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Response of young rats to deprivation of protein or of calories

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Protein deficiency in young children remains one of the major dietary problems of the world. Waterlow, Cravioto & Stephen (1960) reviewed the subject from many points of view, and they summarized what is known about the metabolic effects of protein deficiency on children and the animal experiments which they and other workers have made to study the effects of protein deficiency on metabolism. Widdowson & McCance (1957) analysed the bodies and the livers and skeletal muscle of young rats that had been fed on a diet very low in protein, or on a diet high in protein but limited in quantity to maintain the animals at the same weight as those on the low-protein diet. Well-fed control animals were also analysed, and the effects of rehabilitation were studied. This investigation left several questions unanswered, and no work since that time has entirely solved the problems that were raised. The investigation now described was planned to answer two questions: (*a*) Why did the young animals fed on a high-protein diet in strictly limited amounts die sooner than those on a low-protein diet, although the loss of body-weight in the two groups was the same? (*b*) What is the effect of the two diets on the distribution of nitrogen and other constituents in the body? A preliminary account of this aspect of the results has already been published (Dickerson & Cabak, 1962).

METHODS

Experimental design

Sixty male rats were used. They were about 5 weeks old when the experiment began, and their mean weight was 87 g.

Six animals were killed on the 1st day of the experiment and their bodies analysed as described below. Eighteen animals were fed on a low-protein diet in unlimited amounts. The diet was that described by Widdowson & McCance (1957) and consisted of cassava flour and fresh banana. Eighteen animals were given a high-protein diet consisting of equal parts of full-cream dried milk and fish meal in limited amounts, so that the mean body-weight was kept as close as possible to that of the animals on the low-protein diet. The animals in this group were housed in individual cages. A third group of eighteen animals was fed *ad lib.* on a mixture of the low- and high-protein diets (see below) and these rats served as well-nourished age controls.

After 26 days, six rats in each group were killed, dissected and analysed as described below. The remaining animals were used for a study of the effects of rehabilitation (Čabak, Dickerson & Stanier, 1963).

Diets used during the period of deprivation

Low-protein diet. It consisted of 67% cassava flour, obtained from Uganda, and 33% fresh banana. The dry diet contained 2·1 g protein and provided (calculated) 374 kcal/100 g. The rats ate on the average about 14 g (dry weight) of the mixture daily at the beginning of the experiment and about 18 g towards the end.

High-protein diet. It consisted of 50% full-cream dried milk and 50% fish meal. The dry diet contained 48 g protein and provided (calculated) 437 kcal/100 g. The amount of the mixture required per rat per day to maintain the mean body-weight the same as that of the low-protein group was 6·7 g at the beginning of the experiment decreasing to 4·2 g towards the end.

Control diet. It consisted of 50% cassava flour, 25% banana, 12·5% full-cream dried milk and 12·5% fish meal. The dry diet contained 16 g protein and provided (calculated) 404 kcal/100 g.

Mineral and vitamin supplements. A mineral mixture prepared by British Drug Houses Ltd (Dunn's salt mixture) was added to all the diets throughout the experiment. Vitamin B complex powder (Crookes Laboratories Ltd) was also added. The allowance was 200 mg dissolved in water for eighteen rats each day.

Water. All the animals had a continuous and unlimited supply of drinking water throughout.

Killing and dissection of the animals

The rats were anaesthetized with ether. The abdomen was opened and 1–2 ml blood removed by syringe and needle from the abdominal aorta. The blood was transferred to a weighed tube containing heparin so that the amount withdrawn from the body could be ascertained. The plasma was separated, and the samples from the rats in each group were pooled and stored at $-20°$.

A piece of liver (about 0·2 g) was snipped off and placed in a tube containing 30% (w/v) KOH for the determination of glycogen. Then the dead animals were dissected; the digestive tract was weighed before and after emptying it, and the contents were discarded; the liver, brain, femurs and quadriceps muscles were removed and weighed. The skin was taken off the carcass and the fur shaved from it. The liver, quadriceps muscles, skin, fur, femurs and the remainder of the carcass from each of the animals in a group were pooled, and then each pooled sample was analysed.

Analysis

Plasma. The proteins were precipitated with 10% (w/v) trichloroacetic acid and the N in the precipitate was determined by micro-Kjeldahl distillation after digestion with conc. H_2SO_4 as described by Chibnall, Rees & Williams (1943). Sodium and potassium were determined in an EEL (Evans Electroselenium Ltd) flame photometer. Chloride was determined by the method of Schales & Schales (1941) and urea as described by Lee & Widdowson (1937). Electrophoresis of the plasma proteins was carried out on cellulose acetate strips (Oxoid, Oxo Ltd) in an EEL horizontal tank containing tris–EDTA–boric acid buffer pH 8·9 (Aronsson & Gronwall, 1958) at 0·5 mA per strip of width 2·5 cm.

Muscle and liver. These organs were sampled and analysed as previously described (Dickerson & Widdowson, 1960*a*; Widdowson & Dickerson, 1960). Glycogen was determined in the liver by the method of Good, Kramer & Somogyi (1933).

Carcass and skin. Water was determined by drying the material at 105° until constant in weight. Fat was assumed to be the loss in weight of the dry material after extraction with light petroleum in the cold. The dry fat-free solids were powdered with a power-driven mincer. Total N, and the various minerals, were then determined in the dry fat-free powders by the methods used for muscle and liver. The amount of collagen was determined from the percentage of hydroxyproline obtained by the Neuman & Logan (1950) procedure on the assumption that rat collagen contains 13·4% hydroxyproline.

Fur. The fur was dried at 105° until constant in weight, and N and minerals were determined on the dry material by the methods used for muscle and liver.

Derived values. The distribution of muscle water and the composition of the intracellular phase were calculated as previously described (Dickerson & Widdowson, 1960*a*). In the skin the amount of intracellular water was obtained from the concentration of K in the tissue on the assumption that the concentration of K in the intracellular water of skin is the same as that in muscle cells. The amount of extracellular water was then calculated as the difference between the total water and intracellular water. It was assumed that collagen contains 18% N.

Femur. The femurs were weighed, and then measured, dissected and analysed as described previously (Dickerson & Widdowson, 1960*b*; Dickerson, 1962).

RESULTS

Food intakes

The animals having limited amounts of the high-protein diet ate all their daily ration of food within 2 h after it was put into the cage, and for the remaining 22 h they went hungry. Those having the low-protein diet always had food available in their cages and they were able to continue eating throughout the day and night. Their food intake could only be measured approximately, but Table 1 shows that they must have taken more calories than the rats having the high-protein diet, both per rat and per 100 g body-weight, even though their body-weights followed a similar course (see below). At the beginning of the experiment the animals having the control diet in unlimited amounts took more calories both per rat and per 100 g body-weight than the rats in either of the experimental groups, but at the end their calorie intake per 100 g weight was less than half that of the rats having the low-protein cassava–banana mixture, although they were still gaining weight whereas the low-protein animals were losing it.

At the beginning of the period of deprivation the amounts of protein (N × 6·25) eaten were similar for the animals having the high-protein low-calorie and the control-mixture diet; towards the end the experimental animals were having less per rat, but more per 100 g body-weight. The animals having the low-protein diet always had much less protein than those in either of the other two groups.

The results for the concentration of Na in the muscle (see Table 5) led us to

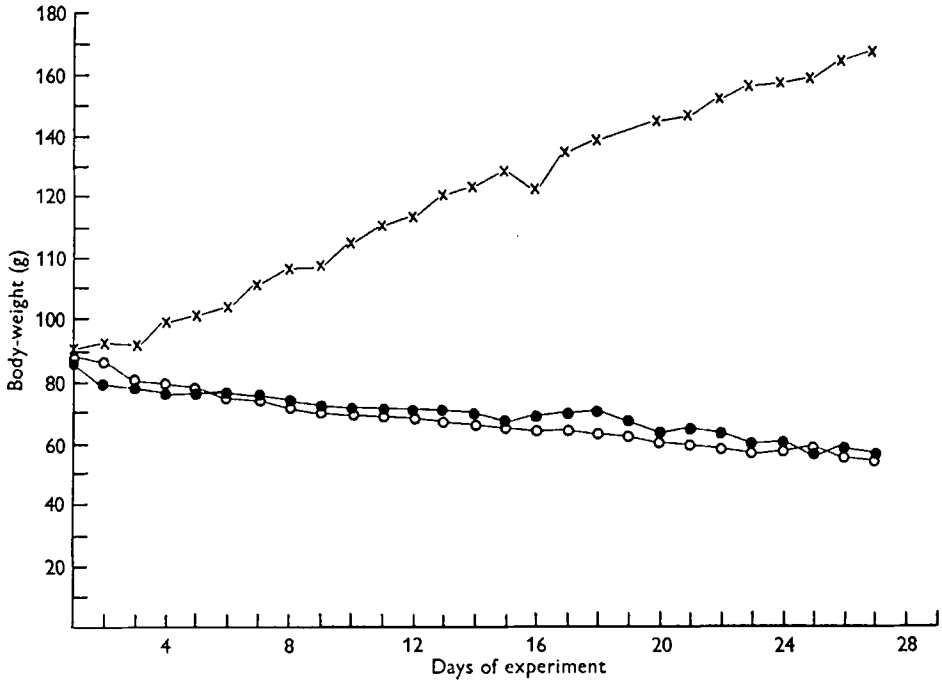

Fig. 1. Changes in mean body-weights of rats. ○—○, low-protein diet; ●—●, high-protein, low-calorie diet; × — ×, control diet.

Table 1. *Daily intake of calories, protein and sodium by the malnourished rats and their well-nourished controls*

	Low-protein group	High-protein, low-calorie group	Controls
	Calories		
At beginning			
kcal/rat	36–53	28	55–79
kcal/100 g body-weight	40–58	32	60–87
Towards end of experiment			
kcal/rat	53–64	18	79–94
kcal/100 g body-weight	94–113	32	40–48
	Protein		
At beginning			
g/rat	0·20–0·28	2·95	2·4–3·2
g/100 g body-weight	0·22–0·31	3·33	2·6–3·5
Towards end			
g/rat	0·28–0·35	1·85	3·2–3·8
g/100 g body-weight	0·50–0·62	3·31	1·7–2·0
	Sodium		
At beginning			
m-equiv./rat	1·7–2·2	2·3	3·1–4·4
m-equiv./100 g body-weight	1·8–2·4	2·6	3·3–4·8
Towards end			
m-equiv./rat	2·2–2·7	1·4	4·4–5·0
m-equiv./100 g body-weight	4·0–4·7	2·6	2·2–2·6

calculate the Na intakes, and these are also shown in Table 1. At the beginning the animals having the high-protein, low-calorie diet took amounts of Na intermediate between those of the other two groups but towards the end the Na intake per high-protein rat was the lowest of all.

Body-weights

Fig. 1 shows the mean body-weights. During the period of deprivation the animals having the low-protein cassava diet lost about 37% of their original weight, and the animals having the high-protein diet had severely limited amounts of it so that they lost weight at approximately the same rate. The weights of the well-fed control animals more than doubled during the same 4 weeks.

Behaviour and appearance of the animals

By the end of the 1st week of the experiment it was evident that the animals having the high-protein diet in restricted amounts were much less active than the others, and the difference in behaviour increased as time went on. When they were being weighed they hardly moved, whereas those having the low-protein diet were as active as normally nourished animals. Those of the high-protein, low-calorie group that were rehabilitated soon became more active when they had more food.

The two groups of malnourished animals differed in appearance. Those that had had limited amounts of the high-protein diet looked thin and emaciated, whereas those that had had the low-protein diet *ad lib.* had full round bellies.

Macroscopic observations at post-mortem dissection

Several points of difference were noted between the two groups of deprived animals and between the deprived animals and the well-nourished controls. (*a*) The livers of the rats that had been maintained for 4 weeks on the low-protein diet were a pale yellowish colour whereas those of the animals that had had the high-protein low-calorie or the control-mixture diet were dark red. (*b*) The long bones of the rats having severely limited amounts of the high-protein diet were a dark-reddish colour, especially at the ends. The marrow cavity was filled with a semi-liquid dark-red material. The bones of the animals in the other groups were normal in appearance. (*c*) The stomachs and small intestines of the animals having limited amounts of the high-protein diet still contained food residue even though the last meal had been given 20 h before death. They were also blown up with gas. The digestive tracts of the low-protein animals contained more food residue but no gas.

Weights of organs and tissues

Table 2 shows the weights of some of the organs and tissues. Skin, muscle and liver were reduced to about half their initial weight in both groups of malnourished animals and all three organs formed a smaller percentage of the body-weight at the end of the period of deprivation than they did at the beginning. The weights of the brain and femurs did not alter significantly while the body-weight was falling, so that these organs

came to form a much larger proportion of the body-weight. The animals having the high-protein, low-calorie diet had twice as much fur at the end as at the beginning.

Table 2. *Weights of some of the organs and tissues of the malnourished rats and of their well-nourished controls*

(g fresh weight except fur)

Group	Whole body excluding contents of digestive tract	Fat-free skin	Quadriceps muscle	Liver	Brain	Femur	Fur (dry weight)
Controls, at beginning	81·3	12·3	0·68	4·7	1·41	0·54	0·87
Low-protein	51·2	5·8	0·34	2·6	1·42	0·58	0·71•
High-protein, low-calorie	49·7	5·0	0·34	2·4	1·37	0·59	1·64
Age controls	187	26·8	1·75	9·5	1·65	0·99	2·44

• Weight unreliable as animals ate fur.

Table 3. *Composition of the whole bodies of the malnourished rats and their well-nourished controls*

	Controls, at beginning	Low-protein group	High-protein, low-calorie group	Age controls
Fat (g/kg fresh tissue)	52·0	22·2	5·9	114
Composition of fat-free fresh tissue (expressed per kg)				
Water (g)	737	720	708	731
Total N (g)	32·6	31·0	31·6	31·8
Collagen N (g)	7·7	10·4	10·9	8·9
Collagen N × 100/total N	23·6	33·5	34·5	27·8
Na (m-equiv.)	66·2	88·8	98·5	62·2
K (m-equiv.)	79·8	63·1	62·1	75·9
Cl (m-equiv.)	37·8	43·4	42·1	33·6
Total P (m-moles)	188	325	347	198
Ca (m-equiv.)	404	920	965	436
Mg (m-equiv.)	31·4	43·9	40·1	33·8
Derived values				
K space• (g/kg)	395	299	297	456
ECW† (= total water − ICW) (g/kg)	342	421	411	275
Intracellular Cl (m-equiv./kg)	1·2	Nil	1·8	5·8
'Excess' Na (m-equiv./kg)	19·7	27·7	36·7	23·4

• Assumed to be a measure of the amount of intracellular water (ICW).
† Extracellular water.

Deaths among the experimental animals

During the 26 days of deprivation six rats having limited amounts of the high-protein diet died and a seventh died during the period of rehabilitation. None was lost from the other groups. Five of the animals that died had an initial body-weight below the average for those that survived, and they all lost a greater percentage of their initial body-weight. Rate of weight loss relative to the initial weight appeared to be one of the causes that contributed to death within this particular group of animals. This is not the

whole story, however, for several animals having the low-protein diet lost an even greater percentage of their initial body-weight in the same time, yet they survived. It was characteristic that the animals that died gained a little weight during the 24 h before death. This gain was partly due to an increase in the amount of residues in the digestive tract resulting from hypotonia of the stomach and small intestine.

Composition of the whole body

The animals on the low-protein diet had more fat per kg body tissue than those on the high-protein, low-calorie diet and, since the body-weights were the same, they had less lean body tissue (Table 3). The amount of water per kg fat-free tissue fell in both groups of malnourished animals, but the fall was somewhat greater in those on the high-protein, low-calorie diet. In both groups the decrease in the amounts of non-collagen N (total N − collagen N) and K per kg fat-free tissue showed a reduction during malnutrition in the proportion of cell mass in the body, and the increase in Cl indicated a rise in the proportion of extracellular fluid. The large increases in the concentrations of calcium, magnesium and phosphorus, and to a lesser extent in those of Na and collagen N were the result of a rise in the proportion of skeleton and of an increase in its degree of calcification (see Table 9).

The K 'space' (assumed to be a measure of the amount of intracellular water) fell, and the amount of extracellular water (calculated as the difference between the total water and the K space) rose to values which were similar in both groups of malnourished animals. The fall in the K space was greater than the rise in the amount of extracellular water. A number of factors were involved in these changes, but the principal ones were probably the dehydration that occurred in the skin (see p. 610) and the large increase in the proportion of the body-weight contributed by the skeleton.

Table 4. *Composition of the plasma of the malnourished rats and their well-nourished controls*

(Values obtained on a pooled sample from each group and expressed per l. plasma)

Group	Protein N (g)	Urea (mg)	Na (m-equiv.)	K (m-equiv.)	Cl (m-equiv.)
Controls, at beginning	7·65	336	135	5·22	97·4
Low-protein	5·65	648	144	5·41	99·5
High-protein, low-calorie	5·45	1070	149	4·92	91·7
Age controls	9·15	318	139	4·72	91·7

Composition of the plasma and blood

Malnutrition, whether brought about by the low-protein diet or the high-protein, low-calorie diet, resulted in a fall in the concentration of protein and a rise in that of urea (Table 4). The concentration of urea was, however, increased more by the high-protein diet (from a control value of 34 to 107 mg/100 ml) than by the diet low in protein (to 65 mg/100 ml). Electrophoresis of the plasma proteins showed that malnutrition on both the diets decreased the proportion of α- and β-globulins, but did not appreciably change the proportion of albumin. The striking similarity of the

electrophoretic patterns of the plasma proteins of the two groups of malnourished animals is shown in Fig. 2. The concentrations of Na, K and Cl were little changed during malnutrition, and the significance of the small differences found is not known, for only the pooled plasma was analysed.

The level of blood sugar (i.e. reducing substances) of animals having the high-protein, low-calorie diet was a little lower than that of the animals on the low-protein diet. Blood obtained from one of the high-protein, low-calorie animals just before it died contained 7 mg 'glucose'/100 ml.

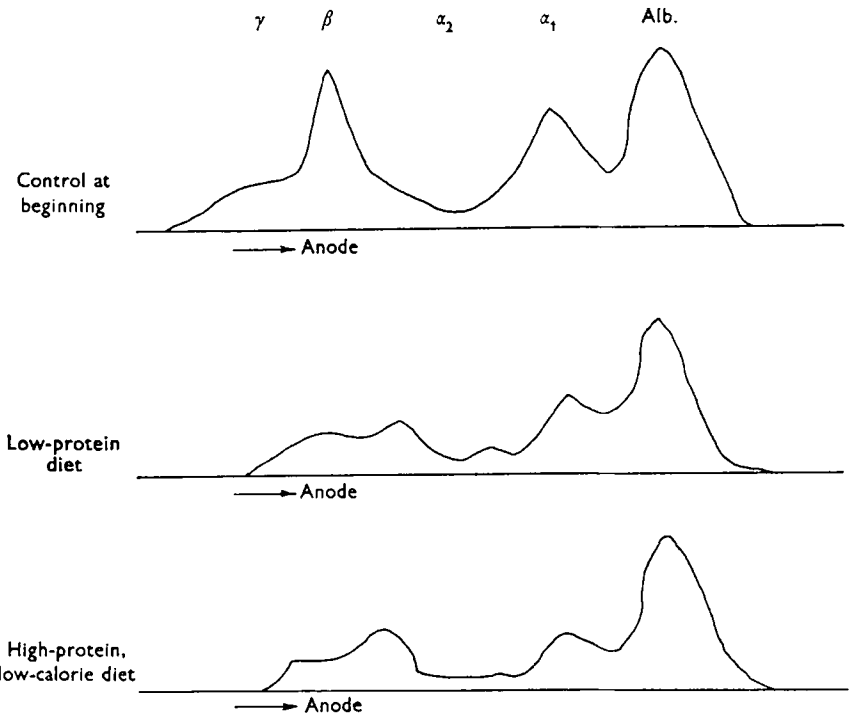

Fig. 2. Electrophoretic pattern of plasma proteins. Electrophoresis carried out on cellulose acetate strips (Oxoid; Oxo Ltd) in tris–EDTA–boric acid buffer pH 8·9 (Aronsson & Gronwall, 1958) at 0·5 mA per strip of width 2·5 cm. Alb., albumin.

Composition of quadriceps muscle

Table 5 shows that the low-protein diet reduced, and the high-protein diet increased slightly, the amount of water/kg muscle. Since pooled samples of muscle were analysed it is not known whether these differences are statistically significant. In both groups there was a large rise in the concentration of Na and Cl and a fall in that of K and P, indicating an increase in extracellular and a decrease in intracellular fluid. These changes were more severe in the animals that had been fed on the high-protein, low-calorie diet. Both diets resulted in an increase in non-protein N and extracellular protein, and a decrease in sarcoplasmic and fibrillar protein, but the reductions were greater in the high-protein, low-calorie group than in the low-protein one; this finding

is in keeping with the larger amount of extracellular water in the muscles of the former group. The amount of intracellular K per unit of intracellular protein N was reduced in both groups of malnourished animals. The similarity between the effects of malnutrition on the two diets is further demonstrated in Table 6, which shows the changes in the absolute amounts of various constituents in the quadriceps muscles. Malnutrition reduced each of the cellular constituents, but resulted in little change in the

Table 5. *Composition of quadriceps muscles of the malnourished rats and their well-nourished controls*

(Values expressed per kg fat-free tissue)

	Controls at beginning	Low-protein group	High-protein, low-calorie group	Age controls
Water (g)	782	770	791	780
Total protein N (g)	26·4	26·3	23·9	28·2
Non-protein N (g)	3·3	4·1	4·3	3·3
Sarcoplasmic protein N (g)	6·1	5·1	4·2	6·0
Fibrillar protein N (g)	19·6	18·4	16·0	20·7
Extracellular protein N (g)	1·3	3·3	3·0	0·8
Na (m-equiv.)	39·2	68·7	92·3	40·7
K (m-equiv.)	127	93·5	71·4	116
Cl (m-equiv.)	16·9	34·5	45·0	20·6
Total P (m-moles)	81·5	68·4	61·5	70·1
Ca (m-equiv.)	8·9	47·8	29·0	6·75
Mg (m-equiv.)	18·9	26·8	13·3	23·0
	Derived values			
Cl space (g/kg)	158	336	460	204
Intracellular protein N (ICPN) (g/kg)	25·7	23·5	20·2	26·7
Intracellular K (ICK) (m-equiv./kg)	126	91·7	69·1	115
ICK (m-equiv./g ICPN)	4·9	3·9	3·42	4·31

Table 6. *Changes in the absolute amounts of cellular and extracellular constituents in the quadriceps muscles of the malnourished rats and their age controls*

	Initial amount	Low-protein group	High-protein, low-calorie group	Age controls
Non-protein N (mg)	2·37	−1·01	−0·84	+3·37
Sarcoplasmic protein N (mg)	4·37	−2·67	−2·87	+6·10
Fibrillar protein N (mg)	14·0	−7·84	−8·26	+22·1
K (m-equiv.)	0·094	−0·06	−0·065	+0·11
Extracellular protein N (mg)	0·93	+0·18	+0·11	+0·46
Cl (m-equiv.)	0·012	0	−0·003	+0 023

Losses indicated by a minus sign and gain by a positive sign.

amount of Cl. The amount of extracellular protein was slightly increased. The amount of sarcoplasmic protein lost from the muscles accounted for 61 and 67% of the amount initially present in the low- and high-protein groups respectively. The loss of fibrillar protein accounted for 55 and 60% respectively of the initial amount.

There was a high concentration of Ca in the muscle of the malnourished rats (Table 5). Widdowson, Dickerson & McCance (1960) observed the same in severely undernourished pigs.

Composition of the liver

There was no measurable glycogen in the livers of the animals that had lived for 26 days on limited amounts of the high-protein diet (Table 7) but the concentration of glycogen in the livers of rats that had had the low-protein diet was as high as that in the livers of the well-nourished age controls.

The amount of fat per kg liver was increased in the animals on the low-protein diet, but not in those on the high-protein, low-calorie diet. On a fat-free basis the amount of water per kg was also increased in the livers of the low-protein animals, and there was a corresponding fall in the amount of protein N. The amount of non-protein N was, however, increased in both groups of experimental animals, the increase being a little larger in the animals on the high-protein, low-calorie diet. Both the low-protein diet and the high-protein, low-calorie diet caused a fall in the concentration of K and a rise in that of Cl. The concentration of Na in the liver rose on the low-protein diet but not on the high-protein, low-calorie diet.

Table 7. *Composition of the liver of the malnourished rats and their well-nourished controls*

	Controls at beginning	Low-protein group	High-protein, low-calorie group	Age controls
Fat (g/kg fresh liver)	3·3	43·8	4·9	6·9
Glycogen (g/kg fresh liver)	49·3	30·2	0	23·0
Composition of fat-free fresh organ (expressed per kg)				
Water (g)	737	788	745	741
Protein N (g)	27·6	21·1	28·5	23·3
Non-protein N (g)	2·2	3·7	4·3	2·4
Na (m-equiv.)	39·7	61·9	44	42·1
K (m-equiv.)	90·3	81·5	78·9	76·1
Cl (m-equiv.)	34·3	45·0	43·7	38·1
Total P (m-moles)	96·5	90·7	96·5	87·3
Ca (m-equiv.)	3·76	5·75	5·07	6·2
Mg (m-equiv.)	16·1	15·9	18·3	16·1

Composition of the skin and fur

Table 8 shows that the skin of the malnourished animals in both groups contained less water, and more total N, per kg fat-free tissue than that of the control animals either at the beginning or at the end of the experiment. The amount of collagen was also increased per kg fat-free tissue, and relative to the amount of total N its value was similar to that in the age controls. Malnutrition arrested the fall in the concentrations of Na and Cl which takes place during normal growth. The amounts of K, P, and Mg were reduced in the animals on the low-protein diet, but increased in those on the high-protein diet. The K space has been calculated and is shown in Table 8. If it may be assumed to be a measure of the amount of intracellular fluid, then the reduction in the water content of the skin on the low-protein diet was the result of a fall in the volume of intracellular fluid, whereas the reduction on the high-protein, low-calorie diet was the result of a fall in the amount of extracellular fluid.

During normal growth the amount of total N per kg dry fur rose (Table 6), and this change took place to some extent during the period of malnutrition. The amount of fur

per animal increased in those animals malnourished on the high-protein, low-calorie diet (Table 2), and the fur in these animals accounted for 46% of the total N in the skin and fur combined.

Table 8. *Composition of the skin and fur of the malnourished rats and their well-nourished controls*

(Values expressed per kg fat-free tissue unless otherwise stated)

	Controls at beginning	Low-protein group	High-protein, low-calorie group	Controls at end
		Skin		
Water (g)	726	676	642	701
Total N (g)	39·6	48·3	54·0	43·4
Collagen N (g)	17·0	28·0	32·1	24·7
Collagen N × 100/total N	43·0	57·9	59·3	57·2
Na (m-equiv.)	77·3	74·2	81·0	66·6
K (m-equiv.)	67·7	56·6	76·6	55·9
Cl (m-equiv.)	65·5	65·5	68·0	57·7
Total P (m-moles)	53·1	35·3	65·1	40·7
Ca (m-equiv.)	9·6	12·2	10·7	8·0
Mg (m-equiv.)	11·7	9·2	14·9	9·1
		Derived values		
K space* (g/kg)	335	268	367	328
		Fur		
Total N (g/kg dry fur)	126	134	141	156

* Assumed to be a measure of the amount of intracellular water (ICW).

Table 9. *Composition of the femur of the malnourished rats and their well-nourished controls*

	Fat (g/100 g fresh bone)	Water	Total N	Collagen	Ca	P	Ratio, Ca:N
		(g/100 g fat-free fresh bone)					
Controls at beginning	0·45	53·9	3·15	8·20	8·6	4·15	2·73
Low-protein diet	8·2	41·1	2·94	10·2	14·3	6·46	4·87
High-protein, low-calorie diet	0	46·9	2·86	9·12	12·3	5·28	4·30
Age controls	0	38·6	3·03	10·35	14·8	6·6	4·89

Composition of the femur

Table 9 shows that the concentration of fat in the femur, like that in the liver, increased when the rats were malnourished on the low-protein diet to a value much higher than that normally found in the rat. This change did not occur in the animals that had had the high-protein, low-calorie diet. The composition of the fat-free bone shows that, although bone growth had almost ceased, chemical maturation of the bone, as indicated by the Ca:N ratio, had continued in both groups of malnourished animals, and the composition of the femur of the animals that had had the low-protein diet was, in fact, the same as that of the age controls. On the high-protein, low-calorie diet maturation was a little retarded. Analysis of the cortex and epiphyses has shown essentially the same result.

Composition of the organs and whole bodies of the animals on the high-protein, low-calorie diet that died during malnutrition

Comparison of the values shown in Table 10 for the composition of the organs and whole bodies of the animals on the high-protein, low-calorie diet that died during the period of malnutrition with those shown in Tables 3, 5, 7 and 8 for those killed at the end of the period of malnutrition shows that in all instances the amounts of water, Na and Cl were higher and the amounts of total N, K, P and Mg were lower in the former. In the quadriceps muscles and the liver the differences in the amount of Na were greater than those in the amount of Cl and it is likely that Na had entered the cells and K had been lost from them.

Table 10. *Composition of the organs and whole bodies of the rats on the high-protein, low-calorie diet that died during the period of malnutrition*

(Values expressed per kg fat-free tissue)

	Quadriceps muscles	Skin	Liver	Whole body
Water (g)	813	662	796	731
Total N (g)	27·1	49·6	26·9	29·3
Collagen N (g)	3·6*	31·0	—	10·6
Collagen N × 100/total N	13·3*	62·4	—	36·2
Na (m-equiv.)	136·0	91·4	85·3	112·2
K (m-equiv.)	47·0	53·4	60·7	55·4
Cl (m-equiv.)	67·2	75·4	59·8	55·8
P (m-moles)	51·5	36·2	73·9	303
Ca (m-equiv.)	22·1	20·4	6·5	887
Mg (m-equiv.)	12·6	10·6	13·3	39·4

* Extracellular protein N.

DISCUSSION

The diets used in this experiment were not intended to represent exactly any diet eaten by man. The low-protein diet was an exaggerated form of the type of diet eaten by children and adults in parts of Africa and other regions of the world, but among those people some form of animal protein is generally consumed from time to time, even if only in very small amounts. The only source of protein for the rats was cassava and banana, and the diet, besides containing very little protein, may well have been deficient in particular amino acids. The high-protein diet given in severely limited amounts was intended to provide an adequate supply of protein, but an inadequate number of calories.

By design the mean loss of weight among the animals in the two experimentally deprived groups was the same, although brought about in two different ways. In some respects the response of the animals to the two diets was similar, and here the undernutrition, which affected both groups of animals, was the predominating feature. In other respects the specific effects of a protein deficiency were different from the more general effects of a shortage of calories.

Fryer, Miller & Payne (1961) described what they called a 'calorie paradox'.

Growing rats and pigs were maintained at a constant body-weight by feeding on restricted amounts of a stock diet, or *ad lib.* on a diet with a low concentration of protein. The calorie intake of both rats and pigs having restricted amounts of the stock diet was much lower than that of the others, and the difference could not be accounted for by differences in the energy content of the faeces or urine, the specific dynamic action of the diets, activity, or body composition. In our experiments there was a difference in calorie intake in the same direction as that observed by Fryer *et al.*, but there was also a striking difference in the behaviour and activity of the two groups of animals. The apathy and misery so characteristic of kwashiorkor were not observed in the protein-deficient animals, and it is possible that the mental changes in kwashiorkor have a specific cause, perhaps allied to the disturbed metabolism of aromatic amino acids, as occurs in phenylketonuria (Dean & Whitehead, 1963).

Death during the period of deprivation occurred only among the animals having limited amounts of the high-protein diet, and the same happened in the previous experiment of Widdowson & McCance (1957). It is true that within the high-protein groups it was the animals that had lost the greatest proportion of their original body-weight that died, but some of the animals in the low-protein group lost an even greater percentage of their body-weight and survived. The changes in weight of the organs of the animals in the two groups were similar, and therefore loss of weight in itself does not seem to have been the cause of death.

Plasma was not obtained from the animals that died, but from the values for the concentration of electrolytes for those that survived (Table 4) it does not seem likely that changes in composition of the extracellular compartment with respect to these constituents contributed in any major way to the cause of death. The concentration of urea in the plasma was, however, 107 mg/100 ml in the animals that survived. This may simply have been the result of the high protein content of the diet, since elevated concentrations of urea were also found in control animals given this diet during the period of rehabilitation (Čabak *et al.* 1963).

From Table 6 it is evident that the concentration of Na was higher and that of K lower in the muscle of animals having the low-protein diet than it was in the controls; there were similar but greater variations from normal in the high-protein, low-calorie group, and the variations were greatest of all in the animals in this group that died (Table 10). There were differences in Cl similar to those in Na, and in P and Mg similar to those in K. The changes in the animals that survived can probably largely be accounted for by an increase in the proportion of the tissue occupied by extracellular fluid and a corresponding decrease in cell mass, but there evidently came a point in the history of animals having the high-protein, low-calorie diet when the cell walls lost their ability to extrude Na and retain K, and when this happened the animals died. The values for the electrolytes in muscle shown in Table 10 could not possibly be accounted for in any other way. These results, together with those indicating the depletion of glycogen stores in the liver and possibly also of sugar in the blood, suggest that those animals that died did so because the small amounts of the high-protein diet provided insufficient energy for cellular metabolism, so that Na entered the cells and K was lost from them. This in turn interfered with cellular enzyme activity. Similar changes may

have occurred in the kidney also. Since one animal died after rehabilitation had begun there may be a stage when the process becomes irreversible.

The use of intracellular protein N as a basis of reference (Dickerson & Widdowson, 1960*a*) did not provide any evidence of increased hydration of the muscle cells in protein deficiency, such as that suggested by the results of Gopalan, Venkatachalam & Srikantia (1953) and Holmes, Jones & Stanier (1954) in malnourished human adults, and inferred in infantile protein deficiency (Trowell, Davies & Dean, 1954).

Experiments with isotopically labelled amino acids (Neuberger, Perrone & Slack, 1951; Neuberger & Slack, 1953) have shown that collagen is metabolically inert compared with other body proteins. The metabolic activity of collagen is, however, not the same in different organs of the body. In both groups of malnourished rats some collagen was metabolized during the period of deprivation. About 60% of the total loss of collagen came from the skin, which lost over a quarter of its initial amount. In the quadriceps muscles, on the other hand, the amount of extracellular protein N (mainly collagen) tended to increase. The large contribution of skin collagen to the total amount lost from the body in our rats is very similar to the result obtained by Harkness, Harkness & James (1958) in adult mice given a protein-free diet for about 20 days. Evidence has been accumulating that the collagen of skin is a mixture of collagens with differing metabolic activity (see Orekhovitch & Shprikiter, 1957), and Gross (1958) found that a fraction of the collagen of the skin of young guinea-pigs soluble in 0·45 M-NaCl was almost halved when the animals were fasted for 2 days.

The femurs of the malnourished animals continued to mature during the period of malnutrition, and in the animals given the low-protein diet were normal in composition for their chronological age. Thus, in these animals there was not the same retardation of skeletal development as has been found radiologically in infants and children suffering from the effects of protein deficiency (Gopalan, 1955; Jones & Dean, 1956, 1959) and undernutrition (Berridge & Prior, 1954).

The loss of the same amount of body-weight on the two very different diets used in these experiments produced more similarities than differences in body composition. It seems likely, therefore, that the shortage of calories, rather than the nature of the diet, was the primary cause of many of the changes that were found.

SUMMARY

1. Young rats were fed on three different diets: (*a*) One made up from cassava and banana containing very little protein was supplied in unlimited amounts, but the animals did not eat enough of it even to maintain their body-weights. (*b*) A high-protein diet made up from fish meal and full-cream milk powder was given in restricted amounts so that the mean body-weight remained the same as that of the animals on (*a*). (*c*) A mixture of the two diets was given in unlimited amounts; the rats eating this diet gained weight, and served as the controls.

2. The animals having the high-protein diet required fewer calories than those having the low-protein diet to keep their body-weight at the same level. Rats in both groups lost on average 37% of their original body-weight.

3. No deaths occurred during the 26 days of the experiment among the animals having the low-protein diet, but six rats died which were having the high-protein diet in limited amounts.

4. In both groups of deprived animals the skin, skeletal muscle and liver were reduced to about half their initial weight, and these organs lost relatively more weight than the body as a whole. The weights of the brain and femurs hardly changed during the period of deprivation in either group.

5. The bodies of the animals that had had the low-protein diet contained more fat and less lean body tissue than those of the rats that had had the high-protein, low-calorie diet. In both groups there was a fall in non-collagen nitrogen and potassium per kg lean body tissue and a rise in sodium and chloride, indicating a decrease in cell mass and an increase in extracellular fluid.

6. Similar changes in the concentrations of Na, Cl and K were found in the quadriceps muscles; the concentrations of sarcoplasmic and fibrillar protein N were reduced, and those of non-protein and extracellular protein N were increased. All these changes were more severe in the high-protein, low-calorie group. On an absolute basis the amount of Cl in the muscles did not change during malnutrition, but the amount of extracellular protein N tended to increase.

7. The concentration of proteins in the plasma fell in both groups of malnourished animals and that of urea rose.

8. There was no measurable glycogen in the livers of the animals that had had restricted amounts of the high-protein diet and their blood sugar content was very low. The percentage of fat in the livers of the low-protein animals was increased.

9. The concentration of the cellular constituents non-collagen N, K and P were reduced in the skins of the animals on the low-protein diet, but increased in those on the high-protein, low-calorie diet. Both types of deprivation led to an increase in the concentration of collagen, but the absolute amount of collagen in the skin fell and the loss accounted for about 60% of the total amount lost from the body.

10. Although both types of malnutrition prevented growth of the bones they did not hinder their chemical maturation.

11. It is concluded that the deaths that occurred amongst the animals on the high-protein diet resulted from an insufficient supply of energy for cellular metabolism so that Na entered the cells and K was lost from them.

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