The Metabolism of Nitrogen, Calcium and Phosphorus in Undernourished Children

2. The Effect of Supplementary Groundnut-milk Curds on the Metabolism of Nitrogen, Calcium and Phosphorus

By GOWRI SUR, SARANYA KUMARI REDDY, M. SWAMINATHAN and V. SUBRAHMANYAN

Central Food Technological Research Institute, Mysore, India

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The previous communication (Murthy, Reddy, Swaminathan & Subrahmanyan, 1955) reported that undernourished children, subsisting on an inadequate and ill-balanced vegetarian diet, showed a remarkable adaptation to low levels of intake, and maintained, on the average, slightly positive nitrogen, calcium and phosphorus balances. The children were stunted, and their rate of growth was very low. In another publication from this laboratory Subrahmanyan, Reddy, Moorjani, Sur, Doraiswamy, Sankaran, Bhatia & Swaminathan (1954) reported that supplementation of the diet of the undernourished children with 12 oz. groundnut-milk curd daily for a period of 6 months produced a marked improvement in their growth and nutritional status, compared with the performance of a control group receiving a supplement of an equivalent amount of calories in the form of maize starch-sugar pudding. In view of the marked improvement observed in the growth and nutritional status of the children, it was considered to be of interest to study the effect of the groundnut-milk curd supplement on the metabolism of nitrogen, calcium and phosphorus. The results of this study are here reported.

METHODS

Subjects. The present investigation was carried out when the institution feeding experiment with groundnut-milk curd (Subrahmanyan *et al.* 1954) had been in progress for 3 months. The children had been in the orphanage for about 3 years before the experiment. Six pairs of children aged 8–11 years were selected from the subjects on the groundnut-curd experiment (Subrahmanyan *et al.* 1954) for the metabolism study. The girls in each pair were comparable in age, weight and height (Table 1). They were examined clinically and were found to be free from any disease likely to interfere with the metabolism study.

Experimental diets and the feeding of the children. The composition of the diets consumed by the experimental subjects is shown in Table 2. The pattern of breakfast, lunch and dinner was the same as that reported in a previous paper (Subrahmanyan *et al.* 1954). The procedure adopted for the feeding of the subjects was similar to that described by Murthy *et al.* (1955). Samples of cooked food preparations were collected daily, dried in an air oven and analysed for nitrogen, calcium and phosphorus.

Some of the foods (rice, ragi, pulses and groundnut-milk curds) used in the experiment were also analysed separately for these constituents.

Plan of the experiment. The experimental period was of 7 days' duration. The first 2 days were treated as a preliminary period in which the children were allowed to get used to the experimental discipline and to the equipment kept for the collection of urine and faeces. During this time the approximate food intake of the children was also noted. The urine and faeces were collected during the next 5 days. The

Table 1. *Age, height, weight, red blood cell count and haemoglobin level of children at the beginning and end of a period of* 6 *months*

Child no.	Age (years)	Height (in.)		Weight (lb.)		Red blood cells (10^6/mm^3)		Haemoglobin (g/100 ml. blood)	
		Initial	Final	Initial	Final	Initial	Final	Initial	Final
				Rice diet					
1	11	49·0	49·3	51·8	50·9	4·23	4·56	11·05	11·05
2	9	47·4	47·6	40·5	40·9	4·10	4·41	11·73	11·05
3	11	51·3	52·3	52·9	54·0	4·16	4·66	11·22	11·56
4	9	45·9	46·1	40·1	40·5	4·30	4·64	11·82	10·71
5	9	43·8	44·4	37·9	38·6	3·47	4·29	10·71	11·39
6	8	43·6	44·3	36·6	38·0	4·24	4·10	11·56	12·24
				Rice-groundnut curd diet					
7	10	49·9	50·5	49·9	53·8	4·49	4·42	10·54	11·22
8	10	47·9	48·1	41·9	41·8	3·92	4·56	11·05	12·24
9	11	49·9	50·5	55·6	58·6	4·14	4·73	11·73	12·75
10	10	45·8	46·6	40·4	42·8	4·32	4·82	12·58	12·75
11	10	42·8	44·3	39·4	43·0	3·91	4·75	10·88	10·88
12	8	43·6	44·6	37·0	38·1	4·21	4·47	10·71	12·07

Table 2. *Mean daily food consumption of subjects on the rice and the rice-groundnut curd diet*

Constituent	Rice diet (g)	Rice-groundnut curd diet (g)
Rice, raw unmilled (husked)	204·0	204·0
Ragi (*Eleusine coracana*)	40·0	40·0
Wheat	8·0	8·0
Red gram dhal (*Cajanus indicus*)	8·8	8·8
Horse gram (*Dolichos biflorus*)	12·0	12·0
Groundnut oil	2·7	2·7
Jaggery (crude cane-sugar)	11·4	11·4
Common salt (crude sea-salt)	7·4	7·4
Tamarind fruit pulp (*Tamarindus indicus*)	2·0	2·0
Pumpkin	10·0	10·0
Radish	15·0	15·0
Brinjal	9·0	9·0
Radish tops	2·0	2·0
Amaranth leaves (*Amaranthus gangeticus*)	8·0	8·0
Meat	4·0	4·0
Milk, cow's	10·0	10·0
Maize starch	34·0	—
Cane sugar	31·0	—
Groundnut curds	—	341·0
Condiments such as chillies, garlic	3·9	3·9

methods adopted for the collection, preservation and analysis of urine, faeces and food were those reported by Murthy, Swaminathan & Subrahmanyan (1954) and Murthy *et al.* (1955).

RESULTS

The results obtained for nitrogen metabolism are given in Table 3, for calcium metabolism in Table 4 and for phosphorus metabolism in Table 5.

It will be observed from the results given in Tables 3–5 that the mean daily intake of calories, protein, calcium and phosphorus of children receiving the rice diet fell short of the recommended allowances suggested by the Indian Council of Medical

Table 3. *Mean daily intake, excretion and balance of nitrogen of children on rice and rice-groundnut curd diets*

Diet	Calorie intake (Cal.)	Nitrogen intake (g)	Nitrogen excretion: Urinary (g)	Faecal (g)	Total (g)	Nitrogen balance (g)
Rice	1297	3·60	1·97	1·35	3·32	0·28
Rice-groundnut curd	1298	5·16	3·26	1·17	4·43	0·73
Difference		1·56	1·29	−0·18	1·11	0·45** ± 0·07

** Significant at 1 % level.

Table 4. *Mean daily intake, excretion and balance of calcium of children on rice and rice-groundnut curd diets*

Diet	Calorie intake (Cal.)	Calcium intake (mg)	Calcium excretion: Urinary (mg)	Faecal (mg)	Total (mg)	Calcium balance (mg)
Rice	1297	382·2	81·3	227·5	308·8	73·4
Rice-groundnut curd	1298	676·4	81·5	361·5	443·0	233·4
Difference		294·2	0·2	134·0	134·2	160·0 ± 70·80

Table 5. *Mean daily intake, excretion and balance of phosphorus of children on rice and rice-groundnut curd diets*

Diet	Calorie intake (Cal.)	Phosphorus intake (mg)	Phosphorus excretion: Urinary (mg)	Faecal (mg)	Total (mg)	Phosphorus balance (mg)
Rice	1297	545·8	160·2	328·8	489·0	56·8
Rice-groundnut curd	1298	766·2	253·7	396·8	650·5	115·7
Difference		220·4	93·5	68·0	161·5	58·9* ± 18·40

* Significant at 5 % level.

Research: Nutrition Advisory Committee (1944). It will also be noted that the intakes of protein and phosphorus in the two groups were slightly lower than the corresponding intakes reported earlier (Subrahmanyan *et al.* 1954). The difference was due to the fact that, whereas the protein, calcium and phosphorus contents of the diets consumed by the subjects in the present study were determined by actual

analysis, the values reported earlier were obtained by calculation, using figures given by Aykroyd, Patwardhan & Ranganathan (1951) for the nutritive value of Indian foods and the average composition of groundnut-milk curds used during a 6-month period. For example, the mean intakes of phosphorus on rice and rice-groundnut curd diet obtained in the present study were 546 and 766 mg respectively. These figures were lower than the 'calculated' values of 712 and 1052 mg reported earlier for similar diets (Subrahmanyan *et al.* 1954). The lower values obtained in the present study were due to the lower phosphorus content of the samples of raw unmilled rice, ragi and horse gram consumed by the subjects during the metabolic experiment as compared with the values reported by Aykroyd *et al.* (1951) and used in earlier calculations (raw unmilled rice 182 as against 230 mg/100 g, ragi 210 as against 270 mg/100 g, horse gram 204 as against 390 mg/100 g). The mean phosphorus content (71 mg/100 g) of the sample of groundnut curds consumed by the subjects during the metabolism study was considerably lower than the mean figure (102 mg/100 g) for samples consumed in a 6-month period and used in earlier calculations (Subrahmanyan *et al.* 1954). These differences account for the discrepancies between the intakes of phosphorus by the subjects on rice and rice-groundnut curd diets observed in the present study and the corresponding intakes reported earlier (Subrahmanyan *et al.* 1954).

Nitrogen metabolism. The mean daily nitrogen intake on the rice diet and on the rice-groundnut curd diet was 3·60 and 5·16 g respectively. The mean excretion of nitrogen in the faeces was of the same order on both diets. The loss of nitrogen in the urine was appreciably greater on the rice-groundnut curd diet than on the rice diet. One out of the six subjects in the rice group maintained a negative nitrogen balance. All the subjects on the rice-groundnut curd diet were in positive balance. The mean quantity of nitrogen retained daily by the subjects on the rice and on the rice-groundnut curd diet was very much higher than on the rice diet, the difference being highly significant ($P < 0{\cdot}01$).

Calcium metabolism. Five of the subjects were in positive calcium balance and one in negative balance on the rice diet. All six subjects were in positive calcium balance on the rice-groundnut curd diet. The mean daily calcium intake on the rice and on the rice-groundnut curd diets was 382 and 676 mg respectively, and the mean retention on the two diets was 73 and 233 mg respectively. In spite of the large mean difference in the calcium balance between the two groups, the difference was not significant, owing to the large variations in the calcium balances of individual children.

Phosphorus balance. The mean phosphorus intake on the rice diet and on the rice-groundnut curd diet was 546 and 766 mg respectively. Five subjects were in positive phosphorus balance and one in negative balance on the rice diet. All the six subjects maintained a positive balance on the rice-groundnut curd diet. The excretion of phosphorus in faeces and in urine was appreciably greater on the rice-groundnut curd diet than on the rice diet. The mean daily retention of phosphorus on the two diets was 57 and 116 mg respectively, the difference being statistically significant ($P < 0{\cdot}05$).

DISCUSSION

The results show that supplementation of a poor vegetarian rice diet with 12 oz. groundnut-milk curds daily brings about an increase in the retention of nitrogen, calcium and phosphorus. The mean calorie intake of the subjects under experiment was only 1297 Cal. This value is very low as compared with the recommended allowance of 1850 Cal. (Aykroyd *et al.* 1951). It is interesting to note that, in spite of the inadequate calorie intake, the addition of extra protein produced a significant increase in the retention of nitrogen. The results obtained in this and previous studies from this laboratory (Murthy *et al.* 1954; Subrahmanyan *et al.* 1954) have shown the gross inadequacy of diets consumed by the children in the orphanage and the beneficial effects produced by a daily supplement of 12 oz. groundnut-milk curds. It is obvious that, since milk and milk products are scarce and costly, the production and consumption of milk substitutes like fortified groundnut milk and curds will go a long way to make up for the deficiencies in poor Indian diets.

SUMMARY

1. The metabolism of nitrogen, calcium and phosphorus was studied in six pairs of girls aged 8–11 years, comparable in age, height, weight and nutritional status, fed on a poor vegetarian diet based on rice or on the same diet supplemented with 12 oz. groundnut-milk curd daily.

2. The mean daily nitrogen intake on the rice diet and on the rice-groundnut curd diet was 3·60 and 5·16 g respectively. One of the six subjects on the rice diet maintained a negative nitrogen balance, whereas all the six subjects on the rice-groundnut curd diet maintained positive balance. The daily nitrogen retention on the rice-groundnut curd diet (0·73 g) was significantly higher ($P<0·01$) than that on the rice diet (0·28 g).

3. The mean daily intake of calcium on the rice diet and on the rice-groundnut curd diet was 382 and 676 mg respectively. One of the six subjects on the rice diet was in negative balance. All the subjects on the rice-groundnut curd diet maintained positive balance. The mean daily retention of calcium on the rice diet and on the rice-groundnut curd diet was 73 and 233 mg respectively; the difference, however, was not statistically significant.

4. The mean daily intake of phosphorus on the rice and on the rice-groundnut curd diet was 546 and 766 mg respectively. One of the six subjects in the rice group was in negative balance. All the subjects in the rice-groundnut curd group maintained positive balance. The mean daily phosphorus retention on the two diets was 57 and 116 mg respectively, the difference being statistically significant ($P<0·05$).

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REFERENCES

Aykroyd, W. R., Patwardhan, V. N. & Ranganathan, S. (1951). *Hlth Bull., Simla,* **23**, 15.
Indian Council of Medical Research: Nutrition Advisory Committee (1944). *Report of the Scientific Advisory Board for the Year* 1944. Delhi: Indian Council of Medical Research.
Murthy, H. B. N., Reddy, S. K., Swaminathan, M. & Subrahmanyan, V. (1955). *Brit. J. Nutr.* **9**, 203.
Murthy, H. B. N., Swaminathan, M. & Subrahmanyan, V. (1954). *Brit. J. Nutr.* **8**, 11.
Subrahmanyan, V., Reddy, S. K., Moorjani, M. N., Sur, G., Doraiswamy, T. R., Sankaran, A. N., Bhatia, D. S. & Swaminathan, M. (1954). *Brit. J. Nutr.* **8**, 348.

Amino-acid Metabolism in the Rumen of the Sheep

By D. LEWIS

Agricultural Research Council Institute of Animal Physiology, Babraham, Cambridge

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During recent years considerable attention has been given to the breakdown of protein in the rumen, and in particular to ammonia production during this process. The first suggestion of the significance of ammonia in the rumen was made by Pearson & Smith (1943) based on the finding that ammonia was produced in vitro during the incubation of rumen contents with protein. McDonald (1948, 1952) recognized the importance of ammonia in the rumen and studied the changes in the ammonia concentration in rumen contents following the feeding of various proteins: he demonstrated the production of ammonia in vivo from ingested protein and also the absorption of significant quantities of ammonia from the rumen. He suggested that urea derived from this ammonia in the liver would partly be excreted and partly returned to the rumen in the saliva.

It seems probable that some of the ingested protein is converted to amino-acids which are deaminated giving rise to ammonia. However, it is not known in fact that amino-acids are intermediates in this reaction, neither have they been shown to be present in rumen contents. Using washed suspensions of rumen micro-organisms El-Shazly (1952*a*, *b*) demonstrated that casein hydrolysate was converted to ammonia, carbon dioxide and volatile fatty acids. The extent to which deamination occurred was found to vary with the diet of the animal; as the protein in the diet was raised so the rate of deamination by the washed suspensions increased, probably owing to an increase in the number of micro-organisms capable of attacking amino-acids. A detailed examination was not made of the products formed during the breakdown of individual amino-acids.

The present work has been concerned with an extension of these findings and in particular with the breakdown of amino-acids in the rumen and by washed suspensions of rumen micro-organisms in vitro. It was first necessary, however, to determine whether amino-acids were to be found in rumen contents.