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BIOCHEMICAL CHANGES IN SERUM OF PROTEIN ENERGY MALNOURISHED CHILDREN FOLLOWING DIETARY PROTEIN REPLETION

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ABSTRACT

The serum levels of total protein, albumin, urea, calcium (Ca^{2+}), sodium (Na^{+}), potassium (K^{+}) where determined during a ten day process of dietary protein repletion in 30 protein energy malnourished (PEM) children and in a control group of thirty healthy children of equivalent age group. The findings show that the total protein, albumin, urea, Ca^{2+}, Na^{+}, and K^{+} were significantly lower ($p<0.05$) in PEM patients when compared with healthy controls. These parameters were significantly increased ($p<0.05$) following the ten-day dietary protein repletion. However, the treated group still had significantly lower values ($p<0.05$) than the healthy controls. The serum levels of total protein, albumin, urea, Ca^{2+}, Na^{+}, K^{+}, were 45.10 ± 2.70g/L; 28.40 ± 2.10g/L; 1.40 ± 0.39mmol/L; 1.30 ± 0.27mmol/L; 128.00 ± 4.30mmol/L; 2.90 ± 0.60mmol/L respectively in the PEM patients and 5.90 ± 2.90g/L; 33.10 ± 2.3g/L; 2.40 ± 0.49mmol/L; 1.90 ± 0.32mmol/L; 131.00 ± 3.30mmol/L and 3.40 ± 0.30mmol/L respectively following a ten day dietary protein repletion. In the healthy controls the values were 66.00 ± 2.20g/L (total protein); 45.54 ± 2.20g/L (albumin); 3.90 ± 0.40mmol/L (urea); 2.40 ± 0.04mmol/L (Ca^{2+}); 140.00 ± 3.30mmol/L (Na^{+}); 4.80 ± 0.70mmol/L (K^{+}). It is concluded that protein repletion normalizes the biochemical parameters listed above.

Key words: Malnourished children, Protein repletion.

INTRODUCTION

In the tropics of Africa, Latin America and Asia, the incidence of PEM, a spectrum of diseases arising from a deficiency of protein in the diet, is on the increase. In Nigeria in particular, it is estimated that 15.25% of rural children and 10-15% of urban children suffer from various forms of malnutrition (Fed. Min. of Health, 1989). The most vulnerable of PEM are known to be children of the poor class and the early weaned. Recently, there has been widespread economic instability in the West African sub region with resultant increase in the number of PEM patients (Fed. Min. of Health, 1989).

Protein Energy malnutrition is characterized by reduced plasma proteins, severe muscle wasting, structural changes in the liver and its attendant clinical manifestations and abnormal electrolyte changes (Fed. Min. of Health, 1989; Bassir, 1959; Whitehead 1969; Olowukene, 1980). Treatment of PEM patients after resuscitation involves feeding of protein rich diets and vitamins. Previous reports have centred mostly on biochemical changes in protein repletion. The present work was undertaken to investigate biochemical changes in serum of protein energy malnourished children during the process of dietary protein repletion.

MATERIALS AND METHOD

Subjects: The subjects were thirty protein malnourished children classified according to Welcome classification (1970) of mean age 1.6 ± 1.2 years, attending the paediatric clinic of various hospitals in Calabar metropolis and a control group of healthy children of the same age group.

DIETARY PROTEIN REPLETION: During the 10 days of rehabilitation, the patients were given food rich in protein and vitamins. The foods include beans, fish, soya milk, vegetables and fruits. In addition, rice, yam and garri were given for adequate energy supplementation.

SAMPLE COLLECTION: About 5ml of blood were collected from each patient by venopuncture from the cubital vein into sample bottles. These were allowed to clot at room temperature, centrifuged at 3,000 rpm for 5 minutes and sera collected. The serum samples were analyzed for total protein, albumin, urea, Ca^{2+}, Na^{+} and K^{+}.

TOTAL PROTEIN: serum protein was determined by the biuret method of Doumas 1981. The biuret reagent contained 0.3 %sulphate, 0.9% tartarate, and 0.55% potassium iodide in 0.6 NaOH. Serum

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(100 ul) was added to 5ml of biuret reagent. The solution was mixed, allowed to stand for thirty minutes at room temperature and their absorbance read at 540 nm. The concentrations of the samples were then calculated from the absorbance readings of standard solutions of Bovine Serum Albumin treated the same way as the serum sample.

ALBUMIN ESTIMATION: Serum albumin concentration was determined using Bromocresol green method of Doumas and Bhris, 1972. The working dye solution (pH 4.2) contained 5.6g succinic acid, 1.0g NaOH, 2.5ml of 25% (w/v) "Brij 35" solution, 0.1g sodium azide, and 5.8mg Bromocresol green dissolved and made up to 1L. To 20ul of sample was added 4ml of working dye solution, mixed and read at 623nm after 30 seconds against a reagent blank consisting of 20ul of water and 4ml of working dye solution. Standards were treated as test samples. The albumin concentration of test samples was calculated using absorbance reading of standards.

BILIRUBIN ESTIMATION: Bilirubin was estimated by the method of
Powell, 1994. Diazo reagent A contained 1.0g of sulphanilic acid dissolved in 15ml conc. HCl and made up to one litre. Diazo reagent B contained 5g sodium nitrite in 100ml of water. The diazo working solution contained 10ml of diazo A and 3ml of diazo B reagent. To 20ul of serum was added 0.2ml of working diazo reagent and 3.6 ml of benzoate urea. The solutions were mixed allowed to stand for 5min and the absorbance read at 540nm. The results were calculated from standard readings.

ESTIMATION OF UREA: Urea was estimated using the diacetyl method of Wybenga et al, 1971.To 10ul of sample and standards were added 1.0ml of 5% TCA, mixed and centrifuged at 3,000 rpm for 5minutes. The supernatants were reacted with Diacetyl monoxime colour reagent and the absorbance read at 530nm. The urea concentration of samples was calculated from the absorbance of standards. The working diacetyl monoxime reagent was prepared by mixing equal volumes of 0.4% diacetyl monoxime in water and acid reagent containing 44ml conc. H_2SO4, 66ml phosphoric acid, 50mg thiosemicarbaziide, 1.6g cadmium sulphate and 1.5ml of 2.5mmol/L urea solution in a final volume of 500ml made up with distilled water.

CALCIUM ESTIMATION: calcium was estimated using the cresolphthalein complexone method Baginski et al, 1973. The cresolphthalein complexone (CPC) reagent contained 40mg CPC, 1.0ml conc. HCl, 100ml dimethyl sulfoxide, 2.5g of hydroquinoline in a final volume of 1l made up with distilled water. To 0.5 ml of sample and standard were added 2.5ml of CPC reagent and 2.5ml of DEA buffer (containing 0.5g KCN and 40ml dimethylamine in 1l solution). The absorbance was read to get the first reading, and excess of ethylene glycol tetracetic acid (EGTA) was added and the absorbance read again. The difference is proportional to calcium level. The sample calcium concentrations were calculated from standard readings.

ESTIMATION OF SODIUM AND POTASSIUM: sodium and potassium were determined by flame photometry using corning 410C flame photometer. The instrument was calibrated using standard solutions. Appropriate dilutions of samples were made before direct reading with flame photometer.

STATISTICAL ANALYSIS: this was carried out using two-tailed analysis of variance to determine whether or not significant changes in biochemistry occur during protein repletion.

RESULTS

The effect of protein repletion in serum total protein, albumin, Ca^{2+}, Na^{+}, K+ and urea are shown on table 1. The mean serum total protein and albumin levels of well nourished controls pre-repletion PEM patients varied significantly ($p<0.05$) from each other. These results showed that the total protein and albumin concentrations in the controls were significantly higher when compared with the PEM patients before and after dietary repletion. However dietary protein repletion led to significant ($p<0.05$) increase in these parameters.

Serum total protein, albumin, Ca^{2+}, Na+, K^{+} and urea levels also varied significantly ($p<0.05$) in the groups studied. The observed values for the well-nourished controls were significantly higher ($p<0.05$) than those of the pre-repletion and post-repletion PEM patients. The results also showed that dietary protein rehabilitation increased the serum levels of these parameters.

TABLE 1: CHANGES IN SOME BIOCEMICAL PARAMETERS IN BLOOD OF PROTEIN ENERGY MALNOURISHED CHILDREN FOLLOWING 10 DAYS DIETARY PROTEIN REPLETION

Nutritional Status (subjects)	n	Protein (g/L)		Electrolytes (mmol/L)			Urea mmol/L
		Total	Albumin	Ca	Na	K	
Well nourished Controls	30	66.00 ±2.20	45.40 ±2.20	2.40 ±0.04	140.0 ±3.30	4.80 ±0.70	3.90 ±0.04
Malnourished	30	45.10 ±2.70	28.40 ±2.10	1.30 ±0.27	128.0 ±4.30	2.90 ±0.60	1.40 ±0.39
Post-repletion	30	51.90 ±2.90	33.10 ±2.30	1.90 ±0.32	131.0 ±3.30	3.40 ±0.30	2.40 ±0.49
		$p<0.05$	$p<0.05$	$P<0.05$	$p<0.05$	$p<0.05$	$p<0.05$

DISCUSSION

The results of this study indicate that serum total protein, albumin, urea Ca^{2+}, Na^{+} and K^{+} in protein energy malnourished children were markedly reduced. The results also clearly demonstrate that dietary protein restriction enhances the levels of these parameters in serum. In this connection, it is noteworthy that a great majority of Africans drift between dietary status of protein deprivation and repletion as a result of poverty, agricultural, cultural, social and religious practices.

Serum total protein, albumin, and urea decreased as a result of PEM and increased following dietary protein repletion. Similar reductions in serum protein and albumin have been reported (Bassir, 1959; Whitehead, 1969; Olowokere, 1980) while increases in serum albumin following dietary rehabilitation have been reported (Whitehead, 1969; Opara, 1995). The reduction in serum protein in malnourished individuals is usually due to impaired synthesis due to lack of precursors as a result of the inadequate protein diet. Felipo and Minna, (1989) observed decreased liver cells, ATP and AMP levels and attributed these to impaired liver functions. These have also been associated with low protein feeding. As such the low serum protein may be due to impaired synthesis due to both amino acid deficiency and tissue damage.

Serum urea, which was low in PEM patients, increased on dietary protein repletion. Reduced blood urea nitrogen (BUN) has been observed in rats with low protein feeding while increased BUN has been reported while feeding a high protein diet (Opara, 1995; Mallet et al, 1990). The trend in serum urea level observed in this study should be expected since urea is a break down product of protein metabolism. The serum Ca^{2+}, Na^{+}, and K^{+} were markedly reduced in the malnourished patients but increased on the dietary protein repletion. Similar reductions in Na^{+} and K^{+} have been reported in earlier studies. (Jeliffe, 1985).

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REFERENCES

Ahern, E D.A: Mitchell M.E., 1989. Liver function in protein energy metabolism measured by cinnamic acid tolerance and benzoic acid tolerance. Effect of carnitine supplementation. *Brit J. Nutr* 61 (2): 209-221

Baginski, E.S.: Clark, W.L. and Zak, R., 1973. *Clin. Chem. Acta* 46: 49

Bassir O., 1959. changes in serum protein during treatment of kwashiorkor. *Nature* 184(2): 2019

Doumas, B. T. and Bhris, H.G., 1972. Determination of serum albumin. Standard Methods of Clinical chemistry. Ed Cooper, C. A. New York. Acad press 7: 175

Doumas, B. T.; Bhris, D. D.; Cartey, R. J.; and Schaffer, R. A., 1981. Candidate reference method for the determination of total proteins in serum. Development and validation. *Clin. Chem.* 27: 1642-1650.

Fed Min of Health, 1989. Nutrition and Statistics Division *J. P. H. C. Nigeria* 1: 200-213

Felipo, V. and Minna, M. D., 1989. Effect of prolonged protein free diet on cell *NMR* 21 (3): 229-235

Jeliffe, D. B., 1985. Sodium supplementation in very low birth weight infants fed on their own mother's milk. *Afr. J. Med. Sc.* 69: 627-630

Mallet, A. K.; Walters, G. G.; Ward, F.; Farmer, F. B.; Rowland, R., 1990. Effects of *Escheria coli* Lipopolysaccharide on nitrate synthesis and on nitrosation of proline in rats. *Food Chem. Toxicol* 28 (8): 547-551

Olowookere, R. G., 1980. Some energy implications of PEM in rat *Ph.D. Thesis*, University of Ibadan, Ibadan.

Opara C. A., 1995. Presence of N-nitrosamines and precursor compounds in human breast milk and their metabolism and excretion in monkeys. *Ph.D. Thesis*, University of Ibadan, Ibadan.

Powell, 1944. The determination of serum bilirubin. *In Fundamentals of Clinical Chemistry* (Ed. Norbert W. Tietz) Philadelphia W. B. Saunders company pp. 337-338.

Wellcome Trust Working Party, 1970. Infantile classification of malnutrition. *Lancet* 11: 302-305.

Whitehead, R. G., 1969. The assessment of nutritional status in protein malnourished children. *Roc. Nutr. Soc* 28: 1-16.

Wybenga, D. R.; Giorgio, D. and Pileggi, J. V., 1971. Urea estimation by the diacetylmonoxime method. *Clin. Chem.* 17: 891-895.