Br. J. Nutr. (1977), 38, 31

Serum proteinase inhibitors and other serum proteins in protein-energy malnutrition

By F. P. SCHELP, P. MIGASENA, PRANEET PONGPAEW, W. H. P. SCHREURS and VENUS SUPAWAN

Department of Tropical Nutrition and Food Science, Faculty of Tropical Medicine, Mahidol University, Bangkok 4, Thailand

(*Received* 18 *June* 1976 – *Accepted* 4 *January* 1977)

1. The concentrations of serum protein albumin, prealbumin and transferrin were determined in twenty-eight cases of protein-energy malnutrition (PEM) with infection, together with the levels of serum proteinase inhibitors (PI), α_1-antitrypsin (AT), α_1-antichymotrypsin (Ach), α_2-macroglobulin (α_2M) and inter-α-trypsin inhibitor (IαI).

2. Albumin, prealbumin and transferrin concentrations, as well as the levels of PI, IαI and α_2M were found to be lower in cases of PEM associated with infection than the corresponding values for a group of healthy Thai preschool children and a group of newborn Thai children, but despite starvation AT and Ach values generally were increased.

3. The results provide support for the hypothesis that PI, especially AT and Ach might limit the synthesis of albumin, prealbumin and transferrin in PEM associated with infection, via the inhibition of the mobilization of body's own protein.

It was observed that infection produced kwashiorkor in children suffering from subclinical chronic, protein malnutrition (Scrimshaw, Taylor & Gordon, 1968). The ultimate pathophysiological factors leading to this phenomenon are not known. It might be speculated that proteinase inhibitors (PI) are involved in this mechanism.

One of the metabolic alterations associated with infection is an increased production of 'acute-phase-reactant' proteins; these proteins are released into the plasma (Beisel, 1972) and they include the proteinase inhibitors (PI), α_1-antitrypsin (AT) and α_1-antichymotrypsin (Ach) (Wiedermann, Wiedermannova, Vaerman & Heremans, 1970; Kueppers, 1971; Aronsen, Ekelund, Kindmark & Laurell, 1972; Johansson, Kindmark, Trell & Wollheim, 1972; Alper, 1974).

In subclinical, chronic, protein malnutrition and in marasmus muscle-wasting is one source of essential amino acids for the synthesis of urgently needed proteins, e.g. albumin and β-lipoprotein, for maintaining homoeostasis (Whitehead & Alleyne, 1972). It might be speculated that in cases of PEM with infection PI may inhibit the mobilization of the body's own proteins.

In the present study the concentrations of plasma proteins albumin, prealbumin and transferrin, proposed as indices of nutritional status (Ingenbleek, De Visscher & De Nayer, 1972; Whitehead, Coward & Lunn, 1973; Ingenbleek, Van den Schriek, De Nayer & De Visscher, 1975), were determined. In addition the levels of those PI which have direct functions in infection, namely the 'acute-phase-reactant' proteins AT and Ach, and the PI which are not 'acute-phase-reactants' α_2-macroglobulin (α_2M) and inter-α-trypsin inhibitor (IαI), were also determined. In order to investigate the response of PI to infection in PEM in relation to those serum proteins whose concentrations are known to decrease in malnutrition, a group of healthy Thai preschool children and a group of newborn children, one infected group and two groups of clinical PEM from two different countries were studied. Haptoglobin, which is also an 'acute-phase-reactant' protein (Alper, 1974) was also determined.

EXPERIMENTAL

Subjects. Twenty-eight children with clinical PEM and suffering from acute infections, mainly upper respiratory tract infection and gastroenteritis were chosen for this study. Fourteen children of Thai origin were admitted to the paediatric ward of Chulalongkorn University Hospital, Bangkok, Thailand. Another fourteen children were admitted to the ward of the Nutrition Research Institute, Bogor, Indonesia.

According to the Wellcome Working Party's suggestion (Dugdale, 1971; Waterlow, 1972) five cases from Indonesia and four cases from Thailand had to be classified as marasmic-kwashiorkor as their weight-for-age was less than 60 % of the Harvard standard (Stuart & Stevenson, 1959) and oedema was present. In the remaining nine cases from Indonesia and in three cases from Thailand liver enlargement was found. In those PEM cases in which only liver enlargement without oedema was present, the classification according to the Wellcome party's suggestion was not possible. Seven cases from Thailand had to be classified as marasmus since they did not show either oedema or liver enlargement. Twenty-eight apparently healthy Thai preschool children from a village about 450 km north-east of Bangkok were also investigated. The children were selected after a careful physical examination. Another twelve children from the same village had haptoglobin levels which were higher than those of the healthy preschool children, because of minor infections, e.g. common colds, but had no fever. This group was chosen because the children had infections but were not malnourished. Twelve healthy newborn children, not more than 1·5 months of age, from the Bangkok Children Hospital were chosen to form a third group, which was a control group for the effect of low age on the serum protein pattern.

Methods. Blood was collected in heparinized capillary tubes, then plasma was separated and stored frozen at −20° before electrophoresis. The method used for quantitative measurement of serum proteins including PI was the electroimmunoassay (rocket immunoelectrophoresis) introduced by Laurell (1972). Rabbit antisera for the different proteins were supplied by Behring Werke, Marburg, West Germany.

The genetic variants of haptoglobin, but not of AT, were determined using the polyacrylamide-gel electrophoresis (Hoffmeister & Schuett, 1972; Pongpaew, Migasena & Schelp, 1975), and the values for haptoglobin determined by rocket immunoelectrophoresis were calculated using correction factors appropriate to the different genetic types.

The results for the five groups of children were analysed using one-way analysis of variance and the statistical significance of differences between the groups was tested as described by Campbell (1967) for groups of unequal size.

RESULTS

The weight-for-age (% expected weight-for-age) for the healthy preschool children and those children with increased concentrations of haptoglobin was approximately 75 and these children were therefore classified as 'undernourished' (Table 1). However, when weight-for-height was measured the values for both groups were approximately 90 % expected weight-for-height. The weight-for-age (% expected weight-for-age) for the PEM cases from Thailand and from Indonesia was 54 and 68 respectively.

When compared with values for healthy preschool children prealbumin and transferrin concentrations for the group of newborn children were significantly lower and albumin, haptoglobin and Ach levels were distinctly, but not significantly, lower. However, the concentrations of albumin, prealbumin and transferrin were significantly lower in both groups with PEM when compared with those of the other three groups, except for prealbumin

Table 1. *Age, weight-for-age (% expected weight-for-age based on the Harvard standard (Stuart & Stevenson, 1959)), serum albumin, prealbumin and transferrin concentrations of healthy preschool children, preschool children with increased haptoglobin levels and newborn children from Thailand and children of Thai and Indonesian origin with protein-energy malnutrition (PEM)*

(Mean values with their standard errors)

Group no.	Subjects	No. of subjects	Age (months)		Weight-for-age		Albumin (g/l)		Prealbumin (mg/l)		Transferrin (mg/l)		Haptoglobin (mg/l)	
			Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1	Healthy Thai preschool children	28	32·9	2·8	76·9	0·3	48	2·0	160	11·1	3034	121	1745	190
2	Thai preschool children with increased haptoglobin levels	12	26·7	3·7	78·1	0·7	51	2·5	127	20·3	2772	240	3305	324
3	Newborn Thai children	12	0·9	0·4	95·4	0·9	42	2·2	84	13·5	2341	239	959	387
4	Children of Thai origin with PEM	14	11·7	2·3	53·9	1·0	21	1·8	40	10·8	1005	189	1974	444
5	Children of Indonesian origin with PEM	14	35·6	4·4	51·4	0·8	29	2·0	53	11·5	1196	241	4202	527

Statistical significance of differences between groups when tested by analysis of variance (Campbell, 1967): age: group 1 *v.* groups 3 and 4, group 2 *v.* groups 3 and 4, $P < 0{\cdot}01$; group 4 *v.* group 5, $P < 0{\cdot}01$; weight-for-age: groups 1 and 2 *v.* group 3, $P < 0{\cdot}01$; groups 1, 2 and 3 *v.* groups 4 and 5, $P < 0{\cdot}01$; albumin: groups 1, 2 and 3 *v.* groups 4 and 5, $P < 0{\cdot}01$; group 4 *v.* group 5, $P < 0{\cdot}05$; prealbumin: groups 1 and 2 *v.* groups 4 and 5, $P < 0{\cdot}01$; group 1 *v.* group 3, $P < 0{\cdot}01$; group 3 *v.* group 4, $P < 0{\cdot}05$; transferrin: groups 1, 2 and 3 *v.* groups 4 and 5, $P < 0{\cdot}01$; group 1 *v*, group 3, $P < 0{\cdot}05$; haptoglobin: group 1 *v.* groups 2 and 5, $P < 0{\cdot}01$; group 2 *v.* group 3, $P < 0{\cdot}01$; group 2 *v.* group 4, $P < 0{\cdot}05$; group 3 *v.* group 5, $P < 0{\cdot}01$; group 4 *v.* group 5, $P < 0{\cdot}01$.

Table 2. *Serum proteinase inhibitors of healthy preschool children, preschool children with increased haptoglobin levels and newborn children from Thailand and children of Thai and Indonesian origin with protein-energy malnutrition (PEM)*

(Mean values with their standard errors)

Group no.	Subjects	No. of subjects	α_1-Antitrypsin (mg/l)		α_1-Antichymotrypsin (arbitrary units*/l)		Inter-α-trypsin inhibitor (arbitrary units*/l)		α_2-Macroglobulin (mg/l)	
			Mean	SE	Mean	SE	Mean	SE	Mean	SE
1	Healthy Thai preschool children	28	2426	101	1001	50	1106	31	4588	184
2	Thai preschool children with increased haptoglobin levels	12	3099	182	1330	87	1299	97	4890	350
3	Newborn Thai children	12	2466	172	882	68	—	—	5003	551
4	Children of Thai origin with PEM	14	2962	276	1860	308	795	86	3192	370
5	Children of Indonesian origin with PEM	14	3452	245	1768	196	1092	90	3336	299

Statistical significance of differences between groups when tested by analysis of variance: α_1-antitrypsin: group 1 *v.* groups 2 and 5, $P < 0{\cdot}01$; group 1 *v.* group 4, $P < 0{\cdot}02$; group 3 *v.* group 5, $P < 0{\cdot}01$; α_1-antichymotrypsin: groups 1 and 3 *v.* groups 4 and 5, $P < 0{\cdot}01$; group 2 *v.* group 4, $P < 0{\cdot}05$; inter-α-trypsin inhibitor: groups 1 and 2 *v.* group 4, $P < 0{\cdot}01$; group 4 *v.* group 5, $P < 0{\cdot}01$; α_2-macroglobulin: groups 1, 2 and 3 *v.* groups 4 and 5, $P < 0{\cdot}01$.

* Value derived from pooled samples from 100 blood donors was set as 1000 arbitrary units/l.

concentration in the group of PEM cases from Indonesia which only differed significantly from the group of healthy preschool children; the age-ranges of these two groups were comparable.

Haptoglobin levels of the group of children with minor infections and in the cases of PEM from Indonesia were significantly higher than those of the healthy Thai preschool children and the group of newborn children. The group of children with minor infections had higher AT and Ach concentrations than those of the control groups but differences were significant only for AT (Table 2). The values for AT and Ach for the cases of PEM were significantly higher, and those of α_2M were lower than the values for the group of healthy preschool children. There were no significant changes in the concentration of IαI between the groups except for PEM cases from Thailand. Unfortunately, values for this PI for the group of newborn children were not available, because of a shortage of serum samples from this group.

From each individual, values for the concentration ratios, PI: protein, except IαI: albumin, IαI: prealbumin, IαI: transferrin, were calculated in order to demonstrate relationships between these serum proteins independent from their actual plasma concentration. The mean values for these ratios for each group are given in Table 3. The values for the ratios, AT: albumin, AT: transferrin, Ach: albumin and Ach: transferrin were higher for PEM groups than for the other three groups but the differences were not as great as that for AT: prealbumin. The values for the ratios, α_2M: albumin, α_2M: prealbumin and α_2M: transferrin for the PEM groups differed from those for the other groups but the differences were not as marked as those for the other PI: protein.

Table 3. *Mean values for the ratios, proteinase inhibitors (PI): albumin (Alb), PI: prealbumin (Prealb), PI: transferrin (Transf) for healthy preschool children, preschool children with increased haptoglobin levels, newborn children from Thailand and children of Thai and Indonesian origin with protein-energy malnutrition (PEM)*

(Mean values with their standard errors)

Subjects ...	Healthy Thai preschool children		Thai preschool children with increased haptoglobin levels		Newborn Thai children		Children of Thai origin with PEM		Children of Indonesian origin with PEM	
No. of subjects ...	28		12		12		14		14	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
AT (mg/l): Alb (g/l)	53·6	6·1	60·9	15·5	60·9	5·4	147·9	13·9	125·8	11·4
Ach (arbitrary units*/l): Alb (g/l)	21·1	1·0	27·0	2·1	21·7	1·5	94·5	1·6	63·3	7·4
α_2M (mg/l): Alb (g/l)	98·1	4·7	99·2	7·9	131·7	17·7	155·5	15·9	121·8	14·6
AT (mg/l): Preabl (mg/l)	17·6	1·6	27·5	3·7	88·8	29·1	138·6	20·4	175·7	45·4
Ach (arbitrary unit/l): Prealb (mg/l)	6·7	0·61	13·2	1·7	30·3	9·9	88·0	15·1	71·5	19·3
α_2M (mg/l): Prealb (mg/l)	32·8	2·5	41·2	4·0	102·2	39·4	140·7	21·5	99·6	34·3
AT (mg/l): Transf (mg/l)	0·8	0·06	1·2	0·14	1·2	0·16	4·0	0·58	4·3	0·67
Ach (arbitrary units*/l): Transf (mg/l)	0·3	0·02	0·5	0·07	0·4	0·06	2·6	0·53	2·4	0·38
α_2M (mg/l): Transf (mg/l)	1·4	0·09	1·8	0·14	2·5	0·41	4·1	0·54	4·0	0·66

AT, α_1-antitrypsin; Ach, α_1-antichymotrypsin; α_2M, α_2-macroglobulin.
* Value derived from a pooled sample from 100 blood donors was set at 1000 arbitrary units/l.

DISCUSSION

The results of this study demonstrated a general increase in concentration of the 'acute-phase-reactant' proteins and of PI, AT and Ach in the serum of individuals suffering from acute infection, despite severe starvation. This could be shown for a group of PEM cases from Thailand as well as for a group of PEM cases from Indonesia, which produced a similar protein pattern compared with the Thai cases. Unfortunately a group of healthy preschool children of Indonesian origin could not be included in this study. Recent published results from Nigeria indicated lower AT values in PEM cases compared with the control group (Razban, Olusi, Ade-Serrano, Osunkoya, Adeshina & McFarlane, 1975); it could be demonstrated in the present study that at least for two groups of PEM cases from two different countries in south-east Asia, AT concentrations were higher in PEM cases than in healthy children.

Albumin, prealbumin and transferrin levels were generally lower for PEM cases compared with the other three groups. The concentration of these proteins for the healthy Thai preschool children were in a range comparable to European values (Sveger & Ekelund, 1975; Vahlquist, Rask, Peterson & Berg, 1975) where results from the same age-range were available.

For the group of newborn Thai children prealbumin and transferrin concentrations were found to be lower than those for the Thai preschool children, but were higher when compared with those for the PEM groups. The concentrations of other proteins under investigation were in general similar to the values for the Thai preschool children.

The group of Thai preschool children with increased haptoglobin levels did not show a significant difference in the protein pattern compared with the group of healthy Thai preschool children except for AT levels, which were increased and comparable to those of the PEM groups. However, this was not associated with a significant decrease in albumin, prealbumin and transferrin for the group of Thai preschool children with high haptoglobin levels, when compared with the PEM groups.

The function of PI has not been clearly defined. Interpretation of changes in their concentrations could only be speculative. In particular, the biological functions of Ach in vivo have not been studied thoroughly. For AT, it is known that the enzymes of polymorphonuclear granulocytes, pancreatopeptidase E (*EC* 3.4.4.7) and a neutral protease, are found to be inhibited by AT. In connexion with inherited variants of AT deficiency, patients may develop a panlobular emphysema due to decreased inhibition of the leucocytal proteinases (Ohlsson, 1971; Orell & Mazodier, 1972). Patients with AT deficiency may also develop liver cirrhosis.

From the results of studies on interactions between endogenous proteases and plasma PI in vitro and in vivo, it was concluded that one of the functions of the α_2-M may be the binding and clearance of proteolytic enzymes in the organism, and it is assumed that α_2M has a key function in the organism in protection against autodigestion (Ohlsson, 1974). The results presented here are in accordance with those reported by other workers in that the level of α_2M does not increase during infection (Aronsen *et al.* 1972). However, it was found in patients suffering from burns that large amounts of α_2M rapidly invade the extravascular space but the serum level remains constant (Farrow & Baar, 1973). This is possible only when there is a clear increase in the synthesis rate (Steinbuch & Audran, 1974). In PEM associated with infection an increase in synthesis might not be possible due to the limited protein intake, and in this instance the serum concentration of α_2M will decrease; this might possibly explain the low levels of α_2M found. At the same time the PI, AT and Ach levels are increased and may interrupt to a certain extent the wasting of the body's own protein.

It should be expected that low albumin, prealbumin and transferrin concentrations are associated with high PI concentrations for AT and Ach. This should be apparent from regression analysis. However, no significant relationship was found between the PI, AT, Ach and α_2M and the proteins albumin, prealbumin and transferrin when a linear regression analysis was done. The groups of PEM cases are too heterogeneous, particularly with respect to the period of malnutrition. Further studies are being undertaken to obtain 'clear-cut' cases of kwashiorkor and marasmus. First results did show that in cases of marasmus higher albumin and transferrin values are associated with lower PI concentrations compared with marasmic-kwashiorkor cases (Schelp, Migasena, Pongpeaw & Schreurs, unpublished results).

However, in this study also, the relationship between the increased levels of PI and the decreased levels of albumin, prealbumin and transferrin is more obvious when values for PI:serum protein are considered. In particular, values obtained for the PEM groups were higher than those for other groups.

These observations might support the hypothesis that certain PI are a part of a mechanism which in PEM associated with infection inhibits the serum proteins like albumin, prealbumin and transferrin.

These studies were partly supported by Research Grant No. T-72-11 and T-74-01 from SEAMEO-TROPMED project, as well as by the German Agency for Technical Cooperation, Ltd (GTZ) Pr. No. 74.2513.5. The authors wish to express sincere thanks to Dr Sonchai Pongsupath and Dr Prakong Poshakrishana of the Department of Paediatrics, Faculty of Medicine, Chulalongkorn University, Bangkok and Dr Darwin Karyadi, Nutrition Research Institute, Bogor, Indonesia for providing the serum from the clinical malnutrition cases; and to Professor C. Harinasuta, the Dean of the Faculty of Tropical Medicine, Mahidol University, Bangkok, for his support. The authors also thank Mrs Niyomsri Vudhivai for skilled technical assistance.

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Printed in Great Britain