THE ABNORMALITY OF TRYPTOPHANE METABOLISM IN CHILDREN WITH MONGOLISM

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There is evidence (Gershoff, Hegsted and Trulson, 1958; Jérôme, Lejeune and Turpin, 1960; O'Brien, Groshek and Streamer, 1960) that children with mongolism have a defect in tryptophane metabolism which is characterized primarily by a diminished urinary excretion of xanthurenic acid.

The purpose of this study was to confirm this observation using more exact techniques and to attempt to specify the alteration in metabolism more precisely by comparing the effects of 1-tryptophane loading on the urinary excretion coefficients ($\mu\text{mols/kg./7 hours}$) of both 5-hydroxy-3-indoleacetic acid and the kynurenine pathway metabolites of tryptophane in a group of children with mongolism and in a control group of non-mongoloid mentally retarded children.

Methods

Twenty-one children with mongolism, aged 9 to 16 years, and 19 non-mongoloid mental defectives of comparable age, sex and intelligence were studied. All the children were active, able to feed themselves and keep themselves clean. Both groups were in the same institution and received an identical diet. At the commencement of the test, fasting subjects voided and then drank 300 ml. of flavoured milk in which was suspended 100 ml./kg. body weight of 1-tryptophane. All urine was collected for seven hours after the tryptophane load, and, to ensure completeness, individual voidings were supervised by one or other of the investigators. This seven-hour collection period was chosen as being the longest period that could be properly monitored.

A measured aliquot of urine was assayed for 5-hydroxy-3-indoleacetic acid (Udenfriend, Titus and Weissbach, 1955), N^1 -methylnicotinamide (Huff and Perlzweig, 1947; Asami, 1957), and 4-pyridoxic acid (Reddy, Reynolds and Price, 1958). Other metabolites of the kynurenine pathway were separated by two-dimensional paper chromatography and identified under ultraviolet light. These were then eluted and estimated either directly by their absorbance in the ultraviolet, or as azo compounds coupled with pyridine or naphthylethylenediamine (Coppini, Benassi and Montorsi, 1959; O'Brien and Ibbott, 1962a). The chromatographic methods were

linear within the experimental range and recoveries for kynurenine, acetylkynurenine, kynurenic acid, hydroxy-kynurenine, xanthurenic acid and hydroxyanthranilic acid gave a mean value of 97.6% (82.5-104.7%) with a mean coefficient of variation of 7.9% (4.6-12.6%). Reproducibility, expressed also as a coefficient of variation, averaged 4.7% for standards and 5.9% for urine samples. These figures were shown to be slightly, but not significantly, better than those for column chromatography techniques (Satoh and Price, 1958) in the cases of kynurenic and xanthurenic acids.

Because intravenous tryptophane loading was not permitted, fasting, one- and two-hour serum samples were obtained for estimation of the tryptophane levels. These were determined as the enol-borate complex after adsorption and elution from a Dowex 50 column (O'Brien and Ibbott, 1962b).

TABLE 1
URINARY EXCRETION COEFFICIENTS OF
4-PYRIDOXIC ACID
($\mu\text{g./kg./7 hour period}$)

	Mongols (20 Observations)	Non-mongols (18 Observations)	Significance*
Mean	2.7	2.4	$p < 0.10$
Standard deviation	1.5	1.7	$p > 0.05$

* Trickett, Welch and James (1956).

Results

The urinary excretion coefficients ($\mu\text{mols/kg./7 hours}$) of 5-hydroxy-3-indoleacetic acid and of the metabolites of the kynurenine pathway are set out in Fig. 1. These show that, whereas the children with mongolism excreted uniformly less of all these metabolites than the non-mongols, the differences were highly significant only for xanthurenic acid and to a lesser degree for acetyl kynurenine. There was no difference (Table 1) between the two groups in the amount of 4-pyridoxic acid excreted in the urine.

FIG. 1.—Urinary excretion coefficients of tryptophan metabolites following an oral loading dose of 100 mg. (490 μmols)/kg. body weight of l-tryptophane (μmols/kg./7 hours).

TABLE 2
MEAN SERUM TRYPTOPHANE LEVELS IN mg./100 ml. AT ONE AND TWO HOURS, FOLLOWING AN ORAL LOADING DOSE OF 100 mg./kg. BODY WEIGHT OF l-TRYPTOPHANE

	Mongols (21 Observations)	Non-mongols (19 Observations)	Significance
Pre-loading			
Mean	1.8	1.8	
Standard deviation	1.3	0.75	p > 0.10
One hour			
Mean	7.1	8.7	
Standard deviation	2.7	2.7	p < 0.10 p > 0.05
Two hours			
Mean	8.5	9.8	
Standard deviation	2.6	2.9	p > 0.10

TABLE 3
THE RISE IN SERUM TRYPTOPHANE LEVELS IN mg./100 ml. FOLLOWING AN ORAL LOADING DOSE OF 100 mg./kg. BODY WEIGHT OF l-TRYPTOPHANE

	Mongols (21 Observations)	Non-mongols (19 Observations)	Significance
One hour			
Mean	5.3	6.9	
Standard deviation	2.1	2.4	p < 0.05 p > 0.02
Two hours			
Mean	6.7	7.9	
Standard deviation	2.5	2.5	p > 0.10

There was no significant difference in the levels of serum tryptophane either before the test or at one or two hours after loading (Table 2). However, when the actual rise in serum tryptophane over and above the fasting levels was calculated (Table 3), it could be shown that this was less in the children with mongolism and that the difference between them and the controls was probably significant (p = <0.05 and >0.02) at one hour.

Discussion

Orally administered l-tryptophane is partly metabolized by the flora of the bowel to indole derivatives. These are absorbed and excreted in the urine principally as indican, indoleacetic and indolelactic acids (Fig. 2), the last two compounds being also the products of endogenous metabolism. Of the l-tryptophane entering the blood stream, a certain proportion will be incorporated into tissue proteins. In adults in nitrogen balance, about 1% of the residue (Udenfriend, Weissbach and Sjoerdsma, 1956) is metabolized via serotonin to 5-hydroxyindoleacetic acid, a further small amount is excreted directly as tryptophane and the greater part of the remainder is broken down to 3-hydroxy-anthranilic acid and then via glutarate and acetate to CO₂ (Gholson, Sanders and Henderson, 1959). Finally, some 3-hydroxyanthranilic acid is converted

FIG. 2.—Metabolism of tryptophane.

to nicotinic acid appearing in the urine as N¹-methylnicotinamide and N-methyl-2-pyridone-5-carboxamide.

In this study the results confirm previous observations (Gershoff *et al.*, 1958; Jérôme *et al.*, 1960; O'Brien *et al.*, 1960) that, in children with mongolism, there is a decreased urinary excretion of xanthurenic acid following an oral dose of tryptophane. Clearly there are a number of possible causes for this finding. One explanation suggested by the serum tryptophane levels at one hour is that of tryptophane malabsorption. However, this appears improbable for two reasons. In the first place, it would be expected to lead to an increased urinary excretion of indole derivatives as is the case in Hartnup disease (Shaw, Redlich, Wright and Jepson, 1960) and other malabsorption states (Haverback, Dyce and Thomas, 1960) instead of to the observed decrease in urinary indoleacetic acid

(Jérôme *et al.*, 1960). Secondly, significant reductions in the urinary excretion coefficients (Fig. 1) in this series are not uniform but appear to be unique for xanthurenic acid and to a less significant extent for acetyl kynurenine, a point that is also against an increased renal clearance of unmetabolized tryptophane.

If the data are examined another way and the product : substrate ratios in the urine are calculated for acetyl kynurenine to kynurenine and xanthurenic acid to hydroxykynurenine, it is apparent that the low urinary excretion of acetyl kynurenine in children with mongolism is not significant considering their lower kynurenine excretion. However, in the case of xanthurenic acid, the decreased urinary excretion in the children with mongolism does not appear to be similarly explicable in terms of a diminished availability of hydroxykynurenine. Thus it appears that in the children with mongolism

TABLE 4
PRODUCT: SUBSTRATE RATIOS IN THE KYNURENINE
PATHWAY

	Mongols	Non-mongols	Significance
<i>Acetylkynurenine: kynurenine</i>			
Mean	0.56	0.71	p > 0.10
Standard deviation	0.38	0.16	
Number of observations	20	19	
<i>Xanthurenic acid: hydroxykynurenine</i>			
Mean	0.40	0.85	p < 0.05
Standard deviation	0.35	0.70	
Number of observations	18	15	
<i>Hydroxyanthranilic acid: hydroxykynurenine</i>			
Mean	0.93	0.68	p > 0.10
Standard deviation	0.34	0.43	
Number of observations	18	14	

there may be a specific abnormality of hydroxykynurenine transaminase resulting in a diminished conversion of hydroxykynurenine to xanthurenic acid. For final proof, however, it would be necessary both to measure urinary xanthurenic acid after the intravenous administration of its precursor hydroxykynurenine, and to demonstrate a normal renal clearance of xanthurenic acid per unit of glomerular filtrate.

Vitamin B₆ deficiency appeared to play no part in these changes in tryptophane metabolism not only because there was no difference between the groups in 4-pyridoxic acid excretion but, more importantly, because the most sensitive index of pyridoxine deficiency and dependency, the ratio of urinary hydroxyanthranilic acid to hydroxykynurenine (Table 4), was not decreased. It remains a possibility, however, that the apoenzyme of hydroxykynurenine transaminase in children with mongolism has an altered affinity for pyridoxal phosphate.

Finally, this study did not confirm previous observations suggesting a defect in the methylation of nicotinamide in children with mongolism (Gershoff *et al.*, 1958). Nevertheless, there is a possibility that these children have other less defined impairments of tryptophane metabolism from the significantly lowered values for urinary hydroxyindoleacetic acid (Jérôme *et al.*, 1960; Gershoff, personal communication) and indoleacetic acid

(Jérôme *et al.*, 1960) found in certain instances after tryptophane loading.

Summary

The seven-hour urinary excretion coefficients of 5HIAA, kynurenic acid, xanthurenic acid, kynurenine, hydroxykynurenine, hydroxyanthranilic acid, methylnicotinamide and 4-pyridoxic acid were measured in 21 children with mongolism and in a comparable group of 19 non-mongoloid mental defectives after an oral load of 100 mg./kg. of l-tryptophane.

The results suggest an abnormality of hydroxykynurenine transaminase resulting in diminished conversion of hydroxykynurenine to xanthurenic acid in the children with mongolism.

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