Tryptophan Metabolism in Rice Moth Larva (Corcyra Cephalonica St.) (20705)

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Interest in the metabolism of tryptophan in the rice moth larva arose from the observation made by Sarma(1) that the larvae maintained on a tryptophan-rich, pyridoxine-deficient diet excreted yellow-colored feces. This revealed a striking parallel between mammals and insects, as regards tryptophan metabolism, since rats, rabbits, dogs and other mammals under similar conditions had been known to excrete the pigment metabolites, kynurenine, 3-hydroxykynurenine, xanthurenic acid etc.(2-5). Xanthurenic acid, the most characteristic metabolite in pyridoxine deficiency in mammals, was shown to be absent in the larval excretory pigment, as the latter gave a negative test with iron salts. Earlier work on tryptophan metabolism in insects has been mainly in connection with its function as a precursor of the pigments, ommochromes, found in the eyes and tissues. Here tryptophan undergoes conversion via kynurenine and hydroxykynurenine to the complex pigment molecule(6-8).

Paper partition chromatography has in recent years greatly facilitated the investigation of tryptophan metabolism in mammals. In the present paper, the larval excretory pigment has been analysed by the technic of circular paper chromatography first developed by Rutter(9) and later adapted to the resolution of amino acids in biological materials by Giri(10). In a preliminary communication Sundaram and Sarma(11) have reported the identification of kynurenine and hydroxykynurenine in the larval feces.

Methods. Whatman No. 1 filter circles, 27 cm in diameter, were used and the method employing the detachable wick described by Giri(10) was followed. Two solvent mixtures were tried, namely, butanol-acetic acid-water (4:1:5)(4) and butanol-methanol-benzene-water (1:2:1:1)(12). The former gave by far the better resolution in the form of compact spots and was used throughout the experiments reported herein. The effective distance traversed was about 10 cm, the time taken varying from 3 to 5 hours. Ultraviolet fluorescence and Ehrlich's reagent(13) were employed for the detection of the metabolites on the chromatogram. Quinolinic acid was located as the yellow chelate compound it forms with ferrous ion. In addition to being chromatographed individually, the metabolites were resolved in various combinations. The experimental technic for the study of tryptophan metabolism in the rice moth larva has been described(1,11). Rice moth larvae were kept on the appropriate experimental diet till the major portion of the diet had been consumed (1-1½ months). The feces excreted by pyridoxine-deficient and pyridoxine-fed larvae on diets supplemented with DL-tryptophan at a level of 1% were collected separately from the residuary diet and thoroughly extracted with distilled water. The extracts were concentrated *in vacuo* at 55°, made 60-70% alcoholic by addition of rectified spirit, centrifuged and the clear supernatant used for paper chromatography. Pure tryptophan metabolites were run alongside for purposes of comparison and identification of the constituents of the larval excretory pigment. After irrigation with the solvent mixture, the chromatograms were developed as mentioned above.

Results. The R_f values of the several tryptophan metabolites with details of identification tests employed are presented in Table I and compared with the values obtained by us according to the method(14) of ascending paper chromatography. In general, it is found that the R_f values obtained by circular paper chromatography are slightly higher. Formylkynurenine gave a second spot (R_f on circle .91) much paler than the main one. Kynurenic acid and 3-hydroxyanthranilic acid left green streaks behind the main spots.

The results of analysis of the extracts of

TABLE I. R_f Values of Tryptophan Metabolites.

Metabolite	Ultraviolet fluorescence	Ehrlich's reaction	R_f value Circular	R_f value Ascending
L-tryptophan	Negligible	Orange-yellow (violet*)	.73	.58
DL-kynurenine	Sky-blue	Yellow-orange (fades*)	.69	.57
Formylkynurenine	"	"	.70	.56
3-hydroxykynurenine	Green	Pink (fades*)	.64	.49
3-hydroxyanthranilic acid	Purple-blue	Light orange-yellow	.95	.90
Xanthurenic "	Violet-blue	Nil	.78	.69
Kynurenic "	Green	"	.74	.64
Quinolinic "†	Nil	"	.55	.50
Anthranilic "	Violet	Orange-yellow	.97	.95

* On exposure to HCl fumes.
† Yellow with ferrous sulphate.
R_f values given represent mean of values obtained in several runs, deviations being within ±.03.

TABLE II. Metabolites Excreted by Rice Moth Larvae.

Spot	R_f	Ultraviolet fluorescence	Ehrlich's reaction	Identity
			Pyridoxine-deficient (L−)	
1	.44	Grey	Nil	†
2	.52	"	"	†
3	.66	Green	Pink (fades*)	3-hydroxykynurenine
4	.71	Sky-blue	Yellow-orange (fades*)	Kynurenine
5	.74	Nil	Orange-yellow (violet*)	Tryptophan
			Pyridoxine-fed (L+)	
1	.67	Violet	Yellow	†
2	.72	Green	Orange-yellow (fades and turns violet*)	Tryptophan and an unidentified metabolite
3	.82	Blue	Nil	†
4	.85	Green	"	†

* On exposure to HCl fumes.
† Not identified.
R_f values given represent mean of values obtained in several runs, deviations being within ±.03.

the larval excreta are summarised in Table II and Fig. 1.

The influence of pyridoxine on tryptophan metabolism, indicated in a qualitative way by the absence of the yellow color in the feces of pyridoxine-fed larvae on a tryptophan-supplemented diet, was further elucidated by chromatography of the extracts L+ and L— (Fig. 1) side by side. The 4 spots given by the L+ extract (Table II) do not appear to correspond to any of the common tryptophan metabolites. They may represent either tryptophan metabolites hitherto unknown or normal excretory products of the larva having their origin in substances other than tryptophan. It is significant, however, that these metabolites are absent in the L— extract. Similarly spots 1 and 2 given by the L— extract have not been identified so far. Similar unidentified metabolites have been reported by Dalgliesh(5) and by Reddi and Kodicek(13) in mammalian excretions and by Mason and Berg(12,15) in their tissue slice experiments on tryptophan metabolites and in the urine of rats dosed with D-tryptophan.

Kynurenine and 3-hydroxykynurenine, which are present in the L— extract but absent in the L+ extract (Table II and Fig. 1). constitute the yellow pigment excreted in pyridoxine deficiency. 3-hydroxykynurenine, identified in the excretion of mammals only very recently(4), has been found by us to be excreted by larvae fed tryptophan in the absence of pyridoxine. It was considered until 1951 as only a hypothetical intermediate in the tryptophan-niacin transformation in mammals in view of its demonstrated role in this series of reactions in *Neurospora*(16) and of

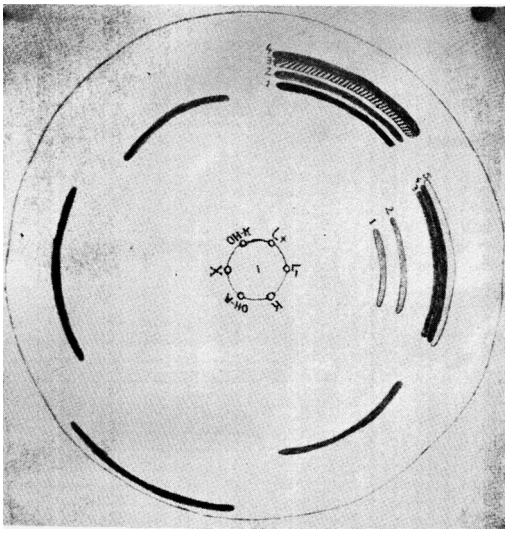

FIG. 1. Diagrammatic reproduction of circular chromatograms. K—Kynurenine; OH-A—3-Hydroxyanthranilic acid; X—Xanthurenic acid; OH-K—3-hydroxykynurenine; L+—Extract of feces of pyridoxine-fed larvae on tryptophan-supplemented diet; L−—Extract of feces of pyridoxine-deficient larvae on tryptophan-supplemented diet. (Vide Table II for details regarding spots given by extracts L+ and L−.)

its identification as the intermediary cn^+ substance formed from the v^+ substance, kynurenine, in the production of the insect pigments, ommochromes, from tryptophan(6-8). A tryptophan-niacin conversion scheme involving kynurenine and 3-hydroxykynurenine as intermediates and the role of pyridoxine therein are thus established also in the case of insects. The accumulation and the excretion of the kynurenines in pyridoxine deficiency by the rice moth larva are both in accord with the accepted mechanism of the metabolism of tryptophan to niacin in other organisms, where pyridoxal phosphate as coenzyme mediates the further conversion of 3-hydroxykynurenine to 3-hydroxyanthranilic acid(4,5). The absence of xanthurenic acid in the larval excretory pigment already reported(1,11) is confirmed.

Summary. 1. The technique of circular paper chromatography has been adapted to the resolution of the various tryptophan metabolites. 2. Kynurenine and 3-hydroxykynurenine have been shown to constitute the yellow pigment excreted by rice moth larva on a tryptophan-rich pyridoxine-deficient diet. The absence of xanthurenic acid in the excretory pigment has also been demonstrated.

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