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Antithrombin activities in childhood malnutrition

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SUMMARY Antithrombin activities in 30 severely malnourished children and 40 normal children were estimated in clotting tests by thrombin neutralisation as anti-Xa and by a heparin antithrombin assay; and by immunodiffusion as α_2-globulin and α_1-antitrypsin. The patients' mean α_2-globulin was severely depressed, and there were less marked depletions in mean values for thrombin neutralisation, anti-Xa, and in the heparin antithrombin assay (which showed the flat curve thought to reflect a thrombotic tendency). The α_1-antitrypsin values were normal. The findings support the concept of antithrombin as the summation of α_2-globulin and α_1-antitrypsin (with α_2-macroglobulin); and the low values may be related to the high incidence of thrombosis reported in childhood malnutrition, although it was not seen in these patients.

Antithrombin III (AT III) (Seegers *et al.*, 1954) is considered to be the most important naturally occurring thrombin inhibitor in man (Abildgaard, 1967b). Acquired and hereditary deficiencies are thought to play an important role in thrombosis and intravascular coagulation (Egeberg, 1965; Abildgaard *et al.*, 1970; Bjarke *et al.*, 1974; Marciniak *et al.*, 1974). The major compartment of AT III is α_2-globulin, while α_2-macroglobulin and α_1-antitrypsin also have some antithrombin activity (Abildgaard, 1967a; Lane *et al.*, 1975; Lane and Biggs, 1977). A close relationship between AT III, heparin co-factor, and anti-Xa has been described (Biggs *et al.*, 1970; Yin *et al.*, 1971; Marciniak, 1973), while others have ascribed heparin co-factor and AT III activities to the same protein (Monkhouse and Milojevic, 1968).

There are few studies on antithrombin activities in childhood, and most of the reports refer to the newborn and infants (Biland and Duckert, 1973; Mahasandana and Hathaway, 1973; Bjarke *et al.*, 1974; Weissbach *et al.*, 1974; Teger-Nilsson, 1975). Children reach adult values at about 6 months of age (Teger-Nilsson, 1975). Low levels might occur in severely malnourished children because of protein deficiency; and a higher incidence of thrombosis has been reported in this group (Loría *et al.*, 1967; Jiménez *et al.*, 1970, 1972). Various antithrombin activities, and the augmentation of antithrombin activity by heparin, have therefore been determined in sera from children with severe protein-caloric malnutrition.

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Material and methods

SUBJECTS

Blood samples from 30 malnourished children (10 girls, 20 boys) aged 6 months to 6½ years were obtained within two days of admission to the Hospital Nacional de Niños, San José. All the children were severely malnourished (18 with marasmus, 12 with marasmus-kwashiorkor), presented abnormal clinical signs, and showed weight deficits for age of more than 40%.

Similar samples were obtained from 40 normally nourished, clinically healthy children of about the same age in other wards of the hospital, who were awaiting minor elective surgery and served as controls. A pool of fresh plasmas from at least three such children was used every day when coagulation tests were performed.

COAGULATION TESTS

Prothrombin time (PT) and activated partial thromboplastin time (PTT) were performed with Hyland reagents. Fibrindex thrombin (Ortho Diagnostics) was used for the thrombin time (TT),

standardised to give a clotting time of 9-11 seconds with normal plasma. Fibrinogen/fibrin degradation products (FDP) were investigated with Thrombowell-cotest (Wellcome). Fibrinogen was measured by the microprecipitation method of Ruíz-Reyes and Jiménez (1965). Anti-Xa was measured in serum by the method of Denson and Bonnar (1973). Factor X was measured in serum with the chromogenic substrate S 2222 (benzoyl-isoleucine-glutamyl-glycyl-arginine-paranitroanilide HCl; Kabi).

ANTITHROMBIN AND HEPARIN-ANTITHROMBIN CLOTTING ASSAYS

The antithrombin assay (ATA) and the heparin-antithrombin assay (HATA) were performed by the methods of Innerfield *et al.* (1976) in sera stored at −70°C for two to three months.

The method for antithrombin assay was as follows:

1 Fibrindex thrombin was diluted with isotonic saline until 0·1 ml clotted 0·2 ml of fresh normal plasma at 37°C in 15-16 seconds.

2 0·2 ml of the control pool plasma was placed in a glass tube at 37°C.

3 0·1 ml of test serum was then added to 0·9 ml of standardised thrombin in a glass tube, mixed thoroughly, and placed in the waterbath to warm for exactly 3 minutes.

4 After 3 minutes' incubation, 0·1 ml was removed from the thrombin-serum mixture and added forcibly to the tube containing 0·2 ml of plasma, and the clotting time was recorded.

5 For comparison (Fig. 1), individual results were converted to percentages of the mean control clotting time.

The method for heparin-antithrombin assay was as follows:

1 Fibrindex thrombin was diluted as above to clot normal plasma in 10·5-11·0 seconds.

2 0·2 ml of the control pool plasma was placed in each of two glass tubes at 37°C.

3 0·1 ml of test serum was then added to each of two tubes containing 0·1 ml of heparin solution of concentrations 0·5 and 1·0 IU/ml, respectively, at bench temperature.

4 0·1 ml of each heparin-serum mixture was then added to 0·9 ml of standardised thrombin, mixed thoroughly, and placed in the waterbath to warm for exactly 2 minutes.

5 After 2 minutes' incubation, 0·1 ml was removed from each thrombin-heparin-serum mixture and added forcibly to one of the tubes containing 0·2 ml of plasma, and the clotting time was recorded. The other thrombin-heparin-serum mixture was then similarly tested. In each case, the 0·5 IU/ml heparin solution was tested first.

6 For comparison (Fig. 1), individual results were converted to percentages of the mean control clotting times.

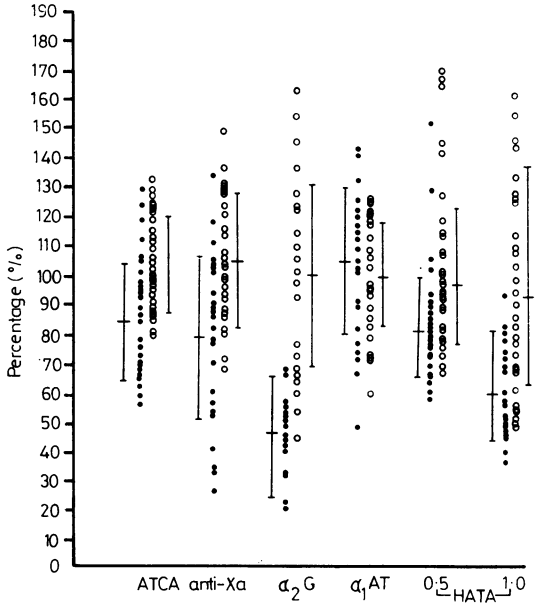

Fig. 1 *Distribution in percentage and mean (±1 SD) in controls (○) and patients (●) of all the antithrombin studies. ATCA = antithrombin clotting activity; anti-Xa = anti-Xa clotting activity; α_2 G=α_2-globulin; α_1 AT = α_1-antitrypsin; HATA = heparin antithrombin assay with two concentrations (IU/ml) of the anticoagulant. The horizontal bars show the mean values and the vertical line the ±SD ranges. The calculations for ATCA, anti-Xa, α_2 G, and α_1 AT were carried out on the percentage values; for the HATA values the calculations were done in logs so the geometric means are shown and the ±SD ranges are asymmetrical.*

IMMUNOLOGICAL STUDIES

α_1-antitrypsin and α_2-globulin (Antithrombin III) were measured in serum by radial immunodiffusion in duplicate using M-Partigen plates (Behringwerke AG, Marburg) against a protein standard from the same commercial source.

OTHER STUDIES

Haemoglobin, PCV, WBC, platelets, serum protein fractionation (Biuret), intestinal parasites, and blood cultures were investigated in all patients. The control group of children were not similarly tested.

Results

In all patients malnutrition was complicated by one or more of the following: mild infections, 25;

intestinal parasites, 17; anaemia, 21 (Table 1). No patient developed thrombosis (geometric mean follow-up, 27·4 days; 2 SD range from analysis in logs, 14-52 days), and all blood cultures were negative. No difference was found in protein fractionation, Hb, WBC, platelet count, or fibrinogen level between the cases of marasmus and marasmus-kwashiorkor (Table 2).

Table 1 *Complications in 30 children with severe malnutrition*

Complications	*No. of cases*
Illness	25
Diarrhoea	18
Bronchopneumonia	8
Otitis media	6
Sinusitis	1
Intestinal parasites	17
Ascaris lumbricoides	4
Trichuris trichiura	13
Protozoa	6
Others	2
Anaemia (less than 10 g/dl)	21

Table 2 *Laboratory features in children with severe malnutrition: mean values (± SD)*

	Marasmus	*Marasmus-Kwashiorkor*	*Total*
Number of cases	18	12	30
Age (months)	31 (±25)	34 (±19)	33 (±23)
Sex (M/F)	12/6	8/4	20/10
Hb (g/dl)	8·2 (±2·0)	9·1 (±2·9)	8·8 (±2·2)
WBC/ml	11·1 (±3·8)	11·8 (±2·4)	11·3 (±3·3)
Platelets (10^9/l)	413 (±167)	385 (±171)	401 (±166)
Fibrinogen (g/l)	2·63 (±1·09)	2·56 (±0·66)	2·60 (±0·93)
Total protein (g/dl)	6·0 (±1·0)	5·5 (±1·0)	5·8 (±1·0)
Albumin (g/dl)	3·3 (±0·5)	3·0 (±0·9)	3·2 (±0·7)
Globulin (g/dl)	2·5 (±1·0)	2·5 (±0·4)	2·5 (±0·8)

The distribution of values in the various antithrombin tests for the patients' and the control sera are shown in Fig. 1, converted to percentages for comparability. The antithrombin and anti-Xa clotting activities show a lower mean value in the patient group, but an even greater difference is seen in the α_2-globulin values. Figure 2 shows the distributions of clotting values in the heparin test; again the patients show lower mean values than the controls. Other coagulation results are shown in Table 3; minor prolongations are seen in the mean PT and TT, but there were no gross differences. There was no correlation between the clotting tests and AT III clotting activity among the patients. The

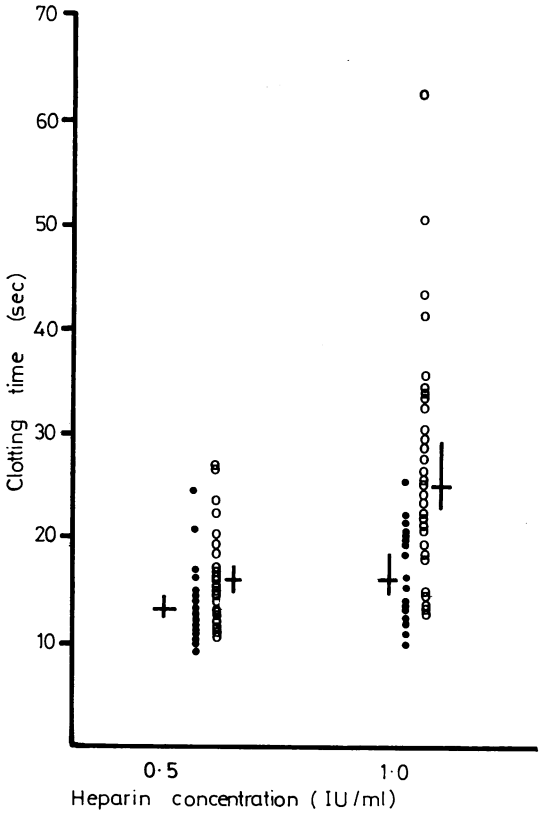

Fig. 2 *Distribution of clotting times in the heparin-antithrombin assay with two different concentrations of heparin (controls (○), patients (●)). The horizontal bars show the geometric means, and the vertical lines the 2 SE mean ranges calculated in logs.*

patients showed no evidence of intravascular coagulation; the platelet counts were within normal limits, and all patients gave normal FDP titres except one which rose only to a titre of 1 in 25. The relation between AT III by clotting assay and the immunological determination of α_2-globulin for patients and controls is shown in Figure 3.

Discussion

The normal progressive inhibition of thrombin in plasma is associated with several substances. Lane *et al.* (1975) found that α_2-globulin accounted for approximately 50%, and α_1-antitrypsin and α_2-macroglobulin each contributed about 25% to the total activity. AT III also inactivates factor Xa in much the same manner as thrombin (Biggs *et al.*, 1970); and the binding of heparin to α_2-globulin

Table 3 *Coagulation results in controls and in malnourished children: mean values (± SD)*

Test	*Controls*	*Patients*	P
Number of cases	40	30	
AT III clotting activity (s)	50·5 (±15·8)	42·7 (±10·1)	*
Heparin AT III assay (s)	16·0	13·2	**
0·5 IU/ml	(± 4·2)	(± 3·1)	
1·0 IU/ml	26·6 (±10·8)	16·6 (± 6·4)	**
Antifactor Xa (%)	105·0 (±22·8)	79·3 (±27·9)	**
α_2-globulin (mg/dl)	22·8 (± 7·2)	10·6 (± 4·6)	**
α_1-antitrypsin (mg/dl)	328·3 (±57·2)	343·4 (±83·1)	ns
Prothrombin time (s)	13·0 (± 0·8)	14·0 (± 1·6)	**
Partial thromboplastin time (s)	38·3 (± 3·4)	43·4 (±18·3)	ns
Thrombin time (s)	9·6 (± 0·8)	10·7) (± 2·5)	*
Factor X (%)	97·4 (±21·2)	94·6 (±29·7)	ns

ns = not significant; *P < 0·05; **P < 0·01 (Student's *t* test for the difference between two means).

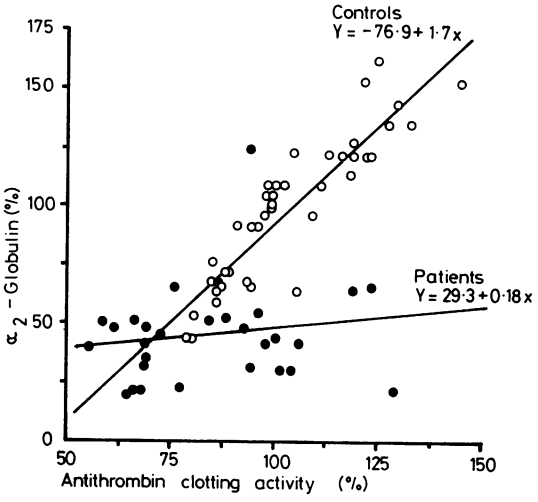

Fig. 3 *Comparison of α_2-globulin and antithrombin clotting activity (thrombin neutralisation) in controls (○) and patients (●). The regression equations are given.*

accelerates the neutralisation of both thrombin and factor Xa (Rosenberg, 1975).

Since antithrombin activity is carried out by at least three proteins, many techniques have been used to measure this activity. We have used clotting assays for the inactivation of thrombin and factor Xa, and immunological measurements of α_2-globulin and α_1-antitrypsin; we estimated HATA with two different concentrations of heparin, following the work of Innerfield *et al.* (1976), who suggested that prethrombotic and thrombotic patterns could thus be identified.

Studies of AT III have not, to our knowledge, previously been reported in severely malnourished children.

All the antithrombin tests, except α_1-antitrypsin, showed significantly decreased mean levels in the malnourished group. Antithrombin clotting activity and anti-Xa results indicate that the inactivation of thrombin and factor Xa was reduced in these children.

In comparing the various results shown in Fig. 1, the following points may be made. Firstly, the antithrombin clotting activity and the anti-Xa measurements agree well, supporting the concept (referred to above) that these activities are referable to the same molecule. Secondly, the discrepancy between these activities and α_2-globulin in the patients is counterbalanced by the normal values found for α_1-antitrypsin; it has been pointed out that antithrombin activity is regarded as the summation of α_2-globulin (*c* 50%) and α_1-antitrypsin and α_2-macroglobulin (each *c* 25%), so that in view of our normal α_1-antitrypsin results it might be anticipated that the mean antithrombin clotting activity in the patients would be higher than their mean α_2-globulin level. It was unfortunately not possible to estimate α_2-macroglobulin levels.

Children in this study did not develop thrombosis, although a prethrombotic pattern of HATA was found. The high incidence of thrombosis reported by others in malnutrition has been mentioned above. Presumably the actual occurrence of a thrombus depends on precipitating factors which did not affect our patients.

Marked alterations in blood coagulation have been reported in children with severe malnutrition (Merskey and Hansen, 1957; Dorantes *et al.*, 1964; Jiménez *et al.*, 1969; Bello *et al.*, 1971), but the present series showed only minor alterations.

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Notes