

Lohmann and Schuster⁴ report that natural co-carboxylase, isolated in highly purified form, from bottom yeast, represents a diphosphoric ester of vitamin B₁. The thiochrome pigment prepared from co-carboxylase differs from that obtained from vitamin B₁ by its phosphorus content. Cathoretic 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 B₁ into co-carboxylase by tissue extracts (liver, brain, intestine) have as yet not been successful.

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AN ACCESSORY PHOTSENSITIVE SUBSTANCE IN VISUAL PURPLE REGENERATION

KÜHNE'S discovery of the regeneration of visual purple in solution¹ 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 ($\log I_0/I$) in a 5 mm absorption

cell was measured at 500 m μ 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 m μ 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 m μ 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.

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⁴ K. Lohmann and Ph. Schuster, *Naturwiss.*, 25: 26, 1937.

¹ A. Ewald and W. Kühne, *Unters. aus dem physiol. Inst. der Universität Heidelberg*, 1: 267, 1878.

² S. Hecht, A. M. Chase, S. Shlaer and C. Haig, *SCIENCE*, 84: 331, 1936.