Abnormal Tryptophan Metabolites in Human Pregnancy and their Relation To Deranged Vitamin B_6 Metabolism. (21183)

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After ingestion of 10 g of Dl-tryptophan, normal individuals excrete only traces of xanthurenic acid (4-8 dihydroxquinoline-2-carboxylic acid). However, in subjects with induced vit. B_6 deficiency the same tryptophan load test results in the excretion of appreciable amounts of this metabolite in the urine(1). A similar disturbance of tryptophan metabolism occurs in pregnancy but can be promptly corrected by the administration of pyridoxine (2,3). Paper chromatographic methods have recently disclosed that after feeding tryptophan, additional metabolites are excreted by vit. B_6 deficient, but not by normal rats(4). We wish to report a similar tryptophan metabolite excretion pattern in normal pregnant women, which is readily corrected by the administration of pyridoxine.

Material and methods. Ten gram doses of Dl-tryptophan were given to 4 normal controls, and to 11 women one to 2 days after delivery. The urine was collected for 24 hours and xanthurenic acid determined quantitatively, as previously described(5). The excretion of the normal controls averaged 16 mg and that of women at term 183 mg of xanthurenic acid. To isolate the tryptophan metabolites, the method of Dalgliesh(4) was used. All urines were treated identically to enable semi-quantitative correlation. Mercuric acetate in slight excess was added to 2/10 of the acidified 24-hour specimen. The formed precipitate was treated with H_2S. Excess H_2S was then expelled from the supernatant containing the released metabolites with the help of nitrogen. Deactivated charcoal was then added to absorb completely all fluorescent compounds. The charcoal was eluted to remove the fluorescent substances—generally 9 washings of 5% phenol were sufficient—and the combined yellow eluates were vacuum concentrated to 2 ml.

Four λ aliquots were chromatographed on Whatman paper No. 1 using the descending technic with a solvent mixture of butanol, acetic acid and water (4:1:5). Available reference substances included L-kynurenine, Dl-kynurenine, 3-hydroxy-knurenine, xanthurenic acid, kynurenic acid and Nα-acetyl-kynurenine. Spots appearing under fluorescent light are recorded in Table I as well as R_f values, results of various color tests and correlation to the spots described for vit. B_6 deficient rats(4). Concentrated urine preparations were also hydrolyzed in a sealed tube with 6 N H_2SO_4 for one hour at 100°C and chromatographed after removal of the acid.

Results. Three spots, No. 6, 7, and 8 were always found in the controls and occasionally a trace of No. 10. In pregnancy urine these spots exhibited stronger fluorescence and at least 6 additional spots numbers 1, 2, 3, 4, 5, and 9 were recognized. Spot 10 was always present. After acid hydrolysis only spots 5, 6, and 8 remained.

Reference to Table I indicates that spots 5 and 6 can be clearly identified as 3-hydroxy-kynurenine and kynurenine, respectively. Available reference standards enabled direct comparison and proof. Spot 6 was composed of 2 distinct parts, the lower part, representing the D-isomer, formed a narrow band on the leading edge of 3-hydroxy-kynurenine. This indicates some conversion of D-tryptophan to d-kynurenine. If only L-tryptophan was given the D-isomer was absent. In pregnancy urines, spot 8 was apparently a mixture of kynurenic and xanthurenic acids, and gave a positive Pauly reaction. In contrast, spot 8 in normal subjects gave a negative reaction, thus confirming the absence of an appreciable amount of xanthurenic acid, as previously established by chemical estimation. Therefore, spot 8 in non-pregnant subjects is probably kynurenic acid only. Spot 9 is identical with spot 1 found by Dalgliesh in vit. B_6 deficient rats and seems to be a conjugated 3-hydroxy-kynurenine. Spot 10, compared with Nα-acetyl-kynurenine as reference material gave identical reactions. Spots 1, 2,

TABLE I. Summary of Experimental Data.

Spot #	Mean Rf	Fluorescence	Color reactions: Ninhydrin	Ehrlich	Ekman	Pauly	Ammoniacal silver nitrate	Reference substance available	Correlation with Dalgliesh spots(4)	Probable identity	Occurrence in: Controls	Occurrence in: Pregnancy following 10 g dl-tryptophan
1	14	Weak bluish-green	Red-purple	Faint pink	—	—	—	No	A?	Deriv. of 3-hydroxy-kynurenine?	—	+
2	18	Strong bluish-green	Purple	Pink	Yellow, on diazotising turning brown	Red-brown	Brown	"	C?	*Idem*	—	+
3	21	Brilliant blue-purple	—	—	—	—	—	"			—	+
4	24	Brilliant blue-purple	—	—	—	Red	—	"		Derivative of xanthurenic acid?	—	+
5	41	Green	Brown-purple	Pink	Yellow, on diazotising turning brown	Red-brown	Brown-black	Yes	E	3-hydroxy-kynurenine	—	+
6	49	Strong pale blue	Purple	Orange	Magenta	—	Brown	"	F	Kynurenine	+	+
7	55	Deep blue-purple	—	—	—	—	—	No			+	+
8	63	Blue-purple	—	—	—	Red	Faint brown	Yes	H	Kynurenic and xanthurenic acid	+*	+
9	83	Green	—	Pink	Yellow, on diazotising turning brown	Red-brown	Brown-black	No	I	N α-acetyl-3-hydroxy-kynurenine	—	+
10	86	Pale blue	—	Orange	Magenta	—	Brown	Yes	J	N α-acetyl-kynurenine	±	+

* Apparently kynurenic acid only.

5. and 9 appear yellow on the untreated chromatogram. On the basis of its color reactions, fluorescence and disappearance on hydrolysis, spot 2 is apparently a derivative of 3-hydroxy-kynurenine. Spot 1 may also be a derivative of 3-hydroxy-kynurenine. Its inconsistent color reactions are probably due to its low concentration. Spots 3 and 4 showed a brilliant blue-purple fluorescence and the strong reaction with Pauly's reagent given by spot 4 would suggest that it is a derivative of xanthurenic acid. Spot 7 had a deep purple fluorescence and gave no typical color reactions.

In 3 women at term the tryptophan load test was repeated after an interval of 24 hours. During this time 50 mg of pyridoxine HCl were given in 2 equal doses 12 hours and ½ hour before the administration of the second dose of 10 g of Dl-tryptophan. In agreement with previous findings, no xanthurenic acid could be detected in the urine after administration of the vitamin(3). Chromatographic analyses of these urines showed complete disappearance of spots 5 and 9 and the merest traces of fluorescence in spots 1, 2, 3, 4, and 10. The intensity of spots 6, 7, and 8 resembled those obtained in non-pregnant normal controls.

Comment. The excretion of various tryptophan metabolites in subjects on a regular hospital diet even without added tryptophan has been reported. Thus, children with congenital hypoplastic anemia excrete large amounts of anthranilic acid(6). Spaček described the increased excretion of kynurenine and of another unidentified substance in conditions associated with cachexia such as cancer(7). In the urine of a patient with severe tuberculosis, Makino and his coworkers demonstrated the presence of kynurenine, 3-hydroxy-kynurenine, kynurenic and xanthurenic acids and conjugation products of these substances(8). Kotaki and Tani found xanthurenic acid and 3-hydroxy-kynurenine in the urine of patients with diabetes(9). Dalgliesh and Tekman investigated a large number of patients with various diseases and found excretion of 3-hydroxy-kynurenine only in patients with febrile states regardless of their etiology(10). These investigators attributed the excretion of abnormal tryptophan metabolites to a high rate of breakdown of body proteins causing a relative insufficiency of the enzyme systems responsible for tryptophan catabolism.

Following the ingestion of 10 g Dl-tryptophan, we found several fluorescent spots in the chromatograms prepared from the urines of our normal controls. Three of these spots could be identified as kynurenine, kynurenic acid and Nα-acetyl-kynurenine, respectively. Dalgliesh and Tekman detected only kynurenine in the urine of 2 control subjects under the same conditions. The finding of kynurenic acid and acetylated kynurenine is, however, of some interest, since besides being attacked by kynureninase, kynurenine formed can be removed in 3 other ways: a) by oxidative deamination or transamination to give, after ring closure, kynurenic acid, b) by conjugation, and c) by ring oxidation to give hydroxy-kynurenine(10).

In pregnancy many more tryptophan metabolites appear in the urine in an excretion pattern strikingly similar to that observed in the vit. B_6 deficient rat. It is of interest to note that abnormal tryptophan metabolites in induced vit. B_6 deficiency occur not only in the rat but also in the rice moth larva(11).

The ability of vit. B_6 to rectify this abnormal excretion pattern strongly suggests that in pregnancy there exists a vit. B_6 deficiency which is probably conditioned by the increased demand of the growing fetus for this essential vitamin(12).

Summary. Following ingestion of 10 g Dl-tryptophan, normal subjects excrete kynurenine, kynurenic acid and occasionally Nα-acetyl kynurenine. In pregnancy many additional metabolites, including 3-hydroxy-kynurenine, occur in the urine. The abnormal tryptophan metabolite excretion pattern in these women is strikingly similar to the one described in vit. B_6 deficient rats. Administration of pyridoxine suppresses to a large degree the excretion of the abnormal substances.

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Preparation of Globin from Human Hemoglobin. (21184)

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Considerable interest has been shown in the preparation of plasma expanders from human globin(2,4,5). For this purpose it is not necessary to utilize so-called "undenatured" globin, but it is desirable to use a colorless globin protein which can then be modified as desired. The usual methods of preparing globin protein from hemoglobin are those of Anson and Mirsky(1), Strumia and Chornock(2) and Strumia and Sample(3). These methods utilize the precipitation of a globin hydrochloride from a reaction mixture obtained by mixing an aqueous hemoglobin solution with acidified acetone. The globin obtained by these procedures is unfortunately contaminated with considerable quantities of heme or heme complexes. The purpose of this communication is to describe a new method of preparing a globin protein which is almost devoid of colored impurities.

This new procedure is based on two findings: 1, a true solution of dissociated human hemoglobin can be obtained in certain mixtures of water, water-miscible solvents and strong acids; and 2, activated carbon will adsorb the heme from such solutions. Thus, the method consists of dissolving hemoglobin in a suitable acid-water-solvent solution, treating the solution with activated carbon, filtering off the carbon, and recovering the globin from the filtrate. One suitable set of conditions is described below.

Materials and methods. Fresh, packed human red blood cells at 10°C were filtered through cheese cloth to remove clots. The filtered cells were lysed by the addition of 2 volumes of distilled water at room temperature and the solution adjusted to pH 5.8 ± 0.1 with 1 N HCl. The precipitated stroma was removed by centrifugation. To 100 ml of stroma-free lysate, 300 ml of acetone containing 5 ml of 37% hydrochloric acid were added slowly with stirring. As soon as a clear solution was obtained, 15 g of Darco G-60* were added and the mixture stirred gently for 10 minutes. Seventy-five ml of distilled water were added to the slurry which was stirred for an additional 20 minutes. The slurry was then filtered with vacuum through a Buchner funnel. One gram of Hyflo Super Cell† was added to the carbon slurry to speed filtration.

The filtrate was adjusted to pH 7.0 with 5% sodium hydroxide. One hundred and fifty ml of acetone were added with stirring to precipitate the globin which was then collected by filtration. The globin can also be obtained

* Obtained from Darco Department, Atlas Powder Co.

† Obtained from Johns Mansville.