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.

solution is a measure of the sulfonamide content of the fluid.

The time for a single determination is exceptionally long, and the color which is formed is stable for no longer than one hour, so that it is impossible to run a large number of samples simultaneously. The nitrogen bubbles which form after the addition of the sulfamate often lead to false readings. The recovery of sulfathiazole from blood is low, and is only about 80 to 85 per cent. when the blood is precipitated in trichloracetic acid in a volume ration of 1 : 20.

Changes in the procedure have removed all the causes for these objections. In the micro method, the blood is precipitated with trichloracetic acid containing a small amount of sulfuric acid. Then sodium nitrite in excess is added to the filtrate, and ethyl alcohol added to the solution of the diazonium salt. The coupling with N-1 naphthyl ethylene diamine is carried out in this solution. It was found unnecessary to add the ammonium sulfamate, the color being more stable in its absence.

It was shown that the blood need not be laked prior to precipitation of the protein in order to obtain complete recovery of the sulfonamides. This is pointed out in Table I, together with comparative results obtained by the Bratton and Marshall procedure.

TABLE I

FREE SULFATHIAZOLE LEVELS—(MG PER CENT.)

Subject and dose	Regular Bratton and Marshall method	Micro method	
		Blood pptd. directly	Blood laked before pptn.
Rabbit— ½ g of STA orally. Blood taken after one hour.	3.0 (trip.)	3.5 (trip.)	..
Man— 2 g of STA taken orally. Blood taken after two hours.	2.9 (dupl.)	3.6 (dupl.)	..
Man— 1 g of STA taken orally. Blood taken after two hours.	2.8	3.3 (dupl.)	3.3 (dupl.)
Rabbit— ½ g of STA taken orally. Sample after one hour.	3.5 (quad.)	4.0 (dupl.)	4.0 (dupl.)
Man— 2 g of STA orally. Sample after one hour.	2.3 (quad.)	2.6 (trip.)	2.6 (quad.)
Man— 2 g STA orally. Sample after 4 hours.	2.7 (trip.)	3.1 (quad.)	3.1 (quad.)

No interference from bubbles was noticed, because the sulfamate-nitrous acid reaction was eliminated. The time for a single analysis is reduced to about eight minutes. This is to be compared with about forty minutes for the Bratton and Marshall procedure, and about twelve minutes for the Werner procedure.[2] Recovery of sulfathiazole added to whole blood was 95 to 100 per cent., at a dilution of 1 : 20, and results on blood of patients who have received the drug were about 15 per cent. higher by the micro method than by the Bratton and Marshall method. This was taken to indicate almost complete recovery of the drug. The color which was formed was stable enough to permit accurate analysis for twenty-four hours.

Substantiating experiments and a discussion of the results will be published elsewhere as soon as the method has been tested under clinical conditions.

The experimental procedure is briefly as follows: Whole blood (0.30 ml) is added dropwise with vigorous shaking to 5.70 ml of "acid mixture" which is prepared by adding 56 ml of 4 N sulfuric acid to one liter of 3.33 per cent. trichloracetic acid. The protein is allowed to coagulate and is filtered through Whatman number 1 or 42 paper. Sodium nitrite solution (0.10 per cent., 0.10 ml) is added to a 2.00 ml aliquot of the filtrate, and three minutes is allowed for diazotization. Ethyl alcohol (1.00 ml) is added, the tube swirled, and 0.10 ml of N-1 naphthyl ethylene diamine (0.10 per cent.) added. The color forms to its maximum intensity in fifteen seconds.

The determinations were carried out in flat-bottomed 10 ml vials, and the color intensities measured in micro cuvettes, using a Coleman Universal spectrophotometer. It was also noted that the values could be found with fair accuracy by visually comparing the developed colors with color standards made from a mixture of fuchsin and methyl violet.

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[2] M. C. Andrews and A. F. Strauss, *Jour. of Lab. and Clinical Medicine*, 26: 888, 1941.

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