Observations on Nicotinic Acid Therapy in Hartnup Disease

PAUL W. K. WONG*, A. M. LAMBERT, P. M. PILLAI, and P. M. JONES

*From the Mental Retardation Research Unit and the University Unit,
Royal Manchester Children's Hospital*

Nicotinamide deficiency is a well-recognized biochemical abnormality in untreated cases of Hartnup disease (Baron, Dent, Harris, Hart, and Jepson, 1956; de Laey, Hooft, Timmermans, and Snoeck, 1964). There is general agreement that nicotinamide or nicotinic acid therapy is effective in the prevention and cure of the pellagrinoïd rash in these patients. There is also some evidence that nicotinic acid may be beneficial in the treatment of the neurological symptoms (Baron *et al.*, 1956; Halvorsen and Halvorsen, 1963). In 1964 de Laey *et al.* reported that nicotinic acid therapy completely corrected the metabolic abnormalities in one patient under their study. This has never been observed in any other case reported in the literature. A confirmation of their observation is of primary importance, not only because nicotinic acid may be a curative treatment in Hartnup disease, but also because it may be a causal factor in the primary defect that has been demonstrated in the transport of tryptophan in these patients (Milne, Crawford, Girão, and Loughridge, 1960; Wong and Pillai, 1966). The present paper describes some biochemical observations before and during intensive nicotinic acid therapy in two cases of Hartnup disease. The clinical and biochemical abnormalities in these two patients have been previously reported (Wong and Pillai, 1966).

Materials and Methods

Oral L-tryptophan loading tests were performed before and during oral nicotinic acid therapy. 100 mg. L-tryptophan per kg. body weight was dissolved in orange squash and was given to the fasting patients in the morning. The patients were given a constant protein diet at regular meal times three days before and during the experiments. Similarly, oral L-tryptophan loading tests were performed in untreated normal children as controls. Heparinized venous blood was used for the estimation of tryptophan and amino acids. Plasma tryptophan was estimated by the method of Duggan

and Udenfriend (1956). Urine was collected under ice without preservative in consecutive periods of 4 hours, for 4 hours before and 24 hours after the loading. Small aliquots from all the urine samples were immediately frozen after collection. Urinary indican (indoxyl sulphate) was estimated by a modification of the method of Sharlit (1933); kynurenine was estimated by a modification of the method of Coppini, Benassi, and Montorsi (1959); indole-3-acetic acid was estimated by the method of Weissbach, King, Sjoerdsma, and Udenfriend (1959); and urine tryptophan was estimated by paper chromatography, using a solvent of butanol-acetic acid-water (12:3:5) and staining with Ehrlich solution. 24-hour urine was estimated for total nitrogen by the micro-Kjeldahl method, for amino nitrogen by the method of Albanese and Irby (1944), and for N'-methylnicotinamide by the method of Carpenter and Kodicek (1950). Both plasma and urinary amino acids were estimated by a modification of the method of Moore, Spackman, and Stein (1958), with a Technicon amino acid autoanalyser. An 8-hour urine collection (midnight to 8.0 a.m.), with blood taken at the end of this period, was used for the estimation of the renal clearance of amino acids. Renal clearance of amino acids was calculated as ml. of blood cleared of the particular amino acid per minute per 1.73 square metres of body surface, according to the following formula:

$$C_m = \frac{U}{P} \times V \times \frac{1.73}{SA}$$

where C_m = ml. blood cleared per minute; U = mg. amino acid/100 ml. urine; P = mg. amino acid/100 ml. plasma; V = ml. urine excreted/minute; and SA = square metre of body surface area.

Results

Both patients (previously reported as Case 1 and Case 2) were treated initially with 50 mg. nicotinic acid orally per day. After 2 months of therapy, an oral tryptophan loading test was performed in Case 2, without any improvement in his biochemical findings. Similarly in Case 1, after 5 months of therapy, repeated tests did not show an improvement in the excessive excretion of amino nitrogen in the urine or the abnormal amino acid pattern in the paper chromatograms of urine.

Received March 20, 1967.

* Present address: Mount Sinai Hospital, Chicago, Ill., U.S.A.

In order to ensure that the patients received adequate doses of nicotinic acid, they were admitted to the Royal Manchester Children's Hospital. Case 1 was given 150 mg. nicotinic acid daily in three divided oral doses at meal times. After 4 days of therapy, a tryptophan load was given. Case 2 was given 300 mg. nicotinic acid daily in three divided oral doses at meal times, for 4 days before the tryptophan load. During these periods of high nicotinic acid intake both cases excreted higher than average amounts of N'-methylnicotinamide, Case 2 more markedly than Case 1; indicating that both patients were no longer deficient in nicotinic acid or nicotinamide at the time of the tryptophan loading tests (see Table VI).

Table I shows the plasma levels of tryptophan in both cases and in 5 controls after oral loading; while there was a doubtful improvement in the intestinal absorption of tryptophan in Case 1, there was no similar improvement in Case 2.

Table II shows the urinary excretion of kynurenine after tryptophan loading. In both patients there was a consistently higher output of kynurenine in the urine during the period of high nicotinic acid

TABLE I
Plasma Tryptophan (mg./100 ml.) After Tryptophan Loading

Dosage of nicotinic acid per day (mg.)	Case 1		Case 2		Mean of 5 Controls
	0	150	0	300	0
0 hour	1.0	1.2	1.7	1.0	1.58
2 hours	2.3	5.0	3.2	2.7	8.56
4 hours	2.0	3.2	4.1	1.8	6.64
6 hours	2.0	2.7	2.5	1.5	2.42
8 hours	1.9	2.2	2.8	1.5	1.70

intake. However, it is seen that the increase was delayed and was less when compared with that observed in the controls.

Table III shows the urinary excretion of indican. No reduction of the abnormally high output of indican was observed in either patient during the treatment periods.

Table IV shows the urinary excretion of indole-3-acetic acid. Both patients appeared to have a reduction of indole-3-acetic acid excretion when

TABLE II
Urinary Kynurenine (μg./kg. per 4 hours) After Loading

Dosage of nicotinic acid (mg.)	Case 1		Case 2				Mean of 3 Controls
	0	150	0	0	50	300	0
- 4 to 0 hours	130	194	170	92	123	176	33
0 to 4 hours	50	150	218	171	303	133	737
4 to 8 hours	170	445	206	177	339	629	483
8 to 12 hours	180	230	157	218	266	114	305
12 to 16 hours	60	153	121	94	76	—*	61
16 to 20 hours	64	180	194	—*	262	193	80
20 to 24 hours	231	160	352	171	273	307	70
μg./kg. 24 hours	755	1318	1248	831	1519	1376	1736

* No urine voided.

TABLE III
Urinary Indican (mg./kg. per 4 hours) After Loading

Dosage of nicotinic acid (mg.)	Case 1		Case 2				Mean of 3 Controls
	0	150	0	0	50	300	0
- 4 to 0 hours	2.83	3.90	3.60	2.55	3.58	3.53	0.760
0 to 4 hours	1.77	1.44	1.52	1.00	5.09	2.40	0.810
4 to 8 hours	1.84	11.50	1.15	1.76	4.61	3.50	0.686
8 to 12 hours	2.13	15.20	0.54	3.12	7.76	0.67	0.334
12 to 16 hours	2.25	3.84	1.00	4.82	4.48	—*	0.455
16 to 20 hours	1.93	3.71	2.90	—*	13.09	3.25	1.680
20 to 24 hours	8.51	15.75	6.42	11.93	9.70	3.14	0.908
mg./kg. 24 hours	18.43	51.44	13.53	22.53	44.73	12.96	4.873

* No urine voided.

TABLE IV
Urinary Indole-3-acetic Acid (mg./kg. per 4 hours) After Loading

Dosage of nicotinic acid (mg.)	Case 1		Case 2				Mean of 3 Controls
	0	150	0	0	50	300	0
- 4 to 0 hours	0.20	0.35	0.95	0.14	0.14	0.09	0.054
0 to 4 hours	0.08	0.05	0.47	0.07	0.22	0.12	0.344
4 to 8 hours	2.12	0.62	0.57	0.65	0.52	0.23	0.208
8 to 12 hours	1.29	0.94	0.65	1.98	1.18	0.15	0.056
12 to 16 hours	0.37	0.20	0.83	1.47	0.87	—*	0.033
16 to 20 hours	0.30	0.17	0.48	—*	1.50	0.50	0.054
20 to 24 hours	2.54	0.80	2.67	2.39	1.64	0.93	0.088
mg./kg. 24 hours	6.70	2.78	5.67	6.56	5.93	1.93	0.783

* No urine voided.

TABLE V
Urinary Tryptophan (mg./kg. per 4 hours) After Loading

Dosage of nicotinic acid (mg.)	Case 1		Case 2				Mean of 2 Controls
	0	150	0	0	50	300	0
- 4 to 0 hours	6.10	0.78	1.10	0.37	1.09	0.08	0.02
0 to 4 hours	10.60	0.80	3.15	0.80	2.06	0.08	0.15
4 to 8 hours	9.20	0.95	1.45	1.04	1.45	0.50	0.37
8 to 12 hours	5.00	0.17	0.73	0.80	1.21	0.15	0.07
12 to 16 hours	2.00	0.13	0.79	0.53	1.09	—*	0.08
16 to 20 hours	1.70	0.23	1.39	—*	2.55	0.73	0.08
20 to 24 hours	8.00	1.18	2.97	1.29	2.30	0.64	0.13
mg./kg. 24 hours	36.50	3.46	10.48	4.46	10.66	2.10	0.88

* No urine voided.

TABLE VI
24-hour Excretion (without tryptophan loading)

Dosage of nicotinic acid per day (mg.)	Case 1				Case 2				Normal Controls
	0	0	150	150	0	0	300	300	0
24-hour urine excretion of N'-methylnicotinamide (mg.) ..	1.2	3.7	—	5.2	1.54	0.94	18.8	20.5	3-4.5
24-hour urine total nitrogen (mg.) ..	5169	5332	3192	5548	2941	4244	3656	3235	3750-5880
24-hour urine amino nitrogen (mg.) ..	543	453	479	787	430	499	469	344	12-42
Amino nitrogen as % of total nitrogen ..	10.5	8.5	15.0	14.2	14.6	11.8	12.8	10.6	0.5-2

they were given large doses of nicotinic acid. However, in both cases the output was in excess of that in the controls.

Table V shows the urinary excretion of tryptophan. High doses of nicotinic acid appeared to have improved the reabsorption of tryptophan in the renal tubules in both cases. However, the total 24-hour excretion was still above that of the controls.

Table VI shows the total 24-hour excretion of N'-methylnicotinamide, total nitrogen, and amino

nitrogen. It is seen that without treatment the patients excreted subnormal amounts of N'-methylnicotinamide, while on high doses of nicotinic acid they excreted higher than average amounts of this metabolite. Both patients excreted 10 times or more amino nitrogen in the urine than the controls. There was no significant reduction in its excretion during the treatment periods. Similarly, the ratios of amino nitrogen to total nitrogen in the urine remained about 10 times higher than the normal.

Table VII shows the clearances of amino acids

TABLE VII

Renal Clearance of Amino Acids (without tryptophan loading) (ml. plasma cleared/min. per 1.73 sq. m.)

Dosage of nicotinic acid per day (mg.)	Case 1				Case 2			
	0	0	150	150	0	0	300	300
Asp	—†	11.8	13.2	—†	—†	5.2	—†	—†
Thr*	38.0	38.8	173.0	86.4	86.5	49.0	184.0	104.5
Ser*	4.4	64.6	61.2	75.7	35.8	61.2	80.4	95.7
Glu	2.9	2.0	11.8	13.2	1.0	4.4	5.1	6.1
Gly	3.3	8.5	14.4	16.9	7.3	11.6	24.3	15.4
Ala	6.0	10.6	17.8	17.9	7.6	10.6	23.9	18.9
Val	2.3	5.3	14.2	18.5	21.3	46.2	45.7	26.2
Cys	—†	2.7	4.2	4.1	—†	33.2	40.3	22.2
Met	—†	7.6	17.6	12.3	—†	—†	8.1	3.3
Ile	—†	12.0	34.4	27.4	15.1	41.7	38.6	16.5
Leu	2.0	3.2	21.8	15.2	11.0	42.2	22.6	12.9
Tyr	33.8	55.0	82.6	55.0	21.5	51.0	98.7	38.7
Phe	6.2	8.7	18.6	21.0	21.5	14.6	—†	30.0
Orn	2.7	4.7	2.6	3.5	—†	—†	—†	—†
Lys	12.6	—†	18.2	13.5	2.6	0.8	3.1	1.0
His	100.7	28.6	18.4	42.4	34.1	54.6	78.2	61.1
Arg	4.2	—†	3.8	2.8	3.4	4.2	4.2	4.4

* Threonine and serine peaks contain glutamine and asparagine.

† Not detected in the urine.

‡ Not detected in the plasma.

in the patients before and during treatment. It should be noted that the method of column chromatography used in this study did not separate the threonine and serine peaks from glutamine and asparagine; they were, therefore, overestimated. It should also be noted that tryptophan was destroyed by this method. It is seen that with the exception of lysine in Case 2, the clearance of the amino acids was abnormally raised. These values are similar to those reported by Evered (1956). Treatment, in the dosage and for the duration given in our 2 patients, did not correct the abnormal amino acid clearances. This confirms the persistent aminoaciduria, as indicated by the continuing high 24-hour excretion of amino nitrogen.

Discussion

In the untreated cases of Hartnup disease, there is a gross generalized aminoaciduria, hypertryptophanuria, hyperindoluria, and a reduced excretion of kynurenine and N'-methylnicotinamide. Due to the small number of cases and the variable and intermittent nature of the clinical signs and symptoms in this disease, assessment of the effectiveness of therapy has been difficult and insufficiently documented. With the exception of one case (de Laey *et al.*, 1964) no one has reported a reversal to normal of the biochemical abnormalities in this disease.

De Laey *et al.* state that in their patient, following 2 weeks' treatment with 30 mg. nicotinic acid daily, 'observations were in favour of a complete normalization of the elimination of tryptophan metabolites'. Oral tryptophan loading tests showed that on nicotinic acid therapy, the excessive urinary excretion of

tryptophan was reduced to normal; there was an increase in the amount of indole-3-acetic acid excreted; a reduction of the hyperaminoaciduria; a disappearance of the indicanuria; and the urinary excretion of kynurenine was the same as that of the treated controls. However, it should be noted that the amount of kynurenine excreted during the period of treatment for both the patient and the treated controls was only one-quarter to one-third of the untreated controls. Under nicotinic acid therapy neither indole nor tryptophan was detected in the faeces. De Laey *et al.* explained their results as follows: (1) nicotinic acid improves the absorption of tryptophan and other amino acids in the intestine and renal tubules; (2) nicotinic acid inhibits the activity of tryptophanase of the intestinal bacteria, resulting in the cessation of the formation of indolic metabolites which are responsible for the inhibition of tryptophan pyrrolase and formylase; (3) nicotinic acid enhances the kynurenine pathway, leading to increased excretion of N'-methylnicotinamide.

Our observations in 2 patients differed from those of de Laey *et al.* in several respects. (1) Nicotinic acid therapy did not bring about normal tryptophan absorption in the intestine or in the renal tubules. This was indicated by the low levels of plasma tryptophan attained after oral loading, and by the higher than normal amount of tryptophan excreted in the urine during therapy. (2) Nicotinic acid did not abolish the formation and the excessive excretion of indican. (3) During nicotinic acid therapy, no improvement in the gross aminoaciduria was observed. On the other hand, there was a suggestion of improvement in the output

of kynurenine, and a reduction in the urinary excretion of tryptophan and indole-3-acetic acid towards normal levels. The data obtained in the present study do not provide any explanation for these changes; nor is there any obvious cause for the difference in the effect of nicotinic acid therapy in our patients and that of de Laey *et al.*

It appears that if the hypotheses of de Laey *et al.* are correct, the high doses of nicotinic acid should not only cure the clinical signs and symptoms but also cause the biochemical picture to become normal. In addition, the high doses of nicotinic acid given to our patients should have been adequate to produce a 'pharmacological effect' and correct the abnormal biochemical findings. The present study failed to confirm the observations of de Laey *et al.*

This difference in the effect of nicotinic acid on the metabolic abnormalities in the patient of de Laey *et al.* and the two cases of our own raises the possibility that there may be different variants of Hartnup disease.

Summary

The effect of nicotinic acid on tryptophan metabolism was studied in two cases of Hartnup disease. No correction of the metabolic abnormalities was observed after an extended period of nicotinic acid therapy, though there was a suggestion of improvement in some of the abnormal findings. Further studies in this disorder are required before we can recommend treatment with higher than replacement doses of nicotinic acid for long periods of time.

The authors wish to thank the Research Committee of the Manchester Regional Hospital Board, the Rag Fund of the University of Manchester, the Wellcome

Trust, and the Swinton and Pendlebury Branch of the National Society for Mentally Handicapped Children for their financial assistance. They also wish to thank Professor Wilfrid Gaisford and Dr. Neil Gordon for their permission to study their cases, Dr. E. Kodicek for his help in the determination of N'-methylnicotinamide, and Dr. G. M. Komrower for his advice and for reviewing the manuscript. One of the authors (P.W. K.W.) is supported by a grant from the Children's Research Fund.

REFERENCES

- Albanese, A. A., and Irby, V. (1944). Determination of urinary amino nitrogen by the copper method. *J. biol. Chem.*, **153**, 583.
- Baron, D. N., Dent, C. E., Harris, H., Hart, E. W., and Jepson, J. B. (1956). Hereditary pellagra-like skin rash with temporary cerebellar ataxia, constant renal amino-aciduria, and other bizarre biochemical features. *Lancet*, **2**, 421.
- Carpenter, K. J., and Kodicek, E. (1950). The fluorimetric estimation of N'-methylnicotinamide and its differentiation from co-enzyme 1. *Biochem. J.*, **46**, 421.
- Coppini, D., Benassi, C. A., and Montorsi, M. (1959). Quantitative determination of tryptophan metabolites (via kynurenine) in biologic fluids. *Clin. Chem.*, **5**, 391.
- de Laey, P., Hoof, C., Timmermans, J., and Snoeck, J. (1964). Biochemical aspects of the Hartnup disease. *Ann. paediat. (Basel)*, **202**, 145, 253, and 321.
- Duggan, D. E., and Udenfriend, S. (1956). The spectrophotofluorometric determination of tryptophan in plasma and of tryptophan and tyrosine in protein hydrolysates. *J. biol. Chem.*, **223**, 313.
- Evered, D. F. (1956). The excretion of amino acids by the human. *Biochem. J.*, **62**, 416.
- Halvorsen, K., and Halvorsen, S. (1963). Hartnup disease. *Pediatrics*, **31**, 29.
- Milne, M. D., Crawford, M. A., Girão, C. B., and Loughridge, L. W. (1960). The metabolic disorder in Hartnup disease. *Quart. J. Med.*, **29**, 407.
- Moore, S., Spackman, D. H., and Stein, W. H. (1958). Chromatography of amino acids on sulfonated polystyrene resins. An improved system. *Analyt. Chem.*, **30**, 1185.
- Sharlit, H. (1933). A method for quantitative estimation of indoxyl compounds in the urine. *J. biol. Chem.*, **99**, 537.
- Weissbach, H., King, W., Sjoerdsma, A., and Udenfriend, S. (1959). Formation of indole-3-acetic acid and tryptamine in animals: a method for estimation of indole-3-acetic acid in tissues. *ibid.*, **234**, 81.
- Wong, P. W. K., and Pillai, P. M. (1966). Clinical and biochemical observations in two cases of Hartnup disease. *Arch. Dis. Childh.*, **41**, 383.