Serum protein fractions from children of differing nutritional status analysed by polyacrylamide gel electrophoresis and electroimmunoassay

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1. The nutritional status of children showing no clinical signs of malnutrition, from the University School of Khon Kaen, Khon Kaen province, north-east Thailand and from two villages nearby, was tested. The children were grouped according to their body-weight expressed as a percentage of expected weight-for-height (Harvard standards (Stuart & Stevenson, 1959), as given by Jelliffe (1966)).

2. The differing prealbumin concentrations indicated that nutritional status differed between the groups.

3. The urinary urea:creatinine ratio was significantly lower in the village children compared with the children from Khon Kaen, indicative of the higher dietary protein intake of the latter.

4. α_1-Acid glycoprotein and the first 'post-albumin peak' (obtained by polyacrylamide gel electrophoresis of serum and containing mainly Gc-globulin, α_1-antichymotrypsin and α_1-B-glycoprotein) were found to be significantly higher in the village children compared with children from Khon Kaen.

5. The three main proteins of the first 'post-albumin peak' from polyacrylamide gel electrophoresis of serum were tested separately using the electroimmunoassay method. There was no significant difference in Gc-globulin between the children from Khon Kaen and the village children. The concentration of α_1-B-glycoprotein from those Khon Kaen children whose body-weight was more than 95% expected weight-for-height was significantly lower compared with that of village children. α_1-Antichymotrypsin concentration was significantly higher in serum from Khon Kaen children than in serum from village children.

With the development of sensitive methods for protein separation, plasma protein determinations are now recommended as a useful aid for studies of certain pathological states (Alper, 1974). Although polyacrylamide gel electrophoresis (PAGE) is widely used in biochemical laboratories, it has not yet proved useful as a substitute for paper or cellulose-acetate electrophoresis or immunoelectrophoresis for human plasma protein separation. This is mainly because of a lack of reproducibility and also a lack of a widely accepted standardized method (Maurer & Allen, 1972). Despite these disadvantages, PAGE might be a useful additional test in the assessment of nutritional status, because several proteins of interest in nutritional research, such as prealbumin (Ingenbleek, de Visscher & de Nayer, 1972), albumin (Whitehead & Alleyne, 1972) and transferrin (Kumar, Chase, Hammond & O'Brien, 1972) can be determined directly at the same time. In addition, the glycoprotein-rich, 'α_1-' range is separated into two different peaks and the haptoglobins can be measured semi-quantitatively as indices of infection (Prokop & Bundschuh, 1963; Pongpaew, Migasena & Schelp, 1975). The method used in this survey allows the simultaneous separation of serum

proteins from twenty individuals, which provides rapid evaluation of a large number of samples with reasonable economy.

Previously it was reported that the first 'post-albumin peak', obtained by PAGE of human serum, was significantly higher in healthy adults from a rural area of north-east Thailand, compared with healthy 'well-to-do' adults from Bankgok (Schelp, Migasena, Saovakontha & Pongpaew, 1974). About 90% of the serum proteins forming this first 'post-albumin peak' are Gc-globulin, α_1-antichymotrypsin and α_1-B-glycoprotein (Schultze & Heremans, 1966; Felgenhauer, 1970; Hoffmeister & Schütt, 1972; Pongpaew *et al.* 1975). It was presumed that this difference in the height of the first 'post-albumin peak' was due to the differences in food intake between the two groups. In order to determine whether there are similar differences in children, we studied the serum protein patterns of children of differing nutritional status attending the University School in the town of Khon Kaen, north-east Thailand, and of children from two villages in the same province.

METHODS

Subjects

The survey was done in the dry season (March 1974), and involved children from the University School, Khon Kaen, Khon Kaen province, north-east Thailand, and children from two villages in the same province. The first village, Resettlement Village 14, is one of a group of standard resettlement villages provided in Khon Kaen province by the Thai government for people who were displaced by the building of a dam nearby. The second village, Non Bua Noy, is one whose inhabitants are of the same ethnic group, and are typical of this area. The resettled people work on a cash-crop system whereas the people from Non Bua Noy are involved primarily in rice farming (Migasena, Thurnham, Pongpaew, Hongthong & Harinasuta, 1974).

Anthropometric measurements and physical examination. Body-weight and height were measured and relative body-weight (% expected weight-for-height) was calculated from the Harvard standards (Stuart & Stevenson, 1959) as given by Jelliffe (1966). A general physical examination was also made.

Age. The exact age was estimated either from information available in the school or from birth certificates.

Faecal examination. Faeces, when available, were examined for parasites using a faecal smear.

Blood samples and their analysis

Capillary blood was collected in heparinized capillary tubes (Harshaw Chemicals Ltd, London). Measurement of haemoglobin and separation of plasma was done soon after collection. The plasma was stored at 4° for no longer than 3 d, then frozen at −70° and thawed only once before electrophoresis. During storage, the fibrinogen clotted, and serum for electrophoresis was obtained after centrifugation.

Haemoglobin and mean corpuscular haemoglobin concentration (MCHC). Whole blood haemoglobin values were determined by the cyanmethaemoglobin method (Aculute reagents; Ortho Diagnostic Corp., Raritan, New Jersey, USA). Packed cell volume

(PCV) values were determined by a microcapillary method and values for MCHC were calculated from the results.

Total protein. Total protein was measured using an ultra-micro-analytical system (Model 150; Beckman Instruments Inc., Fullerton, California, USA) with Biuret reagent, according to the method of Beckman Instruments Inc. (1962), which is based on the method of Kingsley (1939) and Gornall, Bardawill & David (1949).

Electrophoresis. Electrophoresis was done using a vertical flat-bed gradient, 80–60–45 g polyacrylamide gel/kg, with an Ortec® Model 4100/4200 (Ortec Inc., Oak Ridge, Tennessee, USA) (Allen & Moore, 1966; Ortec Inc., 1972; Pongpaew *et al.* 1975). For every gel two references, as standards, were run simultaneously with the samples. The gels were stained with a solution of 0·17 g Amido black 10B (E. Merck AG, Darmstadt, Germany)/l and destained in acetic acid (100 g/l). The destained gels were transported to Bundesgesundheitsamt, West Berlin, Germany, and protein patterns were analysed using a densitometer (Model no. 4310; Ortec Inc.). Bands produced by electrophoresis were identified according to Felgenhauer (1970) and Hoffmeister & Schütt (1972), who used similar techniques. Prealbumin, α_1-acid glycoprotein, albumin and transferrin were determined directly. About 90% of the first 'post-albumin peak' contained Gc-globulin, α_1-antichymotrypsin and α_1-B-glycoprotein. The second 'post-albumin' peak consisted mainly of α_2-HS glycoprotein, and 4·6 S-post-albumin; caeruloplasmin, together with haemopexin, migrated directly in front of the transferrin.

The number of individual measurements for each peak differed because there was slight damage to the gels during transportation, so that not all peaks could be determined in all samples. For determination of caeruloplasmin those with the haptoglobin type 1-1 were excluded, because the peak for haptoglobin of this type overlaps the caeruloplasmin–haemopexin peak.

Electroimmunoassay. The method of electroimmunoassay (rocket immunoelectrophoresis) introduced by Laurell (1972) was used for quantitative measurement of Gc-globulin, α_1-antichymotrypsin and α_1-B-glycoprotein. Antiserum from rabbit for these proteins was supplied by Behringwerke, Marburg, Germany. Agarose gel (15 g/l) was prepared using a 0·024 M-sodium barbital–barbital buffer (pH 8·6). For the electrophoresis buffer, 0·086 M-sodium barbital–sodium acetate trihydrate (pH 8·2) was used, and the electrophoresis was done at low voltage (0·2 V/mm in the gel) for more than 8 h overnight. Because of a lack of serum in some samples, the number of samples analysed differed from protein to protein and also differed from the number analysed by PAGE.

Biochemical tests

Random samples of urine were collected late in the morning and preserved by the addition of hydrochloric acid (0·1 ml concentrated HCl in 30 ml urine).

Creatinine. Creatinine was determined by a modification of the procedure of Folin & Wu (1919) as outlined by Technicon Instruments Corporation (1972*a*).

Urine urea-nitrogen. Samples of 0·5 ml urine were diluted with 9·5 ml distilled water and urea-N was determined using a modification of the method of Marsh,

Table 1. *Mean values for body-weight (% expected weight-for-height (Harvard standards*)) of children from the University School in the town of Khon Kaen, and from Non Bua Noy village and Resettlement Village 14 in north-east Thailand*

(No. of children/group given in parentheses)

Group no.	Range	Children from Khon Kaen	Village children
1	105–95	101·4 (19)	99·6 (16)
2	94·9–85	90·4 (24)	89·9 (35)
3	84·9–75	—	80·6 (6)

The anthropometric distribution for Khon Kaen children and for the village children was compared by a Kolmogoroff-Smirnoff test and no statistically significant difference was found.

* Stuart & Stevenson (1959), as given by Jelliffe (1966).

Table 2. *Mean ages (months) of children from the University School in the town of Khon Kaen, and from Non Bua Noy village and Resettlement Village 14 in north-east Thailand*

(Mean values with their standard errors)

Group no.*	Children from Khon Kaen	Village children
1	107·1 ± 2·4	104·1 ± 9·0
2	104·9 ± 2·2	95·3 ± 5·2
3	—	73·0 ± 14·3

When tested by analysis of variance (Campbell, 1967) no statistically significant difference between the groups was found.

* For details, see Table 1.

Fingerhut & Miller (1965), as described by Technicon Instruments Corporation (1972*b*) for the AutoAnalyzer (Technicon Instruments Corporation, Tarry Town, New York, USA).

The urinary urea:creatinine ratio was calculated as recommended by the Committee on Procedures for Appraisal of Protein-Calorie Malnutrition (1970).

RESULTS

Anthropometric measurements and physical examination

The children were grouped according to their relative body-weight (% expected weight-for-height) (Table 1). None of the children in this survey showed any sign of malnutrition or disease.

There was no significant difference in age between the groups (Table 2), although there was a more marked variation in the age of the village children.

Haematology

Values for haemoglobin and MCHC are given in Table 3. Although there were significant differences between the groups, none of the groups had mean values indicating more than slight anaemia.

Table 3. *Mean blood haemoglobin concentration (g/l) and mean corpuscular haemoglobin concentrations (MCHC) (g/l) of children from the University School in the town of Khon Kaen, and from Non Bua Noy village and Resettlement Village 14 in north-east Thailand*

(Mean values with their standard errors)

	Children from Khon Kaen		Village children		
Group no.* ...	1	2	1	2	3
Haemoglobin	134±1·6	131±1·8	128±2·7	124±1·8	112±6·7
MCHC	36±0·5	35±0·3	34±0·3	34±0·3	32±1·2

Difference between mean blood haemoglobin concentrations of 'group 2' Khon Kaen children and 'group 2' village children was statistically significant ($P < 0.05$).

Difference between haemoglobin concentrations of village children in groups 2 and 3 was statistically significant ($P < 0.01$).

Differences between mean MCHC values of (*a*) 'group 1' Khon Kaen children and 'group 1' village children; (*b*) 'group 2' Khon Kaen children and 'group 3' village children were statistically significant ($P < 0.01$).

* For details, see Table 1.

Prealbumin and urea:creatinine ratio

The prealbumin concentration and the urinary urea:creatinine ratio for each group is given in Table 4. Significant differences in the prealbumin concentration were found only between 'group 1' Khon Kaen children and 'group 2 and 3' village children, with a decreasing trend in the mean values for the different groups ('group 1' Khon Kaen children had the highest value and 'group 3' village children the lowest value).

However, values for the urinary urea:creatinine ratio for the children from Khon Kaen were significantly different from those for the village children, irrespective of groups.

Carbohydrate-rich serum proteins

α_1-Acid glycoprotein concentration (Table 4) reflected the findings for prealbumin concentrations for the groups of village children because there was a decreasing trend in values from group 1 to group 3. The mean values for the two groups of children from Khon Kaen were not significantly different, but they were significantly lower than those for the corresponding groups of village children.

Analysis of the results suggested that the urea:creatinine ratio was inversely related to α_1-acid glycoprotein concentration and to the concentrations of proteins from the first 'post-albumin peak' (Table 4). Those groups having higher values for their urea:creatinine ratio had low protein concentrations, and vice versa.

The concentrations of α_1-antichymotrypsin and α_1-B-glycoprotein for the groups of children from Khon Kaen and the village children are given in Table 4.

The α_1-antichymotrypsin concentrations for children from Khon Kaen were significantly different from those of the village children, irrespective of nutritional status, and were higher than those for the village children.

The α_1-B-glycoprotein concentration for 'group 1' village children was significantly higher than that for 'group 1' Khon Kaen children.

Table 4. *Mean concentrations of prealbumin and carbohydrate-rich serum proteins, and urea:creatinine ratios for children from the University School in the town of Khon Kaen, and from Non Bua Noy village and Resettlement Village 14 in north-east Thailand*

(Mean values with their standard errors; no. of children/group given in parentheses)

	Group no.*	Prealbumin (mg/l)		Urinary urea: creatinine		α_1-Acid glycoprotein (mg/l)		First 'post-albumin peak'† (mg/l)		α_1-Antichymotrypsin (AU/l)‡		α_1-B-glycoprotein (AU/l)‡		Second 'post-albumin peak'§ (mg/l)	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Children from Khon Kaen	1	129	32 (16)	6·9	0·3 (18)	129	14 (11)	743	24 (18)	913	35 (10)	678	149 (6)	430	26 (19)
	2	106	32 (16)	7·4	0·5 (20)	129	13 (12)	658	22 (24)	889	51 (8)	881	32 (8)	473	23 (24)
Village Children	1	112	60 (11)	4·4	0·3 (12)	243	16 (10)	915	27 (15)	664	17 (14)	1051	61 (12)	380	30 (15)
	2	87	26 (29)	4·7	0·3 (31)	206	22 (21)	872	18 (34)	672	16 (29)	875	45 (28)	323	20 (33)
	3	70	12 (6)	3·7	0·7 (4)	193	8 (5)	872	45 (6)	669	28 (7)	726	43 (6)	381	45 (6)

Difference between mean prealbumin concentrations of 'group 1' Khon Kaen children and 'group 2 and 3' village children was statistically significant ($P < 0.05$).

Difference between mean (*a*) urinary urea:creatinine ratios, (*b*) α_1-acid glycoprotein concentrations, (*c*) α_1-antichymotrypsin concentrations of 'group 1 and 2' Khon Kaen children and 'group 1, 2 and 3' village children was statistically significant ($P < 0.01$).

Difference between mean first 'post-albumin peak' concentrations of (*a*) 'group 1' Khon Kaen children and 'group 1' village children, (*b*) 'group 2' Khon Kaen children and 'group 2 and 3' village children was statistically significant ($P < 0.01$, $P < 0.05$ respectively).

Difference between mean α_1-B-glycoprotein concentrations of 'group 1' Khon Kaen children and 'group 1' village children was statistically significant ($P < 0.01$).

Difference between mean second 'post-albumin peak' concentrations of 'group 1 and 2' Khon Kaen children and 'group 2' village children was statistically significant ($P < 0.01$).

AU, arbitrary units.

* For details, see Table 1.

† Gc-globulin, α_1-antichymotrypsin and α_1-B-glycoprotein; for details of electrophoresis procedures for serum, see p. 213.

‡ Values given as AU because no standard serum was available.

§ Mainly α_2-HS glycoprotein; for details, see p. 213.

Table 5. *Mean serum concentrations of total protein, albumin, caeruloplasmin–haemopexin, transferrin and Gc-globulin of children from the University School in the town of Khon Kaen, and from Non Bua Noy village and Resettlement Village 14 in north-east Thailand*

(Mean values with their standard errors; no. of children/group given in parentheses)

	Group no.*	Total protein (g/l) Mean	SE	Albumin (g/l) Mean	SE	Caeruloplasmin–haemopexin (mg/l) Mean	SE	Transferrin (mg/l) Mean	SE	Gc-globulin (mg/l) Mean	SE
Children from Khon Kaen	1	58	1·4 (19)	36	1·1 (19)	643	31·0 (19)	2271	98·0 (19)	307	7·4 (7)
	2	61	1·3 (21)	36	1·0 (24)	663	34·8 (24)	2150	65·4 (29)	284	7·3 (8)
Village children	1	62	1·4 (10)	40	1·2 (15)	632	41·2 (15)	2140	100·8 (15)	282	11·6 (12)
	2	61	0·4 (28)	36	0·9 (34)	633	25·0 (33)	2068	49·7 (34)	279	4·4 (27)
	3	63	1·2 (6)	40	1·2 (6)	650	58·8 (6)	2254	113·5 (6)	293	13·9 (7)
Variance ratio†		1·29		2·07		0·15		1·15		0·90	
Total no. of samples		84		98		97		98		61	

* For details, see Table 1.

† Values indicate no statistically significant difference among the groups when tested by analysis of variance.

Other serum proteins

Values for the second 'post-albumin peak', which mainly consists of an α_2-globulin (α_2-HS glycoprotein), are given in Table 4. Although the differences between the groups were not always significant, the values for the children from Khon Kaen were generally higher than those for the village children, which is the opposite of the findings for α_1-acid glycoprotein and the first 'post-albumin peak'.

There was no significant difference among the groups in total protein, albumin, caeruloplasmin–haemopexin, transferrin, and Gc-globulin concentrations (Table 5).

DISCUSSION

The results suggest that serum proteins reflect current nutritional status. Therefore, the classification of the groups undertaken here, i.e. weight in relation to height, was well-selected, as this index is supposed to be a measure of malnutrition, whereas height in relation to age may be used as a measure of growth retardation (Seoane & Latham, 1971; Waterlow, 1972).

Only apparently healthy children were chosen for this survey. Many children and their parents refused to give samples of urine and faeces because of great shyness. We believe, however, that the results of this survey were not influenced by infection. The children were selected by a careful physical examination, and those with minor colds and with skin lesions were excluded. All the children had haptoglobin levels not more than 'two plus' according to the classification of Pongpaew *et al.* (1975). (An increase in haptoglobin level is a sensitive indicator of infection (Prokop & Bundschuh, 1963). Furthermore, no severe anaemia was found (Table 3).

The values for serum protein concentrations obtained in this survey by PAGE are generally lower than normal values obtained by other methods (Schultze & Heremans, 1966). We are only aware of the results reported by Hoffmeister (1974) and his group, who did quantitative determinations of serum proteins using PAGE. In a previous publication (Schelp *et al.* 1974), we reported values for albumin, transferrin and the combined first and second 'post-albumin peaks' for adult subjects which were comparable with those reported by Hoffmeister (1974); values for prealbumin, caeruloplasmin–haemopexin and α_1-acid glycoprotein were not reported by Hoffmeister (1974). The values for transferrin and caeruloplasmin–haemopexin concentrations for the children in our study (Table 5) are slightly lower than those reported for Thai adults (Schelp *et al.* 1974).

As already expected from physical examination and anthropometric measurements, serum protein values indicated that none of the children had signs of manifest malnutrition. Ingenbleek *et al.* (1972) suggested that prealbumin concentration is a useful index in the detection of protein-energy malnutrition. In accordance with the results of Ingenbleek *et al.* (1972), the decreasing trend of values for prealbumin concentration (Table 4), from 'group 1' Khon Kaen children to 'group 3' village children, seems to indicate poorer nutrition in 'group 2 and 3' children. The results of a study of the plasma protein pattern in subjects with cirrhosis of the liver seem to

indicate that prealbumin is the most sensitive indicator of the impaired liver function (Haellen & Laurell, 1972).

The values for urinary urea:creatinine ratio did not indicate nutritional differences between the groups within the communities. The size of this ratio depends on the dietary protein intake (Committee on Procedures for Appraisal of Protein-Calorie Malnutrition, 1970; Simmons, 1972). Although the values we obtained for the urea:creatinine ratio do not suggest that the difference in the nutritional status between the groups was due to different protein intake, they do suggest that the diet of the Khon Kaen children was richer in protein than the diet of the village children, because there was a significant difference in the urea:creatinine ratio between the groups from the town and from the villages (Table 4).

The results of a dietary survey made in the villages studied here have been published (Migasena *et al.* 1974). The energy intake for the age group from 73 to 96 months was found to be about 4·2 MJ (1000 kcal)/d. This is less than the recommended energy requirements for children of this age, which is stated to be 7·95–9·45 MJ (1900–2260 kcal)/d (FAO/WHO, 1973). The protein intake, mainly from vegetable sources, was between 14·9 and 34 g/d. Assuming the protein quality is 0·60 relative to milk or eggs, the 'safe level of intake' for children in the age range 84–108 months would be about 40 g/d (FAO/WHO, 1973).

No dietary survey results are available for the children from Khon Kaen. The anthropometric and biochemical measurements reported here, however, seem to indicate that at least for the 'group 1' Khon Kaen children, the amount and composition of food was sufficient. The urea:creatinine ratio for the 'group 2' Khon Kaen children was not significantly different from that for 'group 1' Khon Kaen children, so that it is supposed that in this group also the protein content of the food was above the 'safe level of intake'.

There was no significant difference between the groups in the levels of total serum protein and albumin (Table 5). Serum protein and albumin concentrations only begin to decrease when clinical signs of malnutrition appear (Committee on Procedures for Appraisal of Protein-Calorie Malnutrition, 1970), which was not so for any child under study.

There were highly significant differences in the plasma concentration of α_1-acid glycoprotein and the proteins of the first 'post-albumin peak' (Table 4) for the Khon Kaen children compared with those for the village children. These findings are in accordance with previous results from healthy adults in Bangkok compared with healthy adults in a rural area in north-east Thailand; these results indicated that the first 'post-albumin peak' was also significantly higher in the rural group (Schelp *et al.* 1974).

α_1-Acid glycoprotein and the proteins forming the first 'post-albumin peak' during PAGE migrate in the 'α_1-' range on paper and cellulose-acetate electrophoresis (Schultze & Heremans, 1966).

Although most of the proteins in human plasma are glycoproteins (Schwick & Heide, 1973) most of those with a high carbohydrate content migrate in the 'α_1-' range. The total carbohydrate content of acid glycoprotein is 414 mg/g (Heimburger,

Heide, Haupt & Schultze, 1964). Among those proteins forming most of the first 'post-albumin peak' obtained by PAGE, the carbohydrate content of Gc-globulin is comparatively low (42 mg/g) (Schultze, Biel, Haupt & Heide, 1962), but is higher for α_1-antichymotrypsin (227 mg/g) (Schultze, Heide & Haupt, 1962) and for α_1-B-glycoprotein (133 mg/g) (Heimburger *et al.* 1964). The results have been published of several studies of the influence of clinical protein-energy deficiency on serum glycoprotein and total protein-bound hexose (PBH) in man and animals. The results are controversial. Serum glycoprotein and serum PBH are reported to be high in kwashiorkor (Shehata, Abdel Hay, Kamel, Fayad & Talaat, 1965; Fayad, Metwalli, Shukry & Ismail, 1969; Patwardhan, Maghrabi, Mousa, Gabr & El Maraghy, 1971), but some workers found high values also in marasmus (Shehata *et al.* 1965; Patwardhan *et al.* 1971), others found low values in marasmus but high values in kwashiorkor (Fayad *et al.* 1969). In rats, low-protein diets or food restriction decreased the level of seromucoid (α_1-acid glycoprotein) and PBH (Weimer & Nishihara, 1959; Weimer & Hummelbaugh, 1965).

However, the values for α_1-globulin in young baboons were significantly higher than those in the control animals, when they were given a diet consisting only of local staples from Uganda, with a low protein, high carbohydrate content, long before the animals developed signs of malnutrition (Coward & Whitehead, 1972; Coward, Whitehead & Coward, 1972).

The results of the baboon experiments seem to be in accordance with the findings in our survey, in which the village children seemed to have a higher content of carbohydrate and lower protein content in their diet compared with the town children of the same nutritional status.

However, poorer nutritional status in the village children, as represented by groups 2 and 3, was not associated with an increase in those serum protein fractions containing proteins with a high carbohydrate content (Table 4). In this respect our results resemble those from the rat experiments mentioned previously in which PBH and α_1-acid glycoprotein were found to be reduced when a low-protein diet was fed.

Changes in the concentration of PBH and serum glycoprotein in clinical cases of protein-energy malnutrition mentioned previously cannot be explained by the results reported here. It might be theorized that infection in the clinical cases produces the conflicting results in those surveys.

Of those proteins representing mainly the first 'post-albumin peak' from PAGE, Gc-globulin values were not significantly different between the groups (Table 5), although the values were low compared with normal values reported by Schultze & Heremans (1966).

The concentration for α_1-antichymotrypsin and α_1-B-glycoprotein can only be given in arbitrary units (AU), because no standard serum was available. Pooled serum from 100 adult blood donors of Thai origin was taken as 1000 AU/l. As seen in Table 4 the values for α_1-antichymotrypsin concentration were about 900 AU/l for the children from Khon Kaen whereas the values for the village children were significantly lower. α_1-B-glycoprotein concentration was only significantly higher in 'group 1' village children compared with 'group 1' Khon Kaen children (Table 4).

That seems to indicate that the increase in the first 'post-albumin peak' obtained by PAGE of serum from the village children is most probably due to α_1-B-glycoprotein whereas for group 2 the results did not suggest a similar conclusion. It might be assumed that a complex interaction, as yet unknown, of all the proteins forming the first 'post-albumin peak' produces the increase in this peak which is also found in the 'group 2' village children.

α_1-Antichymotrypsin is known as a very fast increasing acute phase reactant (Aronsen, Ekelunol, Kindmark & Laurell, 1972). In that it does not seem to be increased in the children from Khon Kaen and is lower in the village children; it might be taken as further evidence that the results reported here are not influenced by undetected infection. The authors, however, are not aware of any report concerning changes in concentration of this protein in relation to different nutritional status.

Coward *et al.* (1972) reported that α_2-globulin and β-globulin decrease in concentration during the terminal stages of kwashiorkor. Our results indicate significantly lower values for the second 'post-albumin peak' for the village children compared with the children from Khon Kaen (Table 4). It is supposed that the second 'post-albumin peak' is formed mainly from α_2-HS glycoprotein (Felgenhauer, 1970). This may indicate that at least one of the α_2-globulin proteins may react rather sensitively to different dietary intakes. We have no values for haptoglobin and α_2-macroglobulin. These proteins mainly migrate in the α_2-globulin range.

Caeruloplasmin migrates in the α_2-globulin range and haemopexin in the β-globulin range during ordinary paper or cellulose-acetate electrophoresis. The height of the peak representing both these proteins, which migrate together during PAGE, did not differ significantly among the groups.

The results here seem to indicate that α_1-acid glycoprotein and the proteins of the first 'post-albumin peak' obtained during PAGE, consisting mainly of α_1-B-glycoprotein, are initially high in those children on a diet with a higher content of carbohydrate relative to protein compared with those on a diet with more protein.

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