The Metabolism of Nitrogen, Calcium and Phosphorus in Human Adults on a poor Vegetarian Diet Containing Ragi (*Eleusine coracana*)

By V. SUBRAHMANYAN, M. NARAYANARAO, G. RAMARAO AND M. SWAMINATHAN

Central Food Technological Research Institute, Mysore, India

(*Received 24 January 1955*)

Ragi (*Eleusine coracana*) is a grain of considerable importance in the dietary of millions of people who inhabit the Deccan Plateau of India. It is generally raised as a dry (rain-fed) crop, though in certain areas it is also raised as an irrigated crop. The grains are usually red-brown in colour, though white-skinned varieties are also known. The grain is very small and has a diameter of only 1–1·5 mm. In view of this, and the difficulty in separating the seed-coat, the grain is usually cleaned and ground as a whole for use as flour. The flour is generally cooked to a paste with the minimum amount of water and the paste rolled into balls which are eaten with soups, chutneys and cooked vegetables. The cooked balls are somewhat sweet to the taste and, though tasting a little coarse because of the associated skin, they are considered to be quite wholesome. The working classes generally prefer ragi to softer grains like rice, because of both its cheapness and its sustaining qualities.

Ragi is unique among cereal grains in being a very rich source of calcium, containing about 0·33 % as compared to other common cereals whose Ca content ranges from 0·01 to 0·06 % (Aykroyd, Patwardhan & Ranganathan, 1951); but like in other cereals a greater part of the phosphorus in ragi is present in the form of phytate (Sundararajan, 1938; Giri, 1938). Giri (1940) studied the availability of Ca from ragi, kaffir corn (*Sorghum vulgare*) and pearl millet (*Pennisetum typhoideum*) using albino rats, and reported that at the same level of Ca intake the availability of Ca from the different grains was of the same order. The proteins of ragi have been reported to possess a fairly high biological value for the maintenance of nitrogen equilibrium in adult rats and only a moderate value for promoting growth in young rats (Swaminathan, 1938). The total nutritive value of poor vegetarian diets containing ragi as compared with that of diets containing other cereals has been studied by growth experiments on albino rats (Sur, Reddy, Swaminathan & Subrahmanyan, 1954; Subrahmanyan, Kuppuswamy, Ramarao, Swaminathan & Bhatia, 1954). The results of these studies have shown that the total nutritive value of a poor vegetarian diet containing ragi is higher than that of similar diets containing rice or kaffir corn and almost equal to that of a wheat diet. The human metabolic studies so far carried out in India have been confined to diets containing rice or wheat (Basu & Basak, 1939; Patwardhan, Mukundan, Rama Sastri & Tulpule, 1949; Murthy, Swaminathan & Subrahmanyan, 1954). No human metabolic studies have so far been reported with diets containing ragi, kaffir corn or pearl millet. The present paper describes the results of studies on the absorption of N, Ca and P in adult human beings fed on a poor vegetarian diet containing ragi.

EXPERIMENTAL

Subjects. Eight healthy adult males who carried out ordinary duties in the laboratory were selected as experimental subjects. They were first clinically examined and found to be free from any disease. They had not suffered from any debilitating disease in the recent past and their body-weights had remained constant over a period of 1 year before the present experiment. All the subjects were habitual ragi eaters. They were all normally active and healthy and within the age range 22–32 years. Information about their heights and weights is given in Table 1.

Table 1. *Ages, heights and weights of the experimental subjects*

Subject no.	Age (years)	Height (m)	Weight (kg)
1	28	1·62	51·3
2	28	1·72	64·8
3	22	1·71	54·0
4	24	1·61	51·3
5	26	1·60	45·9
6	27	1·64	50·4
7	32	1·64	51·3
8	24	1·65	53·1

Plan of the experiment. The subjects were housed in a building specially designed for human metabolic studies. They were fed on the experimental ragi diet for a period of 12 days. The first 7 days were a preliminary period to allow the subjects to get accustomed to the diet and to the equipment used for the collection of urine and faeces. Urine and faeces were taken for analysis only during the last 5 days.

Experimental diets and the feeding of the subjects. The chemical composition of ragi (variety H.22) used was determined by the methods of the Association of Official Agricultural Chemists (1950). The results are given in Table 2. The mean composition

Table 2. *Percentage composition of ragi* (Eleusine coracana) (*variety H.22*) *used in the experiments*

Moisture	12·0	Crude fibre	3·5
Starch	66·2	Protein (N × 6·25)	6·8
Reducing sugars	1·2	Fat (ether extractives)	1·3
Hemicelluloses and other carbohydrates (by difference)	6·8	Ash	2·2
Calcium (g/100 g)	0·440		
Phosphorus (g/100 g)	0·285		
Calories* (Cal./100 g)	308		

* Calories were calculated with factors of 4, 9 and 4 Cal./g for protein, fat and carbohydrate respectively.

of the diet consumed by the subjects daily is shown in Table 3. The diet was similar to that consumed in normal times by the vast majority of the poorer people in Mysore State. The subjects were fed four times a day, at breakfast, lunch, tea and dinner.

Breakfast and tea consisted of a savoury dish made of ragi flour and a cup of coffee. Lunch and dinner consisted of dumplings made of ragi flour, vegetable soup (a preparation containing pulses, tamarind, chillies, spices, salt and vegetables) and dilute 'buttermilk'. The 'buttermilk' was prepared as follows: a part of the milk powder included in the diet was reconstituted with water and then converted into sour curd by seeding with a small quantity (1 % by volume) of sour curd (as a source of *Lactobacillus*) and incubating at 37° for 16 h; the sour curd thus obtained was diluted with four times its volume of water and agitated with a stirrer for 5 min to yield 'buttermilk'. Records of the food consumed daily by the subjects were kept throughout the experiment. Complete duplicates of all dishes consumed by each subject were collected daily, dried in an air oven and weighed. They were powdered, and analysed for N, Ca and P.

Table 3. *Mean composition and nutritive value of the ragi diet consumed daily by the subjects*

Composition		Nutritive value*	
Ragi (*Eleusine coracana*) (g)	690·5	Calories (Cal.)	3098
Red gram dhal (*Cajanus indicus*) (g)	50·0	Protein (N × 6·25) (g)	66·0
Groundnut oil (g)	50·0	Fat (g)	60·0
Potato (g)	41·0	Carbohydrate (g)	573·5
Brinjal (*Solanum melongena*) (g)	41·0	Calcium (g)	3·38
Amaranth leaves (*Amaranthus gangeticus*) (g)	21·0	Phosphorus (g)	2·29
		Thiamine (mg)	3·1
Whole-milk powder (Nespray)† (g)	9·0	Nicotinic acid (mg)	9·1
Cane sugar (g)	35·0	Vitamin A value (mainly as carotene) (i.u.)	1004
Tamarind pulp (*Tamarindus indicus*) (g)	16·0		
Common salt (refined) (g)	20·6		
Bengal gram dhal (*Cicer arietinum*) (g)	3·3		
Black gram dhal (*Phaseolus mungo*) (g)	3·3		
Curry leaves (*Murraya koenigii* (g)	1·5		
Coriander leaves (g)	1·5		
Onions (g)	8·0		
Chillies (green) (g)	7·5		
Lemon juice (ml.)	3·0		

* All the values (excepting protein, calcium and phosphorus which were determined according to methods referred to in the text) were calculated from figures given by Aykroyd *et al.* (1951).
† See this page, line 4.

Collection and preservation of urine and faeces. The methods were those described by Murthy *et al.* (1954).

Analytical methods. The methods adopted for the estimation of total N, Ca and P in food, urine and faeces were those followed by Murthy *et al.* (1954). Phytate P in the diet and faeces was estimated by the method of McCance & Widdowson (1935). All the analyses were carried out in duplicate.

RESULTS

Nitrogen. The results for N metabolism are given in Table 4. The mean daily intake and retention on the ragi diet were 10·6 and 1·1 g respectively. The mean excretion of N in faeces was very high (5·5 g), amounting to about 50 % of the intake, so that the apparent digestibility of proteins from the ragi diet was about 50%. The mean loss of N in urine was 4·0 g. All the subjects were in positive balance.

Table 4. *Mean daily intake of calories, and intake, excretion and retention of nitrogen by subjects on the ragi diet*

		Nitrogen				
			Excretion			
Subject no.	Calorie intake (Cal.)	Intake (g)	Urine (g)	Faeces (g)	Total (g)	Retention (g)
1	2909	9·88	3·58	4·79	8·37	1·51
2	3267	11·16	4·78	5·93	10·71	0·45
3	3113	10·61	4·03	6·07	10·10	0·51
4	3211	10·96	3·92	5·83	9·75	1·21
5	3064	10·43	5·29	4·79	10·08	0·35
6	3208	10·95	3·10	5·89	8·99	1·96
7	2841	9·64	3·38	4·81	8·18	1·46
8	3175	10·83	3·53	6·16	9·69	1·14
Mean value with its standard error	3098	10·56 ± 0·19	3·95 ± 0·26	5·53 ± 0·22	9·48	1·07 ± 0·21

Table 5. *Mean daily intake, excretion and retention of calcium by subjects on the ragi diet*

		Excretion			
Subject no.	Intake (mg)	Urine (mg)	Faeces (mg)	Total (mg)	Retention (mg)
1	3113	262	2830	3092	21
2	3625	221	3206	3427	198
3	3404	210	3052	3262	142
4	3543	195	3190	3385	158
5	3333	185	3108	3293	40
6	3540	183	3188	3371	169
7	3015	265	2726	2991	24
8	3492	222	3238	3460	32
Mean value with its standard error	3383·0 ± 80·4	217·9 ± 11·2	3067·2 ± 67·2	3285·1	98·0 ± 26·6

Calcium. The results for Ca metabolism are given in Table 5. All the subjects were in positive balance. Though the mean daily intake of Ca was 3·4 g, the mean retention was only 0·1 g, corresponding to about 3% of the intake. The mean loss of Ca in faeces was very high (3·1 g) and accounted for about 90% of the ingested Ca.

Phosphorus. The results for P metabolism are given in Table 6. The mean daily intake and retention were 2·3 and 0·2 g respectively. All the subjects maintained a positive balance. The mean loss of P in faeces was 1·8 g, accounting for about 75% of the ingested P.

Phytate phosphorus. The results for the metabolism of phytate P are given in Table 7. Ragi, which formed the major constituent of the experimental diet, was found to contain 250 mg phytate P/100 g, or 80% of the total P present in ragi. The other constituents of the diet containing phytate P were: red gram dhal (200 mg/100 g), Bengal gram dhal (186 mg/100 g) and black gram dhal (150 mg/100 g). The mean daily intake and excretion in the faeces of phytate P were 1·8 and 0·3 g respectively. The results show that about 85 % of the ingested phytate P was hydrolysed during digestion and absorption of the food.

Table 6. *Mean daily intake, excretion and retention of phosphorus by subjects on the ragi diet*

Subject no.	Intake (mg)	Excretion: Urine (mg)	Excretion: Faeces (mg)	Excretion: Total (mg)	Retention (mg)
1	2118	307	1510	1817	301
2	2449	275	1920	2195	254
3	2307	311	1870	2181	126
4	2398	300	1930	2230	168
5	2261	318	1780	2098	163
6	2395	275	1820	2095	300
7	2055	268	1460	1728	327
8	2364	275	1970	2245	119
Mean value with its standard error	2293·4 ± 49·9	291·1 ± 7·0	1782·5 ± 68·6	2073·6	219·8 ± 30·0

Table 7. *Mean daily intake and excretion, and hydrolysis of phytate phosphorus by subjects on the ragi diet*

Subject no.	Intake (mg)	Excreted in faeces (mg)	Hydrolysed (as percentage of intake)
1	1686	156	91
2	1976	437	78
3	1852	298	84
4	1931	317	84
5	1812	171	91
6	1929	312	84
7	1631	221	86
8	1902	212	89
Mean	1840	266	86

DISCUSSION

Ragi and other millets are generally considered coarse foods as compared to rice, which in the cooked condition is very soft. Sudden change from a rice to a ragi diet generally causes digestive troubles. This may be to some extent connected with the bulkiness of the stools found in the present experiment; they amounted on average to about 150 g (dry weight) daily as compared with 43 g observed on rice diets in an earlier experiment (Murthy *et al.* 1954). It is of interest to note that McCance & Walsham (1948–9) found that the dry weight of faeces on a wholemeal-bread diet was almost twice that found on a white-flour bread diet. Cullumbine (1950) observed that the mean daily dry weight of faeces on an unpolished-rice diet was 72 g as compared with 27 g on a polished-rice diet. The high faecal bulk on the ragi diet may have been due to the high content of cellulose (3·5 g/100 g) and hemicelluloses (6·8 g/100 g) (Table 2) present in ragi. All the subjects of the present experiment were habitual ragi eaters; four of them had been subjects in a previous experiment (Murthy *et al.* 1954).

Nitrogen. The mean intake (10·56 g) of the experimental subjects was slightly higher than that (9·69 g) found on rice diet in the previous experiment (Murthy *et al.* 1954). The mean loss of N in the faeces on the ragi diet was very high (5·53 g) and the apparent digestibility of the protein was very low (50%) as compared with that (72%) reported earlier for the proteins of a milled-rice diet (Murthy *et al.* 1954). It may be of interest to compare the results obtained in the present investigation with those reported by other workers for diets based on wholemeal wheat bread (McCance & Widdowson, 1947; McCance & Walsham, 1948–9; Macrae, Hutchinson, Irwin, Bacon & McDougall, 1942), oats (McCance & Glaser, 1948–9) and unmilled rice (Cullumbine, 1950). McCance & Widdowson (1947) and McCance & Walsham (1948–9) found in human metabolic studies that the apparent digestibilities of the proteins of diets containing breads made of wholemeal flour, 90% extraction flour, and 80% extraction flour were 74, 80 and 83% respectively. Macrae *et al.* (1942) obtained somewhat similar results. McCance & Glaser (1948–9) reported that the proteins of a diet based on oats were poorly digested (69%) by human beings. Cullumbine (1950) observed that the apparent digestibility of the proteins of diets based on undermilled rice (74%) was lower than that of the proteins of a milled-rice diet (80%).

The actual mechanism responsible for the lower digestibility of protein as the fibre content of the diet is raised is not yet clear. McCance & Widdowson (1947) and McCance & Walsham (1948–9) concluded from their investigations that the greater amount of N in the faeces on a brown-bread diet was probably due to an increased amount of digestive secretions called forth by the nature of the diet. This does not appear to be a general happening, however, since evidence from the work of McCance & Glaser (1948–9) indicates that the protein of oatmeal was digested to a much lesser extent than that of whole wheat, even though the fibre content of the oatmeal was less than that of whole wheat. Macrae *et al.* (1942) found that the time required to pass carmine markers was approximately 40 h on the white-bread diet and about 24 h on the wholemeal diets. Hegsted, Tsongas, Abbott & Stare (1946) also have reported similar results. McCance, Prior & Widdowson (1953) found from radiological studies that brown bread was evacuated from the stomach and passed through the small intestine more rapidly than white bread; the residue from the brown bread when in the ileum and colon appeared to be greater than that from the white bread. Thus the time the material is in the gut, and susceptible to digestion and absorption may be a major factor in deciding digestibility.

Calcium. The daily Ca requirements of adult human beings have been estimated as 10 mg/kg body-weight (Steggerda & Mitchell, 1946). According to this estimate, the average daily Ca requirement of the subjects of the present investigation would be about 0·5 g. The average daily intake of Ca by the experimental subjects on the ragi diet was unusually high (3·4 g). The Ca:P ratio in the diet was 1·5:1. A large part (about 90%) of the ingested Ca was voided in the faeces. The results obtained in the present study may be compared with those reported by McCance & Widdowson (1942–3) for Ca-fortified brown-bread diets. They found that adults consuming a diet (supplying about 0·5 g Ca/day) based on brown bread were in negative Ca balance; fortification of the bread to contain 100 mg Ca/100 g, which raised the average daily

Ca intake to about 1·2 g, produced a slight positive balance in some of the subjects. The authors recommended the fortification of whole-wheat flour with calcium carbonate to provide a level of 200 mg Ca/100 g flour to counteract the anticalcifying influence of phytic acid and to promote Ca balance on such diets. The ragi diet used in the present experiment contained more Ca (about 400 mg/100 g) than the level (200 mg/100 g) recommended by McCance & Widdowson (1942–3) for whole-wheat bread diets.

Phosphorus. The extent to which phytates are broken down and their P absorbed depends on many factors, the most important being the intestinal flora and the Ca content of the diet (Lowe & Steenbock, 1936). McCance & Widdowson (1942–3) reported that 40–70 % of the phytate present in wholemeal-bread diets was hydrolysed, and Cullumbine, Basnayake, Lemottee & Wickramanayake (1950) reported that 85 % of phytate P ingested from rice diets was hydrolysed. We have found the same degree of hydrolysis for ragi; despite this the mean daily absorption of P on the ragi diet was only 0·5 g (about 25 % of the intake). The low percentage of absorption of the dietary P may be due to the high Ca intake, since it has been demonstrated by earlier workers that excess of Ca in the gut may precipitate the P as calcium phosphate and thus interfere with the absorption of P (Orr, Holt, Wilkins & Boone, 1924; McCance & Widdowson, 1942–3).

SUMMARY

1. The intake and absorption of nitrogen, calcium and phosphorus were studied in eight men fed on a poor vegetarian diet based on ragi. The composition of the diet was similar to that consumed by the poorer people in Mysore State.

2. All the experimental subjects maintained a positive N balance, the average daily intake and retention being 10·6 and 1·1 g. The excretion of N in faeces was very high (5·5 g); the apparent digestibility of the proteins from the diet was only 50 %.

3. The average daily intake and retention of Ca were 3·4 and 0·1 g. All the subjects maintained a positive Ca balance.

4. The average daily P intake and retention were 2·3 and 0·2 g. All the subjects maintained a positive P balance. The average daily intake of phytate P was 1·8 g, which amounted to about 80 % of the total P ingested. The average daily excretion of phytate P in faeces was only 0·3 g. About 85 % of the ingested phytate was found to have been hydrolysed.

Our thanks are due to Mr A. N. Sankaran for the statistical analysis of the results reported in this paper.

REFERENCES

Association of Official Agricultural Chemists (1950). *Official and Tentative Methods of Analysis*, 7th ed. Washington: Association of Official Agricultural Chemists.

Aykroyd, W. R., Patwardhan, V. N. & Ranganathan, S. (1951). *The Nutritive Value of Indian Foods and the Planning of Satisfactory Diets*, 4th ed. Delhi: Manager of Publications.

Basu, K. P. & Basak, M. N. (1939). *Indian J. med. Res.* **27**, 115.

Cullumbine, H. (1950). *Brit. J. Nutr.* **4**, 129.

Cullumbine, H., Basnayake, V., Lemottee, J. & Wickramanayake, T. W. (1950). *Brit. J. Nutr.* **4**, 101.

Giri, K. V. (1938). *Indian J. med. Res.* **25**, 869.

Giri, K. V. (1940). *Indian J. med. Res.* **28**, 101.

Hegsted, D. M., Tsongas, A. G., Abbott, D. B. & Stare, F. J. (1946). *J. Lab. clin. Med.* **31**, 261.
Lowe, J. T. & Steenbock, H. (1936). *Biochem. J.* **30**, 1991.
McCance, R. A. & Glaser, E. M. (1948–9). *Brit. J. Nutr.* **2**, 221.
McCance, R. A., Prior, K. M. & Widdowson, E. M. (1953). *Brit. J. Nutr.* **7**, 98.
McCance, R. A. & Walsham, C. M. (1948–9). *Brit. J. Nutr.* **2**, 26.
McCance, R. A. & Widdowson, E. M. (1935). *Biochem. J.* **29**, 2694.
McCance, R. A. & Widdowson, E. M. (1942–3). *J. Physiol.* **101**, 44.
McCance, R. A. & Widdowson, E. M. (1947). *J. Hyg., Camb.*, **45**, 59.
Macrae, T. F., Hutchinson, J. C. D., Irwin, J. O., Bacon, J. S. D. & McDougall, E. I. (1942). *J. Hyg., Camb.*, **42**, 423.
Murthy, H. B. N., Swaminathan, M. & Subrahmanyan, V. (1954). *Brit. J. Nutr.* **8**, 11.
Orr, W. J., Holt, L. E., Wilkins, L. & Boone, F. H. (1924). *Amer. J. Dis. Child.* **28**, 574.
Patwardhan, V. N., Mukundan, R., Rama Sastri, B. V. & Tulpule, P. G. (1949). *Indian J. med. Res.* **37**, 327.
Steggerda, F. R. & Mitchell, H. H. (1946). *J. Nutr.* **31**, 407.
Subrahmanyan, V., Kuppuswamy, S., Ramarao, G., Swaminathan, M. & Bhatia, D. S. (1954). *Bull. cent. Fd tech. Res. Inst.* **3**, 187.
Sundararajan, A. R. (1938). *Indian J. med. Res.* **25**, 685.
Sur, G., Reddy, S. K., Swaminathan, M. & Subrahmanyan, V. (1954). *Bull. cent. Fd tech. Res. Inst.* **3**, 111.
Swaminathan, M. (1938). *Indian J. med. Res.* **26**, 113.