Balance studies in malnourished Jamaican infants

1. Absorption and retention of nitrogen and phosphorus

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Several excellent papers have been published on nitrogen metabolism in malnourished infants and children in different parts of the world, notably the Belgian Congo (Holemans & Lambrechts, 1955), South Africa (Pretorius & Smit, 1958; Hansen, 1956), French West Africa (Sénécal, 1957), Mexico (Gómez, Ramos-Galván, Cravioto, Frenk, de la Peña, Moreno & Villa, 1957; Cravioto, 1958) and Guatemala (Scrimshaw, Squibb, Bressani, Béhar, Viteri & Arroyave, 1957). Most workers agree that protein malnutrition, kwashiorkor, or *distrofia policarencial*—to cite only three of the names that are commonly used—presents basically the same features in all the regions where it has been described (Waterlow, 1955*a*). There are, however, local differences. For instance, in our work on malnutrition in Jamaica we have found that the signs tend to develop at an earlier age than in Central America or Mexico; skin changes are less marked, but fatty liver seems to be more common and more severe (Waterlow, Bras & DePass, 1957). Because of these differences, we could not assume that the results obtained by others on N metabolism would apply to our cases. While we were making our observations on it, a number of other questions occurred to us that have not been answered by studies elsewhere.

First, we need to know how far the efficiency of N absorption and retention is affected by the severity of protein malnutrition, and whether it changes as the child gets better. This problem has not been considered explicitly or in detail in the studies mentioned above, some of which have been concerned mainly with the convalescent patient as a test subject for measuring the nutritive value of proteins (Scrimshaw, Bressani, Béhar & Viteri, 1958; Bressani, Scrimshaw, Béhar & Viteri, 1958; DeMaeyer & Vanderborght, 1958). The point is clearly important if different dietary regimes are being compared one after the other.

A second question, related to the first, is whether, in patients who fail to respond to treatment and eventually die, N absorption and retention are severely impaired, and if so, whether this impairment could be considered the cause of death. The information on this point in the literature is scanty and inconclusive.

Thirdly, it seemed to us that from controlled studies on malnourished infants it should be possible to get some information on the difficult problem of protein requirements. If a given intake is adequate for cure, one may suppose *a fortiori* that it is adequate for normal maintenance and growth.

Lastly, there are indications in the literature that infants with kwashiorkor are depleted not only of protein and potassium (Hansen, 1956), but also of phosphorus (Holemans, Lambrechts & Martin, 1955). This problem seemed worthy of further investigation, in view of the importance of phosphate as an intracellular anion, and its central role in so many metabolic processes.

All the work described in this paper is based on the results of treatment with milk mixtures. We have made no study of the efficiency or nutritive value of different kinds of proteins or amino-acid supplements (cf. Gómez et al. 1957; Cravioto, 1958; Scrimshaw et al. 1957, 1958; Bressani et al. 1958; DeMaeyer & Vanderborght, 1958), except for a comparison between human milk and mixtures based on cow's milk which will be referred to briefly as 'cow's-milk mixtures'; their composition is given in Table 2. The results of this investigation are given by Waterlow, Wills & György (1960).

EXPERIMENTAL

Clinical material

Symptomatology. The clinical picture of malnutrition in Jamaican infants has been described in previous reports (Waterlow, 1948; Jelliffe, Bras & Stuart, 1954). The onset tends to be somewhat earlier than in Africa and Central America. Apart from failure of growth, the salient features are oedema and fatty liver. Changes in skin

Table 1. *Mean values for clinical characteristics of malnourished infants*
on admission to hospital

	Non-fatal cases	Fatal cases
No. of patients	32	5
No. with oedema	25	4
No. with hepatomegaly	12	2
Age (months)	12·2 (3–24)	11·0 (6–15)
Body-weight (as percentage of normal weight for age*)	51 (30–73)	52 (43–61)
Serum total protein (g/100 ml)	5·25 (3·7–8·5)	4·7 (4·3–5·1)
Serum albumin (g/100 ml)	2·28 (0·9–3·9)	2·20 (1·8–2·4)
Serum cholinesterase units†	0·27 (0·1–0·8)	0·17 (0·1–0·3)
Haemoglobin (g/100 ml)	8·9 (3·2–12·0)	8·6 (3·2–12·0)
Serum potassium (m-equiv./l.)	3·95 (1·7–5·7)	3·65 (3·1–5·1)
Serum sodium (m-equiv./l.)	133 (124–149)	129 (117–157)
Liver fat (as percentage of wet weight)	21·8 (3–45)‡	44·1 (37–53)§

Figures in parentheses are the ranges.
* Normal weights taken from Nelson (1954).
† Michel (1949); the Michel unit is the Δ pH/h produced when 0·02 ml serum is incubated with buffer under stated conditions.
‡ Sixteen patients.
§ Four patients.

and hair, once thought to be diagnostic (Trowell, 1941; Brock & Autret, 1952), are variable, and bear no relation to the clinical severity. For the purpose of the present study we have made no hard and fast distinction between kwashiorkor and marasmus, but regard both as variants of the more general condition—protein malnutrition

(Waterlow, 1955 *b*). In Jamaica the commonest form is the intermediate type described by Jelliffe *et al.* (1954) as 'marasmic kwashiorkor'.

Thirty-seven patients were studied, of whom five died. Clinical and biochemical findings are summarized in Table 1. Liver fat was measured on biopsy specimens taken as soon as possible after admission and again when the baby was convalescent. In the infants who died the livers were more consistently fatty, and serum levels of cholinesterase and sodium tended to be lower than in those who survived.

Treatment. Vomiting and refusal of food are very common in our patients, and in the first days after admission it is often a problem to get the baby to take anything at all. Sometimes it is necessary to resort to an intragastric drip, and very severely ill infants need intravenous therapy with Darrow's solution (Govan & Darrow, 1946) or plasma. For this reason in the early stages many of the intakes were very low.

Since one of the objects of this work was the comparison of breast milk and a cow's-milk mixture, in the later stages we have used mainly mixtures of rather low protein content. Only a few patients in this series were given high-protein mixtures based on dried skim milk or calcium caseinate, as recommended by Dean & Skinner (1957). The breast milk was pooled breast milk collected from mothers in their homes at all stages of lactation after the 1st week.

Table 2. *Composition, protein content and calorie value of diets used for treatment of malnourished infants*

Diet	Lactogen* (g/l.)	Casilan† (g/l.)	Dried skim milk (g/l.)	Sucrose (g/l.)	Oil‡ (g/l.)	Protein (g/l.)	Calories (kcal/l.)
A§	—	50	50	20	30	57	700
B§	—	50	—	20	30	40	530
P	65	—	—	30	55	12	940
R	65	—	—	30	20	12	630
S¶	—	—	—	—	—	11	650
Lactogen	125	—	—	10	—	21	670
Breast milk	—	—	—	—	—	11	700

 * A proprietary dried-milk preparation (Nestlé Ltd).
 † Calcium caseinate (Glaxo Laboratories Ltd).
 ‡ Either olive oil or arachis oil.
 § From Dean & Skinner (1957).
 ¶ A liquid-milk preparation containing protein 1·1, fat 3·68, carbohydrate 6·8 %, kindly supplied by Wyeth Laboratories Inc., Philadelphia.

The approximate protein content and calorie value of the milks are shown in Table 2. The figures given for the cow's-milk mixtures were calculated from the manufacturers' data and from the conventional values for sugar and fat. Those for breast milk were given by Macy, Kelley & Sloan (1950). The N content of the mixtures and of the breast milk varied slightly from batch to batch and was therefore measured directly in each balance experiment (see below).

The milk diets were supplemented with potassium (20 m-equiv./l. as potassium acetate) in the early stages, with orange juice, and sometimes with iron, but no other food was given. Patients with a megaloblastic bone marrow were treated with folic acid, 10 mg/day (J. MacIver & E. Back, unpublished).

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Procedure in balance studies

A total of fifty-three balance studies were done with the thirty-two babies who did not die. For the purpose of analysis these studies have been divided into three groups, according to the stage of treatment:

Stage 1: the acute phase—from 0 to 10 days after admission to hospital (twenty-one studies).

Stage 2: from 11 to 25 days after admission (seventeen studies).

Stage 3: from 26 days onwards (fifteen studies).

Ideally, each child should have been studied three times, but it was not practicable. Some for one reason or another had to be taken off the experiment, and with others, because of vomiting, balance studies could not be begun until some time after admission. The majority of children were studied twice, some once only, a few three times.

Each balance study consisted of at least two, and sometimes as many as five, consecutive balance periods. In each period, urine and faeces were collected for 2 days, followed by 1 day of rest. We did not consider it advisable to immobilize the babies for more than 2 days without intermission. On the average, measurements were made for 6 days, excluding rest days, in each balance study (minimum 4 days, maximum 10 days). These periods of observation are short, but it was unavoidable with patients who were often very ill, and whose condition was rapidly changing.

Whenever a baby was put on a diet of different protein content, a fore-period of at least 3 days was allowed before balance measurements were resumed.

The milk intakes were measured as accurately as possible, and spot checks were made from time to time by test weighing. Only a few measurements were made on girls, by means of a specially constructed metabolic cot. In boys, urine was collected by a finger-cot attached to the penis. Stools were collected on a pad of cotton-wool placed under the baby, renewed whenever necessary. The cotton-wool was put into a glass jar with sulphuric acid and water, and at the end of each 2-day period the wool was thoroughly stirred and pounded up in the water, and the whole made to a known volume. A pilot experiment showed that N was not adsorbed by the wool and could be extracted quantitatively with water. Stool markers were not used. Most of the babies had at least three motions a day and the error produced by assigning one stool to the wrong balance period was therefore small enough to be neglected.

Milk mixtures were made up in amounts of about 2 l. at a time for each baby and stored in the refrigerator. Each batch was analysed separately. N was estimated by the micro-Kjeldahl method, and P by the method of Taussky & Shorr (1953), after digestion with perchloric acid.

RESULTS

Non-fatal cases

Nitrogen metabolism. The following list of definitions may assist clarity:

N absorbed = N intake − N in faeces;

$$\text{Apparent N absorption} = \frac{\text{N intake} - \text{N in faeces}}{\text{N intake}} \times 100;$$

$$\text{Corrected N absorption} = \frac{\text{N intake} - (\text{N in faeces} - \text{faecal metabolic N})}{\text{N intake}} \times 100;$$

Faecal metabolic N = faecal N on zero N intake;

N retained = N absorbed − N in urine;

$$\text{N retention (as percentage of absorption)} = \frac{\text{N absorbed} - \text{N in urine}}{\text{N absorbed}} \times 100;$$

$$\text{Corrected N retention} = \frac{\text{N absorbed} - (\text{N in urine} - \text{endogenous N})}{\text{N absorbed}} \times 100;$$

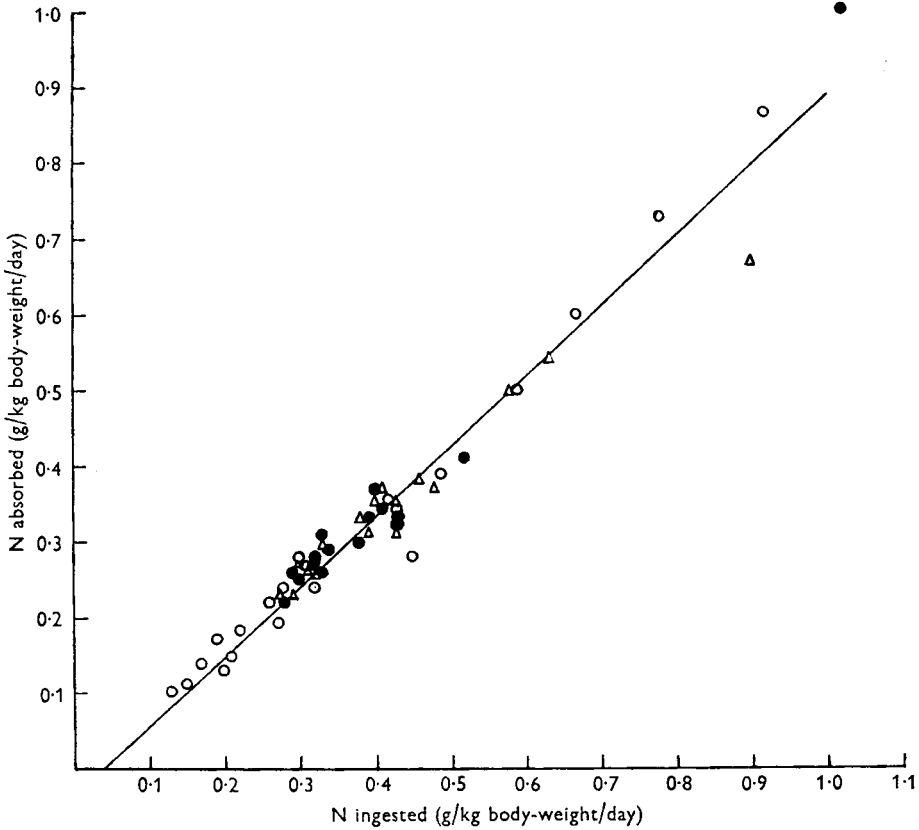Endogenous N = urinary N when N absorbed is zero.

Fig. 1. Relation of N absorbed to N ingested in severely malnourished babies. ○, stage 1; ●, stage 2; △, stage 3 (see p. 186). Regression equation, $y = 0.92x - 0.036$.

The values for apparent N absorption at the three different stages are shown in Fig. 1. The point where the regression line, when projected, cuts the y axis gives an estimate of the faecal metabolic N (Mitchell & Bert, 1954) of 33 mg N/kg body-weight/day. This value agrees well with the figure of 30 mg/kg/day obtained by Holemans & Lambrechts (1955).

Table 3 shows that the corrected absorption did not vary with the stage of treat-

ment. The lower apparent absorption in stage 1 was due to the lower intake. Many of our patients had moderate or severe diarrhoea in the early stages. Our impression agrees with the finding of Cravioto (1958) that diarrhoea has little effect on N absorption. Our method of collecting the stools did not, however, allow us to measure their weight or volume.

The relation of N retained to N apparently absorbed is shown in Fig. 2. As would be expected, there was a tendency for the proportion of N retained to fall off with
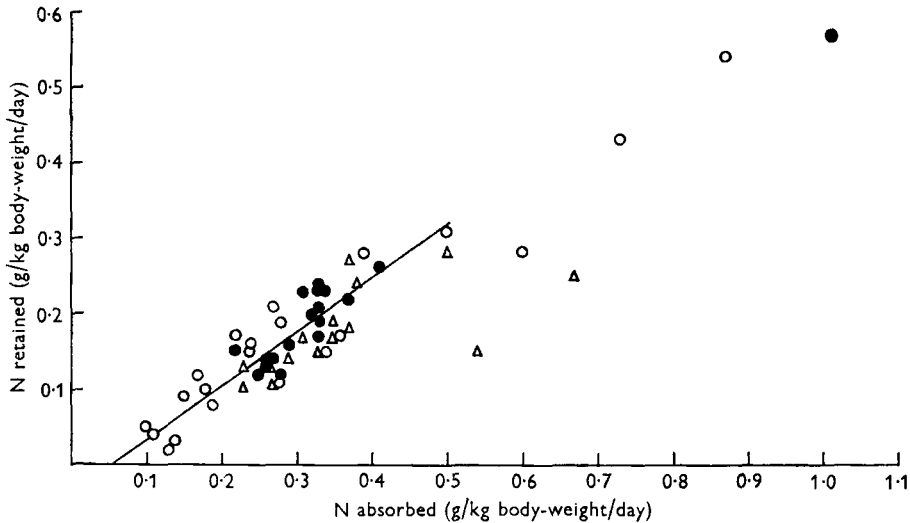

Fig. 2. Relation of N retained to N absorbed in severely malnourished babies. ○, stage 1; ●, stage 2; △, stage 3 (see p. 186). Regression equation, $y = 0.704x - 0.038$.

Table 3. *Mean values with their standard errors for nitrogen intake, absorption and retention by malnourished infants at different stages of treatment who absorbed not more than 500 mg N/kg body-weight/day*

Stage	Days from admission	No. studied	N intake (mg/kg/day)	N absorbed (as percentage of intake)		N retained (as percentage of N absorbed)	
				Apparent*	Corrected*	Apparent*	Corrected*
1	0–10	19	300 ± 88	79·4 ± 1·8	90·3 ± 2·1	53·4 ± 3·9	71·8 ± 3·45
2	11–25	17	373 ± 15	82·3 ± 1·3	90·2 ± 1·6	59·0 ± 2·3	70·5 ± 2·0
3	> 25	15	385 ± 21	83·1 ± 1·3	91·2 ± 1·5	51·9 ± 1·95	63·7 ± 1·8

The difference between retentions in stages 2 and 3 is significant ($0.05 > P > 0.02$ for both corrected and apparent retention). Since the children studied at the two stages were not all the same, the difference found depends on variation between children as well as on variation between stages of treatment. Ten children were studied in both stages 2 and 3. In this subgroup the results were:

	N retained, as percentage of N absorbed (corrected)
Stage 2	72·1 ± 2·26
Stage 3	63·2 ± 1·58

The means are very close to those of the larger group, and the difference between them is significant ($P < 0.01$). The difference between the two stages was therefore not due to individual variation between children.

* See pp. 186, 187.

increasing intake. The relationship, however, seemed to be linear until N absorption reached a level of about 0·5 g/kg/day or more. The regression line has been drawn through points corresponding to amounts of N absorbed up to 0·5 g/kg/day. The intercept of this line, when projected to the *y* axis, gives an estimate of the endogenous N of 37 mg/kg/day. The intercept on the *x* axis represents the minimum amount of N absorbed that will just secure N equilibrium; it comes to 53 mg N/kg/day.

From the values in Figs. 1 and 2 it can be calculated that under the conditions of these measurements the minimal intake needed to secure N balance would be about 96 mg N/kg/day. This figure, corresponding to a protein intake of about 0·6 g/kg/day, fits in with results obtained by us in field surveys. Four infants with intakes of less than 0·8 g protein/kg/day all showed clinical signs of kwashiorkor (V. G. Wills, unpublished).
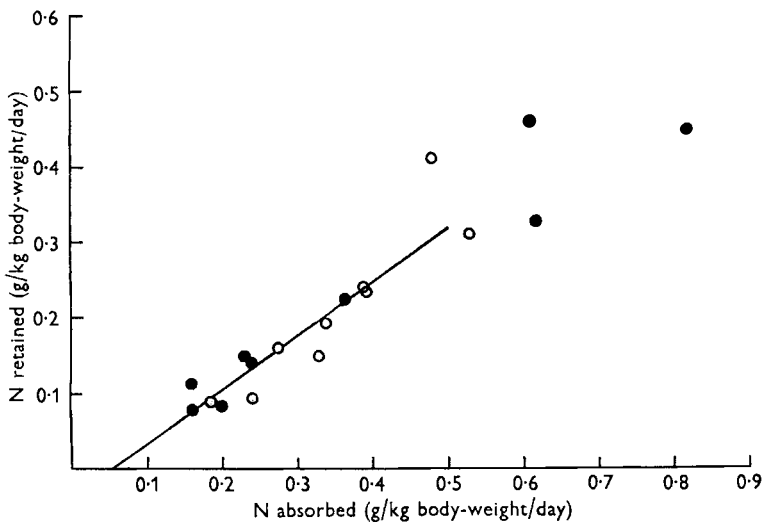

Fig. 3. Relation of N retained to N absorbed in severely malnourished babies; values for Jamaican babies (———) (Fig. 2) compared with values of Cravioto (1958) (○) and of Holemans & Lambrechts (1955) (●).

The slope of the regression line in Fig. 2 indicates the average efficiency of retention after the endogenous or inescapable N losses had been covered. It appears that over the range studied about 70 % of absorbed N is utilized.

In Fig. 3 our results are compared with those of Cravioto (1958) and of Holemans & Lambrechts (1955) for early cases.* It is clear that the agreement is good.

N retention at the three different stages is shown in Table 3. To reduce the effect of variations in intake, results for patients in whom N absorbed was more than 0·5 g/kg/day have been excluded. In the first stage the variability was high, because some of the intakes were very low, which increased the error of measurement (Wallace, 1959). It appears that retention in the third stage was significantly less than in the

* We are grateful to Dr K. Holemans for supplying us with details of his balance experiments in addition to those recorded in his paper.

earlier stages. This reduced avidity for N presumably reflects the filling up of the protein stores (Allison, 1951).

In stages 1 and 2 changes in weight gave little useful information, because a gain in tissue mass may be cancelled out by loss of oedema fluid. Oedema usually disappeared within 10–14 days. However, measurements of total body water with tritiated water have shown very clearly that even after the loss of overt oedema the water content of the body is still abnormally high (Smith, 1960). It is presumably for this reason that even in stage 2 some of the babies lost weight, although they were retaining N and had apparently adequate calorie intakes (Table 4). It was

Table 4. *Mean values with their standard errors for weight gains of malnourished infants at different stages of treatment*

Stage	Days from admission	No. of infants	N intake (mg/kg body-weight/day)	Calorie intake (kcal/kg body-weight/day)	Weight gain (g/kg body-weight/day)
1	0–10	19	300 ± 88	107 ± 12·9	1·9 ± 2·4
2	11–25	17	373 ± 15	135 ± 7·7	3·3 ± 1·2
3	> 25	15	385 ± 21	142 ± 2·1	7·3 ± 0·93

only in stage 3 that consistent weight gains were found. The mean gain was 7·3 g/kg/day, which is three times the rate of gain of a normal child of 9 months growing normally.

The mean N retention during this stage was 176 mg/kg/day. The N retained therefore amounted to 2·4 % of the body-weight gained. This figure is very close to that for the N content of the normal body at 1 year of age (Wallace, 1959). It seems therefore that weight gain at this stage of treatment represents growth, and not deposition of water or fat.

The mean calorie intake during the balance periods of stage 3 was 142 kcal/kg/day. The studies reported here do not allow us to draw any conclusions about the effect of calorie intake on N retention, because they were not designed in such a way that protein and calorie intakes could be varied independently. Measurements of the rate of weight gain in a larger series of infants indicated that maximal gains require an intake of at least 130 kcal/kg/day, and that in practice calories are much more likely to be limiting than protein.

Phosphorus metabolism. P absorption is shown in Fig. 4 and Table 5. The regression line goes virtually through the origin, indicating that there was no constant faecal loss of P corresponding to the faecal metabolic N. On the average, more than two-thirds of ingested P was absorbed. There was possibly slight impairment of absorption during the first days in hospital, but the difference from the later stages was not statistically significant. This finding is in contrast to results obtained in Mexico, in which faecal losses were much greater—more than 70 % of the intake in four out of five cases (López Montaño, 1954).

P retention was much more irregular than N retention (Fig. 5), and showed a clear tendency to fall off with increasing intake. Our results are very similar to those of Holemans & Lambrechts (1959). Under such conditions no regression line can validly

Table 5. *Mean values with their standard errors for phosphorus absorption and retention by malnourished infants at different stages of treatment*

Stage	Days from admission	P absorbed (as percentage of intake)*	P retained (as percentage of P absorbed)†
1	0–10	65·8 ± 3·0 (18)	64·7 ± 3·85 (13)
2	11–25	71·1 ± 2·7 (20)	49·7 ± 7·1 (9)
3	>25	72·6 ± 2·5 (16)	37·1 ± 4·6 (9)

Figures in parentheses are the numbers studied.

* P absorbed: Fig. 4 suggests that P absorbed varied linearly with intake. If so, the different stages can be compared, regardless of intake. This column therefore includes values for all patients studied.

† P retained: Fig. 5 suggests that P retention fell off as increasing amounts of P were absorbed. Hence the retentions at different stages can only be compared if the amounts of P absorbed were approximately equal. This column therefore contains only values for patients in whom the P absorbed was more than 30 mg/kg body-weight/day. All these subjects were receiving cow's-milk mixtures.

The mean amounts of P absorbed in the three groups were: stage 1, 42·4; stage 2, 49·5; stage 3, 46·1 mg/kg body-weight/day.

The difference in retention between stages 1 and 2, and between stages 2 and 3 is not significant. The difference between stages 1 and 3 is highly significant ($P \sim 0\cdot001$). It is unlikely that this difference was due to the small difference in P absorbed.

Five children were studied in both stages 1 and 3. If results for them are analysed separately, they are:

	P retained as percentage of P absorbed
Stage 1	63·2 ± 6·0
Stage 3	31·4 ± 4·75

This difference is significant ($P < 0\cdot01$). It shows that the decrease in P retention was related to the stage of treatment, and not to variations between different children.
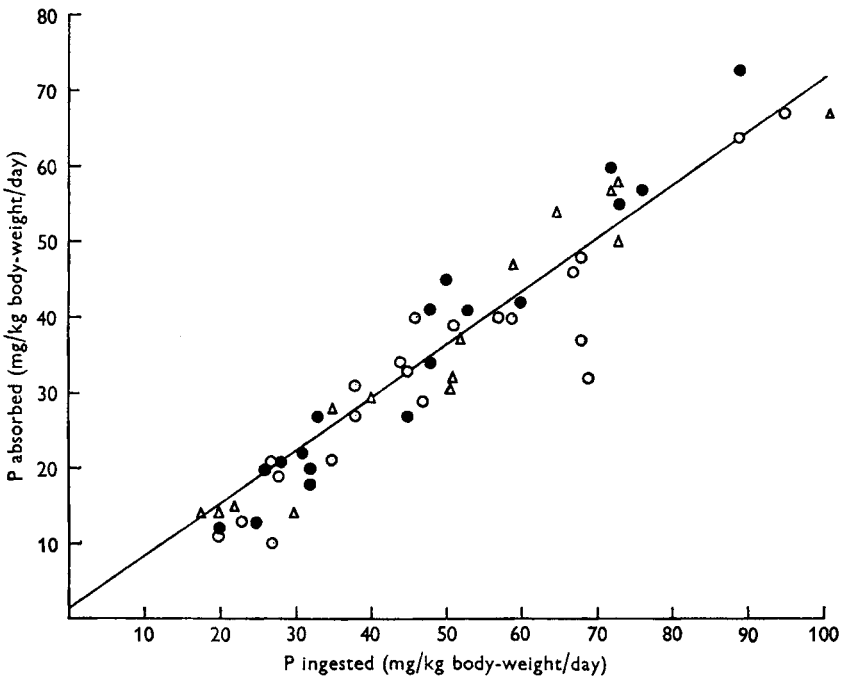

Fig. 4. Relation of P absorbed to P ingested in severely malnourished infants. ○, stage 1; ●, stage 2; △, stage 3 (see p. 186). Regression equation, $y = 0\cdot704x + 0\cdot77$.

be drawn. This effect of intake makes it difficult to compare the efficiency of retention at different stages of treatment. A further complication is introduced by the fact that many of the children with low P intakes were fed on breast milk, which has only about half the P content of cow's milk. The comparison between different stages has therefore been limited to patients in whom P absorbed was more than 30 mg/kg/day. All these babies were receiving cow's-milk mixtures. The results in Table 5 show that in the early stages there was a great avidity for P, which seemed to diminish progressively with treatment.

Further evidence of P depletion is the fact that the majority of patients retained more P in relation to N than would be expected from the known composition of the soft tissues. Baldwin, Robinson, Zierler & Lilienthal (1952) found the mean P content of normal adult human muscle to be 65 mg/g non-collagen N. In infants we have found lower values: the total P content of muscle taken by biopsy from babies fully
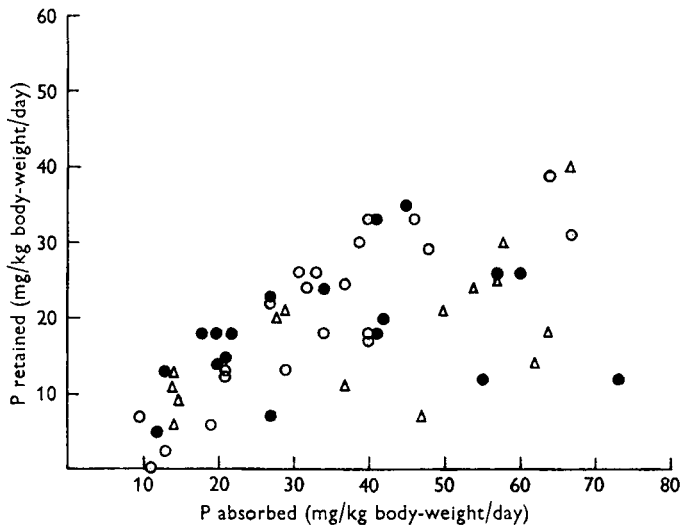

Fig. 5. Relation of P retained to P absorbed in severely malnourished infants.
○, stage 1; ●, stage 2; △, stage 3 (see p. 186).

Table 6. *Ratio of phosphorus (mg) to nitrogen (g) retained by malnourished infants at different stages of treatment*

Stage	Days from admission	Infants fed on breast milk* or mixture B†	Infants fed on cow's-milk mixtures‡
1	0–10	78 (5)	146 (15)
2	11–25	94 (8)	98 (9)
3	>25	81 (7)	121 (8)
All stages (mean value with its standard error)		85·6 ± 7·35	127·2 ± 8·6

Figures in parentheses are the numbers studied.
* P (mg):N (g) in breast milk about 80.
† P (mg):N (g) in mixture B 50.
‡ P (mg):N (g) in cow's milk about 150.

recovered from malnutrition ranged from 40 to 50 mg/g non-collagen N (to be published). The ratio of P to N retained by malnourished infants during treatment was much higher than this value at all stages (Table 6). As would be expected, the ratio of P to N retained was also affected by the P content of the milk.

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Fatal cases

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Nitrogen metabolism. The results for five babies who died are shown in Table 7. The intakes were all very low, except in the second balance period for patient no. 1. For four of the five babies N absorption was almost complete, and the faecal N loss was less than the mean figure for faecal metabolic N derived from non-fatal cases (Fig. 1).

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Table 7. *Absorption and retention of nitrogen and phosphorus in five malnourished infants who died*

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Subject no.	Period after admission (days)	Intake (mg/kg body-weight/day)	Urinary excretion (mg/kg body-weight/day)	Faecal excretion (mg/kg body-weight/day)	Absorption (as percentage of intake)	Retention (as percentage of absorption)
			Nitrogen			
1	0–2	102	116	52	58·5	Neg.
	4–5	445	134	147	67	+39
2	0–2	136	76	25	81·5	+31
3	0–3	40	102	6	85	Neg.
	3–6	140	83	14	90	+34
4	0–2	113	45	28	75	+46
5	0–2	63	38	1	99	+38
			Phosphorus			
1	0–2	18·2	7·6	11·4	37	Neg.
	4–5	22·8	0·3	14·7	36	+96
2	0–2	22·8	0·6	9·1	60	+95
3	0–3	3·4	2·9	0·7	80	Neg.
	3–6	31·0	3·4	3·4	89	+87
4	0–2	17·6	3·7	4·6	74	+74
5	0–2	15·7	10·9	0·6	96	+30

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Neg., negative.

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Two of the babies who died were initially in negative N balance, but in both the balance became positive after a few days. In the other three babies N balance was positive from the beginning. In these positive balance periods, on the average 38% of absorbed N was retained. If it is assumed that the obligatory or endogenous N output was the same in these babies as in those who did not die—i.e. about 0·05 g/kg/day (Fig. 2)—then the N available for retention may be taken as (absorbed N − 0·05) g/kg/day. In the positive balance periods 70–100% of the 'available' N was retained.

Phosphorus metabolism. Table 7 shows also the P intake and output in the five fatal cases. Absorption was satisfactory, except in patient no. 1. All the five babies were either in positive balance from the beginning or came into positive balance before death.

DISCUSSION

The methods used in this investigation are open to the criticism that the balance periods were short. This is a limitation imposed by the condition of the patients and by the object of the work, which was to follow changes occurring over relatively short periods. Lowe & Pessin (1957) have pointed out that the number and duration of balance periods is a variable, to be decided according to the circumstances. Short periods only increase the random error and make it more difficult to establish significant differences.

It is quite otherwise if there is a systematic error, as tends to happen in all balance work. Losses cause intake to be overestimated and output underestimated. Both errors increase the apparent retention. Wallace (1959) has recently pointed out how serious these sources of error may be when intakes are high. It is very difficult to devise any systematic check on the accuracy of these measurements. Test weighings done for 24 h on a number of patients have shown satisfactory agreement with the charted intakes. Measurements of creatinine output have also been done (Waterlow, Wills & Standard, 1959), but can only be relied on to detect a gross error. An external check is the good agreement found with other authors (Holemans & Lambrechts, 1955; Gómez et al. 1957; Cravioto, 1958). Moreover, the N retention is of the order of magnitude that would be expected from the rate of weight gain, and from the rate of increase of total body solids, calculated from measurements of body water (Smith, 1960).

The results obtained answer, at least in part, the questions put in the introduction. It seems that in Jamaica, as in other parts of the world, infants with protein malnutrition absorb and retain N with remarkable efficiency, at least when the source of protein is cow's milk. It is surprising that even in the early stages over 90 % of ingested N should be absorbed, in spite of the fact that, as has been shown in Uganda and Mexico, the trypsin content of the duodenal juice is reduced to one-quarter or one-third of the normal (Thompson & Trowell, 1952; Gómez, Ramos-Galván, Cravioto & Frenk, 1954). It is true that no measurements have been made of the volume of the juice, or of the enzyme content of the succus entericus, but the histological evidence suggests a reduced secretion of enzyme proteins by both pancreas and intestinal glands (Trowell, Davies & Dean, 1954). It seems that either classical ideas about the physiological function of the proteolytic enzymes need to be revised, or that these enzymes must normally be present in great excess. Fisher (1954) writes: 'The existing evidence is certainly insufficient to support the current view that complete proteolysis precedes absorption.'

The interpretation of figures for N retention presents some difficulty. Even though N retained may vary linearly with N ingested and N absorbed, the apparent percentage retention is bound to be lower at low intakes because of the compulsory endogenous loss. It was impracticable to measure the endogenous excretion directly in these infants. The calculated figure obtained by extrapolation (Fig. 2) is a mean that will not apply exactly to each infant. Nevertheless, for comparison between different periods with different intakes it seems fairest to apply this correction.

The results so obtained (Table 3) indicated that after 3–4 weeks of treatment N retention tended to fall off. This decrease is to be expected in the light of Allison's work on protein-depleted dogs: the greater the depletion of the protein stores, the higher the N retention (Allison, 1951). In theory the retention on a fixed N intake might be used as a measure of the degree of depletion. In practice there is probably too much variation between individuals for it to be possible.

Table 3 shows that the apparent N retention was about 7% less in stage 3 than in stage 2. It means that if in convalescents two experimental treatments are being compared, each of which lasts 2 weeks, the second treatment is at a significant disadvantage. Therefore it is clearly very important to alternate the sequence of treatments.

In all the fatal cases studied there was a positive N balance at the time of death. This finding was unexpected, since we had supposed that death might be caused, or at least characterized, by irreversible failure of the protein-synthesizing machinery (Waterlow, 1955 a). N retention by itself is admittedly not proof that protein synthesis is going on; N might be retained as urea or free amino-acids, particularly since these babies at the time of death had oedema and oliguria. With one, however, we have more direct evidence that protein synthesis was still occurring in the last hours of life. Thirty hours before death 4 μc of [^{35}S]methionine ($1\cdot045 \times 10^6$ counts/min) were injected intravenously. Immediately after death samples were taken of blood, muscle and liver. The proteins were precipitated with trichloroacetic acid and washed, and the radioactivity was measured. The results (counts/min/mg protein) were: serum, 26; liver, 30; muscle, 11. If incorporation of the labelled amino-acid can be taken as evidence of protein synthesis, it follows that the synthetic machinery was still working. Moreover, the specific activity of the plasma proteins was similar to that found at the same time interval after injection in infants who recovered (Garrow, 1957). If these preliminary results are representative, failure of protein synthesis cannot be considered the cause of death.

The infants who did not die were for the most part given protein at the level of about 2 g (= 0·3 g N)/kg/day. On this intake about 0·15 g N/kg/day was retained which, if the N content of fat-free tissue is taken as 3%, would correspond to a tissue increment of 5 g/kg/day, or 150 g/kg/month. A normal child 9 months old, growing normally, would weigh perhaps 8·5 kg, and would be gaining about 500 g, or 60 g/kg/month. If one-sixth of this gain is assumed to be fat, there would be a tissue increment of 50 g/kg/month. It appears, therefore, that the undernourished baby is laying down tissue at about three times the rate of the normal baby. It is also gaining weight at more than three times the normal rate. It is doing these things on an intake of about 2 g protein/kg/day, which is undoubtedly much lower than that normally received by a baby in Europe or North America.

Two questions arise from this point, First, it is shown in Fig. 2, and even more clearly by the work of Cravioto (1958), of Holemans & Lambrechts (1955), and of Pretorius & Smit (1958), that N retention increases linearly with intake up to at least 0·5–0·6 g N/kg/day. At higher intakes the efficiency is less, but increased absolute amounts of N are still retained. Kaye, Caughey & McCrory (1954) fed protein to a

group of undernourished infants at the level of 1·0 g N/kg/day; 0·235 g N/kg/day was retained, and 11·8 g/kg/day of weight were gained. These gains are only 1½ times those of our infants, on an intake three times as great. Nevertheless, it is clear that on a daily intake of 0·3–0·4 g N/kg the baby has by no means reached the limit of its power to utilize N and synthesize protein. The problem is, does it do any good to push the organism to its limit, and to give the largest possible amount of protein? This is clearly an important question, and one that is difficult to answer. Dean & Schwartz (1953) found transient rises in blood urea in patients with kwashiorkor at the beginning of treatment with a high-protein diet. On general grounds we consider it wiser to be content with moderate gains, and not to risk overloading the mechanisms of synthesis and excretion. Clinically our impression is that the results obtained in this series, on protein intakes of about 2·5 g/kg/day, are in no way worse than those achieved in the past on intakes nearly twice as great. However, precision in any such comparison is very difficult to achieve.

The second question relates to the problem of requirements. Admittedly, in the malnourished baby the efficiency of protein utilization falls off as the stores are repleted; nevertheless, if utilization can occur at three times the normal rate on an intake of 2 g protein/kg/day, it suggests that the figure of 1·5 g/kg/day put forward by FAO (Waterlow & Stephen, 1957; FAO, 1957) as the requirement for normal growth and maintenance at 1 year is more realistic than the older recommendations of 3–3·5 g/kg/day.

It is perhaps surprising that not much attention has been paid so far to P metabolism in undernourished infants. Holemans et al. (1955) observed that the ratio of urinary to faecal P was low initially, and rose with treatment. They did not, however, express their results in terms of P balances. The large retentions of P found with most of our patients fit in with other evidence of P depletion. We have found that in muscle specimens taken by biopsy from these babies the P content is sometimes low (Waterlow & Mendes, 1957). Serum levels of inorganic P also tend to be low, in the region of 1·5–3 mg/100 ml, compared with the normal range in infancy of 5–7 mg/100 ml. This reduction in serum phosphate has also been noted in Guatemala (Béhar, Arroyave, Tejada, Viteri & Scrimshaw, 1956) and in the Belgian Congo (Dricot, Beheyt & Charles, 1951). In our experience, the fall in serum inorganic P is not accompanied by parallel changes in the esterified P or phospholipid P of either serum or whole blood.

As Table 5 shows, the ratio of P to N retained was higher, particularly in the early stages, than the ratio of P to N in normal tissues. It is true that muscle was even more depleted of P than of N, yet it is difficult to believe that all the extra P retained was taken up by the soft tissues. It seems likely that some of it went into bone. Rarefaction of the bones is a well-recognized characteristic of protein malnutrition. Moreover, many authors have reported a large rise in serum alkaline phosphatase on treatment (Schwartz, 1956). This rise has been observed in our patients also.

We conclude that these malnourished babies are often severely depleted of P as well as of N and of potassium. This depletion probably affects the bones, as well as the soft tissues and serum. It is not the result of excessive loss, e.g. from overactivity

of the parathyroids, because on low P intakes the urine may contain virtually no phosphate. The part played by P depletion in the symptomatology of protein malnutrition is a matter for further investigation.

SUMMARY

1. Nitrogen and phosphorus balances were done on thirty-seven severely malnourished babies, of whom five died. All were treated with either a cow's-milk mixture or human milk. The results have been analysed in relation to the stage of treatment.

2. At all stages of treatment over 80 % of ingested N was absorbed. The corrected absorption, with allowance for faecal metabolic N, was more than 90 %, even in the first days after admission to hospital.

3. On N intakes up to 0·5 g/kg/day, more than 50 % of ingested N was retained. When a correction was applied for endogenous N loss, it was found that N retention progressively fell off as the protein stores were built up.

4. About two-thirds of ingested P were absorbed. Absorption did not vary with the stage of treatment.

5. P retention, like N retention, diminished in the later stages of treatment.

6. In general, more P was retained in relation to N than would be expected from the ratio of these elements in normal muscle. This finding fits in with other evidence of P depletion in these infants. It is suggested that some of the 'extra' P retained may have been taken up by bone.

7. In the five babies who died, absorption of both N and P was satisfactory. All the subjects were in positive balance for both N and P at the time of death, which suggests that death was not caused by an irreversible failure of N utilization.

8. Most of the babies who did not die were treated with cow's-milk mixtures that supplied only 0·3–0·4 g N/kg/day. These intakes were lower than those often used for the treatment of protein malnutrition. At this level of protein intake N retention and gain in body-weight both went on at about three times the rate found in normal infants of the same age. It follows that if such intakes are adequate for repair, they should also be adequate for normal growth and maintenance.

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