SPECIAL ARTICLES

EFFECT OF INDOLE-3-ACETIC ACID ON PHOTOSYNTHESIS

W. MITCHELL and CH. L. HAMNER[1] have recently shown that indole-3-acetic acid (heteroauxin) "stimulates plants to synthesize more solid matter, without causing a corresponding increase in leaf area." The authors' conclusion is based on experiments with young decapitated kidney-beans (*Phaseolus vulgaris*), where a lanoline paste containing indole-3-acetic acid was applied on the cut surface of the stem end. Such plants accumulated in the course of three days almost twice as much dry matter as did the control under identical environmental conditions. The control plants were also decapitated but treated with lanoline paste without heteroauxin.

The most probable explanation of the interesting fact established by Mitchell and Hamner is that heteroauxin from the paste on the cut surface penetrated into the green leaves of the experimental plants and intensified their photosynthetic activity. This assumption is supported by the experiments conducted by the authors during the summer of 1938.

Pairs of leaves were cut off from various plants (lilac, poplar, jasmine, hemp, hydrangea and others), care being taken to choose leaves as similar as possible in regard to age, size and position on the stem. The leaves were placed into two glass chambers, the lower ends of the leaf petioles being immersed in a Knop solution, and the intensity of photosynthesis determined. Electric lighting was employed. The surface of the chambers was cooled by a stream of running water in order to avoid overheating the leaves. The leaves were lighted for about one and three quarters of an hour. After several hours, a second determination was made of the quantity of carbonic acid decomposed by these leaves under the same conditions of lighting and in the course of an equal interval of time. These two determinations made it possible to estimate the photosynthetic activity of the leaves experimented on. It was expressed in milligrams of carbonic acid decomposed by one square decimeter of leaf surface in one hour.

One of the leaves was then immersed with the lower end of the petiole in a Knop solution containing 1 mg of heteroauxin per liter of water; while the other (control) was immersed in a Knop solution without heteroauxin. After several hours both leaves were again placed in the chambers and the intensity of assimilation was determined under the previous environmental conditions.

These experiments, repeated many times with various subjects, showed in the vast majority of cases a considerable intensification of photosynthesis under the influence of the indole-3-acetic acid introduced into the leaf. Thus, the hemp leaf, whose photosynthetic activity was expressed by figures of 10.00 and 9.60 mg in the first two determinations in the Knop mixture (with a four-hour interval between experiments), gave 19.24 mg and, in four hours, 18.00 mg of decomposed CO_2 after being kept in a Knop solution with heteroauxin (1 mg per liter) for nine hours. The control leaf gave the following values on parallel determinations: 12.10; 12.90; 14.73; 13.80. It should be noted that the photosynthetic activity in the control leaf kept in a Knop solution remained at the same level for the subsequent thirty hours; whereas, the experimental plant receiving the same solution with the addition of heteroauxin showed a considerable reduction of photosynthesis towards the end of the experiment (control, 14.83 mg; experimental leaf, 2.10 mg).

In another experiment on hydrangea, where the leaf was kept for 24 hours in a Knop solution with heteroauxin, the intensity of photosynthesis increased from 5.10 to 14.88 mg, while in the control leaf a decrease—from 3.34 to 2.14 mg—was observed.

Using higher heteroauxin concentrations (10 mg per liter) did not cause any changes in photosynthesis in our experiments.

Thus we arrive at the conclusion that indole-3-acetic acid causes a temporary intensification of photosynthesis on being directly introduced into the assimilating tissues of the green leaf in very low concentrations.

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[1] *Botan. Gazette*, March, 1938, p. 569.

GROWTH OF SOME HEMOLYTIC STREPTOCOCCI ON A CHEMICALLY DEFINED MEDIUM

HEMOLYTIC streptococci have previously been grown in complex media containing tissue extracts and have not been successfully cultured on mixtures of pure chemicals. Many problems dealing with the metabolic activity of these organisms would be aided if it were possible to know all the constituents of the medium on which the bacteria were grown. We have been able to obtain luxuriant growth of a representative strain of hemolytic streptococci on a medium containing only pure chemical compounds. The organism used was one of the Lancefield type D group, *S. zymogenes* strain H-6905.

We have recently shown[1] that a wide variety of hemolytic streptococci require riboflavin, pantothenic acid and a suitable reducing substance such as "reduced" iron. In addition, protein hydrolysates and

[1] D. W. Woolley and B. L. Hutchings, *Jour. Bact.*, in press.

aqueous liver extract are also required. We have now found that the protein hydrolysate may be replaced by a mixture of pure amino acids mixed in such proportions as to simulate the composition of casein. The liver extract[2] was purified by adsorption on and elution from lead sulfide, followed by adsorption on and elution from fuller's earth. The resulting concentrate was active at a level of 1 microgram per cc. The properties of the active material revealed by these concentration procedures led us to try various basic growth factors, and it was found that crystalline vitamin B_6[3] was effective.

Representative data illustrating the response obtained on our chemically defined media are shown in Table 1. The amino acid mixture supplied the following amounts of material per cc of medium: glycine 0.1 mg, dl-alanine 0.4 mg, dl-valine 1.6 mg, dl-leucine 1.0 mg, d-isoleucine 0.5 mg, dl-serine 0.2 mg, l-proline 1.0 mg, l-hydroxyproline 0.1 mg, dl-phenylalanine 1.0 mg, l-tyrosine 0.7 mg, l-cystine 0.1 mg, d-arginine hydrochloride 0.8 mg, l-histidine hydrochloride 0.4 mg, d-lysine hydrochloride 1.0 mg, l-tryptophane 0.3 mg, dl-methionine 0.8 mg, l-aspartic acid 0.5 mg, d-glutamic acid 2.5 mg, dl-threonine 0.6 mg. Growth was measured by quantitative determination of turbidity, as previously described. The figures in Table 1 represent the per cent. of the incident light which was transmitted by the culture. The pantothenic acid was supplied as a highly purified concentrate, but a synthetic product derived from di-hydroxy caproic acid was previously shown to replace it.

TABLE 1

Additions to glucose-salts medium	Photometer reading
(1) Riboflavin + reduced Fe + pantothenic acid	100
(2) (1) + amino acids	74
(3) (2) + vitamin B_6 (0.5γ/cc.)	10

It was noted that slight growth usually occurred when the amino acids, but not vitamin B_6, were added. This may possibly be due to contamination of some of the amino acids with the vitamin. It was not possible to supply every amino acid as a synthetic product, and while those that were not synthesized were carefully recrystallized, it may be that they were still contaminated.

The amino acid requirements for *S. zymogenes* have been determined on our medium, and glutamic acid and tryptophane were found to be essential. Details of the work, together with observations on a variety of species, will be published elsewhere.

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[3] We wish to thank Dr. S. Lepkovsky for a sample of crystalline vitamin B_6.

THE PSEUDO-AMNION, PSEUDO-CHORION, PSEUDO-PLACENTA AND OTHER FOETAL STRUCTURES IN VIVIPAROUS CYPRINODONT FISHES

OVOVIVIPARITY or viviparity occurs in all species of four families of the cyprinodont fishes. Most of the specializations for viviparity in embryos and ovaries found in other viviparous fishes occur in this single order, and in addition there are unique features not found elsewhere. Within two of the families (Poeciliidae and Goodeidae) different species show degrees of specialization varying from simple conditions similar to those of oviparous fishes to extremes involving the partial degeneration of the yolk sac, and the development of new structures (pseudo-amnion, pseudo-chorion, pseudo-follicular placenta, trophotaeniae and gut modifications). Simple adaptations occur in the early embryos of extremely specialized forms, but these are ephemeral and are replaced in later stages of gestation by newer and more specialized structures. In the families Jenynsiidae and Anablepidae the living species have become highly specialized and no species retains simple modifications, except in early stages of ontogeny. There are numerous parallel developments in the four families.

The development, function and history of these adaptational structures is parallel roughly to that of the foetal membranes and yolk sac in the amniotes as a whole in the following respects:

(1) The yolk supply is adequate for nutrition up to the time of birth or hatching in some cases, but the yolk sac becomes degenerate in more specialized groups and the function of nutrition is taken over by new structures.

(2) Membranes or specialized organs functioning as respiratory, nutritive and excretory devices are developed from the somatopleure of the pericardial or peritoneal spaces and from the gut. They become highly vascular.

(3) In cases of advanced viviparity the uterus (ovary in fishes) becomes greatly modified.

(4) Foetal membranes or specialized structures are temporary and are discarded at birth.

Poeciliidae. The embryos are retained in the ovarian follicles for the whole period of gestation. In some genera (Mollienesia and others) the yolk sac is large and furnishes an adequate nutritional supply for the whole period of gestation. The somatopleure of the extra-embryonic pericardial cavity becomes vascularized, thus augmenting the respiratory surface, and lateral extensions fuse dorsal to the posterior head region to form a continuous band, a unique feature in this family. Specializations within the family are of two types. (1) *Heterandria formosa* and others have very small yolk sacs. The lateral extensions of the somatopleure of the extra-embryonic pericardial cavity completely envelop the head, forming an outer