Note

Effect of Age and Dietary Phytate on the Availability of Phytate and Minerals in Rats

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The nutritional importance of phytate (myo-inositol hexakisphosphate) in reducing the availability of essential dietary minerals such as Ca, Mg, Fe and Zn has been recognized by many investigators.¹⁾ However, others have shown that phytate has no inhibitory effect on mineral bioavailability.¹⁾ One reason for this disagreement may have resulted from different levels of degradation of phytate in the intestines depending on the ages of animals and their adaptation to phytate intake. There is some indication that the ability of swine²⁾ and poultry³⁾ to utilize phytate-P improves with age. However, Nelson and Kirby⁴⁾ observed a significant decrease in phytate hydrolysis in mature rats. It is speculated that species difference may be involved in this discrepancy. The experiment described in this paper was an attempt to determine whether the effects of age and an adaptive increase in phytate digestibility can be observed in rats given a diet containing natural phytate, and to estimate the bioavailability of N, Ca, Mg and P in rats of different ages in the presence of natural phytate.

Weanling inbred Fischer strain (F344/DuCrj) male rats from Charles River Japan Inc. were used. The rats were randomly distributed into two experimental groups of seven animals each, and were housed individually in stainless steel metabolism cages during the experiment at a temperature of $23 \pm 3^\circ\text{C}$. Experimental diets and distilled deionized water were fed *ad lib.* from 4 weeks of age. The average body weight of the rats at the beginning of the 1st experiment (5 weeks of age) was 86.6 g and that at the beginning of the 2nd experiment (11 weeks of age) was 259.1 g. Body weight and feed intake were recorded for 1 week from the start of each experiment, and the feces and urine were collected daily. The rats were given the sterilized diets L-485⁵⁾ or L-488F.^{6,7)} The former is a practical diet based on corn-soybean meal, and the latter is

TABLE I. N, Ca, Mg AND P CONTENTS OF AUTOCLAVED DIETS (% in dry matter)

Diet	N	Ca	Mg	P	Phytate-P
L-485	4.02	0.639	0.216	0.687	0.285
L-488F	3.90	0.390	0.072	0.431	—

a purified diet based on casein-corn starch. The diets were sterilized by autoclaving for 30 min at 121°C . The chemical compositions of the diets were analysed as shown in Table I. Rats in group A were given diet L-485 throughout the experimental period. Rats in group B were given the diet L-488F from 4 to 9 weeks of age and the diet L-485 from 10 to 12 weeks of age. The feces was dried with circulating air at 60°C after spraying with a 10% HCl solution. The urine was collected with four drops of conc. H_2SO_4 in the bottom of a collecting cylinder. The diets and dried feces were ground finely, and these samples and the urine with a few drops of CHCl_3 added were kept in a refrigerator until the analysis. The semi-micro Kjeldahl method was used for the N estimation.⁸⁾ Determination of Ca and Mg were performed with an atomic absorption spectrophotometer.⁹⁾ Total-P was analyzed by the Allen method,¹⁰⁾ and phytate-P was estimated according to the Oberleas method.^{11~13)} The differences between groups A and B were analysed for statistical significance by Student's *t*-test, and the paired sample *t*-test was used for data from the same animals in the same group. The difference between the means was considered significant if $p < 0.05$.

Because different types of diet were fed to the rats, the values obtained for group B in the 1st and 2nd experiments were not compared during the present experiment. The average body weight gain, feed intake, feed efficiency and phytate hydrolysis for groups A and B during the 1st and 2nd experiments are shown in Table II. There were no differences between group A and B during the two experiments, but the differences were significant when the 1st and 2nd experiments were compared. In group A, growing rats hydrolyzed 85% of the phytate-P whereas mature rats hydrolyzed only 64%. Nelson and Kirby⁴⁾ showed this tendency as well. Several workers have reported the presence of phytase in rat intestines^{14~17)} and microorganisms.^{18,19)} Therefore, it is probable that intestinal or microbial phytase decreases with the age of the rats.

Moreover, the phytate-P digestibility in the 2nd experiment tended to be higher in group A than in group B, although no significant difference was found between the two groups. When rats fed on the phytate-free diet from 4 to 9 weeks of age were given the phytate-containing diet from 50 days of age, they showed a lower ability to hydrolyze phytate-P than rats of the same age fed on the phytate-containing diet from 4 weeks of age. This may show the possibility that adaptation to the hydrolysis of phytate takes place in the rat intestine as shown by Walker

TABLE II. FEED CONSUMPTION, BODY WEIGHT GAIN, FEED EFFICIENCY AND PHYTATE HYDROLYSIS
(per rat per day)

Exp. no.	Group ^a	Feed consumption (g)	Body weight gain (g)	Feed efficiency ^b	Phytate-P			
					Intake (mg)	Fecal excretion (mg)	Apparent digestion ^c (mg)	Apparent digestibility (%)
1st	A	12.0 ± 0.5 ^d	4.64 ± 0.32	38.5 ± 2.9	34.4 ± 1.5	5.2 ± 1.2	29.2 ± 1.9	84.9 ± 3.9
	B	11.1 ± 0.2	4.89 ± 0.14	44.1 ± 2.7	—	—	—	—
2nd	A	15.6 ± 0.4	1.46 ± 0.09	9.4 ± 0.5	44.5 ± 1.3	16.1 ± 2.7	28.4 ± 2.9	63.8 ± 6.1
	B	15.4 ± 0.5	1.77 ± 0.16	11.5 ± 1.0	44.0 ± 1.3	20.3 ± 2.4	23.7 ± 2.5	53.9 ± 5.2
Statistical summary		—	—	—	—	—	—	—
1st (A × B)		—	—	—	—	—	—	—
A (1st × 2nd) ^e		**	**	**	**	*	—	*
2nd (A × B)		—	—	—	—	—	—	—

^a Group A was fed diet L-485 throughout the experimental period, and group B was fed diet L-488F until 9 weeks of age and thereafter the diet was changed to L-485.^b [Body weight gain (g)/feed consumption (g)] × 100.^c Intake (mg) — fecal excretion (mg).^d Mean ± SE.^e Paired sample *t*-test was used.* Significant difference at *p* < 0.05.** Significant difference at *p* < 0.01.

et al.,²⁰⁾ Campbell *et al.*²¹⁾ and Bhaskaram and Reddy,²²⁾ who suggested gradual adaptation in human subjects on high phytate diets. The digestibility of phytate-P obtained in this experiment was higher than those of other researchers.^{4,23,24)} Because the experimental diet in the present study was autoclaved, part of the phytate might have been hydrolyzed¹²⁾ and more easily utilized compared with raw diets.

Data for the apparent digestibility and retention of dietary N, Ca, Mg and P are given in Table III. In the 1st experiment, the digestibility and retention rate of N and three kinds of mineral in group A fed the practical diet were lower than those in group B fed the purified diet. These results agree with our previous study⁷⁾ using mice. The results from the comparison of the 1st and 2nd ex-

periments in group A showed that the growing rats had a higher digestibility of Ca, Mg and P, and a higher retention rate of N, Ca and P than adult rats.

Because the same practical diet was used, the rats in both groups in the 2nd experiment had a similar digestibility, retention and retention rate of N, Mg and P. However, the results showed that the above-mentioned parameters concerning Ca in group B were inferior to those in group A. The similar tendency of Ca balance was also observed in children.²²⁾ It is probable that this difference is due to the lower phytate hydrolysis in group B than in group A, although there was no statistically significant difference. The practical diet (L-485) used for this experiment contained a 5-fold higher concentration of Mg than that of requirement level in rats.²⁵⁾ This may be

TABLE III. DIGESTIBILITY AND RETENTION OF N, Ca, Mg AND P

	Exp. no.	Group ^a	Apparent digestibility (%)	Retention ^b (mg/rat/day)	Retention rate ^c (%)
N	1st	A	79.1 ± 1.6 ^d	307 ± 19	63.3 ± 2.3
		B	92.0 ± 0.5	308 ± 7	71.1 ± 1.0
	2nd	A	78.1 ± 0.8	286 ± 16	45.5 ± 1.7
		B	76.9 ± 0.8	257 ± 18	41.4 ± 2.3
Ca	1st	A	61.0 ± 3.4	46.5 ± 2.5	60.4 ± 3.4
		B	90.9 ± 0.7	39.0 ± 0.5	90.8 ± 0.7
	2nd	A	47.2 ± 2.4	46.3 ± 2.2	46.9 ± 2.4
		B	36.9 ± 3.5	36.0 ± 3.8	35.4 ± 3.5
Mg	1st	A	43.0 ± 2.7	9.5 ± 0.8	37.1 ± 2.7
		B	73.2 ± 3.9	4.8 ± 0.2	61.0 ± 2.6
	2nd	A	36.2 ± 1.9	11.6 ± 0.7	35.4 ± 1.9
		B	34.8 ± 1.1	11.1 ± 0.7	33.2 ± 1.2
P	1st	A	61.4 ± 3.1	43.9 ± 3.6	53.1 ± 5.9
		B	94.6 ± 0.3	38.4 ± 0.9	80.3 ± 0.9
	2nd	A	40.4 ± 2.1	30.6 ± 2.8	28.6 ± 2.4
		B	40.7 ± 1.9	29.0 ± 2.4	27.4 ± 1.8
Statistical summary					
N	1st (A × B)		**	—	*
	A (1st × 2nd) ^e		—	—	**
	2nd (A × B)		—	—	—
Ca	1st (A × B)		**	*	**
	A (1st × 2nd) ^e		**	—	**
	2nd (A × B)		*	*	*
Mg	1st (A × B)		**	**	**
	A (1st × 2nd) ^e		*	*	—
	2nd (A × B)		—	—	—
P	1st (A × B)		**	—	**
	A (1st × 2nd) ^e		**	**	**
	2nd (A × B)		—	—	—

For ^a, ^d, ^e, * and **, see Table II.

^b Intake (mg)—fecal and urinary excretion (mg).

^c Retention (mg)/intake (mg).

one reason why there was no difference in the digestibility, retention and retention rate of Mg between groups A and B.

In our previous study,¹³⁾ it was assumed that phytate-P had a similar digestibility to non-phytate P in mice. This assumption was not confirmed by the present experiment which showed a higher digestibility of phytate-P than total-P in rats. This discrepancy in the results between mice and rats may be attributed to the different abilities to hydrolyze phytate-P in the two animals. Canals *et al.*²⁶⁾ found that rats assimilated far more ³²P than mice from ingested ³²P-labeled phytate.

In conclusion, the results obtained in this investigation show that the age of rats affects the hydrolysis of phytate-P and the apparent digestibility of minerals. A higher digestibility of phytate-P than total-P was also observed in rats. The possibility that an adaptive increase in intestinal phytate hydrolysis occurs within 1 week after commencing the phytate-containing diet cannot be ruled out. Therefore, it is felt that future studies will be needed in this direction.

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