-red and the visible rays have now been shown to be beneficial, except those lying between wave-lengths 585 and 500 $\mu\mu$, which are slightly deleterious; therefore, the chief injury to the diastase in these experiments must have been caused by the violet rays. They do not appear to be so harmful, however, as the ultra-violet, though when allowed to act for a sufficiently long period, they, like the latter, destroy the whole of the enzyme.

It is possible to represent the effects of all the rays of the light by means of such a curve as is shown in fig. 1, the ordinates of which give approximately the percentage increase or diminution of the diastase in the different parts of the spectrum. The curve rises sharply from the infra-red, reaches a maximum in the red, declines steeply to the orange, crosses the base-line in the green, rises to a second maximum in the blue, and cutting the base-line again at the junction of the blue and violet, becomes negative through the violet and ultra-violet, indicating in these regions a destructive effect.

It has been stated that the deleterious effect of the ultra-violet rays continued and increased after the diastase had been removed from its influence. The same progressive effect was noticed in the case of the improvement set up by the blue rays. In both cases the changes initiated during the exposure continued and increased after the light had ceased to act. The effect of the light consequently is to set up changes of a physical or chemical character in the enzyme or its antecedent, which, when once started, can be maintained without further light influence.

Biological Application of these Influences of Light.

Several considerations of importance in the metabolism of the plant may now be discussed.

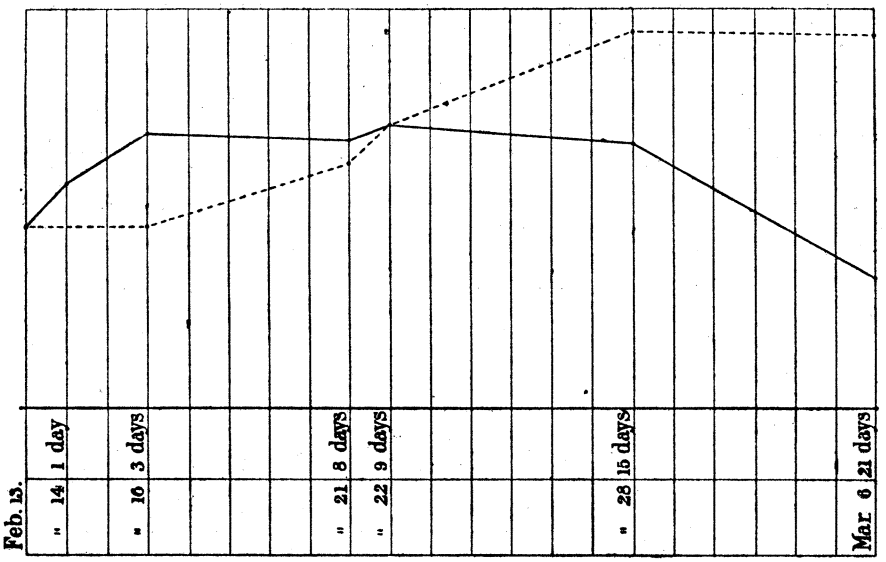
The influence of the beneficial rays has been shown to increase the amount of diastase in an extract of malt or in freshly-secreted saliva, or in saliva that has been preserved from decomposition by antiseptics. The fact that the infra-red rays have a definite power in this direction recalls at once the fact that has been established in

animal physiology that moderate warmth converts many zymogens into enzymes. This has especially been noted in connection with the secretion of the pancreas. Though the existence of zymogens in the cells of plants has not been at all widely established, yet it has been proved more or less certainly in several cases. The zymogen of human saliva has not hitherto been satisfactorily extracted, either from the secretion or from the salivary gland, but all analogy points to its existence.* The following experiments appear to demonstrate the occurrence of zymogen in the saliva of man, and its similarity to that of the pancreatic juice.

Some saliva was secreted, freed from mucin and considerably diluted, ·2 per cent. of potassic cyanide being added as an antiseptic. It was then divided into two portions, one of which was kept for 21 days in an incubator, the temperature of which was maintained at 38° C, while the other remained at the ordinary laboratory temperature. At certain intervals 2 cub. centims. of each were digested with 20 cub. centims. of 1 per cent. starch paste and the products of hydrolysis titrated with FEHLING's fluid.

The results obtained are given in the following table, and are plotted out in the form of a curve in figure 2.

Fig. 2.



Curve showing the diastasic power of saliva during prolonged exposure to a temperature of 38° C. The abscissæ represent days and the ordinates the amount of enzyme present. The black line indicates the activity of the warmed saliva, the dotted one that of a control kept at the laboratory temperature.

The abscissæ in this curve represent days; the continuous line shows the diastasic power of the warmed, and the dotted line that of the unwarmed saliva.

* The zymogen of the saliva of the horse has, however, been shown to exist. *Cf.* GOLDSMIDT in 'Zeitschrift f. Physiolog. Chemie.,' vol. 10, p. 273.

TABLE showing the effect of keeping saliva at 38° C. and at the laboratory temperature.

Time of exposure to 38° C.	Cupric oxide reduced by products of action of 2 cub. centims. of warmed saliva on 20 c.c. starch paste.	Cupric oxide reduced by products of action of 2 cub. centims. of unwarmed saliva on 20 c.c. starch paste.
days	grms.	grms.
1	·0565	·045
3	·0697	·045
8	·067	·062
9	·0706	·0708
15	·066	·095
21	·032	·094

From these experiments, it is probable that the saliva contained, in addition to a certain amount of ptyalin or diastase, a quantity of zymogen, which exposure to a temperature of 38° C. converted into the enzyme completely in about 3 days. Possibly a little remained unchanged until the ninth day. After 15 days at this temperature the enzyme began to change, losing its diastasic power, which at the end of the twenty-first day was much less than at the beginning of the observation. At the temperature of the laboratory, which would be about 18° C., the zymogen did not begin to be converted into the enzyme till after the third day, it then progressed gradually for the rest of the time it was under observation. There was no destruction of the enzyme during the whole of that period, so that the curve of its activity rose somewhat higher than in the case of the warmed extract, in which, during part of the experiment, both processes were at work.

It does not seem from these considerations that it would be rash to infer that the beneficial rays of the infra-red, and hence inferentially of the visible part of the spectrum, convert a zymogen existing in the tissues into diastase.

The rays of the violet and ultra-violet regions are more easily seen to be destructive of the ferment. No other explanation can be admitted, especially when the action of these rays upon bacteria is remembered. The experiments which have been narrated, however, may modify our ideas of what is meant by the term destruction. That the diastasic power is lost is quite clear. The same effect follows when a solution of the enzyme is heated to a temperature of from 70° to 100° C. We have been accustomed to associate this process with a complete decomposition of the molecule of the enzyme. If, however, the conclusions of FISCHER* are sound, that the power of an enzyme to hydrolyse the body on which it acts depends upon the configurations of its molecule and of that of the latter, we need not postulate so

* E. FISCHER, "Einfluss der Configuration auf die Wirkung der Enzyme." 'Ber. d. Deut. Chem. Gesell.,' 27, 2785 (1894).

complete a decomposition. It will be sufficient to explain the cessation of its activity if we imagine the external influence, whether heat or light, to change the configuration of the enzyme, so that it no longer corresponds to the configuration of the molecule of the substance of the hydrolysable material. As FISCHER puts the relation necessary for hydrolysis, the configuration of the two must be related in some such way as a key is related to the lock which it can turn. If, then, this configuration be interfered with, no hydrolytic action will be possible, even if no further splitting up of the enzyme should take place.

This view receives some support from the observations already made, that the diastase is opaque to light after it has been boiled, just as it is before. We should imagine such a change as the complete disruption of the molecule of the enzyme would certainly be accompanied by an increase of transparency. As this does not take place, there seems no difficulty in supposing that whatever change is brought about, it is not so extensive as to involve the disruption of the molecule.

When, therefore, a leaf is illuminated by the whole of the spectrum, the order of events seems to be that the beneficial rays convert the zymogen into diastase, and the latter is speedily and continuously changed by the deleterious ones. A light seems to be efficacious for the first process which is weaker than that required for the second.

The means whereby the diastase is partially or completely protected from destruction in the living plant may well receive some attention. It has been shown above that the rate of destruction is less in the living leaf than in an extract containing diastase. The proteids of the leaf have no doubt a considerable power of screening off the deleterious rays and presumably this power is shared by the protoplasm of the cells. The chlorophyll may also be presumed to have a protective effect, though how far this extends may be disputed. The spectrum of chlorophyll is probably without the ultra-violet rays, though no investigation of this point can at present be made. Still, as the amount of chlorophyll in an alcoholic solution is increased, it cuts off more and more of the violet end of the visible spectrum. Even when very little is present, it blocks out the rays beyond 430 $\mu\mu$, which we have seen to be deleterious. It is true that chlorophyll allows most of the green rays to pass through it and these are apparently deleterious. Their effect is, however, but feeble when compared with that of the violet and ultra-violet regions. A reference to the curve, on p. 181, will show that the effect of the green rays in one direction is but little greater than that of the infra-red rays in the other. The powerfully deleterious rays are those beyond 430 $\mu\mu$.

It is, however, probable that the diastase is not situated in the chloroplastid, but in the cell outside it. The destruction that can be observed in the living leaf is inconsistent with the hypothesis that the location of the diastase is in the plastid, for there is strong presumption, as just stated, that chlorophyll cuts off the rays that are deleterious. The increase in the diastase of the leaf would be difficult to understand

if it were situated behind a chlorophyll screen, as the spectrum of chlorophyll shows that the latter cuts off especially those rays in the red and in the blue which are active in the conversion of zymogen into enzyme. The proteids afford sufficient protection against the deleterious rays, supposing, as it seems probable we may do, that the place of formation of both enzyme and zymogen is the cell-protoplasm outside the chloroplastid.

In this connection it is interesting to refer to a memoir recently published by PICK,* dealing with the question of the function of the red colour of the sap of certain leaves. This author suggests that certain rays of light hinder the translocation of starch and that the red colour is of importance in shielding the leaf from such rays. He points out in the absence of the red colour there is a great accumulation of starch in the chloroplastids. This cannot be due to increased activity in the process of the deposition of starch there, as the red rays which pass the colour are those which the chlorophyll absorbs and utilises. He further notices that when the leaves receive red light, starch is formed in the spongy, but not in the palisade, parenchyma, owing to the red rays allowing more translocation from the cells of the latter. PICK'S conclusions are, however, disputed by EWART,† who thinks that the appearances are due to variations in the rate of carbon assimilation in consequence of alterations in the activity of the chloroplastids under the different conditions PICK describes.

JOHOW‡ has shown that in many tropical plants whose insolation is intense, a red dye is developed in the epidermal cells of the leaf and, in some cases, in their mesophyll cells as well. He proves that the formation of this colouring matter is directly due to the action of the light. When this dye is formed only over the veins and conducting tissues, JOHOW shows that it aids translocation.

The experiments described in this paper indicate the means by which this protective influence, pointed out by both PICK and JOHOW, may be brought about. It is due, not so much to the variation of the action of the chloroplastids, as EWART thinks, but to the effect of the light upon the diastase, the beneficial rays passing to the cells and the deleterious ones being screened off.

In the case of the barley grain, which is the source of the diastase of malt extract, the colouring matter of the ripe grain is a complete screen for the deleterious rays. This was proved by many experiments, in some of which the colouring matter was dissolved in the solution of the diastase, and in others a solution of it was employed as a separate screen superposed during exposure upon the quartz cell containing the extract.

A further consideration of far-reaching importance also arises from the experiments.

* PICK, "Ueber die Bedeutung des rothen Farbstoffes bei den Phanerogamen und die Beziehungen desselben zur Stärkewanderung," 'Bot. Central.,' vol. 16, pp. 9 to 12.

† EWART, "On Assimilatory Inhibition in Chlorophyllous Plants," 'Journ. Linn. Soc.,' vol. 31.

‡ JOHOW, "Ueber die Beziehungen einiger Eigenschaften der Laubblätter zu den Standortsverhältnissen," 'PRINGSHEIM'S Jahrb.,' vol. 15, p. 299.

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There is an absorption of the radiant energy of light, particular rays of which do not pass through the plant-cell, but are absorbed and made available for certain purposes of metabolism. The energy of part of the red and infra-red rays is, as we have seen, very probably employed in converting zymogen into diastase. The absorption of the energy of the ultra-violet and violet rays is equally clear, for they were found not to pass through a solution of diastase so as to affect a second solution placed behind the first. Not only can diastase absorb these rays, but the proteids of the cell have the same power. How they are utilised by the cell the experiments detailed afford no evidence to show, but it does not seem rash to advance the view that we have in vegetable structures a power of absorbing radiant energy which is not connected with the presence and activity of chlorophyll.

The absorption of energy by the vegetable organism without the assistance of a chlorophyll apparatus has already received some attention in recent years. ENGELMANN* has shown that in certain bacteria heat rays are of use in the construction of organic substance. This is, however, probably effected by means of a pigment by which the heat rays are absorbed, much as are the light rays by chlorophyll. There is, at any rate, a fixation of energy in the plant which is derived from the rays of the infra-red region of the spectrum.

PFEFFER† shows further that the energy of pressure and of tension can also be utilised by the vegetable organism. The experiments of WINOGRADSKI‡ on the nitrifying bacteria also show the formation of organic material from inorganic bodies without the agency of chlorophyll. The conditions under which this is carried out point to the absorption of energy from the chemical decompositions which accompany or precede the nitrification. The sulphur bacteria appear to carry on a similar constructive process, obtaining energy from the oxidation of sulphur or sulphuretted hydrogen, and the iron bacteria by oxidation of ferrous oxide.

The absorption of the energy of an electric current appears possible also, though but few observations have been made upon it. SPESCHNEW cultivated some cruciferous and umbelliferous plants in a bed of earth, at one end of which a copper plate, and at the other end an iron one, had been buried a little below the surface, their faces being parallel to each other. When these plates were connected by a wire, a current passed and traversed the earth between them. Plants growing in the course of the current were much more vigorous than others grown for the purpose of a control in an adjoining part of the same bed. It is at least possible that the greater growth in the former case was due to the fixation of a portion of the energy of the current. §

* ENGELMANN, 'Bot. Zeit.,' 1888.

† PFEFFER, "Zur Energetik der Pflanze," Leipzig, 1892.

‡ WINOGRADSKI, "Recherches sur l'organisme de la nitrification," 1890, 1891. 'Annales de l'Institut Pasteur.'

§ [Since this paper was put in type my attention has been called by Professor MARSHALL WARD to an

It is possible, further, that we may base an argument on these experiments against the proteid nature of diastase. The composition of the enzymes is a much-disputed question, and only recently OSBORNE* has advanced the view that the diastase of barley is probably to be identified with one of the proteids of the grain. The change brought about in the diastase by the ultra-violet rays militates to a certain extent against that view. So far as we know, light brings about no change in the composition of proteids, while it evidently does alter, at least, the configuration of diastase to a very marked extent.

Summary.

It has now been established by BROWN and MORRIS that there is a diminution in the amount of diastase in foliage leaves after a period of bright illumination. This has been explained by them as connected with variations in formation and consumption under different conditions, but the possibility remains that there may be a destruction of the enzyme by light comparable to the destruction of bacterial life under the same conditions.

Various diastasic solutions and living leaves were exposed by the writer for different times to the light, either of the sun or the electric arc, and their diastasic power ascertained subsequently by digesting measured quantities with starch solutions, the experiments being in all cases carefully controlled. Various means of exposure were adopted, so that either the total rays, or those of the infra-red, or those of the whole or definite parts of the visible spectrum were tested independently.

The effect of the whole spectrum was to diminish the diastase in the solution. Malt diastase lost on the average 68 per cent. in 14 hours; saliva 45 per cent., and leaf diastase in solution 8 per cent. In the living leaf there was a destruction of diastase amounting to 15 per cent.

When the ultra-violet rays were excluded by using glass vessels the first effect was to increase the diastase from 15 to 20 per cent. This increase was succeeded, after prolonged exposure with antiseptic precautions, by almost complete destruction.

The two ends of the spectrum were thus found to have opposite effects. The

important memoir recently published by MM. LAURENT, MARCHAL, and CARPIAUX, in which the authors detail some experiments made in 1895 and 1896, which prove not only the absorption of the energy of the rays of the violet and ultra-violet regions, but further, that these rays are concerned especially in the construction of nitrogen compounds from the nitrates or ammonia compounds absorbed by the plants, while the intervention of the chlorophyll apparatus is unnecessary for this purpose. "Recherches expérimentales sur l'assimilation de l'azote ammoniacal et de l'azote nitrique par les plantes supérieures." LAURENT, MARCHAL et CARPIAUX. 'Bull. de l'Acad. Roy. de Belgique.' Third series, vol. 32, No. 12, pp. 815–865, 1896.—J. REYNOLDS GREEN.]

* OSBORNE, "The Chemical Nature of Diastase," 'Jour. of the Amer. Chem. Soc.,' vol. 17, 1895, p. 587.

deleterious rays were not, however, confined to the ultra-violet, but some of them were found to be present in the visible region.

On the whole the solar rays were more deleterious than those of the electric arc.

In the living leaf a certain protection is afforded from the action of the light by the proteids in the cells. The effect of adding proteid to diastasic solutions before exposure to the electric light is to reduce the destruction, the protection being greater as the amount of proteid is increased. It is not improbable that chlorophyll may exert the same protective influence, but this cannot be definitely ascertained as the solvents which extract it are largely opaque to the deleterious rays.

The latter rays do not pass through the diastasic solution unchanged, whether the diastase is active or has been destroyed by boiling. There is thus an absorption of radiant energy. No statement can be made as to the fate of the deleterious rays.

The rays which are beneficial are of use to the plant in transforming zymogen into enzyme. In the paper, experiments are quoted bearing on this point, the effect of light being compared with that of prolonged exposure to a temperature of 38° C., with which it coincides.

By a series of experiments the beneficial rays were localised chiefly in the red, orange and blue regions, the green and violet having a similar effect to those of the ultra-violet region, but being much less efficient. The relative increase or diminution of diastasic power caused by the different regions is as follows :—Infra-red, + 10·8 red, + 53·5 ; orange, + 4·75 ; green, — 15·7 ; blue, + 20·8. Those of the violet and ultra-violet regions were deleterious, but their energy could not be measured. The paper contains a curve giving a representation of the light effects and showing the delimitation of the several regions examined.

The effect of the different rays is not produced only during the action of the light, but continues after the extract is removed from illumination.

The experiments lend support to the view that in the cells of the leaf the enzyme is located in the protoplasm or the vacuole, and not in the chloroplastids.

They go further to establish the conclusion that in the vegetable cell there is a power of absorbing the radiant energy of the solar rays apart from the existence of the chlorophyll apparatus.

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