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Abnormal tryptophan metabolism in congenital erythroid hypoplastic (Diamond-Blackfan) anaemia

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Tryptophan loading was performed in a child with Diamond–Blackfan anaemia as well as in his parents and two control persons. There was a five-fold increase of anthranilic acid and kynurenine excretion beside a marked generalized aminoaciduria in the proband; in the parents and the controls no such phenomenon could be demonstrated. The importance of the finding is discussed and investigation of tryptophan metabolism before the first blood transfusion is recommended. The finding cannot be used as a heterozygote test.

Congenital aplastic anaemia caused by bone marrow deficiency restricted to depressed formation of erythrocytes was described as "pure red cell anaemia" by Joseph [14]. Diamond and Blackfan [8] investigated the disorder in detail and termed it congenital erythroid hypoplastic anaemia. With the increasing number of described cases, synonymes have become numerous [4].

The diagnosis rests on the following criteria: (a) severe, normochromic, normocytic anaemia from early infancy; (b) strongly reduced erythropoietic activity of the bone marrow; (c) unaffected leucocyte and platelet production.

Although the condition is rare, its clinical features are well-defined. Therapeutic results are equivocal; in the individual case prognosis can be established after a certain duration of the disease. Aetiology, pathomechanism and genetics of the disorder are still debated. Our data may cast light on some of these problems.

Report of a case

F. L., a male infant showed all the clinical features mentioned above. His anaemia, appearing shortly after birth, could be corrected only by repeated blood transfusions. These caused high plasma iron levels, between 150 and 200 μg/100 ml. Urinary iron excretion could be increased by administration of deferoxamine. In spite of this there were no clinical or laboratory signs of haemochromatosis.

At the patient's age of 22 months studies concerning tryptophan metabolism were performed. Results are shown in Table I.

Loading with L-tryptophan was performed orally. The proband and a child of the same age received 1.0 g, the proband's parents and a healthy adult 2.0 g each, in three equal doses within 24 hours. Twenty-four hour urine specimens were collected in each case before and after loading and the daily creatinine output was measured.

TABLE I

Laboratory findings at the age of 22 months

Erythrocytes	1 700 000
Haemoglobin	5.1 g/100 ml
Leucocytes	6 800
Platelets	140 000
Reticulocytes	less than 1/10000
Serum iron	192 μg/100 ml
Serum copper	115 μg/100 ml
Serum aldolase	5 U
LDH	90 U
GOT	16 U
GPT	10 U
Bromsulphophthalein retention	2%
Serum bilirubin	0.6 mg/100 ml
Thymol turbidity test	3.5 U
Gold sol	++++
Thymol flocculation test	+++
Urinary aminoacid chromatography	generalized aminoaciduria
Urinary iron/creatinine ratio before deferoxamine	2.6
after 200 mg deferoxamine	20.0
Bone marrow	erythropoiesis nearly absent

TABLE II

Daily urinary excretion of tryptophan and its metabolites

	TRY load g		TRY mg/creatinine	KYN mg/day	AA mg/day	Chromatography TRY	KYN	AA
Father	2.0	before	120	47.1	17.1	—	—	—
		after	60	38.2	16.6	—	—	—
Mother	2.0	before	75	31.8	11.4	—	—	—
		after	68	24.6	9.2	—	—	—
Adult control	2.0	before	56	50.7	21.6	—	—	—
		after	60	46.0	15.4	—	—	—
Proband	1.0	before	450	7.5	2.7	+	+	+
		after	525	36.6	14.4	++	++	++
Child	1.0	before	285	10.8	5.1	±	±	±
		after	360	9.7	7.0	±	±	±

TRY □ Tryptophan; KYN □ Kynurenine; AA □ Anthranilic acid

Tryptophan and kynurenine were determined by Jepson's semiquantitative paper-chromatographic method [13]. Kynurenine and anthranilic acid were measured quantitatively by the method of Otani et al [18]. Results are presented in Table II.

DISCUSSION

Altman and Miller [1] found a bluish-fluorescent compound in the urine of their patient suffering from

Diamond–Blackfan anaemia. This turned out to be anthranilic acid. Later, Marver [15] and Chitiyo et al. [4] observed increased urinary excretion of anthranilic acid and kynurenine in their patients. Price et al. [21] showed a high output of anthranilic acid and hydroxy-kynureine. These compounds are intermediary products of a disturbed or reduced enzymatic activity somewhere along the tryptophan pathway. The well-known fact that tryptophan and nicotinic acid play an important role in haemoglobin synthesis and that their deficiency causes anaemia [26], seemed to corroborate this hypothesis.

Pyridoxin and riboflavin are important co-ferments in the enzymatic system metabolizing tryptophan and anaemia has been reported to develop in pyridoxin deficiency and to be accompanied by disturbed tryptophan metabolism [19]. In pyridoxin deficiency, after a tryptophan load, increased excretion of tryptophan metabolites was observed in both man and animal [5, 20]. Riboflavin deficiency can also lead to anaemia [22], and administration of riboflavin in Diamond–Blackfan anaemia was shown to reduce the pathologically high excretion of anthranilic acid [1]. Although vitamin B_2 and B_6 are able to moderate the high excretion of tryptophan metabolites, they are not capable to influence favourably the inactivity of the erythropoietic system [1, 5, 6].

No clinical sign of any avitaminosis can be detected in patients affected by Diamond–Blackfan anaemia. Since no difference in 4-pyridoxic acid excretion could be found between healthy and affected persons, pyridoxin deficiency has been ruled out as a possible cause of Diamond–Blackfan anaemia [21, 23].

Musajo et al. [17] investigated the excretion of tryptophan and its metabolites in severe haematologic diseases (chronic myeloid leukaemia, lymphoid leukaemia, lymphogranulomatosis), and found a higher excretion in these conditions than in healthy persons or subjects affected by other diseases. It is difficult to find anything common in Diamond–Blackfan anaemia and the haemoblastoses. Perhaps the repeated blood transfusions, inevitable in both disease groups, lead to increased haem supply, this in turn would necessitate increased globin synthesis involving exaggerated activity of the enzymes of tryptophan metabolism. Thus, the disturbed tryptophan metabolism may be the result rather than the cause of the disease. Price et al. [21] concluded that the investigation of tryptophan and its metabolites is of moderate value in the diagnosis of Diamond–Blackfan anaemia.

Our own patient after tryptophan loading exhibited large spots of kynurenine and tryptophan on the chromatogram, in sharp contrast to the controls. The quantity of tryptophan excreted after loading was the highest in the proband, a slight increase was present in the control child, while the parents and the adult control subject showed no change.

Similarly, anthranilic acid and kynurenine excretion increased to a fivefold value after loading in the proband; the other persons exhibited no change in this respect (Table I).

Thus, there was a striking difference between the results for the patient and those for the controls. In interpreting these results, the patient's hepatic function has to be taken into account, since tryptophan metabolism takes place in the liver. Haemochromatosis must sooner or later ensue because of the repeated blood transfusions; hepatitis B is also very likely to develop. In this patient there was, however, no evidence for disturbed liver function, hepatitis or haemochromatosis and no hepatitis associated antigen could be detected. We think therefore that the metabolic disturbance is inherent in the basic pathologic processes of Diamond–Blackfan anaemia. It would be desirable to investigate patients suffering from Diamond–Blackfan anaemia before they have received any transfusion of blood, and enzyme activities involved in tryptophan metabolism would have also to be determined. The generalized aminoaciduria observed by some workers [6] and ourselves also needs elucidation.

Not all patients with Diamond–Blackfan anaemia exhibit disturbed tryptophan metabolism. Tartaglia et al. [24] found no increased excretion of anthranilic acid; and Ibrahim et al. [12] reported on normal xanthurenic acid and anthranilic acid excretion after tryptophan loading. These and other data have justly raised doubts concerning the primary role of tryptophan metabolism in the development of Diamond–Blackfan anaemia, but other efforts to elucidate the aetiology have also been fruitless [2, 11, 24].

The inheritance pattern is equally ill-defined. Recessive inheritance has been suspected [3] but there are observations in favour of dominant inheritance as well [9, 10, 16]. Congenital pure red cell hypoplasia has been observed in identical male twins [25], but in our case no abnormality could be found in any of the parents. This was at variance with the observation [15] of an increased kynurenine and hydroxy-kynurenine excretion after tryptophan loading in the mother of a patient affected by Diamond–Blackfan anaemia.

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