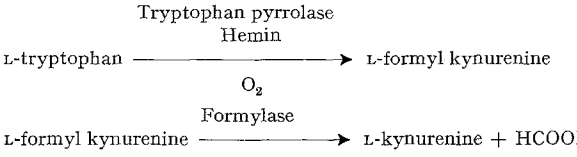
Improved Biochemical Preparation of *L*-Kynurenine

In the liver L-tryptophan is enzymatically oxidized to L-kynurenine as shown in this schematic drawing.



We were able to prepare L-kynurenine by following this same enzymatic pathway, the product being purified by chromatography on a cationic resin and crystallized as sulfate.

Material and methods. a) Purification of tryptophan pyrrolase and formylase. These enzymes are purified from rat liver with a slight modification of KNOX's technique[1]. Intraperitoneal administration of cortisone hemisuccinate, with a dose of 37.5 mg/100 g body wt., increases the concentration of tryptophan pyrrolase in the liver[2]. Four h after this injection, the rats are sacrified and the livers are removed.

The rat livers are homogenized in the Potter-Elvehjem homogenizer in 2 volumes by weight of an isotonic KCl solution containing 2 µmoles/ml of DLα-methyltryptophan, which is a tryptophan pyrrolase stabilizer[3]. The homogenate is centrifuged at 4 °C (18,000 *g*, 15 min). Tryptophan pyrrolase and formylase are precipitated from 60 ml of the supernatant by acidification to pH 5.5 with 6 ml of 0.75 *M* potassium phosphate monobasic and 3 ml of 0.17 *N* acetic acid. The precipitate is separated by centrifugation at 4 °C (18,000 *g*, 10 min), then suspended by homogenization in 40 ml of 0.1 *M* potassium phosphate buffer (pH 7.0) containing 1 µmole/ml of L-tryptophan. No kynureninase activity was detected in this enzymatic suspension.

b) Kynurenine synthesis. The incubation medium contains: 1.5 mmoles of L-tryptophan dissolved in 200 ml of 0.1 *M* potassium phosphate buffer (pH 7.0), 1.88 µmoles of hemin chlorhydrate dissolved extemporarily in 25 ml of 0.04 *N* NaOH and 40 ml of the enzymatic preparation. This mixture is gently shaken at 37 °C on a magnetic stirrer.

To follow the progress of the reaction, 0.5 ml aliquots of the incubation mixture are added to 2 ml of 10% metaphosphoric acid. After centrifugation, readings of the supernatant are taken at 280 nm (maximum absorption of tryptophan) and 360 nm (maximum absorption of kynurenine).

After about 18 h these two readings reach constant values: L-tryptophan is totally converted to kynurenine. 100 ml of 2.5 *N* perchloric acid are added to precipitate all the proteins. After centrifugation the supernatant is stored at 4 °C.

c) Purification of L-kynurenine. The supernatant of the preceding step is chromatographed on resin Dowex 50 × 12 (200–400 mesh) form H^+ on a 7 × 3.25 cm column. Contaminants are washed down with 80 ml fractions of HCl with increasing normality: N/10, N/3, N, 2.5 *N*, 5 *N*. L-kynurenine is eluted with the 5 *N* HCl fraction.

Organic microanalysis of the L-kynurenine sulfate

Values (%)	C	H	N	S
Theoretical	37.04	4.97	8.64	9.89
Found	37.43	5.37	8.76	9.82

1.2% mineral ashes as Na_2CO_3 are substracted to obtain found values.

This eluate is evaporated in vacuum to a dry residue which is taken up in 30 ml of distilled water in a water-bath for 48 h. After addition of 3 ml of NH_2SO_4, this solution is concentrated under reduced pressure to about 3 ml. After standing at 4 °C for 48 h, white crystals of kynurenine sulfate are formed. Recrystallization can be carried out with small amounts of 0.1 *N* H_2SO_4.

Results and discussion. The final yield of this enzymatic preparation is usually about 56%. Organic microanalysis of the product gives satisfactory values for the sulfate hydrate ($C_{10}H_{12}O_3N_2$, H_2SO_4, H_2O), as can be seen on the Table. Kynurenine sulfate darkens and decomposes at 167 °C.

The purity of the newly synthesized product was tested by chromatography on Whatman No. 1 paper with several solvents: butanol, acetic acid and water (4:1:2); isopropanol, ammonia (sp. gr. 0.880) and water (20:1:2); 8% (W/V) aqueous NaCl and glacial actic acid (100:1)[4]. Examination of the chromatograms under UV-light followed by treatment by some location reagents (Ehrlich reagent, Ekman reagent, ninhydrin reagent) assessed the purity of the product. In every solvent, the Rf of our product and of DL-kynurenine Sigma were identical.

Hypothetic kynurenine isomerization during the synthesis, is examined by chromatography on Whatman No. 1 paper with methanol, *n*-butanol, benzene, water, glacial acetic acid (40:20:20:20:1)[5]. After treatment of the chromatogram by Ehrlich reagent, one single spot was seen. Chromatography of DL-kynurenine Sigma under the same conditions shows two spots corresponding to the D- and the L-kynurenine.

The enzymatic synthesis of L-kynurenine previously suggested by HAYAISHI[6] uses cell-free extracts of tryptophanadapted cells of *Pseudomonas* sp (No. 11, 299 of A.T.C.C.) as an enzyme source. The bacteria must be grown on a medium overloaded with L-tryptophan. After the enzymatic transformation of tryptophan into kynurenine under conditions very similar to the one we used, the purification of the product is ensured only by crystallization in sulfuric medium.

That enzymatic preparation is very time-consuming and the kynurenine obtained is strongly contamined with non-oxidized tryptophan. In the technique we are proposing, the use of induced liver tryptophan pyrrolase provides a very active, quickly prepared enzymatic suspension. The inclusion of column chromatography improves the purification of the final product.

Résumé. Préparation biochimique de L-kynurénine avec le L-tryptophane et des enzymes du foie de rat semi-purifiés.

C. LEMOYNE, H. JÉROME and P. KAMOUN[7]

Laboratoire de Biochimie Génétique, Hôpital des Enfants Malades, 149, rue des Sèvres, F-75015 Paris (France), 9 January 1972.

[1] W. E. KNOX, in *Methods in Enzymology* (Ed. S. P. COLOWICK and N. O. KAPLAN; Academic Press, New York 1955), vol. 2, p. 242.
[2] O. GREENGARD, J. biol. Chem. *238*, 1548 (1963).
[3] M. CIVEN and W. E. KNOX, J. biol. Chem. *235*, 1716 (1960).
[4] I. SMITH, in *Chromatographic Techniques* (Ed. HEINEMAN; 1958).
[5] M. MASON and C. P. BERG, J. biol. Chem. *188*, 783 (1951).
[6] O. HAYAISHI, ni *Biochemical Preparations* (Ed. E. E. SNELL; 1953), vol. 3, p. 108.
[7] Acknowledgments. This present work was supported by C.N.R.S. E.R.A. No. 47 and by I.N.S.E.R.M. The authors wish to thank Mr. PESSON for his excellent help in organic analyses.