Reasons why hypoalbuminaemia may or may not appear in protein-energy malnutrition

By W. A. COWARD, R. G. WHITEHEAD AND P. G. LUNN

Dunn Nutrition Unit, University of Cambridge and Medical Research Council, Cambridge CB4 1XJ

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1. Investigations have been carried out in experimentally-malnourished rats in an attempt to explain the reasons for the development of the two main forms of protein-energy malnutrition in children, kwashiorkor and marasmus.

2. Isoenergetic diets with values for protein:energy (P:E) of 0·21 (control diet; C) 0·032 (low-protein diet; LP) and 0·005 (very-low-protein diet; VLP) were fed to groups of twenty-six rats either *ad lib.* or in restricted amounts from 5 weeks of age. Rats were killed at the start of the experiment and 1, 2 and 3 or 4 weeks later. Estimations were made of plasma albumin, insulin, corticosterone and amino acid concentrations and of the total protein content of the gastrocnemius muscles and liver.

3. Rats given diet LP *ad lib.* gained weight slowly and by week 1 plasma albumin concentration was slightly reduced. Rats given diet VLP *ad lib.* gradually lost weight and plasma albumin concentrations decreased continuously.

4. In contrast the major effect of dietary restriction during the first 2 weeks of the experiment was to maintain plasma albumin concentrations at normal values, irrespective of the diet given.

5. At later stages, however, when the 'restricted' animals had become very severely wasted, albumin concentrations decreased rapidly to values approaching those found in rats given diet VLP *ad lib.*

6. When diets LP and VLP were given *ad lib.* body protein was proportionally distributed in favour of muscle rather than liver. For 'restricted' rats the reverse was true, at least up to the time when plasma albumin concentration began to decrease.

7. Plasma corticosterone concentrations increased and insulin concentrations decreased when diets LP and VLP were fed in both an *ad lib.* and a 'restricted' regimen but the effects were significantly greater in the latter situation.

8. *Ad lib.* feeding of diets LP and VLP produced a distorted plasma amino acid pattern resembling that of kwashiorkor, but although dietary restriction resulted in a decrease in total amino acid concentration, the plasma concentration ratio, non-essential amino acids:essential amino acids was virtually unaffected.

9. It was concluded that whilst the lower the protein concentration in the diet the greater is the extent of hypoalbuminaemia which develops, dietary restriction with an increase in plasma glucocorticoid concentration and body-wasting can initially delay the development of the hypoalbuminaemia. However, in the final stages of wasting which ensue, low plasma albumin concentrations can appear because of a failure of the mechanisms which had earlier been able to preserve them at normal levels. It is possible that these two separate and distinct routes to hypoalbuminaemia observed in this study may have parallels in human situations in developing countries.

It is recognized that, for clinical purposes, it is possible to distinguish two basic forms of protein-energy malnutrition, kwashiorkor and marasmus (*Lancet*, 1970). The presence of oedema in kwashiorkor is considered the crucial difference, but it is also possible to make distinctions at a biochemical level. Severe hypoproteinaemia and hypoalbuminaemia are universally found in kwashiorkor and are significant determinants in the development of oedema (Coward, 1975), but in severely-wasted marasmic children plasma protein and albumin concentrations can be relatively normal.

These and other differences in the pathophysiology of kwashiorkor and marasmus have been taken to indicate separate dietary aetiologies, namely that kwashiorkor results from a diet which is primarily inadequate in protein but may provide a sufficiency of energy, whilst marasmus is the product of energy deficiency in what could otherwise be a balanced diet; these ideas have been reviewed by Brock & Autret (1952), McCance & Widdowson (1966) and Annegers (1973).

More recent work, however, has indicated that not all cases of kwashiorkor and marasmus can be explained so simply. Gopalan (1968), for example, has shown that in Indian children there need be no essential difference between the values for protein:energy (P:E) in the diets consumed by those who develop kwashiorkor and those who become marasmic. The two syndromes can apparently appear in children for whom there are no demonstrable differences in dietary protein content. This view has since received wide support (Arroyave, 1975; Scrimshaw, 1975*a*; Waterlow, 1975).

A number of workers have introduced hypotheses to reconcile these opposing views. An interpretation of the situation in an area of Uganda where kwashiorkor was most frequently encountered was proposed by Whitehead (1971) and discussed further by Whitehead & Alleyne (1972). It was suggested that the hypoalbuminaemia accompanying the development of kwashiorkor in that country resulted from the high carbohydrate content of the staple foods which, by stimulating insulin release caused a preferential distribution of the limited amino acid supply in favour of muscle rather than liver. If the intake of food happened to be reduced however, because of food shortage, anorexia or illness, it was postulated that the hormonal balance would become reversed and high plasma cortisol concentrations would induce muscle-wasting. Amino acids thus made available might allow a more adequate rate of hepatic albumin synthesis and plasma albumin concentration might be maintained even though body-wasting now became clinically apparent. The final result, if this continued for long enough, would be the appearance of marasmus rather than kwashiorkor. Although Lunn, Whitehead, Hay & Baker (1973) subsequently showed that alterations in hormonal balance of this type did occur in Ugandan children, it was never definitely established that they could be responsible for a change in the metabolic response from one leading to kwashiorkor to one that would result in marasmus.

It was recognized, however, that even if such a mechanism were able to limit the appearance of hypoalbuminaemia this situation might not be sustained indefinitely and thus even in marasmus, hypoalbuminaemia, to some extent, might eventually appear. Thus, conceivably, more than one route to hypoalbuminaemia and hence oedema could exist: either a gradual depletion of plasma albumin concentration arising as an uncompensated response to a poor diet or a more rapid decrease caused by the ultimate failure of the metabolic mechanisms which had been able to maintain plasma albumin concentrations.

The aim of this study was to substantiate these various concepts by studying young rats given diets differing in their P:E values, with and without dietary restriction, for increasing periods.

EXPERIMENTAL

Animals and diets

Male hooded rats from a specific pathogen-free colony were used in the experiment. The diets fed to these animals were the control (C) and low-protein (LP) diets described by Lunn, Whitehead & Baker (1976) which contained 0·210 and 0·032 of the metabolizable energy as protein respectively; in a third very-low-protein diet (VLP) even more protein was removed and replaced on a weight basis by equal amounts of starch and sucrose to give a value for P:E of 0·005. Mineral and vitamin supplements in each diet were as described by Lunn, Whitehead & Baker (1976) with the addition of 3 mg pteroylmonoglutamic acid/kg diet. The diets were virtually isoenergetic containing 16·60, 16·64 and 16·66 MJ/kg in diets C, LP and VLP respectively.

Experimental design

The rats were all weaned, at 3 weeks of age, onto diet C. When they were 5 weeks old, seven animals were killed for analysis and from the remaining 156 rats, pairs of littermates were randomly placed on one of the three diets. One animal from each pair received

the diet *ad lib.* and the dietary intake of the other was restricted. Mean body-weights and mean food intakes (g/kg body-weight) were calculated daily for the animals in each of the *ad lib.* feeding groups; rats in the 'restricted' groups were also weighed daily and given (g/kg mean body-weight) half the intake of the corresponding *ad lib.* groups. The animals were subjected to a 12 h light-12 h dark, lighting regimen with the light period from 0·7.30 hours–19.30 hours. They were always killed between 10.00 hours and 12.00 hours and were not subjected to an overnight fast.

Analytical techniques

The animals were anaesthetized with diethyl ether and blood samples, taken by cardiac puncture, were collected into heparized syringes. The liver and gastrocnemius muscles from each animal were also removed and weighed. Plasma samples and tissues were stored at $-20°$ until analysed.

Plasma albumin concentration was estimated using an AutoAnalyzer (Model AA II; Technicon Instruments Co. Ltd, Basingstoke, Hants). The method was a dye-binding procedure employing bromocresol green (Northam & Widdowson, 1967; Technicon Instruments Co. Ltd, 1972). The routine standard used was a human control serum (Q-PAK^R Chemistry Control Serum 1; Travenol Laboratories, Thetford), but rat albumin has a different binding capacity for bromocresol green as compared with human albumin and appropriate corrections were made using a factor derived from separate albumin determinations, using bromocresol green, in serial dilutions of a freeze-dried rat serum preparation (Miles Laboratories, Stoke Poges, Bucks) in which the albumin concentration had been determined by electrophoresis. Plasma corticosterone concentrations were measured by an adaptation of the protein-binding radioassay described by Murphey (1967) and insulin was estimated using the radioimmunoassay method 'C' of Hales & Randle (1963), with materials supplied in kit form by the Radiochemical Centre, Amersham, Bucks. Amino acid concentrations were estimated using an AutoAnalyzer (TSM amino-acid analyser; Technicon Instruments Co. Ltd).

The protein content of liver and muscle was determined as described by Lowry, Rosebrough, Farr & Randall (1951).

Statistical methods

Statistical significance was assessed by Student's *t* test using log values because standard deviations in the different groups were closely proportional to mean values for the groups. An estimate of standard deviation was obtained by pooling the separate group values for standard deviations.

RESULTS

It had been intended, at the start of the experiment, to kill seven animals from each group at weeks 1, 2 and 4 and to kill the remaining five in each group at a later stage. There was, however, a high mortality among the 'restricted' rats remaining after week 2. Therefore at week 3 only six animals remained in the group receiving restricted amounts of diet VLP and they were killed at this stage. By week 4, four of the animals receiving restricted amounts of diet C had also died and the remaining eight were killed together with seven animals from each of the other groups. At this stage the experiment was terminated.

Voluntary food intakes and dietary restriction

Energy intakes calculated from food consumption for the preceding week for each group of animals killed are shown in Table 1. Intakes within the *ad lib.* or 'restricted' groups were not exactly identical but in relation to the amounts of restriction imposed these differences were small.

Table 1. *Energy intakes (MJ/kg body-weight per d) in rats fed* ad lib. *or restricted amounts of diets with protein:energy values of* 0.21 *(diet C),* 0·032 *(diet LP) or* 0·005 *(diet VLP)*

(Mean values with their standard errors, calculated from the intakes the preceding week for seven animals in each group, except for restricted rats given diets C and VLP at 3 or 4 weeks in which there were eight and six animals respectively; for details see p. 117)

	Diet					
	C		LP		VLP	
Feeding regimen	Mean	SE	Mean	SE	Mean	SE
			Week 1			
Ad lib.	1·98	0·06	2·01	0·08	2·12	0·08
Restricted	0·94	0·02	0·98	0·03	0·83	0·02
			Week 2			
Ad lib.	1·73	0·03	1·91	0·04	1·62	0·03
Restricted	0·90	0·01	0·92	0·01	0·87	0·02
			Weeks 3 or 4			
Ad lib.	1·44	0·04	1·80	0·05	1·45	0·04
Restricted	0·76	0·04	0·88	0·02	0·70	0·04

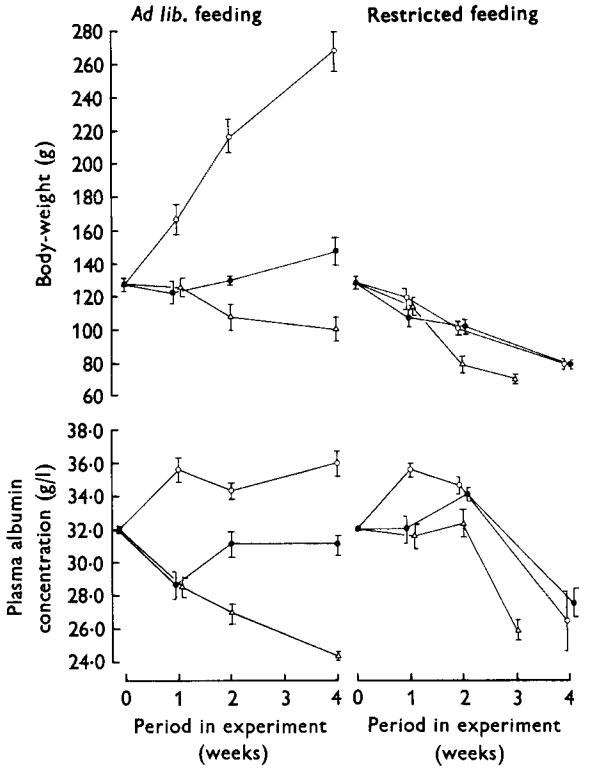

Fig. 1. Body-weight (g) and plasma albumin concentrations (g/l) in rats fed *ad lib.* or restricted amounts of diets with protein:energy values of 0·21 (○, diet C), 0·032 (●, diet LP), or 0·005 (△, diet VLP). Values are means with their standard errors, for seven animals in each group, except for 'restricted' rats given diets C and VLP at 3 or 4 weeks in which there were eight and six animals respectively; for details see p. 117.

Table 2. *Paired gastrocenemius muscle weight* (g) *and protein content* (*mg*) *in rats fed* ad lib. *or restricted amounts of diets with protein:energy values of* 0·21 (*diet C*), 0·032 (*diet LP*) *or* 0·005 (*diet VLP*)

(Values are Means values with their standard errors, for details of numbers of animals in each group see Table 1)

		Diet					
		C		LP		VLP	
	Feeding regimen	Mean	SE	Mean	SE	Mean	SE
		Week 0					
Weight	*Ad lib.*	1·46	0·06				
Protein content	*Ad lib.*	221	16				
		Week 1					
Weight	*Ad lib.*	1·76	0·11*	1·52	0·10	1·55	0·12
	Restricted	1·61	0·07	1·40	0·09	1·51	0·07
Protein content	*Ad lib.*	266	16	232	14	235	21
	Restricted	213	9†	163	$12^{***}_{†††}$	189	12†
		Week 2					
Weight	*Ad lib.*	2·65	0·14***	1·58	0·04	1·40	(0·10
	Restricted	1·40	0·05†††	1·40	0·06	1·03	$0·09^{***}_{†††}$
Protein content	*Ad lib.*	377	30***	322	9***	293	13**
	Restricted	224	11†††	148	$10^{***}_{†††}$	96	$8^{***}_{†††}$
		Weeks 3 or 4					
Weight	*Ad lib.*	3·34	0·16***	1·71	0·11	1·28	0·07
	Restricted	0·93	$0·04^{***}_{†††}$	0·96	$0·05^{***}_{†††}$	0·85	$0·05^{***}_{†††}$
Protein content	*Ad lib.*	544	27***	286	15*	205	10
	Restricted	110	$8^{***}_{†††}$	127	$5^{***}_{†††}$	120	$22^{***}_{†††}$

Values significantly different from those at week 0: * $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$.
Values for restricted groups significantly different from those for corresponding groups fed *ad lib.*: † $P < 0·05$; †† $P < 0·01$; ††† $P < 0·001$.

Body-weight and plasma albumin concentrations

The effects of the various dietary regimens on body-weight and plasma albumin concentration are shown in Fig. 1.

The animals with unrestricted access to diet C showed the expected increases in body-weight and plasma albumin concentration. Rats given diet LP *ad lib.* showed small increases in body-weight, but after 1 week plasma albumin concentrations were significantly less than those at week 0 ($P < 0·01$); thereafter, although no further decrease occurred, at every stage in the experiment they were less than plasma albumin concentrations found in rats given diet C. Rats receiving diet VLP *ad lib.* slowly lost weight during the experiment and plasma albumin concentrations were also found to decrease progressively, differences were always significant compared to values at week 0 ($P < 0·01$ at week 1, $P < 0·001$ at weeks 2 and 4).

In contrast, although dietary restriction produced weight loss in all animals, irrespective of the diet fed, plasma albumin concentrations were maintained at, or above, starting values for the first 2 weeks of the experiment. During this period rats given restricted amounts of diet LP and VLP always had significantly higher albumin concentrations than those given the same diets *ad lib.* (for rats given diet LP: $P < 0·01$ at week 1, $P < 0·05$ at week 2; for rats given diet VLP: $P < 0·05$ at week 1, $P < 0·001$ at week 2).

Table 3. *Liver weight (g) and protein content (mg) in rats fed* ad lib. *or restricted amounts of diets with protein:energy values of* 0·21 *(diet C),* 0·032 *(diet LP) or* 0·005 *(diet VLP)*

(Mean values with their standard errors; for details of numbers of animals in each group see Table 1)

		Diet					
		C		LP		VLP	
	Feeding regimen	Mean	SE	Mean	SE	Mean	SE
		Week 0					
Weight	*Ad lib.*	6·81	0·25				
Protein content	*Ad lib.*	1141	57				
		Week 1					
Weight	*Ad iib.*	8·30	0·39**	5·42	0·39***	5·26	0·16***
	Restricted	4·15	0·19***†††	3·58	0·15***†††	3·54	0·14***†††
Protein content	*Ad lib.*	1345	49*	812	57***	788	37***
	Restricted	874	41**††	737	39***	737	19***
		Week 2					
Weight	*Ad lib.*	9·92	0·41***	5·30	0·22***	4·29	0·33***
	Restricted	3·03	0·09***†††	3·19	0·14***†††	2·39	0·08***†††
Protein content	*Ad lib.*	1798	94***	878	27***	611	11***
	Restricted	741	23***†††	750	23*††	541	25*†**
		Weeks 3 or 4					
Weight	*Ad lib.*	10·15	0·52***	6·21	0·36	3·87	0·29***
	Restricted	2·06	0·10***†††	2·19	0·10***†††	2·56	0·13***††
Protein content	*Ad lib.*	1588	66***	839	52***	534	33***
	Restricted	421	30**††	536	24***†††	434	20***†††

Values significantly different from those at week 0: * $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$.
Values for restricted groups significantly different from those for corresponding groups fed *ad lib.*: † $P < 0·05$; †† $P < 0·01$; ††† $P < 0·001$.

At the later stages in the experiment, when the condition of the 'restricted' animals had deteriorated considerably, reductions in albumin concentration were found. Thus at week 3 in the rats receiving restricted amounts of diet VLP differences compared to week 0 were significant ($P < 0·001$) and at week 4 concentrations in rats given restricted amounts of diets C and LP were also low and significantly different from those at week 0 ($P < 0·001$).

Protein content of liver and gastrocenemius muscles

Details of changes in paired gastrocenemius muscle and liver weights, together with their protein contents, are shown in Tables 2 and 3. Total muscle protein content in the *ad lib.* groups was always higher than that in any of the restricted groups. In animals given diets LP and VLP, there was no significant net loss of muscle protein. At weeks 2 and 4 in rats given diet LP *ad lib.*, and at week 2 in animals given diet VLP *ad lib.*, the protein content was significantly higher than starting values. In all 'restricted' rats, however, muscle protein content was eventually reduced.

All malnourished animals had lower total liver protein contents than at the start of the experiment and while liver weights were also reduced with both *ad lib.* and 'restricted' feeding, this effect was much more marked with the latter treatment.

The fundamental difference in the response of muscle and liver, in terms of protein loss or accumulation, to *ad lib.* and 'restricted' feeding is illustrated in Fig. 2, where total organ

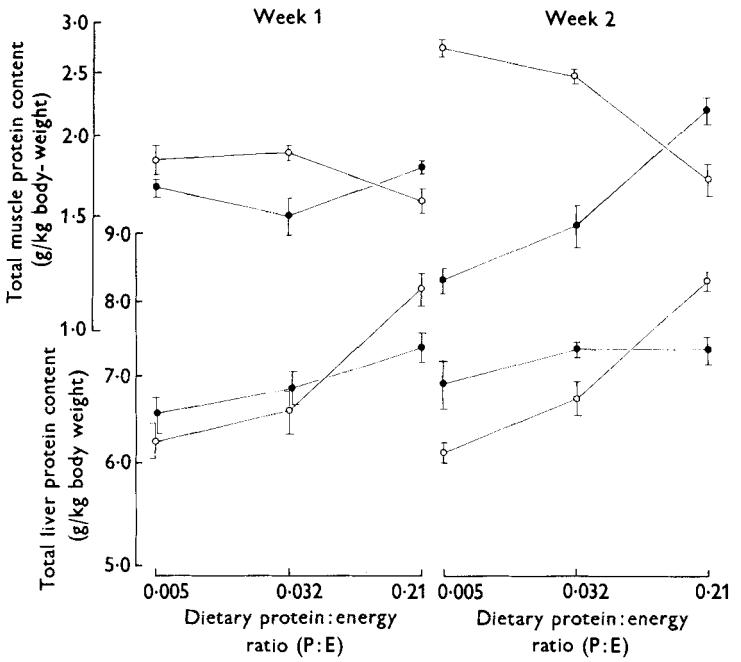

Fig. 2. Relationships between log values for protein:energy (P:E) of diets fed *ad lib.* (○), or in restricted amounts (●), and log values for muscle (g) and liver protein (g) content (/kg body-weight) after 1 and 2 weeks of the dietary treatments. Mean values for seven animals in each group except for 'restricted' rats given diets C and VLP at 3 or 4 weeks in which there were eight and six animals respectively; for details see p. 117.

protein content has been expressed on the basis of body-weight. While relationships could be derived between P:E values of the diets and muscle and liver protein content expressed on a per kg body-weight basis with both *ad lib.* and 'restricted' feeding, the slopes of the regression lines were quite different. With *ad lib.* feeding reductions in values for P:E increased the protein content of muscle on a per kg body-weight basis but decreased liver protein content. For dietary restriction the situation was reversed for muscle protein content and reductions in values for P:E had little effect on liver protein content.

The results could be combined by introducing interaction terms into the regression equations, so that the regression slopes for muscle or liver protein content *v.* P:E changed with energy intake. The significance of the interaction terms were similar for muscle and liver protein at week 1 (t 2·6, $P < 0{\cdot}02$), but at week 2 when both interaction terms were greater, the term for muscle protein was more highly significant (t 8·0, $P < 0{\cdot}001$ for muscle protein; t 3·7, $P < 0{\cdot}001$ for liver protein).

Plasma hormone concentrations

The mean plasma insulin and corticosterone concentrations in the different dietary groups are shown in Table 4. Plasma insulin concentration in the rats given diet C *ad lib.* decreased with age but corticosterone concentrations tended to increase.

The general effect of feeding diets LP and VLP both in *ad lib.* and in restricted amounts was to reduce plasma insulin concentrations, but restriction tended to produce the greater reduction. In contrast, corticosterone concentrations were higher in both the *ad lib.*-fed, LP and VLP groups and in 'restricted' animals, compared with animals given diet C but, as with the effects on insulin concentration, dietary restriction resulted in a greater effect.

Table 4. *Plasma insulin (μU/ml) and corticosterone (μg/l) concentrations in rats fed* ad lib. *or restricted amounts of diets with protein:energy values of* 0·21 *(diet C),* 0·032 *(diet LP) or* 0·005 *(diet VLP)*

(Mean values with their standard errors; for details of numbers of animals in each group see Table 1)

		Diet					
		C		LP		VLP	
	Feeding regimen	Mean	SE	Mean	SE	Mean	SE
				Week 0			
Insulin	*Ad lib.*	91·2	11·5				
Corticosterone	*Ad lib.*	29	8				
				Week 1			
Insulin	*Ad lib.*	86·0	21·7	21·3	4·3***	16·8	5·9***
	Restricted	13·3	3·0***†††	16·9	5·8*†**	10·8	2·3***
Corticosterone	*Ad lib.*	46	17	78	16*	154	37***
	Restricted	240	25***†††	160	18***††	166	34***
				Week 2			
Insulin	*Ad lib.*	72·5	11·4	19·3	2·8***	12·2	0·8***
	Restricted	11·9	1·3***†††	14·6	2·6***	9·8	0·7***
Corticosterone	*Ad lib.*	41	11	115	30**	133	33**
	Restricted	249	53**†††	195	5***	245	39***
				Weeks 3 or 4			
Insulin	*Ad lib.*	51·3	5·1**	20·3	3·4***	6·7	1·0***
	Restricted	7·0	0·8***†††	4·8	0·8***†††	5·6	0·6***
Corticosterone	*Ad lib.*	68	18	194	40***	179	32***
	Restricted	242	43***†††	192	45***	167	14***

Values significantly different from those at week 0: * $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$.
Values for restricted groups significantly different from those for corresponding groups fed *ad lib.*: † $P < 0·05$; †† $P < 0·01$; ††† $P < 0·001$.

Plasma amino acids

The pattern of concentration of plasma amino acids is dependent on various factors including changes in the dietary supply of amino acids, the relative amounts of dietary energy and on hormonal balance. This has been reviewed by Lunn *et al.* (1973). It was of interest, therefore, to study plasma amino acid patterns associated with the various dietary manipulations and the changes which occurred in hormonal balance.

Total, essential and non-essential plasma amino acid concentrations and values for the concentration ratio, non-essential amino acids:essential amino acids (N:E) are shown in Table 5. Essential and non-essential amino acids were classified according to the criteria described by Lunn, Whitehead & Baker (1976).

The early effects of the various dietary regimens on plasma amino acid concentrations are illustrated by the values for week 2. Feeding diets LP and VLP *ad lib.* produced a decrease in the concentration of essential amino acids which became greater with the lower-protein diet (VLP). The concentration of the non-essential amino acids was unaffected by these dietary changes and thus values for N:E increased.

On the other hand, restriction of food intake resulted in a large reduction in the concentration of both the non-essential and essential amino acids and the net effect was that the value for N:E was not significantly different from normal even in animals given diets LP

Table 5. *Plasma total, non-essential (N) and essential (E) amino acid concentrations (mM/l) and non-essential amino acids:essential amino acids (N:E) in rats fed* ad lib. *or restricted amounts of diets with protein:energy values of* 0·21 *(diet C),* 0·032 *(diet LP) or* 0·005 *(diet VLP)*

(Means values with their standard errors; for details of animals in each group see Table 1)

	Feeding regimen	Diet: Mean (C)	SE (C)	Mean (LP)	SE (LP)	Mean (VLP)	SE (VLP)
Total	*Ad lib.*	4·45	0·23	3·14	0·17***	3·20	0·19***
	Restricted	2·55	0·10***†††	2·85	0·12***	2·44	0·10***††
				Week 2			
N	*Ad lib.*	1·68	0·15	1·57	0·08	1·80	0·09
	Restricted	0·92	0·05***†††	1·20	0·10*†	0·81	0·06***†††
E	*Ad lib.*	2·78	0·10	1·57	0·10***	1·40	0·12***
	Restricted	1·63	0·08***†††	1·65	0·05***	1·63	0·10***
N:E	*Ad lib.*	0·60	0·04	1·01	0·03***	1·31	0·07***
	Restricted	0·57	0·03	0·73	0·06†††	0·51	0·05†††
				Weeks 3 or 4			
Total	*Ad lib.*	4·31	0·08	3·10	0·19***	1·80	0·15***
	Restricted	2·20	0·31***†††	2·19	0·07***†††	1·87	0·11***
N	*Ad lib.*	1·62	0·06	1·61	0·08	1·14	0·14***
	Restricted	0·69	0·06***†††	0·86	0·11***†††	0·99	0·05***
E	*Ad lib.*	2·69	0·04	1·49	0·10***	0·66	0·08***
	Restricted	1·51	0·25***†††	1·32	0·06***	0·88	0·07***
N:E	*Ad lib.*	0·53	0·03	1·21	0·14***	1·63	0·22***
	Restricted	0·48	0·03	0·68	0·11†††	1·14	0·07***

Values significantly different from those for rats given diet C *ad lib.* and killed at the same stage in the experiment: * $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$.

Values for restricted groups significantly different from those for corresponding groups fed *ad lib.*: † $P < 0·05$; †† $P < 0·01$; ††† $P < 0·001$.

and VLP. Thus a normal pattern of plasma amino acids as well as a normal albumin concentration was produced by dietary restriction.

Continued feeding of diet LP *ad lib.* had no further significant effect on the concentration of essential amino acids and the low values found after 2 weeks were maintained at week 4; likewise non-essential amino acids remained unaffected in concentration and thus the extent of distortion in the plasma amino acid pattern as measured by N:E was the same. With diet VLP, however, there was a considerable further reduction in essential amino acid concentration and also, to a lesser extent, in non-essential amino acids. The N:E values became even higher.

Restricting diet LP still produced a normal N:E value at week 4, as it had done at week 2, but in the animals given restricted amounts of diet VLP the value was no longer maintained at week 3. At this stage animals in this group were severely ill, had low plasma albumin concentrations and, indeed, some had died.

DISCUSSION

The importance of the interaction between the dietary intake of protein and energy has been recognized for some time. Diets high in energy content but low in protein can simultaneously produce an over-all improvement in nitrogen balance but still have an adverse effect on liver protein content (Munro, 1964). The present results illustrate the consequence of this phenomenon in relation to the development of hypoalbuminaemia in rats given the diets LP and VLP *ad lib.* In the first 2 weeks of the experiment, *ad lib.* feeding resulted in a proportional distribution of body protein in favour of muscle rather than liver and plasma albumin concentrations decreased. With dietary restriction, muscle protein was not preferentially protected and this may have resulted in plasma albumin concentrations being maintained despite an over-all loss of weight.

Changes in the total protein content of tissues indicate the long-term effects of the balance between rates of protein synthesis and breakdown, thus in the muscles of growing animals the former exceeds the latter and protein deposition occurs. Millward, Garlick, Nnanyelugo & Waterlow (1976) studied the effects on protein synthesis of several treatments that might have been expected to produce growth suppression or muscle-wasting and found that although reductions in rates of synthesis of muscle protein usually occurred there was generally a decrease in the rate of breakdown that was entirely compensatory and there was no net loss of protein. This situation was maintained even in rats given a protein-free diet for 9 d, a dietary regimen that was more severe in terms of weight loss than any of the *ad lib.* regimens used in the present experiments. On the other hand, the severe stress of starvation for 4 d, or feeding a protein-free diet for 30 d, or the injection of glucocorticoids were the only treatments that resulted in uncompensated reductions in rates of protein synthesis and net catabolism. It is likely that changes of this type were responsible for loss of muscle protein in the severely-restricted rats studied in the experiments described here, when plasma corticosterone concentrations were often higher than those found in rats given the diets *ad lib.*

Garlick, Millward, James & Waterlow (1975) have recently shown that in its response to nutritional stress the liver does not behave in the same way as muscle. When protein-free diets were fed to rats there was an immediate loss of protein with increases in rates of synthesis and breakdown. Initially rates of breakdown considerably exceeded rates of synthesis, but with prolonged deprivation, although rates of synthesis and breakdown remained high, the imbalance and rate of net catabolism became very much reduced; this was at a time when protein loss from the muscle began to occur. Since in the present experiments there appeared to be inverse relationships between the effects of protein or energy malnutrition on liver and muscle, the release of amino acids from muscle during protein breakdown in that tissue could conceivably allow increased rates of liver protein synthesis. Evidence for a central role of glucocorticoids in this process has been provided by Lunn, Whitehead, Baker & Austin (1976), who injected cortisone acetate into protein-deficient rats and produced muscle-wasting, deposition of protein in the liver and increased plasma albumin concentrations.

Results obtained from children (Lunn *et al.* 1973) have implicated increased plasma insulin concentration as a possible cause of preferential shunting of available amino acids towards muscle rather than liver when staple diets are relatively high in carbohydrate content. In the present work plasma insulin concentrations were not increased when diets LP and VLP were fed *ad lib.* and therefore the significance of this effect in rats must be open to question. However, restricted feeding of diets LP and VLP often produced further reductions in insulin concentration and this would at least enhance the effect of the high corticosterone concentrations on net muscle protein catabolism.

Within the limits imposed by the measurement of plasma albumin concentration and its indirect links with total body albumin mass (Coward & Sawyer, 1977) it is possible to conclude that when loss of muscle protein occurred in the 'restricted' rats during the first 2 weeks of the experiments, rates of albumin synthesis and breakdown were in balance; this could not have been the situation in the rats given diets LP and VLP *ad lib.*, when albumin concentrations decreased. Evidence exists that the pattern as well as the availability of amino acids is important (Kirsch, Saunders, Frith, Wicht & Brock, 1969; Rothschild, Oratz, Mongelli, Fishman & Schreiber, 1969) and the grossly distorted plasma amino acid pattern found in rats fed diet VLP *ad lib.* might have been incompatible with an adequate rate of albumin synthesis.Restriction of the same diet, however, restored the balance between plasma non-essential and essential amino acids, and although the over-all concentration of amino acids was reduced rates of albumin synthesis must have been sufficient to maintain plasma albumin concentration.

These results are almost certainly relevant to the aetiology of different types of protein-energy malnutrition in children since two apparently contradictory sets of observations must be reconciled in order to explain why kwashiorkor predominates in some circumstances but in others the main form of protein-energy malnutrition is marasmus. The present experiments demonstrated clearly that dietary restriction, producing a marked increase in plasma corticosterone concentration, had a powerful effect on the kwashiorkor-like response that otherwise occurred in animals fed diets LP and VLP *ad lib.* In children, dietary restriction *per se* need not, however, be the only stimulus for increased plasma glucocorticoid concentrations. Lunn *et al.* (1973) have emphasized the significance of stress, in particular that associated with frequent infections. Taking into account the continuous interplay between infection and dietary intake (Whitehead & Alleyne, 1972; Scrimshaw, 1975*b*) it is not surprising that often it is impossible to establish, especially retrospectively, a dietary difference between children who present with kwashiorkor and those with marasmus.

It is clear, nevertheless, that if rats are fed *ad lib.* the lower the value for P:E of the diet the greater is the extent of hypoalbuminaemia that gradually develops. Ultimately rats given diets with a value for P:E of 0·005 develop oedema (Edozien, 1968; Enwonwu & Sreebny, 1971; Philbrick & Hill, 1974; Anthony & Edozien, 1975; Coward, unpublished observations) and analogous situations do exist in areas where kwashiorkor is commonplace.

These considerations apart, our results illustrate that there is more than one route to a condition of hypoalbuminaemia in protein-energy malnutrition and our findings could explain why one encounters oedematous malnutrition in areas of the world in which marasmus would be the form most frequently expected. Plasma albumin concentrations did eventually decrease rapidly in animals given restricted amounts of the diets. This final pathological event in a disease course previously characterized by severe body-wasting but the maintenance of plasma albumin concentrations is similar to findings at present being obtained in an epidemiological study in The Gambia.

There are various reasons why the processes of adaptation which have maintained plasma albumin concentration may no longer be able to cope in a state of advanced malnutrition (and their relative importance both in the animal and human situation is being studied): (*a*) muscle-wasting may be so extensive that this tissue can no longer provide an adequate source of amino acids for the hepatic synthesis of albumin; (*b*) liver metabolism may have become so deranged that it no longer has an adequate synthetic ability; (*c*) since the concentration of vascular albumin is normally buffered in protein-energy malnutrition by the extravascular component principally in muscle and skin (Coward & Sawyer, 1977) the reduced amounts of such tissues might limit this capacity; (*d*) in children there can be an actual loss of albumin, for example into the gut following measles (Axton, 1975; Dossetor & Whittle, 1975).

In conclusion it must be stressed that the reasons for the appearance or non-appearance of hypoalbuminaemia and hence the different forms of protein-energy malnutrition are many. Consideration of just one single factor, such as the composition of the diet, is likely to be misleading when other factors can substantially modify the individual's response. In this study some of these variables have been reproduced and their interactions investigated. In the case of children, especially when malnutrition is viewed in a world context, the situation is even more complex; protein-energy malnutrition is an environmental syndrome, as the environment varies so can the final form of the disease which ultimately presents.

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REFERENCES

Annegers, J. F. (1973). *Ecol. Fd Nutr.* **2**, 225.
Anthony, L. E. & Edozien J. C. (1975). *J. Nutr.* **105**, 631.
Arroyave, G. (1975). In *Protein Calorie Malnutrition*, p. 343 [R. E. Olson, editor]. New York and London: Academic Press.
Axton, J. H. M. (1975). *Br. med. J.* iii, 79.
Brock. J. F. & Autret, M. (1952). *FAO nutr. Stud.* no. 8.
Coward, W. A. (1975). *Br. J. Nutr.* **34**, 459.
Coward, W. A. & Sawyer, M. B. (1974). *Br. J. Nutr.* **37**, 127.
Dossetor, J. F. B. & Whittle, H. C. (1975). *Br. med. J.* ii, 592
Edozien, J. C. (1968). *Nature, Lond.* **220**, 917.
Enwonwu, C. O. & Sreebny, L. M. (1971). *J. Nutr.* **101**, 501.
Garlick, P. J., Millward, D. J., James, W. P. T., & Waterlow, J. C. (1975). *Biochim, biophys Acta* **414**, 71.
Gopalan, C. (1968). In *Calorie Deficiencies and Protein Deficiencies*, p. 49 [R. A. McCance and E. M. Widdowson, editors]. London: J. & A. Churchill.
Hales, C. N. & Randle, P. J. (1963). *Biochem. J.* 88, 137.
Kirsch, R., Saunders, S. S., Frith, L., Wicht, S. & Brock, J. F. (1969). *S. Afr. med. J.* **43**, 125.
Lancet (1970). *Lancet* ii, 302.
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *J. biol. Chem.* **193**, 265.
Lunn, P. G., Whitehead, R. G. & Baker, B. A. (1976). *Br. J. Nutr.* **36**, 219.
Lunn, P. G., Whitehead, R. G., Baker, B. A. & Austin, S. (1976). *Br. J. Nutr.* **36**, 537.
Lunn, P. G., Whitehead, R. G., Hay, R. W. & Baker, B. A. (1973). *Br. J. Nutr.* **29**, 399.
McCance, R. A. & Widdowson, E. M. (1966). *Lancet* ii, 158.
Millward, D. J., Garlick, P. J., Nnanyelugo, D. O. & Waterlow, J. C. (1976). *Biochem. J.* **156**, 185.
Munro, H. N. (1964). In *Mammalian Protein Metabolism*, vol. 1, p. 381 [H. N. Munro and J. B. Allison, editors]. New York and London: Academic Press.
Murphey, B. E. P. (1967). *J. clin. Endocr.* **27**, 973.
Northam, B. E. & Widdowson, G. M. (1967). *Ass. Clin. Biochem. Tech. Bull.* no. 11.
Philbrick, D. J. & Hill, D. C. (1974). *Am. J. clin. Nutr.* **27**, 813.
Rothschild, M. A., Oratz, M., Mongelli. J., Fishman, L. & Schreiber, S. S. (1969). *J. Nutr.* **98**, 395.
Scrimshaw, N. S. (1975*a*). In *Protein Calorie Malnutrition*, p. 345 [R. E. Olson, editor]. New York and London: Academic Press.
Scrimshaw, N. S. (1975*b*). In *Protein Calorie Malnutrition*, p. 353 [R. E. Olson, editor]. New York and London: Academic Press.
Technicon Instruments Co. Ltd. (1972). Technicon Clinical Method no. 509-72E. Basingstoke, Hants: Technicon Instruments Co. Ltd.
Waterlow, J. C. (1975). In *Protein Calorie Malnutrition*, p. 348 [R. E. Olson, editor]. New York and London: Academic Press.
Whitehead, R. G. (1971). *Proceedings*: 13*th International Congress of Paediatrics*, vol. 2, pt 1: *Nutrition and Gastroenterology*, p. 231. Vienna: Wiener Medizinischen Akademie.
Whitehead. R. G. & Alleyne, G. A. O. (1972). *Br. med. Bull.* **28**, 72.

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