Serum colloidal osmotic pressure in the development of kwashiorkor and in recovery: its relationship to albumin and globulin concentrations and oedema

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1. Serum colloidal osmotic pressure was measured in children 'at risk' to kwashiorkor, in others with frank signs of the disease and during recovery. Simultaneous estimations of serum albumin and globulin concentrations and assessments of the extent of oedema were also made.

2. During the development of kwashiorkor, serum colloidal osmotic pressure did not decrease significantly until albumin concentration was 25·1–27·5 g/l. Above 30·0 g/l, colloidal osmotic pressure was maintained at normal levels during which time a significant reciprocal relationship existed between albumin and globulin concentrations. These findings provide support for suggestions that there may be an oncotic regulation of albumin synthesis.

3. Low albumin concentrations were mainly responsible for the low colloidal osmotic pressures found in children with kwashiorkor and in agreement with previous findings the threshold for the formation of oncotic oedema was found to be about 2·35–2·65 kN/m².

4. Values for colloidal osmotic pressure calculated from serum albumin and gobulin concentrations using empirical formulas did not agree well with measured values and no constant correction factor suitable over the whole range of albumin concentrations found in rural Ugandan children could be devised. In many hypoalbuminaemic children only direct measurement of serum colloidal osmotic pressure will indicate the true extent of risk to an episode of oedema.

Starling (1896) described the physical forces operating between the intravascular and extravascular circulation. From his work it is evident that since hypoproteinaemia such as that seen in kwashiokor results in a reduction of colloidal osmotic pressure, the consequent decreased osmotic difference between plasma and extravascular fluid must lead to increased capillary filtration, decreased venous absorption and oedema. Salt and water retention are additional complicating factors (Alleyne, 1966; Srikantia, 1968); indeed, Srikantia (1968) minimizes the importance of colloidal osmotic pressure changes in the development of oedema in kwashiorkor.

Plasma colloidal osmotic pressure represents the combined osmotic effects of the large number of different plasma proteins. Values can be derived from measurements of the concentration of plasma proteins using theoretical formulas (Marrack & Hewitt, 1927; Kesselman, 1950) or empirical formulas (Verney, 1926; Fishberg, 1929; Wells, Youmans & Miller, 1933; Wies & Peters, 1937; Keys, 1938; Scatchard, Batchelder & Brown, 1944; Meyer, 1951; Ingerslev, Larsen & Lassen, 1966). Such calculations are, however, recognized as being inadequate, particularly in pathological states when the plasma protein fractionation pattern is abnormal (Armstrong, Kark, Schoenberger, Shatkin & Sights, 1954; Ingerslev *et al.* 1966). It is generally accepted that colloidal osmotic pressure must be measured.

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In studies of the changes of serum protein patterns in the development of kwashiorkor, Coward, Whitehead & Coward (1972) found, in individual children, reciprocal relationships between serum albumin and total globulin concentrations. The increases in total globulin concentration that occurred were mainly the result of increases in γ-globulin concentrations. The situation was similar in experimentally malnourished baboons; increases in globulin concentration occurred at the same time as decreases in albumin concentration but in addition to increases in the amount of γ-globulin, increases in α_1- and α_2-globulin concentrations were also noted.

Bjørneboe (1946) and Rothschild, Oratz, Evans & Schreiber (1966) have suggested that albumin synthesis is, in some way, influenced by plasma colloidal osmotic pressure and subsequently Coward et al. (1972) reached the conclusion that the early reductions in albumin concentration during the development of kwashiorkor could be partly caused by increases in globulin concentration. Albumin synthesis might be reduced to compensate for the increases in colloidal osmotic pressure that would otherwise occur when globulin concentration increased due to a high incidence of infections.

The purpose of this study was to obtain direct information about serum colloidal osmotic pressure during the development of kwashiorkor in relation to the corresponding changes in albumin and globulin concentrations. In addition colloidal osmotic pressure has been related to the appearance of oedema, to the extent of oedema in children admitted to our metabolic ward, and to its disappearance during recovery.

<div align="center">EXPERIMENTAL</div>

Children

The study was done using subjects from two different sources. One group consisted of forty-six children attending an out-patient clinic 20 km north of Kampala. The other twenty children were patients admitted to our metabolic ward and treated using the procedures described by Staff (1968).

Analytical methods and clinical observations

Blood samples were obtained by venepuncture. Colloidal osmotic pressure measurements were made on serum samples using a Knauer Membrane Osmometer (Type MOM; Dr H. Knauer, 1 Berlin 37-Zehlendorf, Holstweg 18, Germany). This instrument uses a capacitive pressure-measuring system to determine the negative pressure beneath a cellulose acetate membrane, impermeable to substances of a molecular weight greater than 10000, when a 50 μl serum sample is introduced above the membrane. The measuring cell is thermostatically controlled and all measurements were made at 37° using physiological saline (9 g NaCl/l) as the permeating solution.

Concentration of serum total protein, albumin and globulin were determined by an automated colorimetric micro-method (Coward, Sawyer & Whitehead, 1971).

Assessments of the extent of oedema were classified as follows: grade 0, no oedema; grade 1, oedema just detectable below the knee; grade 2, marked oedema below the knee; grade 3, general oedema; grade 4, massive oedema.
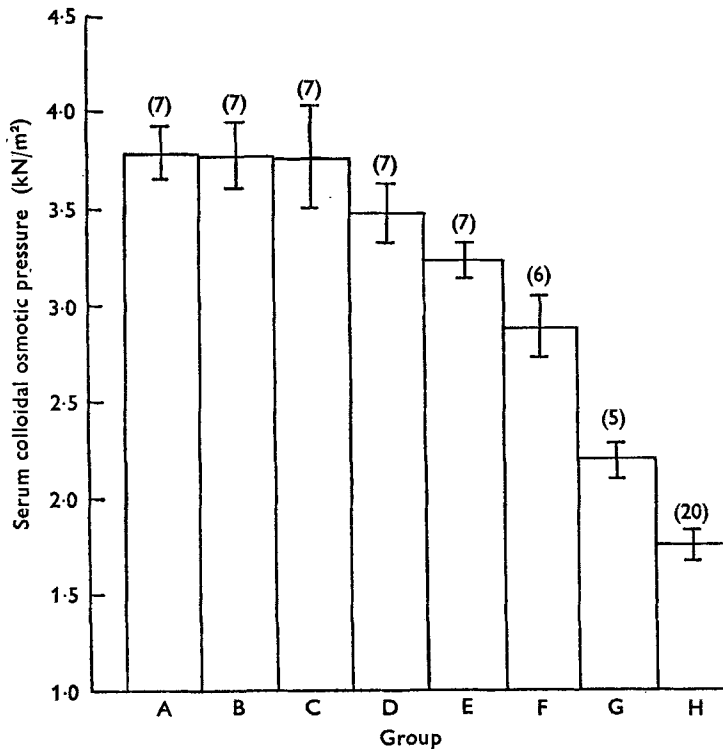
Fig. 1. The relationship between serum albumin concentration and colloidal osmotic pressure in rural Ugandan children 'at risk' to kwashiorkor and in other children with frank signs of the disease. Children are grouped according to their serum albumin concentrations (g/l): *A* > 35·0, *B* 35·0–32·6, *C* 32·5–30·1, *D* 30·0–27·6, *E* 27·5–25·1, *F* 25·0–22·6, *G* 22·5–20·1, *H* < 20·1. Vertical bars represent the standard errors of the means; values in parentheses are the no. of children in each group. Mean values for the following groups were significantly different from that for group A: E, F, $P < 0.01$; G, H, $P < 0.001$.

RESULTS

Fig. 1 shows the relationship between serum albumin concentration and colloidal osmotic pressure in rural Ugandan children attending the out-patient clinic and in other children admitted to the metabolic ward for treatment of kwashiorkor. Mean colloidal osmotic pressure values are shown for serum samples grouped according to albumin concentration. The values for colloidal osmotic pressure at albumin concentrations greater than 30·0 g/l were similar to the normal values quoted by many other workers (for a review, see Schultze & Heremans, 1966). Progressive decreases in serum albumin concentration did not result in significant decreases in colloidal osmotic pressures until albumin concentration had reached 25·1–27·5 g/l.

Albumin and globulin concentration and colloidal osmotic pressure

The extent to which albumin and globulin each contributed towards colloidal osmotic pressure is indicated by the results in Table 1; linear correlation coefficients have been calculated for the relationships between colloidal osmotic pressure, albumin and globulin concentrations. A significant reciprocal relationship existed between

Table 1. *Relationships between serum colloidal osmotic pressure (π) and albumin (A) and globulin (G) concentrations in children 'at risk' to kwashiorkor and in other children with frank signs of the disease*

(Correlation coefficients have been calculated for serum samples grouped according to their albumin concentrations (g/l))

Albumin concentration range	No. of serum samples	Correlation coefficients		
		A v. G	A v. π	G v. π
> 30·0	21	−0·473*	−0·097	0·430
20·1–30·0	25	−0·230	0·758***	0·107
⩽ 20·0	20	0·285	0·789***	0·430

Relationship statistically significant: * $P < 0·05$, *** $P < 0·001$.

Table 2. *Serum colloidal osmotic pressure and albumin and globulin concentrations in nine children recovering from kwashiorkor*

(Mean values with their standard errors on admission are compared with those when oedema was first no longer detectable)

	Admission	Oedema no longer detectable
Colloidal osmotic pressure (kN/m²)	1·76±0·14	2·45±0·15***
Albumin concentration (g/l)	13·6±1·4	20·9±1·7***
Globulin concentration (g/l)	27·8±2·0	32·0±2·1

Mean values significantly different from those on admission: *** $P < 0·001$.

the concentrations of albumin and globulin only for albumin concentrations above 30·0 g/l. Only at lower albumin concentrations was there a statistically significant correlation between albumin concentration and colloidal osmotic pressure.

Colloidal osmotic pressure and the presence of oedema

The relationship between oedema and colloidal osmotic pressure on admission and in recovery is shown in Fig. 2. Obviously there are difficulties inherent in assessing the amount of oedema, but in twelve children admitted to the ward, those with the lowest serum colloidal osmotic pressures generally had the most extensive oedema, and mean osmotic pressures for the five grades of oedema showed progressive increases as oedema disappeared. Only slight (grade 1) oedema remained at a mean colloidal osmotic pressure of 2·15 kN/m² and oedema was never seen at values greater than 2·51 kN/m², although in many cases it had disappeared when the colloidal osmotic pressure was lower than this. In nine children who recovered completely, colloidal osmotic pressure increased as both mean serum albumin and globulin concentrations increased, but during this time only the increase in albumin concentration was statistically significant (Table 2). The mean colloidal osmotic pressure when oedema had finally disappeared was 2·45 kN/m².

Values obtained during the recovery of an individual child (shown in Fig. 3) also indicated that changes in serum albumin concentration closely followed the increase in colloidal osmotic pressure.
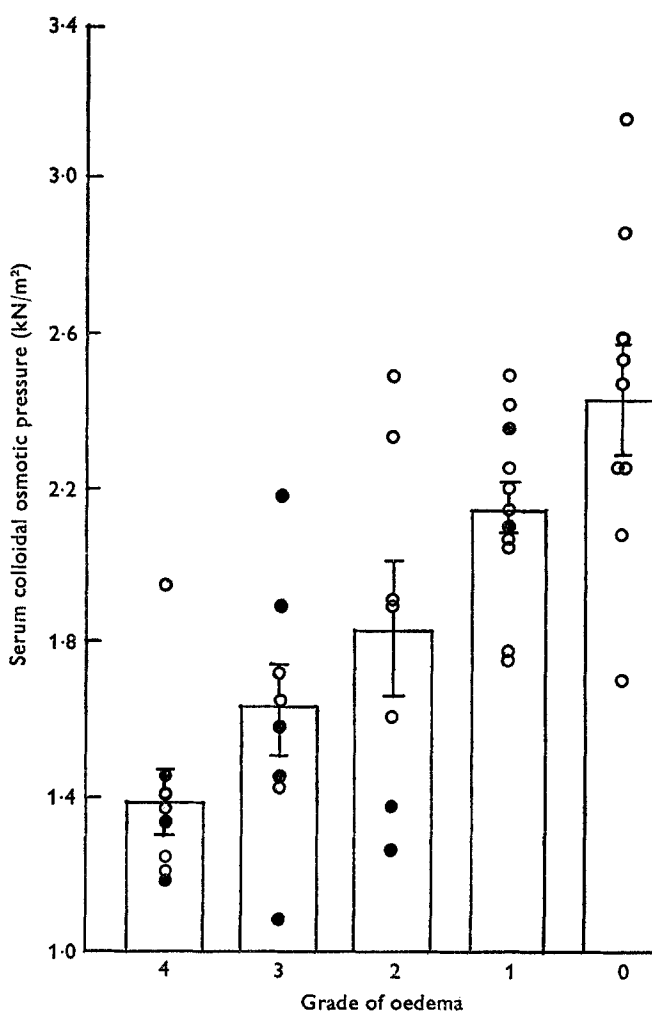
Fig. 2. The relationship between grades of oedema and serum colloidal osmotic pressure in twelve children recovering from kwashiorkor. Grade o, no oedema; grade 1, oedema just detectable below the knee; grade 2, marked oedema below the knee; grade 3, general oedema; grade 4, massive oedema. Vertical bars represent the standard errors of the means. ●, Samples taken at admission; ○, samples taken during recovery. Three children died before they had lost all their oedema. Mean values for the following groups were significantly different from that for the grade 4 group: grade 2, $P < 0.05$; grades 1 and 0, $P < 0.001$.

Empirical formulas for colloidal osmotic pressure calculations

The relationships between measured serum colloidal osmotic pressures and those derived by calculation from serum albumin and globulin concentrations using the formulas of Scatchard *et al.* (1944) and Ingerslev *et al.* (1966) are shown in Table 3. Measured colloidal osmotic pressures were usually higher than the calculated ones and at albumin concentrations greater than 30·0 g/l, there was no significant relationship between measured and calculated values. At lower albumin concentrations significant
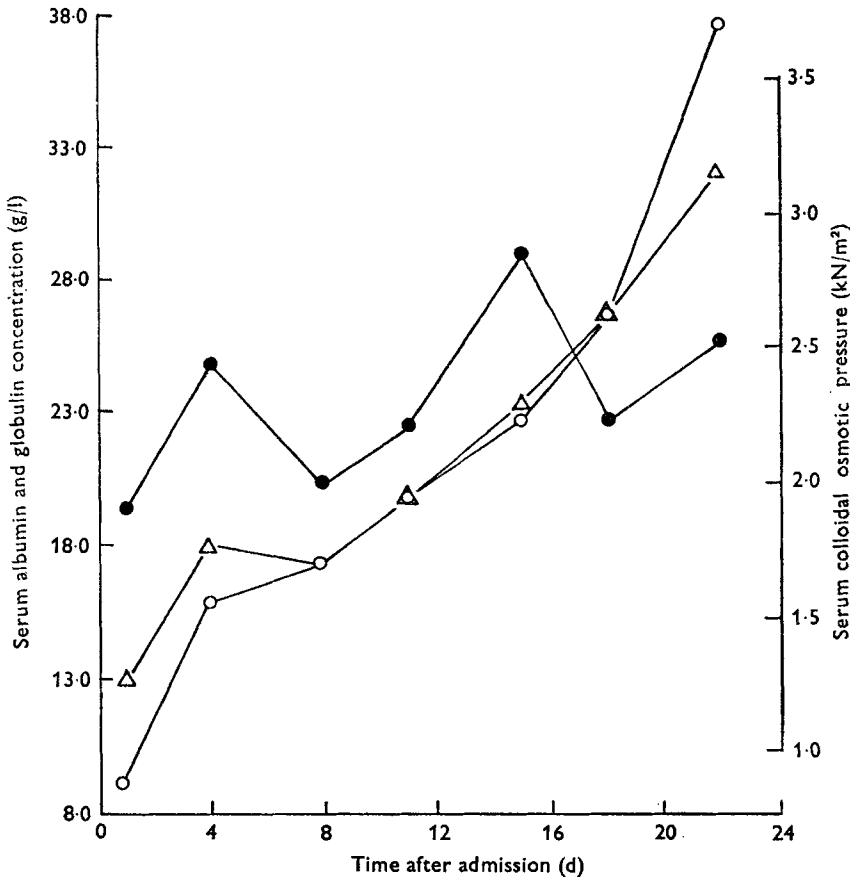
Fig. 3. Serum albumin (○) and globulin (●) concentrations and colloidal osmotic pressure (△) for an individual child recovering from kwashiorkor in hospital.

Table 3. *Relationships between measured colloidal osmotic pressure* (π_M) *and colloidal osmotic pressure calculated using the formulas of Scatchard, Batchelder & Brown* (1944)† (π_S) *and Ingerslev, Larsen & Lassen* (1966) (π_I)

(Correlation coefficients have been calculated for serum samples grouped according to their albumin concentrations (g/l))

Albumin concentration range	No. of serum samples	Correlation coefficients	
		π_M v. π_S	π_M v. π_I
> 30·0	21	0·279	0·179
20·1–30·0	25	0·620***[a]	0·722***[b]
≤ 20·0	20	0·835***[c]	0·841***[d]

a, b, c, d, Regression equations for these relationships are (standard errors of the slopes are given in parentheses): *a*, $\pi_S = 0·37\pi_M + 1·18$ (0·10); *b*, $\pi_I = 0·24\ \pi_M + 1·34$ (0·05); *c*, $\pi_S = 0·72\ \pi_M - 0·11$ (0·11); *d*, $\pi_I = 0·60\ \pi_M + 0·21$ (0·09).

Relationship statistically significant: *** $P < 0·001$.

† In the original publication the formula contains a typographical error subsequently corrected (Scatchard, 1951; Armstrong, Kark, Shoenberger, Shatkin & Sights, 1954).

relationships did exist but calculations of the slopes and intercepts of the regression lines indicate that in different albumin concentration ranges the nature of the relationship alters.

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DISCUSSION

Physiological considerations

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Reductions in the concentration of serum albumin are an early feature in the development of kwashiorkor (Coward *et al.* 1972); because it has a relatively low molecular weight and high concentration in plasma it is the protein that makes the most important individual contribution to plasma colloidal osmotic pressure in normal subjects (Schultze & Heremans, 1966), but in rural Ugandan children early decreases in albumin concentration did not in fact affect the serum colloidal osmotic pressure. In the children studied, serum colloidal osmotic pressure did not decrease significantly until albumin concentration was 25·1–27·5 g/l and was only significantly correlated with albumin concentration when the latter was low. At normal albumin concentrations, greater than 30·0 g/l, the only statistically significant relationship found was the negative correlation between albumin and globulin concentrations.

It is possible to explain the reciprocal relationship between albumin and globulin quite simply. Hypoalbuminaemia is often associated with periods of increased nutritional stress due to partial anorexia induced by infections (Frood, Whitehead & Coward, 1971); globulin concentrations would increase at the same time as part of the immune mechanism. However, the lack of a significant positive correlation between serum albumin concentration and colloidal osmotic pressure during early decreases in albumin concentration would be consistent with the existence of a homoeostatic adjustment of the type suggested by Bjørneboe (1946) and Rothschild *et al.* (1966) who postulated an oncotic regulation of albumin synthesis. The rate of albumin synthesis decreases after hyperimmunization (Rothschild, Oratz, Franklin & Schreiber, 1962; Rothschild, Oratz, Mongelli & Schreiber, 1965) and after infusions of dextran (Rothschild, Oratz, Wimer & Schreiber, 1961). In rural Ugandan children early reductions in serum albumin concentration might therefore be explained as homoeostatic adjustments to maintain a normal colloidal osmotic pressure in the presence of high globulin concentrations caused by frequent infections. A full explanation of the reasons why serum albumin concentrations decrease in the development of kwashiorkor would therefore be that two distinct processes are operative. When both albumin concentration and colloidal osmotic pressure decrease in the final stages of the development of the disease, the pattern and availability of serum amino acids, to which albumin synthesis is extremely sensitive (Kirsch, Saunders, Frith, Wicht & Brock, 1969; Rothschild, Oratz, Mongelli, Fishman & Schreiber, 1969), is most important. In the early stages homoeostatic mechanisms may be more dominant.

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Practical implications

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In a review of previous work Schultze & Heremans (1966) considered that colloidal osmotic pressure in the range 2·35–2·65 kN/m² represented the threshold for the formation of oncotic oedema. The results presented here would tend to confirm this

view. In the observations made in relation to the development of kwashiorkor only two of twenty-seven children with serum colloidal osmotic pressures below 2·65 kN/m² showed no signs of oedema and in recovery, oedema was never seen when values were greater than 2·51 kN/m². Although there were inherent difficulties in making clinical assessments of oedema, in addition to the complicating role of salt and water retention (Alleyne, 1966; Srikantia, 1968), the results of measurements made in children admitted to the ward and during recovery suggested that colloidal osmotic pressure values related well to the amount of oedema described clinically. It is likely, therefore, that serum colloidal osmotic pressure measurements can be used to indicate the extent of susceptibility to oedema in children 'at risk' to kwashiorkor.

Rural Ugandan children with serum albumin concentrations of less than 25·0 g/l will usually have serum colloidal osmotic pressures in the range where the probability of oedema appearing is great. This conclusion is consistent with that of Whitehead, Frood & Poskitt (1971) who related albumin concentration to the presence of oedema in children 'at risk' to kwashiorkor. These workers also commented on the difficulties in interpreting the significance of serum albumin values in the range 28·0–35·1 g/l, which have been described as 'low' ((US) Interdepartmental Committee on Nutrition for National Defense, 1963). The results presented here would indicate that because the relationship between serum albumin concentration and colloidal osmotic pressure is poor at these albumin levels it may often be necessary to measure colloidal osmotic pressure in order to assess the extent of risk to oedema.

Direct measurements of serum colloidal osmotic pressure are not often made and so it is relevant to consider the suitability of empirical equations for its calculation. The formulas tested, those of Scatchard et al. (1944) and Ingerslev et al. (1966), are typical of many others. Both predict non-linear relationships between colloidal osmotic pressure and protein concentration in which the former increases proportionately more rapidly than the latter. However, they did not give satisfactory results. Measured values were usually higher than those calculated and, in addition, the relationship between measured and calculated values changed for various albumin concentration ranges. This finding is not surprising because each formula assumes a globulin fraction of constant composition, but in the development of kwashiorkor the fractionation pattern of the globulins changes considerably as albumin concentration decreases (Coward et al. 1972). The lack of a statistically significant relationship between measured and calculated colloidal osmotic pressures at serum albumin values greater than 30·0 g/l is less easily explained and will be studied further. One possibility would be that there might be considerable variations in the serum concentration of relatively low-molecular-weight components of the globulin fraction in normal Ugandan children.

The results presented here could be used to calculate factors to convert values for serum colloidal osmotic pressures calculated from serum albumin and globulin concentrations to more realistic values and these might be useful in Uganda, but unless serum protein patterns in the development of kwashiorkor in children in other environments are like those seen in rural Ugandan children their application will be limited. When it is not possible to measure serum colloidal osmotic pressure directly, in

order to obtain an estimation of extent of susceptibility to oedema, there is little alternative other than to use local experience and measurement of serum albumin concentration as Whitehead *et al.* (1971) suggested.

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