CXLV. THE METABOLISM OF TRYPTOPHAN.

III. THE MODE OF FORMATION OF KYNURENIC ACID FROM TRYPTOPHAN.

By WILLIAM ROBSON.

*From the Department of Physiology, King's College,
University of London.*

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It has been shown by the author in a previous paper [1924] that light may possibly be thrown on the mechanism of the formation of kynurenic acid from tryptophan by feeding to an animal a tryptophan substituted in the benzene nucleus and recovering from the urine a substituted kynurenic acid. For the purpose of carrying out an investigation on these lines, the author selected *Bz*-3-methyltryptophan [Robson, 1924]. In a subsequent paper [Robson, 1928], the synthesis of the possible end-products of the metabolism of this substituted tryptophan, *i.e.* 6-methyl- and 8-methyl-kynurenic acids, has also been described. The final stage of the investigation consisted in the administration, either by feeding or by injection, of these three compounds to a dog or to a rabbit, followed by an examination of the urine for possible end-products of their metabolism.

The animal selected for the study, a rabbit, was placed on a diet of oats and water. A sample of the oats used for this purpose gave no visible reaction with the Hopkins-Cole reagent and was considered to contain very little, if any, tryptophan. After the second day, a 24 hours' sample of urine was collected and an attempt made to isolate kynurenic acid from it by Capaldi's method. The urine (75 cc.) was treated with half its volume of a solution containing barium chloride (10 %) and ammonium hydroxide (5 %). After half an hour, the voluminous precipitate which had formed was filtered off and the filtrate evaporated to half its original volume (40 cc. approx.). Careful neutralisation of the concentrate with hydrochloric acid (4 %) produced no precipitate even after 48 hours. It was inferred, therefore, that kynurenic acid was absent from the urine.

Exp. 1¹. Injection of tryptophan. Tryptophan (1 g.) in the form of its sodium salt was injected subcutaneously into the rabbit. The urine, collected during the next 24 hours, was treated according to Capaldi's method. The kynurenic acid thus obtained weighed, on recrystallisation from alcohol, 0.13 g., and melted at 282°.

¹ The injections described in the present paper were carried out by Dr F. R. Curtis, to whom the author's thanks are due.

Exp. 2. Injection of Bz-3-methyltryptophan. Bz-3-Methyltryptophan (1 g.) in the form of its sodium salt was injected subcutaneously into the same rabbit, and the urine collected for 24 hours. This measured 70 cc. and was treated according to Capaldi's method. The concentrated urine was acidified with 4 % hydrochloric acid, and was set aside for 48 hours. No precipitate was deposited. The urine, which did not give the Hopkins-Cole reaction in the cold, was consequently freed from barium, made acid to Congo red and shaken with ether and then with ethyl acetate. Evaporation of the ethereal extract gave a very small quantity of an oily residue, reddish yellow in colour, which did not crystallise after standing 14 days in a desiccator. The ethyl acetate extract on evaporation gave a residue consisting mainly of urea; analysis of this residue failed to show the presence of any compound of indole.

A second experiment with Bz-3-methyltryptophan (0.6 g.) was carried out on the same animal and the urine collected for 48 hours. Treatment of the urine as in the previous experiments failed to reveal the presence of either of the methylkynurenic acids in the small amount of flocculent precipitate which formed on the addition of the hydrochloric acid at the final stage. The concentrated urine was then freed from barium and sulphuric acid, neutralised to litmus and shaken several times with ether. The combined ethereal extracts were washed with water and evaporated to dryness on the water-bath. The oily residue resembled in appearance that obtained in the previous experiment. It did not crystallise on long standing in the desiccator and there was not a sufficient quantity of it available for purposes of identification. It would appear from the results of these last two experiments that the Bz-3-methyltryptophan was completely burnt in the rabbit.

Exp. 3. Injection of 6-methylkynurenic acid. For the purpose of Exps. 3 and 4 a second rabbit was used. This animal, on the diet of oats and water, had been shown to give kynurenic acid in the urine when tryptophan was injected subcutaneously.

6-Methylkynurenic acid (0.8 g.) was injected subcutaneously and the urine collected during the next 48 hours. Submitted to Capaldi's method of analysis, it gave 0.65 g. of a substance which, on purification, melted at 277°. A melting-point determination of a mixture of the isolated substance and pure 6-methylkynurenic acid gave a reading of 277°, showing that the unknown substance was undoubtedly 6-methylkynurenic acid. It was apparent, therefore, that 6-methylkynurenic acid had passed through the organism of the animal unchanged.

Exp. 4. Injection of 8-methylkynurenic acid. This acid (0.9 g.) was injected into the rabbit under the same conditions as those already described. The urine, collected for 48 hours, on being treated according to Capaldi's method, gave no precipitate on standing for 2 days. The neutralised concentrate on steam-distillation gave a strongly smelling liquid, an investigation of which failed to reveal the presence of any quinoline derivative. The urine was therefore again concentrated, acidified and shaken with ether. The ethereal extracts on evapo-

ration gave a small quantity of a reddish oil which did not crystallise on long standing in the desiccator.

A second experiment with 1.2 g. of 8-methylkynurenic acid was carried out. Analysis of the urine collected failed to indicate the presence either of the methylkynurenic acid which had been fed, or any of its possible derivatives. It would appear, therefore, that 8-methylkynurenic acid is completely burnt in the animal organism.

DISCUSSION.

From the results of the feeding experiments described above it is seen that, whereas 6-methylkynurenic acid was recovered almost quantitatively from the urine of the animal, no determinable end-products were obtained in the case of *Bz*-3-methyltryptophan and of 8-methylkynurenic acid. In one sense, since no derivative was isolated from the urine when *Bz*-3-methyltryptophan was injected, the results of the experiments with this compound can scarcely be regarded as wholly satisfactory.

On the other hand, however, if reference is made to the diagram [Robson, 1928] illustrating the possible ways in which kynurenic acid may be formed from tryptophan it will be seen that, according to Theory II, *Bz*-3-methyltryptophan gives rise to 8-methylkynurenic acid. The fact that both *Bz*-3-methyltryptophan and 8-methylkynurenic acid are completely burnt in the animal organism strongly suggests that the latter compound is an intermediate metabolite of the former. Such a transformation would involve the elimination of the pyrrole ring according to the following diagram:

Evidence in the literature in favour of the transformation occurring in this way is not entirely lacking. For example, Ellinger and Matsuoka [1914] and Barger and Ewins [1917] on feeding *Pr*-2-methyltryptophan to an animal failed to detect the occurrence in the urine of a corresponding methylated kynurenic acid, and as a result the latter workers concluded that such a transformation did not take place because the pyrrole ring had been rendered more stable by the presence of the methyl group in the α -position. Again, such nitrogen-ring formation by the utilisation of the side chain of an amino-acid—which the above formation of kynurenic acid would demand—is not without precedent in the literature. For instance, Raper [1927] has definitely shown that tyrosine may give rise, under the action of tyrosinase, to 5 : 6-dihydroxyindole and 5 : 6-dihydroxyindole-2-carboxylic acid, where the amino-nitrogen is the precursor of the nitrogen of the pyrrole ring.

It would appear, therefore, that the results of the feeding experiments with *Bz-3-methyltryptophan*, 6-methylkynurenic acid and 8-methylkynurenic acid form additional evidence in favour of the theory, originally brought forward by Ellinger, that the formation of kynurenic acid in the animal organism involves the elimination of the pyrrole ring of the tryptophan molecule.

SUMMARY.

Bz-3-methyltryptophan, when injected into a rabbit, is completely burnt. In view of the fact that a like fate is suffered by 8-methylkynurenic acid in the organism of this animal, whilst 6-methylkynurenic acid can be recovered from the urine of the animal almost quantitatively, it is suggested that in the formation of kynurenic acid from tryptophan the pyrrole-nitrogen of the latter compound is eliminated and the side chain with its amino-nitrogen forms the new pyridine ring.

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