Esterases: Nachmanson[7] has shown that acetyl choline esterase is inhibited by mild oxidizing agents and iodoacetic acid. In agreement with these findings, 3-amino-4-hydroxyphenyl arsine oxide (6.6×10^{-5} *M*) produced 57 per cent. inhibition. In contrast with this esterase, the hydrolysis of mono-n-butyrin by human serum esterase was inhibited by ClHg benzoic acid by only 18 per cent., addition of glutathione bringing partial reactivation; hog liver esterase was inhibited by 31 per cent. with p-carbamyl phenyl arsine oxide, and by 9 per cent. with ClHg benzoic acid; pancreatic esterase was not affected by the -SH reagents.

Proteins containing no -SH groups essential for enzymatic activity: The following enzymes were not affected by the above-mentioned -SH reagents: polyphenol oxidase, arginase, citric oxidase, uricase, catalase, lactic oxidase, liver alcohol oxidase, histaminase, potato phosphorylase, carbonic anhydrase, acid phosphatase, peanut fat oxidase, pepsin, cytochrome oxidase and flavoproteins.

Since such a large number of enzyme systems contain in their protein moiety -SH groups essential for enzyme activity, the role of glutathione becomes of great importance. Glutathione, by maintaining these groups in their reduced form would maintain the enzyme activity of those systems possessing essential -SH groups.

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AUXIN ACTION

MUCH is already known about the results of the action of auxins and similarly behaving compounds in inducing roots to grow on cuttings, in producing parthenocarpy and in affecting the growth of the whole plant, especially at the tips of herbaceous stems where growth activity is most intense. However, the fundamental mechanism is still to be revealed. The purpose of this article is to make known that discovery and to support it with sound evidence.

The mechanism is fundamentally the release of diastase, and possibly sucrase and other enzymes, from the protein colloidal substances to which they are normally attached. Enzymes are partly and considerably inactivated by adsorption onto a colloid as shown by Eyster.[1] A 100 ml solution containing 50 ml 1 per cent. soluble starch and 5 ml 1 per cent. diastase (Merck) and enough water to make a total volume of 100 ml required only 15 minutes for digestion past the last iodine staining stage as shown by the I_2KI test. A similar solution with 1 gram of dry activated charcoal, added immediately after the diastase was added, required 234 minutes (almost four hours). While ethyl alcohol, ether and chloroform decreased slightly the enzymatic activity of diastase in the absence of a colloidal carrier, they markedly accelerated the activity in the presence of charcoal. This indicates that the narcotics had a stronger influence in releasing the diastase from the charcoal (*i.e.*, enzyme from colloid) than it had in reducing the actual digestive action of the unbound enzyme. It was concluded that associated colloids dominate the effects of chemical agents and of environmental factors on enzymes.

Indole-3-acetic acid, β-(indole-3)-propionic acid, γ-(indole-3)-n-butyric acid and α-naphthaleneacetic acid increase the action of diastase when associated with activated charcoal in proportion to their concentrations. Table 1 presents the data for indole-propionic acid, which was being used at the time of the discovery of the mechanism of growth substances. In each case 50 ml of 1 per cent. soluble starch solution, 5 ml of 1 per cent. diastase solution, and enough indole-propionic acid to give the stated concentration were diluted to 100 ml with distilled water. The concentration of indole-propionic acid is based on the final solution volume of 100 ml. The diastase solution was added last and then there followed immediately the addition of exactly 1 gram of dry charcoal. Each mixture was placed in 125 ml bottles. The temperature of the room and of the component solutions before mixing was close to 25° C. The experiment was done in the evening in the presence of four functioning 100-watt electric light bulbs at an average distance of 8 feet. The influence of light will be clarified later in this article.

TABLE 1

INFLUENCE OF VARIOUS CONCENTRATIONS OF INDOLE-PROPIONIC ACID ON THE ENZYMATIC ACTION OF DIASTASE ASSOCIATED WITH CHARCOAL

Concentration of indole-propionic acid	Time required for digestion of soluble starch past the last iodine staining stage
0 parts per million	265 minutes
5 " " "	252 "
10 " " "	245 "
25 " " "	230 "
50 " " "	188 "
75 " " "	160 "
100 " " "	126 "
150 " " "	60 "

Table 2 presents data to show the effect of indole-propionic acid on isolated diastase; *i.e.*, diastase in the absence of charcoal. In this case 50 ml of 1 per cent. soluble starch solution, 1 ml of 1 per cent. diastase solution and enough indole-propionic acid to give the stated concentration were diluted to 100 ml. No charcoal was added. The conditions of temperature and light were the same as in the preceding ex-

[7] D. Nachmanson and E. Lederer, *Bull. Soc. Chem. Biol.*, 21: 797, 1939.

[1] *Plant Physiology*, 18: in press.

periment. Indole-propionic acid retards the action of the diastase in direct proportion to its concentrations. This explains the inhibiting effect of auxin in starch or sucrose free organs, such as some roots. Roots containing starch or sucrose would be expected to, possibly, show a stimulation in growth upon the addition of auxin. It is also expected that organs containing starch or sucrose but with an ample supply of unbound enzyme are inhibited in their growth in the presence of additional auxin.

TABLE 2

INFLUENCE OF VARIOUS CONCENTRATIONS OF INDOLE-PROPIONIC ACID ON THE ENZYMATIC ACTION OF DIASTASE NOT ASSOCIATED WITH CHARCOAL

Concentration of indole-propionic acid	Time required for digestion of soluble starch past the last iodine staining stage
0 parts per million	105 minutes
5 " " "	112 "
10 " " "	117 "
25 " " "	130 "
50 " " "	150 "
75 " " "	172 "
100 " " "	195 "
150 " " "	235 "

Phototropism can be demonstrated by running a series of charcoal-diastase-soluble starch mixtures in varying light intensities, inasmuch as the charcoal system is extremely sensitive to light. Light accelerates the rapidity with which the enzymes are bound to the colloid. The mixtures were similar to those

TABLE 3

INFLUENCE OF VARIOUS INTENSITIES OF LIGHT ON THE ENZYMATIC ACTION OF DIASTASE ASSOCIATED WITH CHARCOAL

Condition of light	Time required for digestion of soluble starch past the last iodine staining stage
Total darkness	234 minutes
Artificial light at night	265 "
Weak diffuse natural light in day	415 "
Strong diffuse natural light in day	605 "

used in Table 1, except no auxin was added. Table 3 presents the data.

Daylight with its ultra-violet and blue-indigo-violet light components is very potent in accelerating the rapidity with which enzymes are bound to a colloidal carrier, consequently making the enzymes less free to act. Artificial light has very little influence, as expressed also in its reaction on growth of plants. Since there was more rapid digestion by the charcoal-bound diastase in the dark than in the light, this explains the greater growth of the stem tip on the shaded side than on the illuminated side. Growth substances merely aid in releasing the enzyme from the colloid, especially after it has been rather securely adsorbed by the influence of many continuous hours of strong natural illumination. This explains why growth substances are not specific, but include a great variety of substances from the indole compounds to ethylene and carbon monoxide. Any substance which releases the digestive enzyme from its colloidal carrier, or slows the rate at which the enzymes are being bound to the colloid, without unduly upsetting any vital process, can apparently act as a growth substance. The indole compounds are more satisfactory because they are milder in their effect. They liberate sufficient enzyme to bring about the formation of digested foods in a quantity large enough to prevent suitable forms of food from being a limiting factor, and still just mildly affect membrane permeability and other cellular properties and functions.

Light is effective in building up food reserves for the plant, and in causing the digestive enzymes to be bound to their colloidal carriers more securely. Auxin releases the enzyme from its colloidal base and makes it free to act. Indications are both that in phototropism the auxin does not shift from the illuminated side to the shaded side, and that it is neither metabolically used up nor destroyed in the light, but that correspondingly more auxin is needed in cases where the enzyme has been more strongly adsorbed—a condition which is directly proportional to light exposure.

A more complete discussion and additional presentation of data will follow elsewhere.[2]

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A RAPID AND ACCURATE MICRO METHOD FOR THE ESTIMATION OF THE SULFONAMIDES

THE analysis of body fluids for the sulfonamides is a subject of clinical importance. The method of Bratton and Marshall[1] is at present most widely used, though it has several shortcomings. The method consists of precipitating the protein of the fluid with trichloracetic acid, filtering, diazotizing the aryl amine in the filtrate with nitrous acid, adding ammonium sulfamate to destroy the excess nitrous acid and coupling the diazonium salt with N-1 naphthyl ethylene diamine. The color intensity of the azo dye

[1] A. C. Bratton and E. K. Marshall, Jr., *Jour. Biol. Chem.*, 128: 537, 1939.

[2] Subsequently, it has been found that the auxin, too, is adsorbed in a manner similar to the adsorption of the enzyme.