SERUM PROTEIN PATTERN IN INFANTS WITH NUTRITIONAL DISORDERS

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Paper electrophoresis for studying the serum protein pattern is of particular value in infancy because of the small volume of blood required. Despite improved methods of diagnosis and increased knowledge of the aetiology of wasting disorders in infancy, some cases of failure to thrive still present diagnostic difficulties. Cases in which diarrhoea, respiratory infection and wasting are associated are common, and in many such cases investigation of pancreatic function shows this to be depressed. After suitable treatment, which may include pancreatin, these infants are found on reinvestigation to have normal pancreatic function. Fibrocystic disease of the pancreas can then be excluded.

The present investigation was undertaken to find out if the serum protein pattern would be of any diagnostic value in wasted infants in whom the exact diagnosis was in doubt.

Methods and Material

Electrophoretic separation was carried out in a vertical tank of the type described by Flynn and De Mayo (1951), using a barbiturate buffer of pH 8·6. The samples of 0·05 ml. serum were run for 16 hours at a current of 0·25 mA per centimetre width of paper. Whatman paper No. 31M extra thick was used throughout. After drying, the strips were stained for 16 hours in 0·01% bromophenol blue according to the method of Block, Durum and Zweig (1955). The individual fractions were estimated by the method of Levin and Oberholzer (1953), which involves the estimation of the nitrogen in each fraction after the strips have been divided. A correction for the nitrogen in the paper was made by estimating the nitrogen in a measured length of paper from protein-free areas at the ends of the strips. The sum of the individual fractions was checked with the total serum protein as determined by the micro Kjeldahl method.

The sera of 67 infants were examined. Twenty were normal infants known to be taking a satisfactory diet; they were free from infection and of normal weight. These infants served as controls and ranged in age from

7 weeks to 10 months. The other 47 infants were divided into four groups according to the main presenting symptom. Sixteen were wasted infants whose failure to thrive was attributed to underfeeding. Infection was not a complicating factor in these cases which were usually the result of maternal ignorance, though in a few cases actual neglect was involved. The second group consisted of nine infants with acute diarrhoea. The sera of these cases was examined as soon as their clinical condition permitted, in all cases after adequate re-hydration. Only severe cases with marked loss of weight were included. Thirteen were cases of chronic or recurrent diarrhoea. Most of these had a history of repeated bouts of diarrhoea over the previous few months. No single attack was usually of sufficient severity to warrant hospitalization, but advice was eventually sought on account of the frequent recurrence of symptoms and the failure to gain weight, or actual loss of weight, over a long period of time. The remaining nine infants suffered from chronic or repeated respiratory infections of varying severity. The attacks were usually accompanied by pyrexia and were often of sufficient severity to require treatment with antibiotics. In general this group of infants had maintained their weight somewhat better than the other groups and their average age was greater.

An attempt to assess the pancreatic function in these infants was also made by means of amino-acid absorption curves after a gelatin meal and when possible by duodenal intubation and measurement of the tryptic activity of the duodenal contents (Bate and James, 1956; unpublished data). The amino-acid curves were performed according to the method of West, Wilson and Eyles (1946). The criterion of normality was a rise in blood amino nitrogen to a level of at least 3 mg. per 100 ml. above fasting values at some time during the first one and a quarter hours after the gelatin was given. This increase in amino nitrogen was sustained for two and a half hours after the feed in normal infants, after which the level fell towards the fasting values.

Results

The serum protein patterns in normal infants and in those with chronic nutritional disturbances are

TABLE 1
SERUM PROTEIN LEVELS IN NORMAL INFANTS AND IN INFANTS WITH NUTRITIONAL DISTURBANCES

	Total (g. %)	Albumin (g. %)	Globulins				Average Age	Cases
			α_1 (g. %)	α_2 (g. %)	β (g. %)	γ (g. %)		
Normals	6.392	3.825	0.327	0.837	0.775	0.617	7 mth.	20
S.D.	0.66	0.41	0.06	0.10	0.11	0.15		
Undernutrition	6.171	3.125*	0.477†	0.902	0.865	0.802§	7 mth.	16
S.D.	0.71	0.45	0.28	0.30	0.19	0.42		
Acute diarrhoea	5.669	3.016*	0.378	0.872	0.767	0.634	7 mth.	9
S.D.	0.69	0.49	0.13	0.15	0.25	0.12		
Chronic diarrhoea	5.980	2.955*	0.504*	0.980§	0.807	0.732	6 mth.	13
S.D.	0.63	0.53	0.17	0.25	0.18	0.27		
Respiratory infections	6.308	3.357‡	0.394	0.970	0.778	0.806	13 mth.	9
S.D.	0.73	0.46	0.13	0.18	0.41	0.41		

* $P=0.001$; † $P=0.01$; ‡ $P=0.02$; § $P=0.05$

shown in Tables 1 and 2. Analysis of the results showed no significant variation from the normal in the total protein or beta globulin and only slight variations in the alpha 2 and gamma globulin. In all groups the albumin levels were significantly lower than in the controls. The alpha 1 globulin was significantly raised in the cases with chronic diarrhoea and undernutrition. The pattern was similar in all the abnormal groups; a depression in albumin levels was the most striking feature with a less pronounced increase in alpha 1 globulin. The rather higher albumin levels in the infants with chronic respiratory infection was probably due to the older age in this group.

The group with chronic diarrhoea also showed the most marked loss of weight as measured by the percentage of expected weight at the time of their first attendance.

The distribution of the amino-acid curves and the titre of duodenal trypsin in the various groups were also established, and are shown in Table 3. Only two infants with chronic diarrhoea had normal

curves and all had trypsin titres of under 1:200. The findings in cases with acute diarrhoea markedly resembled those in chronic respiratory infection and these cases did not show the marked impairment of pancreatic function of the infants with chronic diarrhoea. Unfortunately not all the cases of undernutrition were intubated and only 13 had amino-acid

TABLE 3
DISTRIBUTION OF TRYPSIN TITRE AND RESULTS OF AMINO-ACID ABSORPTION CURVES

No. of Cases	Amino-acid Curve		Trypsin Titre		
			Under 1:200	1:200	Over 1:200
	Normal	Abnormal			
16 Undernutrition* ..	4	9	2	1	5
9 Acute diarrhoea ..	4	5	—	2	7
13 Chronic diarrhoea ..	2	11	13	—	—
9 Respiratory infection ..	4	5	—	3	6

* In this group eight infants were intubated and 13 had amino-acid curves.

TABLE 2
SERUM PROTEIN PATTERN IN NORMAL INFANTS AND IN INFANTS WITH NUTRITIONAL DISTURBANCES (PERCENTAGE VALUES)

	Albumin	Globulins					% of Expected Weight
		α_1	α_2	Total α	β	γ	
Normals	59.8	5.1	13.2	18.3	12.1	9.6	
S.D.	1.4	1.3	1.4	2.1	1.7	2.3	
Undernutrition	50.8	7.7	14.4	22.1	14.0	13.0	73.3
S.D.	6.5	3.1	4.2	5.7	2.9	6.7	
Acute diarrhoea	53.1	6.6	15.4	22.0	13.4	11.2	79.3
S.D.	6.7	2.2	2.8	4.0	3.6	1.7	
Chronic diarrhoea	49.2	8.6	16.3	24.9	13.7	12.0	69.9
S.D.	5.5	3.4	3.9	5.8	4.0	4.3	
Respiratory infections	52.7	6.2	15.4	21.6	12.5	12.7	82.2
S.D.	4.8	1.9	4.6	4.4	2.8	5.6	

curves. From the available results it appears that with regard to pancreatic function this group lay between those infants with chronic diarrhoea and the other two groups. In only one case of chronic diarrhoea was tryptic activity present in a titre of over 1:50 and in four infants no activity could be detected.

The relationship of the percentage of serum albumin to the percentage of expected weight and to the titre of duodenal trypsin is shown in Table 4.

TABLE 4
RELATIONSHIP OF PERCENTAGE SERUM ALBUMIN TO PERCENTAGE OF EXPECTED WEIGHT AND TITRE OF DUODENAL TRYPSIN

	% Albumin	No. of Cases
<i>% Expected weight</i>		
Below 70%	48.8	12
70-80%	52.7	25
Over 80%	50.1	10
<i>Titre of trypsin</i>		
Below 1:200	48.5	15
1:200	50.3	6
Over 1:200	53.5	18

The figures for the relation between the titre of duodenal trypsin and the percentage of serum albumin are somewhat misleading. From them it might be assumed that the two bore a direct relation to each other. Whilst in general infants with very low or absent trypsin had low albumin levels there were exceptions to this and some infants with satisfactory trypsin titres had albumin levels of under 50%. The level of serum albumin does not therefore appear to be directly related either to the weight loss or to the degree of pancreatic dysfunction.

Discussion

Consideration of the results makes it clear that whatever the primary cause of the failure to thrive, the changes in the protein pattern are similar, the differences being of degree rather than of quality. The albumin showed a marked fall in all groups, alpha globulin showed a slight increase over normal values and gamma globulin tended to increase. These findings are in agreement with those of other authors who have determined the serum protein pattern in chronic nutritional disturbances. Gollan (1948) reported low albumin and raised globulins in chronic malnutrition in infants but he did not fractionate the globulins. Burgio and Giacalone (1953), investigating infants with nutritional atrophy, reported low albumin values with a striking increase in alpha globulin. Yoshida (1953) found low albumin levels and raised alpha and gamma globulin fractions. Jaso, Iturriaga and Roldan (1954)

studied two groups of infants, cases of toxicoses (i.e. acute gastro-enteritis) and cases of nutritional dystrophy. In both groups the albumin was low and alpha and gamma globulins raised compared with the normal. Carletti (1955) found low albumin and increased alpha globulins in both acute and chronic nutritional disturbances. The gamma globulin was either normal or elevated. Hallman, Kauhito, Louhivuori and Uroma (1952), Homolka and Mydlil (1955), and Schiavini and Sacconaghi (1956) investigated cases of acute gastro-enteritis, and these workers all found lowered albumin, raised alpha globulin and occasionally raised gamma globulin. Lubchez (1948) described the serum protein changes in a variety of infections in childhood. These were similar to those reported above. The changes in the serum protein pattern seem to be essentially similar in infants with nutritional disturbances whatever the primary cause. Grossman, Sappington, Burrows, Laviates and Peters (1945) and Peters (1946) have stated that pyrexia *per se* does not cause nitrogen loss. Nevertheless, large amounts of nitrogen are lost during acute illnesses, and this loss is continued after the temperature has returned to normal. This factor cannot be ignored when considering the hypoalbuminaemia of acute infection. Madden, Winslow, Howland and Whipple (1937) found that sterile turpentine abscesses markedly inhibited the regeneration of plasma proteins in animals depleted by plasmaphoresis even when given an adequate diet. They suggested a disturbance of the synthesizing mechanism as a cause for this failure.

There is considerable controversy over the state of pancreatic function in nutritional disorders in infancy. All authorities agree that enzyme secretion is depressed in severe protein malnutrition (Veghelyi, 1948; Thompson and Trowell, 1952; Dean and Schwartz, 1954; Gomez, Galvan, Cravioto and Frenk, 1954; Mukherjee and Werner, 1954; Scrimshaw, Behar, Perez and Viteri, 1955). From reports dealing with infants with other conditions it appears that pancreatic enzymatic activity is often depressed in acute and chronic diarrhoea and sometimes during respiratory infections. This depression is often selective, one enzyme being present in normal amounts whilst the other two show lowered values or are absent (Hess, 1913; Davison, 1925; Farber, Schwachman and Maddock, 1943; McDougall, 1950). This demonstrates the need to examine the activity of all three pancreatic enzymes in the duodenal juice in suspicious cases and not to rely merely on a trypsin estimation as is the procedure in many centres. McDougall (1950) reported normal enzyme activity in 18 children with

severe malnutrition. She did not report on the serum protein patterns but Gomez *et al.* (1954) and Mukherjee and Werner (1954), both working with infants with protein malnutrition, correlated the level of pancreatic enzyme activity with the serum albumin level. Gomez *et al.* noted no correlation, but Mukherjee and Werner found a significant correlation between amylase activity and serum albumin, and at albumin levels below 2.5 g.% a linear regression existed between the two. The bulk of evidence suggests that pancreatic exocrine function may be depressed in a variety of chronic nutritional disturbances in infancy, especially if these are associated with alimentary or respiratory infections. Such pancreatic depression is less likely to occur in acute disorders of sudden onset. Matsaniotis (1957) criticized the use of amino-acid curves after a protein meal as a test for pancreatic function because abnormally low values were not necessarily due to diminished output of pancreatic enzymes but might reflect impaired absorption or abnormal rates of amino-acid uptake by the tissues. In a series of infants with simple inanition he found normal absorption after a meal of casein hydrolysate, and normal removal rates of amino-acid from the blood after intravenous hydrolysates. As a result of this work he stated that the proteolytic activity in the gastro-intestinal tract of infants with this type of undernutrition was not impaired.

The infants in the present series had evidence of chronic protein deficiency as shown by depression of the serum albumin, muscular wasting and recovery on a high protein diet. Prolonged inadequacy of the diet from whatever cause must lead to lowered protein intake, and this in turn is responsible for diminished output of proteolytic enzymes and thus available dietary protein becomes even less adequate. Confirmatory evidence that these infants were protein-deficient lay in the fact that the serum protein patterns remained abnormal until satisfactory weight gains had been made. This often took several weeks of high protein feeding. Platt (1958) demonstrated clearly the difference in the clinical picture in cases of protein malnutrition with a high carbohydrate intake where the picture of kwashiorkor is seen, and in cases where protein deficiency accompanies a low carbohydrate intake and a marasmic type of malnutrition results. The type of malnutrition seen in this series and in most cases in this country does not lead to florid clinical signs and for this reason often passes unrecognized; a symptomatic diagnosis of chronic gastro-intestinal disorder is applied and the progress is slow and unsatisfactory. The serum protein pattern shows non-specific changes in these disorders and the level

of serum albumin does not bear a direct relationship to the pancreatic enzyme activity, though in general the most severely affected infants showed the lowest levels.

Summary

The serum protein pattern in 20 normal infants and in 47 infants with nutritional disturbances was examined by paper electrophoresis. The albumin fraction was significantly lower than normal in all the infants who had failed to thrive regardless of the aetiology of their condition. The alpha and gamma globulins tended to be higher than normal, the beta globulin and total protein showed no change. The group of infants with chronic diarrhoea showed the most pronounced changes in the protein pattern and also the most severe loss of weight. This group also had the most marked depression of pancreatic function but no definite correlation between serum albumin, percentage of expected weight or titre of duodenal trypsin could be established. The serum protein pattern is of no help in specific diagnosis in infants who fail to thrive, the changes being non-specific.

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