SERUM PROTEINS IN INFANTS WITH SEVERE GASTRO-ENTERITIS

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'On a worldwide basis it is estimated that diseases and disorders in which diarrhoea is an outstanding manifestation account for 5,000,000 deaths of infants and children each year. These diseases have received remarkably little study in relation to their enormous importance, and views regarding aetiology vary from the conviction that all diarrhoea is due to acute enteric infection to the belief that these conditions are predominantly caused by or related to preceding nutritional disease.'[1]

In Cape Town in 1959 the infant mortality rate from diarrhoea and enteritis was more than 100 times higher in non-Europeans than in Europeans (Table I). The present non-European rate is the same as the European rate was 50 years ago. In the intervening years the gastro-enteritis death rate has been reduced to 1% of the earlier figure in European infants, while in non-Europeans it has only been halved.

TABLE I. INFANT MORTALITY RATE FROM DIARRHOEA AND ENTERITIS IN CAPE TOWN[2] (PER 1,000 LIVE BIRTHS)

	European	*Non-European*
1916–1920	28·8	58·7
1959..	0·27	28·1

The hundredfold discrepancy between the infant mortality rates from gastro-enteritis in the 2 main racial groups in Cape Town becomes even more striking when compared with other diseases. For example, in 1959 the infant mortality rate from all causes was only 4·6 times higher in non-Europeans, and total tuberculosis mortality for all ages was only 3 times higher.[2]

The larger number of severe cases[3] and deaths[4] from gastro-enteritis in the summer months, emphasizes the importance of enteric infection as a precipitating cause of this disease. It is a common experience that European children have one or two attacks of diarrhoea in the summer, but these are always relatively mild and seldom require parenteral fluid therapy. Why is gastro-enteritis so much more severe in one group than in another in the same city where the same pathogens abound? Although the importance of different standards of hygiene cannot be denied, there is evidence from three sources that suggests that an additional factor operating in non-European children may be malnutrition. These sources of evidence are:

1. *Diarrhoea which Appears to be Non-infective can Occur in Malnutrition*

Diarrhoea is a well-known feature of kwashiorkor. Even if the mother denies this symptom it is our experience[5] that stool weights are almost invariably excessive. Intestinal pathogenic bacteria and parasites are no more frequent in the stools of kwashiorkor patients than in those of normal children.[6,7] The non-infective diarrhoea of severe protein deficiency can be explained by atrophy of pancreatic exocrine cells,[8-11] with reduction of digestive enzymes in the duodenal juice,[12,13] and also by atrophy of the intestine.[8,11] In animals, the small intestinal mucosa has the highest protein turnover of all organs,[14-18] and the pancreas has a turnover rate similar to that of the liver.[14,16]

It is therefore possible for function of these organs in children to be impaired before the more florid manifestations of protein deficiency appear. During the siege of Budapest, when milk was not available, Véghelyi[19] found a reduction of duodenal digestive enzymes before children developed oedema or hepatomegaly. Pancreatic enzyme production was restored to normal by treatment with milk. Badr El-Din and Aboul Wafa[20] in Egypt have reported similar cases.

2. *Gastro-enteritis is more Severe in Malnourished Children*

In a previous study of 100 consecutive outpatients with gastro-enteritis,[21] it was shown that 47% of the severely ill children were underweight as against only 6% of those mildly ill. In a large Mexican series,[22] mortality from gastro-enteritis increased stepwise from 14% in children of normal weight, to 52% in children whose weights were less than 60% of the standard for their age. Levin and Slone[23] reported a similar phenomenon in children with gastro-enteritis and hypertonic dehydration.

3. *Signs of Preceding Malnutrition in Children with Gastro-enteritis*

Once established, gastro-enteritis itself is likely to lead to malnutrition as a result of anorexia, vomiting, malabsorption and the widespread belief that milk must never be given to a child with diarrhoea. But if malnutrition is a predisposing cause of severe gastro-enteritis, it ought to be possible to find signs which indicate that the child was malnourished *before* the diarrhoea started. These signs include:

Body weight. In severe gastro-enteritis there is a rapid loss of weight from dehydration. The increase of weight during rehydration may be as much as 17%, and averages 10%.[21] Therefore, in acute gastro-enteritis the original body weight can only be estimated by allowing for dehydration, and if diarrhoea is prolonged an additional allowance has to be made for wasting of tissues. The ideal is to have actual measurements of children's weights taken shortly before the gastro-enteritis started. The only available figures of this kind show that of 242 Cape Town children who subsequently died of gastro-enteritis, 83% were below average weight for age before the onset of the diarrhoea.[24]

Height is less often measured, but has the advantage that it is not affected by dehydration. Of 600 African children with diarrhoea studied in Johannesburg, 94% were shorter for their age than the American average.[25] Very little of the growth retardation can be explained by genetic influence, for the same authors subsequently reported[26] that children of privileged Africans were very nearly the same height as American children.

Clinical signs of malnutrition were frequent in Kahn's

series.[25] The 200 children admitted to the wards were presumably a highly selected group. However, even in 400 unselected outpatients, one quarter had marasmus or signs of kwashiorkor.

Serum proteins. Very little work has been done on biochemical indices of nutrition in gastro-enteritis. There have been a few reports on the serum proteins in children with this disease, all from researchers in Europe. Most of them have concentrated on electrophoretic fractionation,[27-30] and have either not measured absolute protein concentrations[28,29] or omitted to control timing of blood samples. Some patients were dehydrated when blood was taken, others had been given parenteral fluids, including plasma infusions.[27,29] In one series[27] the first blood sample was taken anything from 1 to 19 days after admission. The data in these reports cannot be used to infer the babies' state of nutrition before they developed diarrhoea.

Some information of the sort we need has recently been published by Coles.[31] In 9 infants with severe acute diarrhoea in London, the mean serum-albumin level in blood drawn after adequate rehydration was 3·0 G. per 100 ml. — significantly lower than the normal 3·8 G. per 100 ml. for infants. In chronic diarrhoea serum-albumin concentrations were further reduced. The small size of his sample and uncertainty whether the fluids infused during rehydration had been fully equilibrated throughout the total body water makes it difficult to generalize from Coles' data. The Medical Research Council memorandum on *Treatment of Acute Dehydration in Infants*[32] stated that 'low levels (of haemoglobin, haematocrit or serum protein) after the relief of dehydration may indicate either haemodilution from retention of sodium salts in the extracellular fluid and an overexpansion of the plasma volume, or true anaemia and/or hypoproteinaemia. The initial levels, amount of weight gained, and the presence or absence of oedema, will help to differentiate these conditions'.

On the other hand, dehydration increases the concentration of protein in the serum[33] so that in blood taken at this stage minor degrees of hypoproteinaemia will not be apparent.

To allow for the effects of the state of hydration we have measured serum proteins in children with severe gastro-enteritis both before and after rehydration. The results and other relevant observations are reported here.

MATERIAL AND METHODS

Clinical

Altogether, 55 children with gastro-enteritis were studied between October and Christmas (summer months) 1961, in the outpatient department of the Red Cross War Memorial Children's Hospital. Their predominant clinical manifestation was diarrhoea, with or without vomiting and anorexia. Only severe cases were included. All were clinically estimated to be dehydrated and were treated with parenteral (usually intravenous) fluids in the resuscitation room. The patients were taken from a much larger number at random, depending to some extent on the pressure of work. Children in whom parenteral infection predominated, or who had frank kwashiorkor (defined as oedema with or without skin lesions), or who were desperately ill, were excluded. Patients were not selected on the basis of length of history, so that some were seen at the beginning of their first attack, while others had chronic or recurrent diarrhoea.

The children's ages ranged from 3 weeks to 42 months (mean age 10·2 months). Only 16 (29%) were older than 1 year. There were 39 males and 16 females in the series. All the children were non-European.

Blood was taken from a jugular vein from all 55 children *before* rehydration. An intravenous drip was then set up and run for 12 - 24 hours. The fluid given was usually 2·5% dextrose in half-strength Darrow's solution.[3] In no case was plasma or albumin given. A few hours after discontinuing the drip, a second blood sample was taken from half the children (29 out of 55).

For controls, blood was taken from 35 non-European children in the same age range (1 - 42 months). Their serum was analysed by the same methods at the same time. The control children were judged to be relatively well nourished and not dehydrated. They came mainly from 3 groups: convalescent children, children in an orthopaedic home with non-inflammatory deformities, and 'cold' surgical patients awaiting operation.

All the patients and most of the controls were recumbent at the time blood was taken. All the patients can be considered as fasting when their blood was taken; most of the controls were not fasting.

For standardization of the Coloured control children, the same chemical measurements were made on sera of 6 healthy European adults — doctors and technicians.

Laboratory

Serum proteins were measured with the biuret reagent of Wolfson *et al.*,[34] standardized by Kjeldahl. Serum albumin was separated by the ether-centrifugation method[35] after precipitating the globulins with 27% sodium sulphate at 37° C.[36]

In addition, the first (dehydrated) gastro-enteritis sera and all the control sera were subjected to electrophoresis[37] on cellulose acetate strips ('oxoid' — 2·5 cm. × 18 cm.) in barbitone buffer (pH 8·6, 0·06M). Serum was applied a quarter of the way from the cathode end and current was run at 0·4 ma. per cm. width for 4¾ hours. The dried strips were stained with 0·2% ponceau S (Gurr) in 3% aqueous trichloracetic acid. Duplicate strips were run for each serum:

(*a*) One strip of each pair was cleared with liquid paraffin and scanned using a green filter. Areas under the curves were quantitated by planimetry. (*b*) From the other strip of each pair, the 5 protein fractions and paper blanks were cut out and each eluted in 5 ml. of 0·1 NaOH at room temperature and neutralized with 0·8 ml. of 40% acetic acid before reading in a Beckman DU spectrophotometer at 516 mμ. For both methods of quantitation it was assumed that albumin has the same binding capacity as gloublin for ponceau S.[38]

All sera with any suspicion of a pink colour were analysed for haemoglobin (Hb.) by a benzidine method.[39] Strongly haemolysed sera were excluded from the series, but slightly haemolysed ones were included. Only 3 sera in the patients and controls combined had over 0·10 G. of Hb. per 100 ml. In preliminary experiments we confirmed that Hb. increases the biuret readings of total protein by a factor of 1·9 G. of apparent protein for each gram of Hb. added.[40] However, stepwise additions of Hb. to a known serum did not increase the readings of albumin separated by 27% sodium sulphate until the Hb. exceeded 0·2 G. per 100 ml. With the electrophoretic method Hb. migrates between the α_2- and β-globulins (i.e. with the haptoglobins) and likewise does not increase the albumin.

A few of the control sera were mildly lipaemic; but with the chemical method the albumin-containing solution was clear. Sodium was measured in an EEL flame photometer. Osmolarity was measured by depression of the freezing point in a Fiske osmometer.

RESULTS

Because the serum-albumin level is not affected by lipaemia or slight haemolysis and because it is a more direct and sensitive index of nutritional status than total serum proteins,[41-43] we have concentrated on serum albumins in analysing the results.

Serum Albumin in Dehydrated Gastro-enteritis Patients

Serum-albumin levels in the children with gastro-enteritis while dehydrated (1st blood samples) are shown in Table II. The mean levels are somewhat lower than in the control

TABLE II. SERUM ALBUMIN IN DEHYDRATED GASTRO-ENTERITIS PATIENTS AND IN CONTROLS—CHEMICAL METHOD

	Mean (G. per 100 ml.)	*Range (G. per 100 ml.)*
55 Dehydrated gastro-enteritis patients	$3{\cdot}45 \pm 0{\cdot}73$	1·77—4·72
35 Coloured control children	$3{\cdot}66 \pm 0{\cdot}29$	3·09—4·13
6 Healthy European adults	4·32	4·16—4·52

children, but the most striking difference is the much larger range in the gastro-enteritis group. Although the highest albumin levels in the group were above the highest in the control children (and even in the healthy adults), other gastro-enteritis patients had very low serum-albumin levels — down to 1·77 G. per 100 ml.

The dividing line separating definite hypoalbuminaemia from higher albumin levels was taken as 2 standard deviations below the mean of the control Coloured children. With the chemical method this happened to coincide exactly with the bottom of the control range — 3·09 G. per 100 ml. — and 15 of the 55 gastro-enteritis patients (27%) had albumin concentrations below this level *while they were dehydrated.*

By the electrophoretic methods there were similar numbers of gastro-enteritis children with hypoalbuminaemia. The methods agreed closely (Table III). Strips which were

TABLE III. COMPARISON OF MEAN SERUM-ALBUMIN BY ELECTROPHORESIS AND BY THE CHEMICAL METHOD

	Electrophoresis and scanning (G. per 100 ml.)	*Chemical (G. per 100 ml.)*	*Electrophoresis and elution (G. per 100 ml.)*
Gastro-enteritis ..	$3{\cdot}13 \pm 0{\cdot}62$	$3{\cdot}45 \pm 0{\cdot}73$	$3{\cdot}75 \pm 0{\cdot}87$
Control children	$3{\cdot}42 \pm 0{\cdot}27$	$3{\cdot}66 \pm 0{\cdot}29$	$3{\cdot}86 \pm 0{\cdot}39$
Adults	3·98	4·32	4·42

scanned gave lower absolute albumin values than eluted strips. Twelve children had abnormally low albumin values by all 3 methods. For further analysis, the patients with hypoalbuminaemia by the chemical method are taken as the hypoalbuminaemic group.

The frequency distribution of serum albumin in the dehydrated gastro-enteritis patients is shown in Fig. 1. The histogram looks skewed to the left, which suggests that 2 'populations' may be represented in the sample. However, analysis of moments, as recommended by Fisher[44] and by Snedecor[45] gave a value for g_1 of −0·582. With a standard error of 0·322 this was not quite significant at the 5% level.

Clinical Correlation with Hypoalbuminaemia

Five of the 15 children with hypoalbuminaemia while dehydrated had skin lesions without oedema, ranging from dry skin to lesions more suggestive of kwashiorkor. Three other hypoalbuminaemic patients were clinically marasmic and/or very undersized. Thus, approximately half this group showed one of these signs of protein malnutrition. These signs were less common in the children without hypoalbuminaemia, occurring in only 7 of the 40.

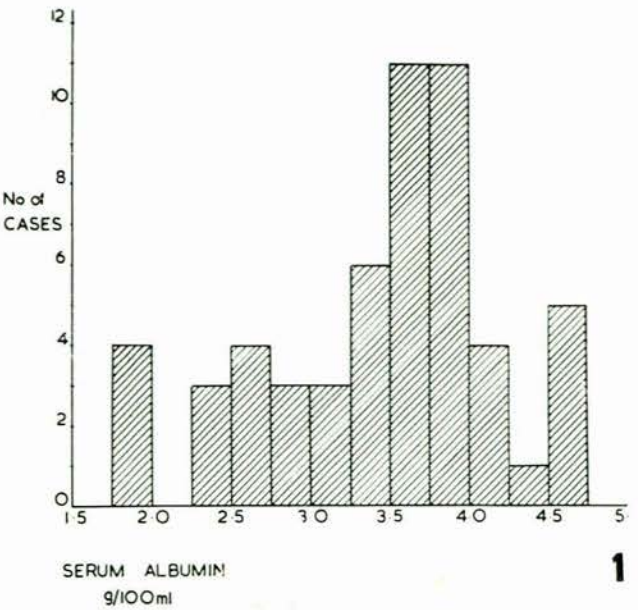

Fig. 1. Frequency distribution of serum-albumin values in dehydrated gastro-enteritis patients.

Patients presenting with oedema were excluded. Oedema was noted after fluid therapy in 3 of the whole series, and 2 of these had normal serum-albumin values.

Hypoalbuminaemia was less common in the younger infants with gastro-enteritis. It was found in only 10% (2) of the 19 infants less than 6 months old, as against 30 - 40% of children older than this.

An attempt was made to see if the serum-albumin concentration of the blood samples taken before rehydration varied with the degree of dehydration, either as estimated clinically or as indicated by the increase of weight during recovery, but there was no relationship. However, clinical grading was not estimated by the same doctor throughout, and weights at complete recovery were only available for 32 of the 55 children.

In order to assess how much of the hypoalbuminaemia was a result of gastro-enteritis, and how much preceded it, the patients have been grouped in Table IV according

TABLE IV. INCIDENCE OF HYPOALBUMINAEMIA IN DEHYDRATED GASTRO-ENTERITIS PATIENTS GROUPED ACCORDING TO HISTORY*

	Present history 7 days or less	*Present history more than 7 days*
No previous illness in last 2 months	5/33 (15%)	2/9 (22%)
One or more previous illnesses in last 2 months	3/7 (43%)	4/5 (80%)

* No history available for 1 child.

to the length of the history of their presenting attack of diarrhoea, and whether they had recent preceding illness(es). The preceding illness was diarrhoea and vomiting in 2/3, measles in 1/3.

Although hypoalbuminaemia appeared to be more frequent when the present history was longer than a week and the child had been ill beforehand, the numbers are very small except in the sub-group with a short history

TABLE V. ELECTROPHORETIC PATTERNS IN GASTRO-ENTERITIS PATIENTS AND CONTROLS. MEAN (AND RANGE) CONVERTED TO G. PER 100 ML. ELUTION METHOD

	n	*Albumin*	*Globulins*				*Total Protein*
			α_1	α_2	β	γ	
Gastro-enteritis	53	3·75	0·22 (0·06–0·53)	0·78 (0·19–1·48)	0·43 (0·12–0·74)	0·70 (0·18–1·90)	5·90
Coloured control children	35	3·86	0·12 (0·04–0·22)	0·58 (0·29–0·85)	0·47 (0·17–0·75)	0·67 (0·15–2·14)	5·70
European adults	6	4·42	0·13 (0·09–0·17)	0·45 (0·27–0·62)	0·39 (0·22–0·48)	0·74 (0·65–0·89)	6·12

TABLE VI. GLOBULIN FRACTIONS IN GASTRO-ENTERITIS. PATIENTS WITH HYPOALBUMINAEMIA COMPARED WITH PATIENTS WITH SERUM-ALBUMIN LEVELS IN THE CONTROL RANGE.* MEAN (AND RANGE) IN G. PER 100 ML.

	n	*Albumin*	*Globulins*			
			α_1	α_2	β	γ
Hypoalbuminaemia	13	2·60	0·22 (0·11–0·53)	0·71 (0·40–1·35)	0·32 (0·16–0·61)	0·64 (0·33–1·13)
Albumin in control range	40	4·12	0·22 (0·06–0·42)	0·80 (0·19–1·48)	0·46 (0·12–0·74)	0·72 (0·18–1·90)

* Control range down to mean −2 SD for electrophoresis and elution method.

and no recent preceding illness. The histories were not always reliable because some of the patients with gastro-enteritis were brought to hospital by a neighbour or older child when the mother was unable to come. A few of the African mothers could only speak Xhosa, and details had to be obtained through an interpreter.

Electrophoretic Patterns

Fractions quantitated by elution in 53 dehydrated gastro-enteritis patients and in controls are expressed in G. per 100 ml. in Table V. There was very little difference in any of the globulin fractions between the Coloured children and the healthy European adults, except that the children had a larger range of γ-globulin concentrations.

In the gastro-enteritis patients there were significantly higher α_1- and α_2-globulins than in the control children (χ^2 13·97 for α_1, 10·96 for α_2). The two α-globulins were nearly always elevated together (correlation coefficient 0·67, t = 6·403). The α_1-globulins were rather more elevated than the α_2.

The increased α-globulins cannot be explained by the rather larger number of slightly haemolysed sera in the gastro-enteritis group (29% as against 20% in the controls).

The 37 gastro-enteritis sera that had no trace of haemolysis had a mean α_1-globulin of 0·23 and α_2 of 0·84.

There were no significant differences in β- and γ-globulins between gastro-enteritis patients and controls. No patient with gastro-enteritis had less than 180 mg. of γ-globulins per 100 ml.

In Table VI, 53 gastro-enteritis patients have been subdivided into those with hypoalbuminaemia and those with albumins in the control range. The patients with low albumins still have high α-globulins. However, their β-globulins are significantly lower than in the remaining patients with gastro-enteritis (t = 9·34). Their γ-globulins are also a little lower.

Osmolarity

Serum sodium was measured in 52 of the dehydrated gastro-enteritis patients. Serum osmolarity was measured in half of these (26 cases). Osmolarity correlated well with serum sodium (r = 0·74). The range of serum-sodium levels in 22 Coloured control children was 132 - 144 mEq. per litre (mean 138·5 ± 3·5). This was a little lower than the range in the healthy adults, which was 134 - 154 mEq. per litre.

In the gastro-enteritis group only 3 out of 52 (6%) had serum-sodium levels equal to or more than the upper limit of the range in the control children. Hyponatraemia was much more common, and as many as 27 of the 52 patients (52%) had serum-sodium levels lower than the lower control limit while they were clinically dehydrated.

Similar proportions of hyper- and hypo-osmolarity were found in the sample in which osmolarity was measured. In the control children the range was 175 - 190 mOsm. per litre. Of the gastro-enteritis patients, 8% had hyper-osmolarity and 62% had hypo-osmolarity.

There was a tendency for low serum-sodium levels to be associated with low serum-albumin levels, but this was not significant (r = 0·24 or χ^2 = 1·95).

Effect of Parenteral Fluid Therapy on Serum-albumin Levels

In 29 of the gastro-enteritis patients, *serum proteins* were measured in the second blood sample taken at the conclusion of the parenteral fluid therapy. In comparison with the sera taken before treatment from the same patients, the albumin concentration fell in 27 out of 29 (χ^2 = 9·1, P < 0·01). Nine (31%) of these gastro-enteritis patients had hypoalbuminaemia before intravenous fluids; the serum-albumin levels fell to below the control range in a further 6 patients during rehydration, so that 15 out of 29 (52%) had hypoalbuminaemia at this stage.

The mean serum-albumin level while dehydrated was 3·41 G. per 100 ml.; after fluid therapy it had fallen to 2·84 G. per 100 ml. The mean fall was 0·57 G. per 100 ml. In individual cases it ranged up to a fall of 1·48 G. per 100 ml.

During rehydration the mean albumin/globulin ratio rose from 1·46 to 1·53. Though small, this was statistically significant (χ^2 = 4·17, P < 0·05). The change of albumin/

globulin ratio was hardly any less (1·17 - 1·22) in the 9 patients with hypoalbuminaemia before fluid therapy.

Body Weights

At the visit when the first blood sample was taken, body weights were reduced by dehydration. In 42 patients, additional weights were available from the 12 days before or after this visit. Not all of them indicated the fully hydrated weights because some children still had diarrhoea (or oedema) at the other visits. However, the highest of these weights for a particular child was his 'best observed weight'. In a previous survey of outpatients with gastro-enteritis it was found that dehydrated children had lost an average of 10% of their normally hydrated weight.[21] So an alternative way of correcting for dehydration was to bring the child's weight up to 111% of its dehydrated weight (i.e. the correction for a child that has lost 10% of its healthy weight from dehydration).

The weight multiplied by 1·11 and the best observed weight (when available) were both worked out for each child and *whichever was the higher* was taken as the weight corrected for dehydration. The weights were thus deliberately somewhat over-corrected. The mean corrected weight for all the patients allowed for a dehydration of 11% (range 10 - 18·4% of the hydrated weight).

In Fig. 2 these corrected weights are plotted against the 2·5 and 50 percentiles of weight of healthy Cape Town Coloured children recently reported by Robertson.[46] Twenty-eight patients (51%) had weights on or below the

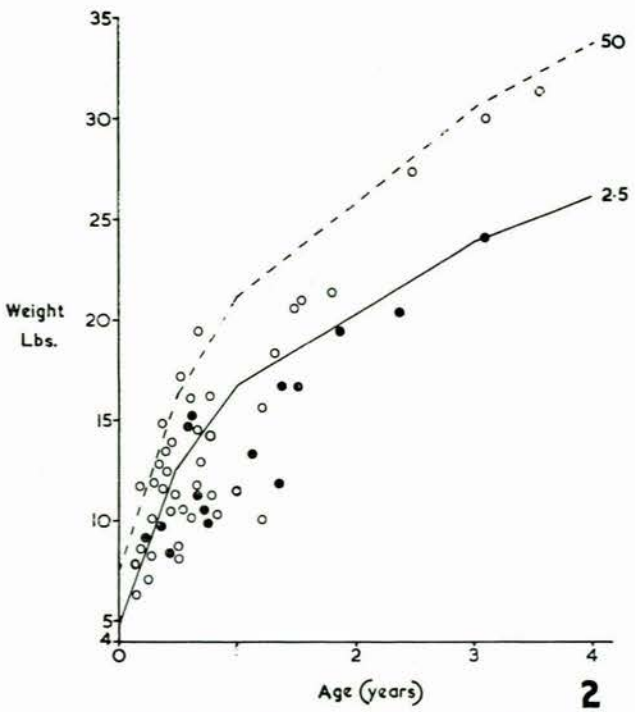

Fig. 2. Corrected weights of children with gastro-enteritis (for explanation see text).

2·5 percentile line after maximal correction for dehydration. Only 4 of the 55 corrected weights were above the 50 percentile line. In the figure, black circles indicate children who had hypoalbuminaemia. Twelve out of 15 hypoalbuminaemic children (80%) had weights on or below the 2·5 precentile, as against 17 out of 40 (42%) children with serum-albumin levels in the normal range (white circles). The association of underweight with hypoalbuminaemia is significant ($\chi^2 = 5{\cdot}48$, $P < 0{\cdot}05$).

Unlike hypoalbuminaemia, underweight was significantly associated with low serum-sodium levels ($\chi^2 = 4{\cdot}93$, $P < 0{\cdot}05$), while underweight did not appear to be more frequent in patients with long histories or recent preceding illnesses. The proportion underweight was 17 out of 33 (52%) in the sub-group with present history less than one week and no other illness in the preceding 2 months.

As a yardstick for comparison with the weights of the children with gastro-enteritis, Robertson's standards are probably the most appropriate. However, these may not be the optimal weights for Cape Town Coloured children. They are somewhat lower than the Boston standards. Gómez' division into first, second and third degree malnutrition[47] has achieved some measure of international acceptance.[48] The gastro-enteritis patients have been subdivided by their corrected weights according to this classification in Table VII.

TABLE VII. DEGREE OF UNDERWEIGHT IN GASTRO-ENTERITIS PATIENTS BY GÓMEZ CLASSIFICATION

	Number of cases	
Standard weight * or above	3	
1st degree malnutrition (75–99% of standard)	19	
2nd degree malnutrition (60–74% of standard)	18	33 (60%)
3rd degree malnutrition (less than 60% of standard weight)	15	33 (60%)
Total	55	

* Boston 50 percentile weight for age. The grouping was worked out separately for boys and for girls.

Rickets and Anaemia and Summary of Incidence of Signs of Malnutrition in the Sample

Rickets was noted in 4 of the children (proved by X-ray in one). Severe anaemia was noted in 2 patients, without a history of bleeding (Hb. levels 2·3 and 6·4 G. per 100 ml.). The patient with a Hb. level of 2·3 G. per 100 ml. had a hypochromic smear and responded to blood transfusion and iron therapy.

The number of patients with 4 major manifestations of malnutrition are added up in Table VIII.

TABLE VIII. TOTAL OF 4 SIGNS OF MALNUTRITION IN THE 55 GASTRO-ENTERITIS PATIENTS

Low albumin	3
Low albumin and underweight	12
Underweight	13
Underweight and rickets	2
Underweight and anaemia	1
Rickets	2
Anaemia	1
Total	34 (62%)

DISCUSSION

The electrophoretic results support the specificity of separation of albumin by the 27% sodium sulphate method which has been in use in our laboratory for several years. Cellulose acetate was used for electrophoresis because it

is claimed that it allows clearer separation of albumin from α_1-globulin than is obtained on filter paper.[37] The new supporting medium came up to expectation in this respect, which makes it valuable for nutrition surveys. Lower albumin values when the electrophoretic strips were scanned suggest that transmission of light through dye on cellulose acetate does not follow Beer's law, but flattens off in heavily stained zones. It is well known that this happens with filter paper because of its micro-porous structure.[49,50] Under the microscope, cellulose acetate is more uniform than filter paper, but nevertheless it has a honeycomb appearance.

The high incidence of hypoalbuminaemia in the gastro-enteritis patients cannot be explained by any of the following factors: *Slight haemolysis and lipaemia* do not affect albumin values (see Methods) and there were rather fewer haemolysed sera in the controls. Our controls were in the same *age* group. A minority of the controls and none of the gastro-enteritis patients were *ambulant*. The serum-albumin level is higher in adults when they are standing than when they are in bed.[51,52] Hydrostatic pressure in the legs must be less in children, and the dividing line from hypoalbuminaemia was the lower limit of the control range. All blood samples were taken from a jugular vein. Venous stasis[53] is unlikely to have been as high as when a sphygmomanometer cuff is applied around an arm vein, and the same technique was used in patients and controls. Environmental *temperature*[52] was the same for the controls, since their blood was taken during the gastro-enteritis survey.

The 27% incidence of hypoalbuminaemia in the sera of dehydrated gastro-enteritis patients is almost certainly an *underestimate*. Firstly, the Coloured children give an idea of the average, rather than the optimal serum-protein levels in the community from which the gastro-enteritis patients come. The mean serum-albumin level of these control children was only 85% of what was found in healthy European adults, whereas in the literature the normal serum-albumin level of infants of 1 year ranges from 92%[54,55] to 100%[56,57] of adult values measured in the same laboratory. Secondly, dehydration produces an elevation of the serum-protein concentration.[33] As many as 7 out of 55 gastro-enteritis sera taken before rehydration had serum-albumin levels above the upper end of the range in the control children.

The incidence of hypoalbuminaemia rose to 50% after parenteral fluid therapy. This figure may give an *over-estimate* of the frequency of protein deficiency because the second blood sample was taken at or soon after the conclusion of the intravenous drip and there may not have been sufficient time for equilibration of the infused water with the total body water. A third blood sample the day after parenteral fluid would be necessary to settle this point. On the present data it can be concluded that in our sample of children with gastro-enteritis at least 25% and possibly as many as 50% had hypoalbuminaemia.

Harroun *et al.*[58] and Ariel[59] showed that when saline infusions are given to normal adults there is an inflow of serum protein — mostly albumin[58,60] — from the extra-vascular space into the circulation. As a result, the serum-protein concentration does not fall as much as would be anticipated. This phenomenon did not occur in post-operative patients or when 5% dextrose was infused.[59] The results in undernourished patients were conflicting.[58,59]

In our studies the intravenous fluid was infused into a pathologically reduced plasma volume and the fluid given was half-strength Darrow's solution with 2·5% dextrose. Despite this, there was a small but significant rise in the albumin/globulin (A/G) ratio, indicating some diffusion of albumin from the extravascular space. The rise of the A/G ratio was hardly any less in the patients who showed hypoalbuminaemia while dehydrated. Although the *absolute* increase in total circulating albumin must have been smaller in this group, it was almost proportional to the intravascular albumin. This is of interest because in kwashiorkor[61,62] and in adult subjects experimentally depleted of protein,[63] recent studies have indicated that extravascular plasma albumin falls before intravascular albumin.

On electrophoretic fractionation, the only additional difference between the gastro-enteritis group and the control children was an increase of the α-globulins, especially the α_1 fraction. This is a non-specific change, seen in many acute and chronic infections and other inflammatory conditions[64,65] and previously reported in infantile gastro-enteritis.[27,29,31] In general, increased α-globulins may be associated with decreased serum-albumin levels, but our gastro-enteritis patients with hypoalbuminaemia had the same α-globulin levels as the rest of the patients (Table VI).

It is of interest that hypogammaglobulinaemia was not found in the gastro-enteritis patients. The possibility that these children might have some sort of dysgamma-globulaemia[66] remains to be tested.

In the gastro-enteritis group as a whole, the β-globulins did not differ significantly from the controls. However, the patients with low albumin had significantly lower β-globulin. This pattern resembles the rather characteristic pattern seen in kwashiorkor. The conspicuous reduction of β-globulin was first commented on by Lahey *et al.*[67] We have calculated the serum-protein fractions in kwashiorkor as percentages of values in recovered or normal subjects measured in the same laboratory in 12 reports suitable for the purpose.[67-78] The grand mean figures are: albumin 45%, β-globulin 55%, α-globulins 77% and γ-globulin 94% of normal.

Having found a frequency of hypoalbuminaemia between 25 and 50% in this sample of children with severe gastro-enteritis, we are faced with two possibilities:

1. All the patients may have been depleted of protein, but only those with more severe depletion showed it in their serum-albumin levels. It is well known that the serum-albumin level only falls below the normal range after there has been considerable loss of body protein.[79-82] Since the intestinal mucosa has a faster turnover rate for protein than for serum albumin,[15] protein deficiency could predispose a child to diarrhoeal disease before hypoalbuminaemia develops.

2. Alternatively, our sample may include 2 different types of diarrhoeal disease in children, a type that is associated with protein deficiency and a type that is not. The histogram (Fig. 1) looked suggestive of a bimodal

frequency distribution of serum albumin, but statistical analysis for skewness was not significant. Since our numbers are small at present, it would seem wiser to leave the two possibilities open.

In either case it is valid to list the other clinical features that were more common in the gastro-enteritis patients with hypoalbuminaemia: (*a*) Skin lesions compatible with pre-kwashiorkor, (*b*) underweight, (*c*) age over 6 months (i.e. post-weaning), (*d*) prolonged diarrhoea, and (*e*) recurrent attacks of diarrhoea.

Since several of the hypoalbuminaemia patients had short histories, our data are compatible with the hypothesis that protein deficiency may be a predisposing cause of severe gastro-enteritis. There can be no doubt that it is an important *continuing* cause. If a child is mildly depleted of protein when an attack of gastro-enteritis starts, the depletion can rapidly become serious from anorexia, vomiting and malabsorption. The resulting intestinal and pancreatic atrophy in turn favour continuation or recurrence of the diarrhoea.

We still see infants in this vicious circle, whose mothers have on their own initiative been giving glucose water, or sometimes Darrow's solution originally obtained at a clinic or hospital, as the sole feed for days or even weeks. **We believe it is dangerous to continue protein-deficient feeds for more than 48 hours even if some diarrhoea is still present.[83] We would urge every doctor, nurse or dispenser who hands a bottle of glucose-electrolyte solution to the mother of a child with gastro-enteritis to make sure she understands that there is a fixed limit to this type of feeding.**

In addition to hypoalbuminaemia, 3 other syndromes of malnutrition were observed in this survey of severe gastro-enteritis (Table VIII). Since Hb. values and wrist X-rays were not obtained systematically, it would not be profitable to guess the frequency of anaemia and rickets in gastro-enteritis from our data. However, their occurrence in this series does suggest lines for future investigation, especially since the gastro-intestinal tract is so sensitive to deficiency of iron[84,85] and folic acid.[86] It is possible that other deficiencies may be as closely related to severe gastro-enteritis as we have found protein deficiency to be.

A Note on Electrolytes

In our series 6% of patients had hypernatraemia and 52% had hyponatraemia. The serum-sodium levels were much lower than those found in gastro-enteritis in the USA and Britain, where hypernatraemia averages 21% and hyponatraemia occurs in 9 - 14% in different series.[87] A higher frequency of low serum-sodium levels was reported by Bowie *et al.*[88] from this hospital, but 34% of their cases had hyperosmolarity. Since Bowie was dealing with the population that our sample came from and even used the same flame photometer, the difference between his series and the present one must be the result of selection. Our patients were outpatients, whereas all his had been admitted to the wards, where it is only possible to admit the most seriously ill children. The association of neurological signs with severe gastro-enteritis seen in over half Bowie's hypernatraemic patients would favour concentration of hyperosmolarity in a ward series.

The figures given by Slone and Levin,[89] from Johannesburg, are very similar to ours. In 1,000 ward cases 26·5% had hyponatraemia and 13% hypernatraemia. If children treated as outpatients are included, they estimate the incidence of hypertonic dehydration would probably be 5 or 6% of the total number. They explained the comparative rarity of hyperosmolarity in malnourished children[89,23] by referring to Mexican work showing that malnourished infants have low serum-sodium levels[90] and osmolarity[91] and appear to have increased intracellular water.[90]

Our findings support the observations at Baragwanath Hospital.[89] Hyponatraemia was significantly associated with underweight and there was a suggestive association with hypoalbuminaemia.

SUMMARY

In Cape Town, gastro-enteritis is a much more severe disease among non-European children than among Europeans, although the same pathogens must be circulating in the community. The evidence suggesting that malnutrition is an important aggravating factor in gastro-enteritis is reviewed.

We have studied a sample of 55 children, aged 1 - 42 months, with gastro-enteritis. All were dehydrated and were treated while they were outpatients. Children with kwashiorkor or predominant parenteral infection were excluded, but otherwise the series was unselected. In comparison with control children of the same age, one-quarter of the gastro-enteritis patients had hypoalbuminaemia *while dehydrated*. After parenteral fluid therapy the incidence of low serum-albumin levels rose to one-half. These findings suggest that protein deficiency may have been a reason for the severity of the gastro-enteritis in at least a proportion of the cases.

On electrophoresis, the serum proteins of gastro-enteritis patients had a non-specific increase of α-globulins; γ-globulins did not differ from the controls. The sub-group with hypoalbuminaemia had decreased β-globulins and thus had a pattern similar to that seen in kwashiorkor.

Underweight, hyponatraemia, rickets and anaemia were also observed in the sample. The inter-relationship and significance of some of these to infantile gastro-enteritis is discussed.

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