CONVERSION OF TRYPTOPHAN TO KYNURENINE, AND SEROTONIN TO KYNURAMINE

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The effect of ultraviolet light on tryptophan and serotonin breakdown awaits further studies as to the rate of hydrolysis and the hydrolysis products.

The production of formyl kynurenine and kynurenine as the metabolic products of tryptophan was suggested by (Heidelberger et al, 1949), and by a number of other investigators (Amano et al, 1950, Stanier and Hayashi, 1951). The conversion of tryptophan to kynurenine by ultraviolet light was reported by (Hakim, 1959).

The ultraviolet light source was minerlight, model V-41, short ultraviolet (2537 angstrom units) six-bar, quartz tube and special short wave filter producing 45 milliwatts per square foot source.

Aqueous solutions of L-. DL- and D-tryptophan, as well as solutions of serotonin-creatin-sulfate complex, were exposed to ultraviolet light for 5, 15, 30, 60, 120 and 180 minute intervals.

After irradiation, the resulting mixture was poured into ice-cold ethanol. The supernatant obtained after centrifugation was concentrated 10 fold in vacuo; and the clear, yellow solution obtained was

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exposed to electrophoretic and chromatographic analysis. It contained formyl kynurenine (from tryptophan) or formyl kynuramine (from serotonin).

Chromatograms of tryptophan or serotonin solutions before and after ultraviolet light irradiation were developed in the following solvents: Equal volumes of 95 per cent ethanol and 0. 1 M acetic acid as described by (Zatman, Kaplan and Colowick, 1953), methanol-water (9:1), methanol-isopropanol-water (2:1:1), Butanol-acetic acid - water (4:1:5) and in phenol-water (9:1).

The chromatograms were examined under ultraviolet light. They were then treated with p-dimethyl aminobenzaldehyde; tryptophan, formylkynurenine, kynurenine, formyl kynuramine and kynuramine produced different color spots. Dried chromatograms not exposed to previous treatment, were sprayed with ninhydrin in butanol ; tryptophan, serotonin and their derivatives were visible after drying the paper at 100° C. for five minutes.

Chromatograms sprayed with ninhydrin or p-dimethyl amino-benzaldehyde reagent were used to guide the location of the various compounds on chromatograms not exposed to previous treatment. From duplicate chromatograms, untreated, the located spots were cut, eluted in 5 ml. of 0. 05 per cent sodium bicarbonate, 0. 05 N sodium hydroxide.

Ultraviolet irradiation of L-, D-, DL-tryptophan, and of serotonin or serotonin-creatin-sulfate solutions resulted in changes of their characteristic ultraviolet spectra and electrophoretic mobility. The pH of the solution decreased and the solution became colored. Resolution of the products by paper chromatography showed the production of formyl kynurenine, D-kynurenine (L-kynurenine from L-tryptophan,

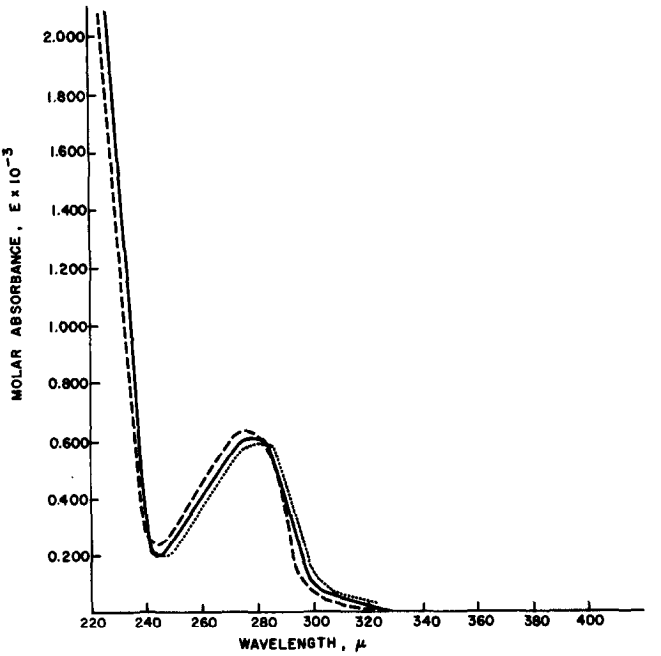

Figure 1a. Ultraviolet spectrum of tryptophan at different pH. At pH 7.5 (———), at pH 5.5 (– – – –), at pH 8.5 (·····).

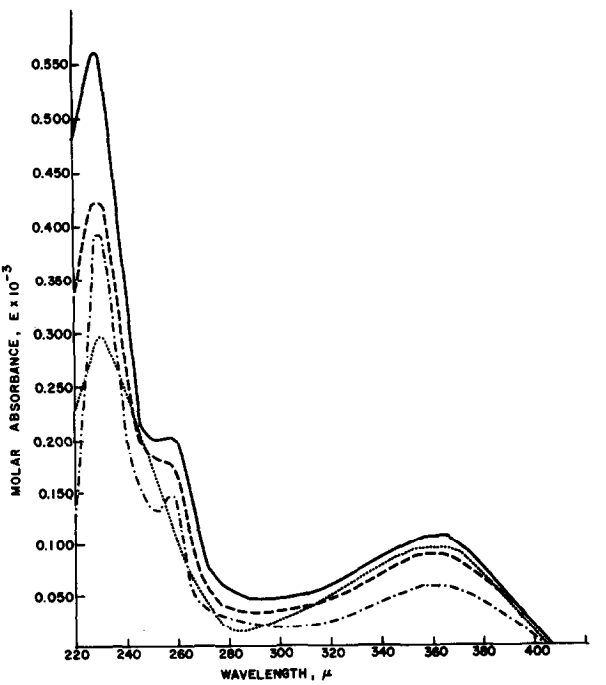

Figure 1b. Ultraviolet spectrum of serotonin at different pH. At pH 7.5 (———), at pH 5.5 (– – – –), at pH 8.5 (·····), in normal saline (—·—·—).

DL-kynurenine from DL-tryptophan) and melanin-like compounds from irradiated tryptophan solutions (Figure 1 a, b).

Resolution of the products, from irradiated serotonin solutions (0. 2 M), on paper chromatography showed the production of formyl kynuramine (0. 2 M), kynuramine (0. 2 M), and pigment-like material (Figure 1 c).

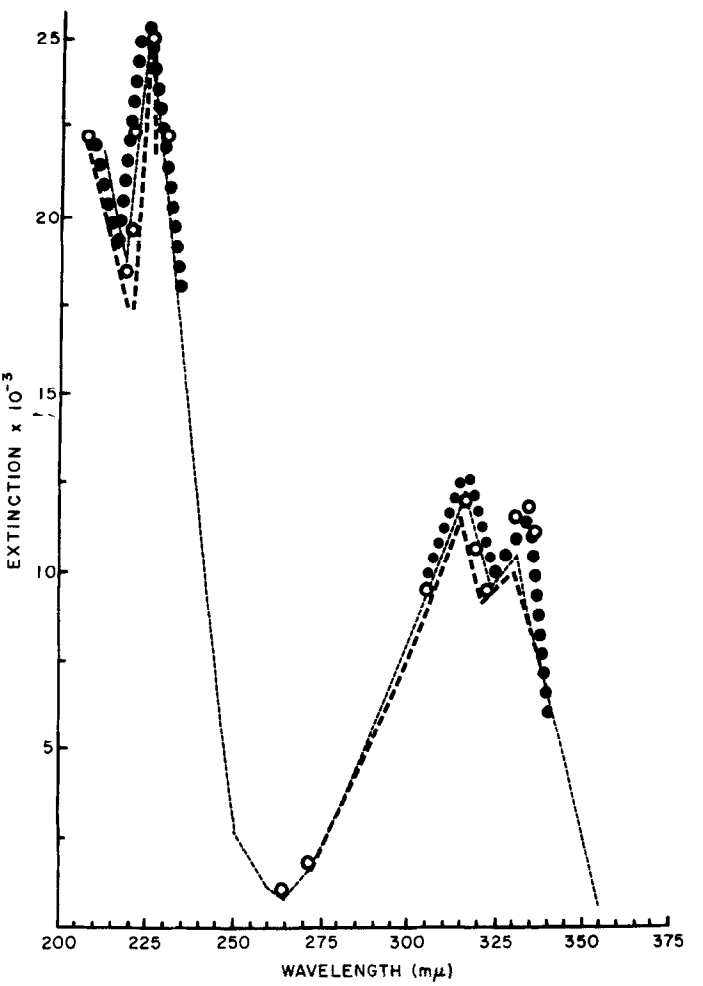

Figure 1c. Ultraviolet spectrum of kynurenine and kynuramine. Authentically pure kynurenine (– – – –), kynurenine obtained after ultraviolet irradiation of tryptophan (●●●●●), kynuramine authentically pure (-------), kynuramine obtained from serotonin after ultraviolet irradiation (○○○○○○).

Aqueous solutions of triphosphopyridine nucleotide (TPNH) and adenosine triphosphate (ATP) were exposed to ultraviolet light for 5, 15, 30, 60, 120 and 180 minutes. The separation of the products of irradiation of TPN or of ATP was attempted in several solvent systems. The resolution of TPN, ATP or the phosphorylated breakdown products was attained in the pyridine water (2:1) (Burton and San Pietro, 1954).

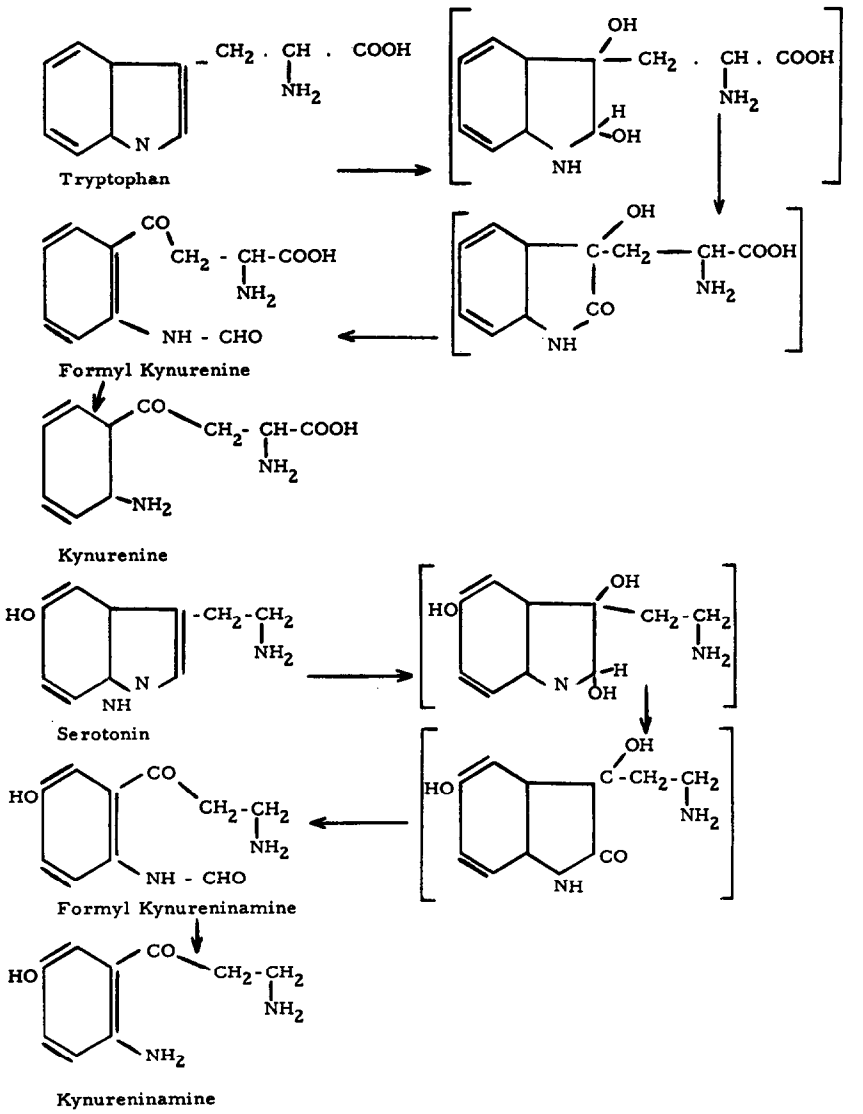

Figure 2. Metabolic Pathway of Tryptophan and Serotonin

Nicotinamide, adenosine-2',5'-diphosphate and 2'-phosphoadenosine diphosphate were identified as breakdown products of TPN. Ultraviolet light irradiation of tryptophan or serotonin, or serotonin in presence of TPN,formed nicotinamide, adenosine-2',5'-diphosphate and 2'-phosphoadenosine diphosphate. Neither kynurenine nor kynuramine were identified. Triphosphopyridine nucleotide prevented the action of ultraviolet light on tryptophan or serotonin.

The effect of pH, salt concentration as well as tryptophan, serotonin and TPN concentration on the conversion of tryptophan to kynurenine, or of serotonin to kynuramine will be reported in detail shortly. These results suggest that ultraviolet light irradiation induces the metabolism of tryptophan and serotonin in the metabolic pathway presented in Figure 2.

References

C. Heidelberger, M. E. Gulberg, A. F. Morgan and S. Lefkovsky, J. Biol. Chem., 179, 143 (1949).

T. Amano, M. Torri, H. Tritani, Med. J. Asake University, 2, 45 (1950).

R. Y. Stanier, and O. Hayashi, J. Bacteriol., 62, 691 (1951).

A. A. Hakim, Fed. Proc., 18, 240 (1959).

L. J. Zatman, W. O. Kaplan, and S. P. Colowick, J. Biol. Chem., 200, 197 (1953).

R. M. Burton and A. San Pietro, Arch. Biochem. Biophys., 48, 184 (1954).

A. Butenandt, W. Weidel and H. Schlossberger, Naturforschg., 4b, 242 (1949).