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Effect of long-term feeding of albino rats on rice and on rice-tapioca diets on the blood and on the liver

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In a previous paper, Subrahmanyam, Murthy & Swaminathan (1954) reported that replacement of 25% of rice in a poor rice diet by tapioca flour enhanced significantly the growth of young rats. This effect was shown to be due to the supplementary value of the extra calcium present in the tapioca flour. Since partial replacement of rice by tapioca flour caused a slight lowering in the protein content of the diet, the possibility remained that the ill effects, if any, due to it, may not be observed in a period of 8 weeks. It was therefore thought desirable to conduct long-term feeding experiments and to study the effect of prolonged feeding of such diets on the concentration of haemoglobin in the blood, on the red-blood cell count, and on the composition and histological appearance of the liver. A third group of rats on the control rice diet, supplemented with the same amount of calcium (as calcium lactate) as that supplied by tapioca in the rice-tapioca diet, was also included with a view to ascertaining the effect of the calcium supplement on growth and on the liver and blood of rats.

EXPERIMENTAL

Three groups of freshly weaned albino rats weighing between 40 and 48 g (ten in each group and distributed equally according to sex, body-weight and litter) received the diets *ad lib.*; the composition of the diets is given in Table 1. The methods of prepara-

tion of the diets and of feeding the experimental animals were the same as those described in an earlier paper (Subrahmanyam *et al.* 1954). The rats were weighed weekly. At the end of 30 weeks' feeding, the haemoglobin concentration and the red-blood cell count were determined in blood drawn from the tail. Haemoglobin was determined by the acid-haematin method in a Sahli-Hellige haemometer (Hepler, 1950) and the red blood cell count was made by standard methods with a Neubauer's haemocytometer.

Table 1. *Percentage composition of experimental diets**

Ingredient	Rice diet (group A)	Rice-tapioca diet (group B)
Raw milled rice	78.5	58.9
Tapioca flour	—	19.6
Tur dhal (<i>Cajanus indicus</i>)	5.0	5.0
Non-leafy vegetables (potato and brinjal (<i>Solanum melongena</i>))	8.2	8.2
Leafy vegetables (<i>Amaranthus gangeticus</i>)	2.1	2.1
Groundnut oil	5.0	5.0
Skim-milk powder	0.9	0.9
Common salt	0.3	0.3

* Animals in group C received the rice diet supplemented with 70 mg calcium lactate (supplying about 11 mg Ca/100 g diet).

When these two measurements had been made, the animals were anaesthetized with Amytal (Eli Lilly and Co. Ltd, Basingstoke, Hants) and were bled through the abdominal aorta to ensure a minimum and relatively uniform amount of residual blood in the liver. The liver was then quickly removed, washed first with normal saline and then with distilled water to remove any adhering blood. The adhering water was absorbed on filter-paper and the liver immediately weighed in a glass dish and dried to constant weight at 90–95°. It was then ground to a fine powder and used for determination of total nitrogen by the micro-Kjeldahl method and of total lipids by the method of Tyner, Lewis & Eckstein (1950).

For histological studies two groups of freshly weaned albino rats weighing from 40 to 48 g (four males and two females in each group equally distributed according to weight and litter) were given *ad lib.* the rice and rice-tapioca diets (Table 1) respectively for a period of 68 weeks. The animals were weighed weekly. The mean body-weights were 161 ± 12.2 and 212 ± 19.3 g respectively at the end of the experiment. The animals were then anaesthetized with Amytal and bled through the abdominal aorta. The livers were removed and weighed. The mean weights of the livers of animals on the rice and the rice-tapioca diets were 6.3 ± 0.41 and 7.1 ± 0.43 g, respectively. The livers were washed in normal saline to remove adhering blood and were fixed in 10% formol saline. They were then embedded in paraffin and the numbered, embedded samples were sent to Dr C. G. Pandit, Director, Indian Council of Medical Research, who kindly arranged for the histological examination of the tissues by three pathologists.

Table 2. *Effect of the long-term feeding of rice and rice-tapioca diets on the growth of albino rats*

Group	Diet	Protein in diet† (%)	Calcium in diet† (mg/100 g)	Initial body-weight (g)	Gain in body weight* (g)				Daily food intake† (g)	
					In 8 weeks	In 30 weeks	In 8 weeks	In 30 weeks	In 8 weeks	In 30 weeks
A	Rice (control)	8.2	42	44.3	55.7	118.4	7.2	8.3		
B	Rice-tapioca	7.0	53	45.1	59.3	130.9	7.9	8.9		
C	Rice-calcium	8.2	53	44.3	63.3	122.0	7.8	8.7		

(Mean values for groups of five males and five females per group)

* Significance

8 weeks	30 weeks
Not significant	Significant at 5 %
Significant at 5 %	Not significant
Not significant	Not significant

† On moisture-free basis.

RESULTS

Growth rate. Table 2 shows that the mean gain in weight of rats receiving the rice-tapioca and the rice-calcium diets for 8 and 30 weeks was greater than that of the rats on the rice diet. It should be noted, however, that the difference in the mean gain in weight of the rats on the rice and the rice-tapioca diets was significant ($P < 0.05$) at 30 weeks but not at 8 weeks, whereas that of the animals on the rice and the rice-calcium diets was significant ($P < 0.05$) at 8 weeks but not at 30 weeks. On the other hand, there was no significant difference in the mean gain in weight of the rats on the rice-tapioca and the rice-calcium diets at 8 or at 30 weeks.

Table 3. *Effect of long-term feeding of albino rats on rice and rice-tapioca diets on the haemoglobin and red blood cell count and the composition of liver (duration of experiment 30 weeks)*

(Mean values for groups of five males and five females per group)

Group	Diet	Body-weight (g)	Haemoglobin (g/100 ml. blood)	Red-blood cell count (10^6 /cu. mm blood)	Fresh weight of liver (g)	Composition of liver*		
						Moisture (%)	Total nitrogen (%)	Fat (%)
A	Rice (control)	162.7	14.72	8.74	5.80	71.3	2.75	4.80
B	Rice-tapioca	176.0	14.82	8.94	6.39	71.8	2.78	4.26
C	Rice-calcium	166.3	14.69	8.77	5.71	71.4	2.90	4.80

Standard error of the difference between means of diets with results of test of significance

A~B	0.28 N.S.	0.25 N.S.	0.33 N.S.	0.52 N.S.	0.13 N.S.	0.36 N.S.
A~C	0.28 N.S.	0.25 N.S.	0.32 N.S.	0.51 N.S.	0.13 N.S.	0.36 N.S.
B~C	0.28 N.S.	0.25 N.S.	0.32 N.S.	0.51 N.S.	0.13 N.S.	0.39 N.S.

* Livers of one female rat in each of the groups A and B and one male rat in group C were lost. On the livers of the remaining rats, fat estimation could not be done for one male in each of groups B and C. The missing values were estimated and the standard errors of comparison between any two diet means were calculated by the method described by Cochran & Cox (1950).

N.S. = not significant.

Composition of blood and liver. The results given in Table 3 show that there was no significant difference in the moisture, nitrogen and fat content of the liver or in the haemoglobin and red blood cell count of the blood of the rats receiving the rice, rice-tapioca and the rice-calcium diets for 30 weeks.

Histological changes in the liver. The livers of the rats fed on the rice diet for 68 weeks showed evidence of mild to moderate parenchymal damage of the mild protein-deficiency type. The lobular pattern was generally well preserved, though liver cord disarray was a prominent feature. No appreciable fatty change was observed. Areas of patchy intralobular necrosis infiltrated with mononuclear cells were present in most of the sections. The portal areas were characterized by moderate to marked mononuclear cellular infiltration. Mesenchymal activity was not observed in any of the liver specimens. Livers of the rats receiving the rice-tapioca diet also showed evidence of diffuse parenchymal damage of almost the same degree. It was, however, suggestive that the livers of the rats on the rice-tapioca diet showed in general a slightly less degree of parenchymal damage than those of the rats on the rice diet.

In view of this observation, further studies on the composition and histological structure of livers of rats fed on the rice, rice-tapioca and rice-calcium diets for varying periods ranging from 2 months to 1 year are being undertaken.

SUMMARY

1. Replacement of 25% of rice in a poor vegetarian rice diet by tapioca flour enhanced the growth of rats. Supplementation of the rice diet with the same amount of calcium (11 mg/100 g) as that supplied by tapioca in the rice-tapioca diet also enhanced the growth of rats.

2. The difference in the mean gain in weight of the rats on the rice and the rice-tapioca diets was significant ($P < 0.05$) at 30 weeks but not at 8 weeks, whereas the difference in the mean increase in weight of the rats on the rice and the rice-calcium diets was significant ($P < 0.05$) at 8 weeks but not at 30 weeks. There was no significant difference, however, in the mean gain in weight of the rats on the rice-tapioca and the rice-calcium diets, in spite of a slightly lower protein content of the former.

3. Histological examination of the livers of the rats fed for 68 weeks on the rice and the rice-tapioca diets, assessed independently by three pathologists, showed in both sets of livers parenchymal damage of the mild protein-deficiency type. It was however, suggestive that the livers of the rats fed on the rice-tapioca diet showed slightly less parenchymal damage than those of rats on the rice diet.

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