

SOME HUMAN "CARBOHYDRATE-RICH" SERUM PROTEINS IN PROTEIN-ENERGY MALNUTRITION

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INTRODUCTION

Human serum proteins are glycoproteins in general. Among those with more than 10 per cent of carbohydrate portion in the molecule are α_1 -acid glycoprotein (41.4%), α_1 -antichymotrypsin (22.7%), α_2 HS-glycoprotein (13.4%) and α_1 B-glycoprotein (13.3%) (Schultze *et al.*, 1962; Heimburger *et al.*, 1964).

Serum glycoprotein and total protein-bound hexose (PBH) in clinical protein-energy malnutrition (PEM) have been studied by several investigators with controversial results. Patwardhan *et al.*, (1971) demonstrated that these were high in kwashiorkor and marasmus. In rats with low protein diets or food restriction the level of glycoproteins and PBH decreased (Weimer and Nishihara, 1959; Weimer and Hummelbough, 1965).

Special staining procedures were used for detection of serum glycoproteins in preceding investigations. In this study the technique of polyacrylamide gel electrophoresis (PAGE) for serum protein separation is used. α_1 -acid glycoprotein, α_1 - and α_2 -globulins with a high carbohydrate portion are present as an individual single band.

The purpose of this study was to investigate some of the single fractions of carbohydrate rich serum proteins in clinical P.E.M.

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MATERIALS AND METHODS

Thirteen children with clinical PEM suffering from infectious diseases, mainly upper respiratory tract infection and gastroenteritis were chosen for this study. They were admitted at the pediatric ward of Chulalongkorn University Hospital, Bangkok. Twenty-four apparently healthy Thai preschool children from a village about 450 km north-east of Bangkok were taken as a control group. For the low age range of the PEM cases a second control group of ten healthy newborn children, not older than one and a half month, from the Children's Hospital, Bangkok, were selected.

Blood was collected in heparinized capillary tubes, then plasma was separated and stored frozen at -20°C before electrophoresis. For this the molecular sieving effect of 8 - 6 - 4, 5% polyacrylamide vertical flat-bed gradient gel was used as described by Pongpaew *et al.*, (1975). Electrophoresis was performed in using an Ortec^R (Ortec, Tennessee, U.S.A.) model 4200 electrophoresis tank and cells and model 4100 pulsed constant power polarization source. After electrophoresis the gels were stained with 0.017% amido black and destained in 10% acetic acid. The protein pattern was read by the manual device of an Ortec model 4300 integrating microdensitometer system. A human standard serum derived from Behring Werke, Marburg, Germany was run simultaneously in each gel. The protein pattern in the gels were identified according to Felgenhauer (1970) and Abraham *et al.*, (1970). Prealbumin, α_1 -acid gly-

coprotein, albumin and transferrin form a single peak. In the range of the first postalbumin band mainly Gc-globulin, α_1 B-glycoprotein and α_1 -antichymotrypsin is found, whereas the second postalbumin peak is mainly composed of α_2 HS-glycoprotein and 4, 6 S-glycoprotein. The normal serum concentration of 4, 6 S-glycoprotein can be neglected because it is comparably low.

The serum concentration of the three main proteins forming the first postalbumin peak were determined additionally in using the electroimmuno assay (rocket immunoelectrophoresis) technique according to Laurell (1972). Antiserum from rabbit for those proteins were obtained from Behring Werke.

RESULTS

Age, weight for age according to the Harvard standard (Stuart and Stevenson, 1959), albumin, prealbumin and transferrin levels derived by PAGE are shown in Table 1.

The age range of the PEM children are significantly below the healthy preschool

children but above the age of the newborn. Weight for age for the healthy preschool children is rather low, but when weight in relation to height was measured then this group were about 90%. The values for albumin, prealbumin and transferrin are significantly lower in the PEM group compared with the healthy preschool children group. Albumin and transferrin in the PEM group were also significantly lower than the values of the newborn.

The serum concentration of α_1 -acid glycoprotein from the PEM group is significantly increased above the control groups (Table 2). The peak containing mainly α_2 HS-glycoprotein is found to be the lowest in the PEM group but the difference to the control group is not significant.

The total concentration of the proteins of the first postalbumin peak did not differ between the groups. Therefore, the proteins Gc-globulin, α_1 B-glycoprotein and α_1 -antichymotrypsin were measured separately (Table 3). Unfortunately there was not enough serum left from the newborn group in order to evaluate these serum proteins.

Table 1

Mean \pm S.E.M. of age, percentage expected weight for age (Harvard standard) and serum albumin, prealbumin and transferrin from a group of healthy preschool children, newborn children and children suffering from protein-energy malnutrition associated with infection.

Groups	No. of cases	Age month	Weight for age %	Prealbumin mg/100 ml	Albumin mg/100 ml	Transferrin mg/100 ml
1. Healthy preschool children	24	32.9 \pm 2.8	76.9 \pm 0.3	26.9 \pm 2.6	4.6 \pm 0.1	224.9 \pm 9.6
2. Newborn	10	0.9 \pm 0.4	78.1 \pm 0.7	14.2 \pm 3.7	4.7 \pm 0.1	272.9 \pm 28.5
3. Children suffering from protein-energy malnutrition	13	11.7 \pm 2.3	53.9 \pm 1.0	11.3 \pm 2.1	3.6 \pm 0.2	103.8 \pm 12.1

Statistical significance between groups when tested by analysis of variance:

Age: Group 1 compared to group 2 and 3: P < 0.01.

Weight for age: Group 1 compared to group 2 and 3: P < 0.01; group 2 compared to group 3: P < 0.01.

Prealbumin: Group 1 compared to group 2 and 3: P < 0.01.

Albumin: Group 1 and 2 compared to group 3: P < 0.01.

Transferrin: Group 1 and 2 compared to group 3: P < 0.01.

Table 2

Mean \pm S.E.M. of carbohydrate rich serum proteins from a group of healthy preschool children, newborn children and children suffering from protein-energy malnutrition associated with infection.

Groups	No. of cases	α_1 -acid glycoprotein mg/100 ml	Gc-globulin, α_1 B-glycoprotein, α_1 -antichymotrypsin mg/100 ml	α_2 HS-glycoprotein, 4, 6 S-glycoprotein mg/100 ml
1. Healthy preschool children	24	24.8 \pm 2.7	77.5 \pm 6.0	89.3 \pm 6.0
2. Newborn	10	13.0 \pm 2.8	84.4 \pm 9.4	78.2 \pm 16.3
3. Children suffering from protein-energy malnutrition	13	35.8 \pm 6.2	81.3 \pm 8.2	64.7 \pm 8.2

Statistical significance between groups when tested by analysis of variance:

α_1 - acid glycoprotein: Group 1 compared to group 3: $P < 0.05$; group 2 compared to group 3: $P < 0.01$.

Table 3

Mean \pm S.E.M. of Gc-globulin, α_1 B-glycoprotein acid α_1 -antichymotrypsin from a group of healthy preschool children and children suffering from protein-energy malnutrition associated with infection.

Group	No.	Gc-globulin mg/100 ml	α_1 B-glycoprotein Au/100 ml**	α_1 -antichymotrypsin* Au/100 ml**
Healthy preschool children	24	28.2 \pm 1.0	142.1 \pm 4.5	100.1 \pm 5.1
Children suffering from protein-energy malnutrition.	13	18.1 \pm 1.6***	95.9 \pm 9.7***	186.0 \pm 30.8***

*Values from Schelp *et al.*, (1976).

**Arbitrary units: Values derived from a pool of 100 blood donors was set as 100 Au/100 ml.

***Statistical significance between groups when tested by Student t-test: $P < 0.001$.

Nevertheless there is a significant decrease for Gc-globulin and α_1 B-glycoprotein below the values of the preschool children. α_1 -antichymotrypsin of the PEM children increase significantly above the control group.

DISCUSSION

The results here for albumin, prealbumin and transferrin are in accordance with other investigators who also found those proteins are low in PEM (Ingenbleek *et al.*, 1975).

The proteins with a carbohydrate portion in the molecule above 10 per cent do not react uniformly in PEM α_1 - acid glycoprotein and α_1 -antichymotrypsin increased significantly in the PEM cases above the controls. These two proteins are known as "acute phase reactant" proteins, which show elevated levels in acute infection and other stress situations as trauma (Aronsen *et al.*, 1972; Johansson *et al.*, 1972). According to the results of this study they also increase in infection in spite of starvation. However,

Gc-globulin and α_1 B-glycoprotein, as a carbohydrate rich protein in PEM cases, decrease significantly below the controls. The peak in which mainly α_2 HS-glycoprotein is present does not increase in the PEM group.

These findings may explain the conflicting results that serum glycoprotein was found to be high in human individuals suffering from PEM (Patwardhan *et al.*, 1971) but decreased in rats under starvation (Weimer and Nishihara, 1959; Weimer and Hummelbough, 1965). Usually PEM is associated with infectious diseases. Then the "acute phase reactant" proteins, with a relatively high portion of carbohydrate in the molecule increase in a high extent above the other "glycoproteins", which either do not change in concentration or decrease. However, in animal experiments rats are kept free from infection. It might be assumed that under energy and protein depletion without infection the plasma glycoprotein level decrease uniformly.

SUMMARY

The human serum proteins with a carbohydrate portion of more than 10 per cent in the molecule, α_1 -acid glycoprotein, α_1 -antichymotrypsin, α_1 B-glycoprotein and α_2 HS-glycoprotein were studied in thirteen cases of protein-energy malnutrition. The "acute phase reactant" proteins α_1 -acid glycoprotein and α_1 -antichymotrypsin were found to be increased above the controls, whereas the other "glycoproteins" either decreased or did not change significantly. Also albumin, prealbumin and transferrin were found to be low in the cases of protein-energy malnutrition.

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