AMINOACIDURIA AND MEGALOBlastic ANAEMIA

BY

D. FOWLER, E. V. COX, W. T. COOKE, AND M. J. MEYNELL

From the General Hospital, Birmingham

(RECEIVED FOR PUBLICATION NOVEMBER 17, 1959)

Urinary amino-acid chromatograms from 23 patients with megaloblastic anaemia have been studied before and after therapy. The most consistent abnormality was an increased taurine or increased taurine/glycine ratio. This was not related directly to deficiency of vitamin B₁₂ or folic acid or to the degree of anaemia.

The occurrence of aminoaciduria in patients with untreated pernicious anaemia was reported by Weaver and Neill (1954). Keeley and Politzer in 1956, using one-dimensional chromatography, confirmed its presence in two patients with pernicious anaemia. They found none in seven patients with megaloblastic anaemia of pregnancy and two infants with malnutrition and megaloblastic anaemia, so they therefore suggested that aminoaciduria was a further method for differentiating pernicious anaemia from the other megaloblastic anaemias. Crane, Hayes, and de Gruchy (1958) studied nine patients with pernicious anaemia and concluded that there was no characteristic pattern. Nevertheless, Weaver and Neill (1954) noted in particular a large increase in taurine concentration in five of their patients with pernicious anaemia and this finding was confirmed by Todd (1959) in the most extensive study to date on this subject. He showed that the mild aminoaciduria would occur in any of the megaloblastic anaemias in relapse and suggested that a pronounced increase of taurine was more common in pernicious anaemia and most likely to be found in the megaloblastic anaemias due to deficiency of vitamin B₁₂.

The purpose of this communication is to record our observations on the aminoaciduria in 23 patients with megaloblastic anaemia due to a deficiency of vitamin B₁₂ or to a disturbance of the metabolism of the pteroylglutamates. Control observations were made in normal subjects and patients with hypochromic anaemia due to iron deficiency.

Clinical Material

Fifteen patients with megaloblastic anaemia and deficiency of vitamin B₁₂ were studied. This group consisted of 10 patients with pernicious anaemia, having a macrocytic anaemia, megaloblastic erythropoiesis, serum level of vitamin B₁₂ less than 105 µg./ml. (Meynell, Cooke, Cox, and

Gaddie, 1957), histamine-fast achlorhydria, normal excretion of faecal fat, and normal radiology of the stomach and small intestine together with an optimal haematological response to vitamin B₁₂. The remaining five patients had a megaloblastic anaemia in association with total gastrectomy, partial gastrectomy, adult coeliac disease, or regional enteritis (two patients). The eight patients with a megaloblastic anaemia but without evidence of deficiency of vitamin B₁₂ included five patients with adult coeliac disease fulfilling criteria laid down elsewhere (Cooke, 1958), and three in association with pregnancy, two a week before and one a week after delivery and all remitting completely with folic acid. Control observations were provided by 10 normal subjects working in hospital without anaemia or evident disease and five patients with hypochromic iron deficiency anaemia, four due to chronic blood loss and one due to nutritional causes.

Methods

All subjects were on a normal hospital diet. Urine specimens were collected for two to three days before therapy and thereafter intermittently in the patients up to 15 days. Random observations on some of the patients were made subsequently up to eight months. Specimens were passed on rising, preserved with thymol and stored in the refrigerator until used, when they were run on two-dimensional paper chromatograms. A urine volume containing 250 µg. total nitrogen (determined by the Kjeldahl method) was applied to the paper (Whatman No. 1). A mixture of ascending and descending chromatography was performed as suggested by Block (1950), using a glass tank 15½ in. by 10½ in. by 9½ in. and paper chromatograms 13 in. by 11 in. This procedure required little laboratory space and no additional temperature control. The solvents and developing reagent were in accordance with Dent's method (1948), solvent (1) phenol saturated with water in the presence of ammonia and potassium cyanide, and solvent (2) lutidine 2: water 1 in the presence of diethylamine. The

TABLE
NUMBER OF CASES WITH STRONGER THAN NORMAL AMINO-ACID EXCRETION

	Total No. Cases	Amino-acids Usually Detected in Normal Urine							Amino-acids Not Usually Detected in Normal Urine				
		Glycine	Taurine	Taurine Relative to Glycine	Serine	Ala-nine	Glutamine + Glutamic Acid	Histi-dine	Ly-sine	Ethanol-amine	Threo-nine	Traces of Valine, Leucine, Phenylalanine, Tyrosine	Cystine Qualitative Test
Pernicious anaemia	10	3	5	5	5	4	3	1	5	2	1	4	5
Other B ₁₂ -deficient megaloblastic anaemia	5	1	0	2	2	3	0	0	2	0	1	3	0
Non-B ₁₂ -deficient megaloblastic anaemia	5	0	3	5	1	1	2	0	1	1	0	3	2
Megaloblastic anaemia of pregnancy	3	2	0	0	1	2	1	1	0	0	0	2	1
Hypochromic anaemia	5	2	1	1	1	3	1	2	2	0	0	0	2

Methylhistidine and β -amino isobutyric acid excretion normal in all the above cases.

chromatograms were run in each solvent overnight, dried, sprayed with 0.1% ninhydrin in n-butanol, and the colours allowed to develop slowly overnight. The squares were then heated for a few minutes at 95–105° C.

By using this standard developing procedure the strength of the spots (numbered on an arbitrary colour scale 1–10) was compared visually with that obtained on the chromatograms of urines from normal subjects. Cystine was detected qualitatively in the urine by the cyanide-nitroprusside test. In some instances, this was confirmed by running oxidized chromatograms (Dent, 1948).

Results and Discussion

The results of 140 chromatograms are summarized in the Table.

In agreement with other workers, the most consistent abnormality in the urine amino-acids in Addisonian anaemia was an increased taurine excretion (five out of 10 patients). In five patients with non-Addisonian pernicious anaemia and a megaloblastic anaemia due to deficiency of vitamin B₁₂, none had an increased taurine excretion, but in two of them taurine excretion relative to glycine was increased. In normal controls taurine excretion was usually less and never more than that of glycine. This incidence in Addisonian anaemia is less than that reported by Crane *et al.* (1958), five out of seven patients, by Neill and Weaver (1958), 12 out of 16, and Todd (1959), 22 out of 24 patients. A possible explanation is that the urine specimens studied by us were obtained in a fasting state whereas pooled 24-hour specimens were used by other investigators. The daily diet providing an extra load may have accentuated the underlying defect.

The increased taurine excretion and the increased taurine-glycine ratio in the seven

patients out of 15 with a megaloblastic anaemia due to B₁₂ deficiency were not related to the degree of anaemia. Furthermore, the increase was not directly related to deficiency of vitamin B₁₂ since it occurred in all five patients with adult coeliac disease who had no demonstrable deficiency and also in one of the five patients with hypochromic anaemia. It is unlikely that the increase is solely due to the disordered metabolism resulting from megaloblastic erythropoiesis since the taurine spot on the urine chromatogram of the three patients with megaloblastic anaemia of pregnancy was very faint. Todd (1959) did not detect any taurine in six of nine patients with this condition.

Other changes in the urinary amino-acids detected were relatively small and, in view of their inconsistency, are difficult to interpret and of doubtful significance. In all but one patient the abnormal urine amino-acids had returned or were returning to normal within five to 15 days of beginning specific therapy, whether this was vitamin B₁₂, folic acid, or iron. At present, urine amino-acid chromatography cannot offer any specific diagnostic aid in the differential diagnosis of anaemia.

We are indebted to the Endowment Research Fund of the United Birmingham Hospitals for financial support and to the physicians of the General Hospital for permission to investigate their patients.

REFERENCES

- Block, R. J. (1950). *Analyt. Chem.*, **22**, 1327.
- Cooke, W. T. (1958). *Brit. med. J.*, **2**, 261.
- Crane, C. W., Hayes, R. A., and Gruchy, G. C. de (1958). *J. clin. Path.*, **11**, 162.
- Dent, C. E. (1948). *Biochem. J.*, **43**, 169.
- Keeley, K. J., and Politzer, W. M. (1956). *J. clin. Path.*, **9**, 142.
- Meynell, M. J., Cooke, W. T., Cox, E. V., and Gaddie, R. (1957). *Lancet*, **1**, 901.
- Neill, D. W., and Weaver, J. A. (1958). *Brit. J. Haemat.*, **4**, 447.
- Todd, D. (1959). *J. clin. Path.*, **12**, 238.
- Weaver, J. A., and Neill, D. W. (1954). *Lancet*, **1**, 1212.