and that some of this diffused into the xylem above the cut. A considerable quantity of the active element must be present before detection is possible by the electroscope.

To determine what may be the amount of active phosphorus in a plant with intact xylem as compared with one that has the xylem removed, experiments were performed with *Sedum praealtum*. The wood was removed from one plant, as in the *Bryophyllum*, and another similar plant with xylem intact was selected as control. The two plants were placed in the same solution and in one experiment kept there for 40 hours. They were both cut up at the same time, sections taken at the same levels and the quantity of active phosphorus determined alternately. Table III gives the results.

TABLE III

TIME REQUIRED TO DISCHARGE THE ELECTROSCOPE BY 2 CM-LONG PIECES OF SEDUM PRAEALTUM STEM

Distance above solution, cm	Phloem		Wood and pith	
	exp. plant	control	exp. plant	control
3	19.0 min.	15.5 min.	26.0 min.	19.0 min.
7	21.0 "	17.25 "	wood removed	
11	26.75 "	20.25 "	32.0 min.	24.25 "
15	30.0 "	26.5 "	35.5 "	29.0 "
19	38.0 "	32.5 "	39.0 "	37.75 "

The difference in quantity of active phosphorus, level for level, between the two plants is remarkably small.

Experiments with well-rooted geranium cuttings have also been performed. The results are essentially the same as for *Sedum*.

These experiments show beyond a doubt that the radioactive phosphorus, in form of phosphate, is transported up the stem of a plant through the phloem.

The writers take this opportunity to express their thanks to Professor J. M. Cork, of the Physics Department, for his kindness in supplying them with the radioactive material, and also to Miss Alice Huse for her help with some of the preliminary experiments.

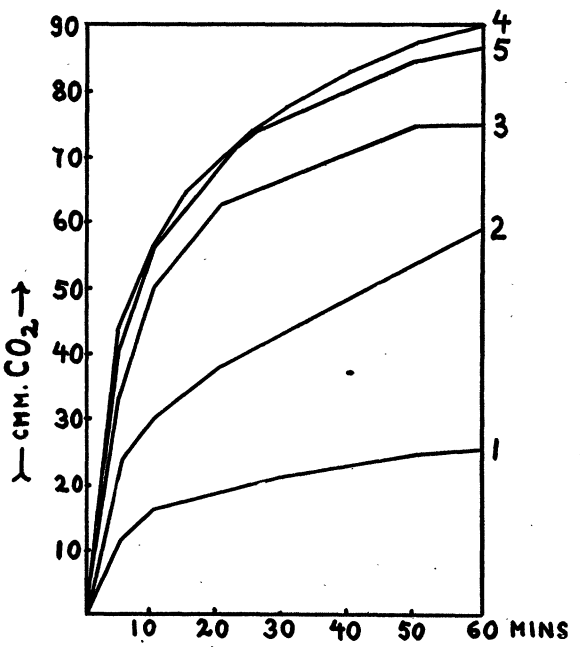
FELIX G. GUSTAFSON
MARJORIE DARKEN

DEPARTMENT OF BOTANY
UNIVERSITY OF MICHIGAN

SYNTHESIS OF CO-CARBOXYLASE FROM VITAMIN B_1

IF synthetic, crystalline vitamin B_1 is treated in the cold with phosphorus oxychloride in a molecular ratio of at least 1:2, a compound is formed exhibiting the properties of co-carboxylase.

The activity of the synthetic co-enzyme of carboxylase is tested in a system containing bottom yeast freed from natural co-carboxylase by extensive washing, pyruvic acid as the substrate, and magnesium as activator. The carbon dioxide formed by decarboxylation of the substrate, as determined in Warburg manometers, serves as the measure of activity. Up to the present the yield of synthetic co-carboxylase, as judged from the comparison with boiled yeast juice, has not exceeded 1.5 per cent. of the theory. The same results have been obtained using crystalline, synthetic vitamin B_1 preparations from two different sources,[1] one prepared by the synthesis of Williams and Cline[2] and the other by that of Andersag and Westphal.[3] A typical experiment is shown in the figure.



The main room of the Warburg vessels (total volume about 17 cc) contained 1 cc of washed dry yeast (*cf.* Lohmann and Schuster[4]), corresponding to 100 mg dry weight, varying amounts of synthetic co-carboxylase or boiled yeast juice, and 0.1 M. phosphate buffer, pH 6.2, to make a volume of 3 cc. After attainment of equilibrium there were added from the side bulbs of the vessels 0.3 cc of sodium pyruvinate solution, pH 6.2, equivalent to 5 mg pyruvic acid, containing 0.1 mg magnesium as $MgCl_2$. In addition to the foregoing, vessel No. 2 contained 0.2 cc of boiled yeast juice, corresponding to 20 mg of bottom yeast, vessel No. 3 contained 0.3 cc, vessels No. 4 0.9 cc, and vessel No. 5 1.8 cc of the synthetic co-carboxylase preparation No. II (1 cc equivalent to 2.2 mg vitamin B_1 hydrochloride). Vessel No. 1, containing only yeast suspension and buffer in the main room, served as the control. Atmosphere: Air; temperature 28°.

[1] The authors are indebted to Merck and Company and to the Winthrop Chemical Company for the supply of synthetic vitamin B_1.

[2] R. R. Williams and J. K. Cline, *Jour. Am. Chem. Soc.*, 58: 1504, 1936.

[3] Andersag and Westphal, cited by R. Grewe, *Zeits. physiol. Chem.*, 242: 89, 1936.

Lohmann and Schuster[4] report that natural co-carboxylase, isolated in highly purified form, from bottom yeast, represents a diphosphoric ester of vitamin B_1. The thiochrome pigment prepared from co-carboxylase differs from that obtained from vitamin B_1 by its phosphorus content. Cataphoretic experiments performed on the thiochrome derived from our synthetic product indicate that ester formation with phosphoric acid has occurred. The present experiments thus offer additional proof for the validity of the results of Lohmann and Schuster.

Attempts to effect a transformation of vitamin B_1 into co-carboxylase by tissue extracts (liver, brain, intestine) have as yet not been successful.

KURT G. STERN
JESSE W. HOFER
YALE UNIVERSITY, SCHOOL OF MEDICINE

AN ACCESSORY PHOTOSENSITIVE SUBSTANCE IN VISUAL PURPLE REGENERATION

KÜHNE'S discovery of the regeneration of visual purple in solution[1] has recently been confirmed and investigated quantitatively.[2] In repeating some of the measurements it was observed that in solutions bleached with a photoflood lamp the subsequent regeneration was greater than in those bleached by an ordinary 100-watt lamp, although the visual purple had completely disappeared in both cases. Because the photoflood lamp emits much energy in the blue, this suggested the existence of a blue-sensitive substance whose decomposition was essential for visual purple regeneration. If this is the case, visual purple solutions bleached by violet and blue light should show much more regeneration than solutions bleached by green, yellow and orange light. This turns out to be true, and an experiment illustrating it will now be described.

Two mutually exclusive parts of the spectrum were secured by passing the light from a heat-screened photoflood lamp on 110 volts either through a yellow filter (Corning No. 350) or through a blue one (Corning "lantern blue" No. 554). Tests showed that these two lights were almost equally effective in bleaching visual purple. A freshly prepared visual purple solution buffered at pH 7.7 was divided into two parts. One was illuminated with the blue light 10 cm away for 30 minutes, which was three times longer than necessary to bleach the visual purple completely. Its photometric density (log I_o/I) in a 5 mm absorption cell was measured at 500 mμ during the next 30 minutes in the dark, in the course of which the density increased by 0.0330. The other identical sample was similarly treated with the yellow light; its density increased only 0.0037 in the same time. To show that the yellow-bleached solution was nevertheless capable of more regeneration, it was then illuminated for 10 minutes with the blue light and its density again measured during 30 minutes in the dark. This time there was an increase in density of 0.0330. (The precise agreement is obviously accidental.)

The density was also measured at 450 mμ during these manipulations, and showed that the yellow-bleached sample had decreased considerably in density during its 10-minute exposure to blue. Apparently, the marked regeneration found at 500 as well as at 450 mμ occurred only after this density decrease in a blue-absorbing substance had taken place.

Whether this photolabile blue-absorbing substance is present in the unbleached visual purple solution or is a product of visual purple break-down is not decided by these data. Dr. E. L. Smith of this laboratory has suggested that it may be a flavin and is investigating this possibility at present. It is also uncertain whether the new material plays a primary rôle in vision in the same sense that visual purple does, or is important only for the resynthesis of visual purple in the dark.

The visual purple extractions which gave these results were obtained from winter frogs by the procedure that has already been described.[1] The photometric density measurements were made with a very sensitive photoelectric spectrophotometer designed by Dr. Simon Shlaer. The work was aided by a grant from the Rockefeller Foundation.

AURIN M. CHASE
LABORATORY OF BIOPHYSICS
COLUMBIA UNIVERSITY

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