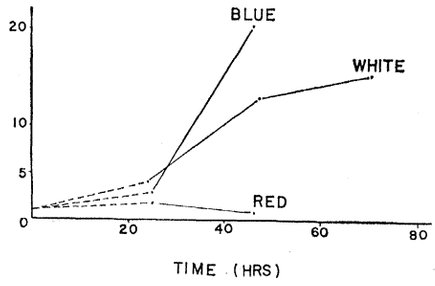

Fig. 1. The effects of red, blue, and white light on HeLa cells. Nonviability was defined by the ratio: (percentage of experimental cells that absorbed trypan blue)/(percentage of control cells that absorbed trypan blue). Ordinate, nonviability; abscissa, time (hours).

colors of light when erythrocytes were present. He described the cellular changes and suggested that the presence of red-cell pigment or its degradation products might be a possible requisite for the occurrence of the photodynamic phenomenon. Menke (*4*) described similar effects of light, in the presence of phloxine, on normal rat tissue and malignant rat sarcoma. Klein and Goodgal (*5*) described the photodynamic inactivation of monkey kidney cells grown in monolayers exposed to white light, or to light filtered through solutions of methylene blue and trypan blue.

We have observed the photodestruction of HeLa cells, grown in monolayers, exposed to white light and blue-filtered light (Fig. 1) and have searched for the cause of this photodynamic destruction.

Monolayers of HeLa cells were grown in Eagle's minimum essential medium (*6*) without phenol red, in Leighton tubes placed in a water bath (37°C) equipped with Corning glass filters, one of which passed red-orange light (maximum transmittance 672 mμ) and the other passed blue-violet light (maximum transmittance 458 mμ).

Cellular destruction was determined by viability tests with trypan blue, in which dye exclusion was the criterion of a viable cell.

Studies of the cells and components of the medium revealed that the human serum component of the medium was required for this phenomenon to occur. Cells maintained in direct light did not undergo degeneration if the medium used had no serum.

Examination of the literature indicates that a pigment is required for the photodynamic destruction described. Pigments present in human serum in relatively large quantities include bilirubin and carotenoids; riboflavin, which acts as a photosensitizer (*7*), is also present. If any of these substances were acting as photosensitizers, a more rapid and more pronounced destructive effect should occur if a higher than normal concentration were present. Thus, relatively high concentrations of bilirubin (from the serum of a hospital patient with 5 mg of bilirubin per 100 ml of serum), carotenoids (0.1 mg of vitamin A palmitate per milliliter), and riboflavin (2 μg/ml) were added separately to the growth medium of cells which were grown in blue light (Fig. 2).

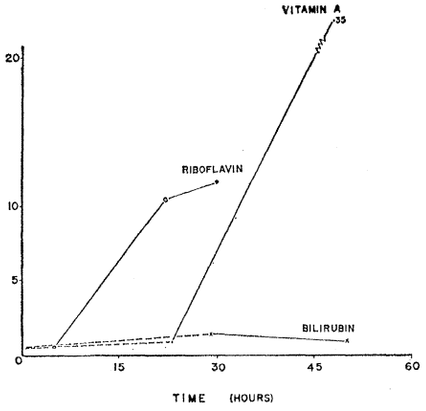

Fig. 2. The effects of blue light on HeLa cells grown in media enriched with riboflavin, vitamin A, and bilirubin. Ordinate, nonviability; abscissa, time (hours).

The increases in concentration of these substances were between 10 and 50 times the concentrations occurring in the normal medium. The curve representing the effect of high bilirubin concentration shows that there is no pronounced effect from exposure to blue light. Vitamin A enrichment produced a very pronounced destruction, but only after 48 hours of exposure to blue light. An increased concentration of riboflavin produced an early and definite cytopathogenic effect.

Therefore, it seems unlikely that bilirubin is the photosensitizer since it is unable to increase cellular destruction. Vitamin A satisfies one criterion, that of increased effect. However, this effect was not significantly rapid, and it might be somewhat different if it were acting as a sensitizer. Since vitamin A is very easily oxidized, it might be possible that the toxicity of a peroxide (*8*) would account for the greatly increased effect of exposure to blue light.

Riboflavin induced a very early destruction. After 30 hours of exposure to blue light, 100 percent of the riboflavin-treated cells were nonviable, whereas only 5 to 20 percent of the cells were nonviable in cultures treated with vitamin A enriched, elevated-bilirubin, and normal serum (*9*).

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9. Supported by Damon Runyon grant 722 and U.S. Public Health Service grant CR-2326. The original idea for this study resulted from discussions with Dr. F. D. Woidich, who designed and built some of the apparatus. We thank Dr. L. K. Alpert for encouragement and Miss H. Lee for technical assistance.

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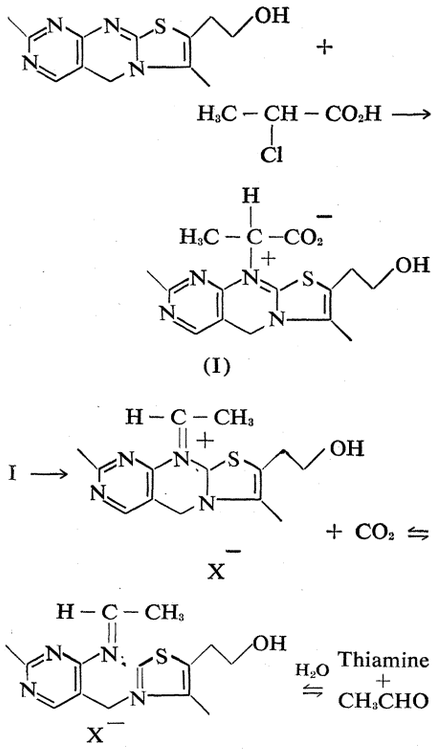
Thiamine: A Novel Conversion from Thiochrome

Abstract. *Reaction of thiochrome with α-chloropropionic acid yields thiamine and acetaldehyde. The reaction seems to proceed by way of a zwitterion intermediate that undergoes decarboxylation and hydrolysis. The biological significance of the reaction cannot yet be evaluated.*

Although the formation of thiochrome in the potassium ferricyanide oxidation of vitamin B_1 has been used for the quantitative assay (*1*) of the vitamin, the chemistry of the heterocycle has not been studied extensively (*2*). In the course of investigating the chemistry of thiochrome, we discovered an interesting decarboxylation. Under mild conditions, the reaction of thiochrome with α-chloropropionic acid yielded thiamine and acetaldehyde as the major products. The conditions for the reaction were as follows: thiochrome (250 mg, 0.9 mmole) and α-chloropropionic acid (98 mg, 0.9 mmole) were suspended in water (5 ml). Ethyl alcohol was then added to dissolve the mixture completely. Sodium hydroxide (40 mg in 2 ml of

water) was added to the solution, and the mixture was stirred and heated in an oil bath at 50°C for 5 hours. Then a portion was acidified to *p*H 2 with dilute hydrochloric acid and analyzed by paper chromatography. The chromatogram showed the presence of acetaldehyde, thiamine hydrochloride, thiochrome, and thiochrome hydrochloride. The relative frequencies, R_F, of these compounds in a mixture of alcohol, acetic acid, and water (1:1:1) were 1.0, 0.57, 0.92, and 0.78, respectively; in a mixture of isobutyl alcohol, acetic acid, and water (4:5:1) R_F's were 1.0, 0.26, 0.84, and 0.49. The reaction sequence can be formulated in these terms:



At present the biological significance of this reaction to the thiamine-catalyzed decarboxylation of pyruvic acid is not known.

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Indoleacetamide as an Intermediate in the Synthesis of Indoleacetic Acid in Pseudomonas savastanoi

Abstract. *When* DL-*tryptophan-2-C*^{14} *was incubated with washed cells or cell-free preparations of* Pseudomonas savastanoi, *two radioactive metabolites were formed. One was identified as indoleacetamide and the second, indoleacetic acid. The amount of indoleacetamide in the reaction mixture increased rapidly during the early stages of incubation; it reached a peak after 15 minutes and declined steadily thereafter. Indoleacetic acid, on the other hand, accumulated slowly throughout the incubation period. Cell-free preparations preferentially utilize the* L-*isomer of tryptophan for the synthesis of indoleacetamide and indoleacetic acid. The results of these experiments suggest, therefore, that* P. savastanoi *synthesizes indoleacetic acid by the following reactions:* L-*tryptophan→ indoleacetamide→indoleacetic acid.*

The bacterium, *Pseudomonas savastanoi*, incites the production of tumorous outgrowths on plants. Indoleacetic acid accumulates in the tumors and, presumably, plays some part in their formation (*1*). Relatively large quantities of indoleacetic acid accumulate in culture media in which the bacteria are growing. Since tryptophan is known to be a precursor of indoleacetic acid in the metabolism of a number of organisms (*2*), studies were made on its metabolism in *P. savastanoi*.

When DL-tryptophan-C^{14} was incubated with extracts of cells of *P. savastanoi* that had been broken up by high frequency sound, indoleacetic acid and an unknown compound with properties resembling those of indoleacetamide were produced. We are reporting the identification of the unknown compound as indoleacetamide and its role as an intermediate in the conversion of tryptophan to indoleacetic acid by this bacterium.

Cells of *P. savastanoi* were washed from a solid proteose peptone-glycerol medium with sterile, distilled water. Cell-free suspensions were prepared by disrupting the bacterial cells, at 2°C under hydrogen, in a Raytheon sonic oscillator. The suspension was then centrifuged at 15,000*g* for 10 minutes at 0°C and passed through a Millipore filter (pore size 0.45 μ) to remove cellular debris. The reaction mixtures consisted of an enzyme preparation containing 26 μg of protein, 0.1 μc of DL-tryptophan-2-C^{14} (1.1 mc/mmole), 2.5 ml of 0.01*M* tris buffer (*p*H 7.4), and water with a final volume of 3.1 ml. The reactions were carried out at 27°C for specified periods of time and were terminated by heating in boiling water for 5 minutes. The denatured protein in the heated reaction mixtures was removed by centrifugation.

The formation of the products of the reactions was followed by separating the components on chromatographic paper by a mixture of isopropanol, concentrated NH_4OH, and water (10:1:1, v/v). Radioactive components on the chromatograms were detected by a chromatographic-strip counter and then radioactivity was determined with a Packard tri-carb liquid scintillation counter (*3*). In addition, radioactivity was determined with a Tracerlab gas flow counter.

Chromatograms thus prepared and treated bore three radioactive areas with R_F values of 0.23, 0.36, and 0.73. These values corresponded with the R_F values of tryptophan, indoleactic acid (IAA), and indoleacetamide (IAM), respectively. The unknown radioactive compounds at R_F 0.36 (unknown A) and at R_F 0.73 (unknown B) were eluted from chromatograms. Unknown A was cochromatographed with unlabeled, authentic IAA and unknown B with unlabeled, authentic IAM. The chromatograms were then developed in the first dimension with 70-percent ethanol and in the second dimension with the mixture of isopropanol, ammonia, and water. Radioautograms were prepared to determine the positions of the radioactive compounds and the chromatograms were then sprayed with Salkowski's reagent (*4*). In both cases radioactive areas corresponded exactly in size and position with the Salkowski-positive areas.

In another experiment, radioactive unknown A and unknown B were eluted from chromatograms with 95-percent ethanol and the eluates diluted with water to give a final solution of 30-percent ethanol. Then 17 mg of authentic IAA were dissolved in the solution of unknown A and 40 mg of authentic IAM were dissolved in the solution of unknown B. After acidification to *p*H 3.0, the solutions were shaken with ether and the ether fractions were separated. The ether was evaporated and the residues were crystallized from water. Weighed samples of the crystalline compounds were measured for radioactivity and their specific activities were calculated. The