3. THE INFLUENCE OF DIETARY FIBRE ON SECRETORY ACTIVITIES OF THE ALIMENTARY TRACT

OBSERVATIONS ON FAECAL PHOSPHATASE EXCRETION AND CALCIUM AND NITROGEN BALANCES OF RATS

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Some years ago, during an investigation of the influence of rate of growth on the development of rickets in sheep, parallel studies were undertaken using the rat. Owing to the difficulty of obtaining from rats blood samples sufficiently large for phosphatase determination, and having in mind the observations of Heymann [1930; 1931; 1933] on the faecal excretion of phosphatase in rickets, the concentration of this enzyme in the faeces of the rats was determined. It was observed that wide fluctuations occurred in the elimination of phosphatase when animals were transferred from one diet to another. This was especially noticeable in the case of weanling rats transferred to stock diets. The tentative conclusion was drawn that the augmentations in excretion were caused by increased bulk of ingesta which, it was felt, must increase secretory activity within the alimentary tract. The large increases observed for the weanlings were, no doubt, the result of the sensitivity to stimulus of an immature tract accustomed to a diet mainly of milk. These results were not published.

More recently investigations have been in progress in which domestic animals received larger quantities of fibre in the diet than is customary, and certain questions regarding the influence of bulk of ingesta on activities within the alimentary tracts were raised. The connexion between this problem and earlier investigations led to the undertaking of the present study.

Experimental

Six virgin female albino rats aged 105–140 days and weighing 199–225 g. were housed in pairs in three Hopkins metabolism cages which permit of the separate collection of urine and faeces. The animals were fed daily at 10 a.m. after collection of the urine, faeces and unconsumed food. Food and distilled water were offered in excess of appetite. The composition of the low fibre basal diet is recorded in Table 1.

Table 1. *Low fibre diet*

	g.		g.
Sugar	250	$NaHCO_3$	28
Casein	800	$FeSO_4.(NH_4)_2SO_4, 6H_2O$	20
Starch	2200	KH_2PO_4	108
Cottonseed oil	250	NaCl	20
Yeast	160	$MgSO_4, 7H_2O$	20
$CaCO_3$	100	Radiostoleum	4 ml.
KCl	40		

The experiment was divided into five periods, the first of 10 days' duration and the remainder of 6 days each. During period 1 the basal diet was offered. During period 2 the basal diet was supplemented with 10 % of acid-washed Ca-free paper pulp. In period 3 20 % and in period 4 30 % of paper pulp was added. Period 5, in which 40 % of paper pulp was added to the basal diet, ended abruptly owing to failure of appetite. The day's ration was made up in a stiff dough to minimize scattering and selective feeding.

There were no intervals for adjustment between the periods, the balances being continuous.

Methods of analysis

Calcium. Samples of urine and faeces were dried and ignited at a dull red heat until all traces of carbon had disappeared. The ash was dissolved in hot dilute HCl and made up to known volume. Aliquots were transferred to conical centrifuge tubes, made just alkaline with dilute NH_4OH and then just acid with dilute CH_3COOH using methyl red as an indicator. The tubes were placed in a boiling water bath and, after a few minutes, an excess of a boiling solution of 4 % $(COONH_4)_2$ was added. The tubes were then held at about 70° for 1 hr. to induce the formation of granular precipitates and allowed to stand at room temperature overnight. The precipitates were centrifuged and washed with 2 % NH_4OH. Supernatant liquors were removed by siphoning to avoid loss of precipitate. The oxalate, dissolved in dilute H_2SO_4, was titrated with permanganate.

Nitrogen. All estimations were performed by the macro-Kjeldahl method.

Phosphatase. The day's faeces were well mixed and a sample taken. The sample was placed in a bottle with 200 ml. distilled water and a little toluene and shaken in an end-over-end shaker for about 4 hr. by which time disintegration was complete. The sample was left at room temperature overnight and then analysed according to Bodansky's [1933] method for serum phosphatase. All results are expressed as Bodansky units.

Results

The Ca balances are recorded in Table 2 and the N balances in Table 3. In Table 4 are given the daily phosphatase excretions in the faeces and the units of phosphatase per g. of fresh faeces. All values given are for the group as a whole.

The animals remained in good health throughout the experiment. During period 1 no diarrhoea occurred but occasionally large, moist boli were passed. Throughout the experiment body weight increased from a mean value of 203 g. at the beginning to a mean value of 229 g. at the end. A certain amount of this increase can be attributed to greater weight of intestinal contents during the periods of high fibre intake.

The inclusion of increasing amounts of fibre caused progressive reductions in the intakes of basal ration. These reductions were moderate, the addition of the maximal quantity of fibre (30 % in period 4) causing a fall of about 20 % in the basal diet intake from the period 1 level.

Urinary Ca values were low, only about 1 % of ingested Ca being excreted by the kidneys. There were slight erratic variations in output, but the range was not wide and the mean excretions for each period were unrelated to changes in fibre intake. The major portion of the Ca was found in the faeces. The balances were positive throughout, the best retention occurring during the period when 10 % of fibre was included in the diet. In periods 3 and 4 the retentions were about the same and, in magnitude, about two-thirds of the period 2 retention.

Table 2. *The effect of adding to the diet increasing amounts of fibre on the daily Ca balances of rats*

Period	Day	Ca intake mg.	Ca output mg.	Ca balance mg.	Ca in urine mg.	Ca in faeces mg.
1	1	1040	1003	+ 37	10·7	992
	5	983	867	+116	6·3	861
	6	1026	1081	− 55	5·4	1076
	7	1004	921	+ 83	7·6	913
	8	1000	922	+ 78	8·4	914
	9	971	982	− 11	6·7	975
	10	913	939	− 26	6·2	933
2	1	960	961	− 1	7·1	954
	2	960	—	—	—	—
	3	1074	953	+121	7·4	946
	4	1002	888	+114	8·2	880
	5	944	888	+ 56	9·3	879
	6	880	835	+ 45	7·1	828
3	1	851	839	+ 12	6·6	832
	2	827	788	+ 39	5·6	782
	3	848	817	+ 31	5·3	812
	4	814	771	+ 43	5·4	766
	5	829	788	+ 41	9·7	778
	6	913	799	+114	7·9	791
4	1	794	853	− 59	6·1	847
	2	795	707	+ 88	5·5	702
	3	815	712	+103	6·9	705
	4	731	697	+ 34	6·4	691
	5	836	782	+ 54	7·9	774
	6	755	723	+ 32	11·4	712
			Averages			
1	—	991	959	+ 32	7·3	952
2	—	972	905	+ 67	7·8	897
3	—	847	800	+ 47	6·8	794
4	—	788	746	+ 42	7·4	738

Urinary N values declined from period to period as the N intake fell. The faecal excretion increased from period 1 to period 2 and again in period 3. The value found for period 4 was somewhat below the period 2 level. The mean retentions were positive in all periods, higher retentions being found in periods 1 and 3 and the lowest in period 4. Negative daily balances preponderated in period 4 but were absent in period 1. Occasional negative daily balances occurred during periods 2 and 3.

During period 1 there were moderate fluctuations in the daily excretion of phosphatase but, although the range was wider than might have been expected, the inclusion of 10 % of fibre in the diet caused a large increase in excretion well beyond the upper limit of period 1 range. In period 3 the excretion of phosphatase was again greater than in period 2 and the values for period 4 were higher than in period 3. The increases with each addition of fibre were progressively less. The mean values showed a more than sevenfold increase between periods 1 and 4. The units of phosphatase excreted per g. of fresh faeces increased in periods 2 and 3 over period 1, but in period 4 the value fell to a point midway between the two extremes. In each of the fibre periods the sudden increase in fibre intake led to maxima in faecal phosphatase excretion, after which the animals tended to adjust themselves to the new conditions.

Table 3. *The effect of adding to the diet increasing amounts of fibre on the daily N balances of rats*

Period	Day	N intake mg.	N output mg.	N balance mg.	N in urine mg.	N in faeces mg.
1	1	3016	2745	+271	2392	353
	5	2852	2544	+308	2260	284
	6	2979	2720	+257	2400	320
	7	2914	2806	+108	2504	302
	8	2901	2660	+241	2350	310
	9	2930	2655	+275	2349	306
	10	2654	2604	+ 50	2328	276
2	1	2743	2436	+307	2092	344
	2	2744	2607	+137	2264	343
	3	3101	3015	+ 86	2665	350
	4	2876	2563	+313	2250	313
	5	2513	2634	−121	2278	356
	6	2312	2575	−263	2272	303
3	1	2458	2223	+235	1738	485
	2	2388	2430	− 42	2090	340
	3	2450	2242	+208	1900	342
	4	2391	2289	+ 62	1990	299
	5	2393	2216	+177	1822	394
	6	2640	2464	+176	2130	334
4	1	2281	2292	− 11	1950	342
	2	2286	2188	+ 98	1839	349
	3	2347	2387	− 40	2062	325
	4	2095	2155	− 60	1870	285
	5	2408	2213	+195	1906	307
	6	2166	2213	− 47	1874	339
			Averages			
1	—	2891	2676	+215	2369	307
2	—	2715	2638	+ 77	2303	335
3	—	2447	2311	+136	1945	366
4	—	2264	2241	+ 23	1917	324

Table 4. *The effect of adding to the diet increasing amounts of fibre on the daily faecal excretion of phosphatase by rats*

Period	Day	Total faecal phosphatase	Phosphatase per g. of faeces	Period	Day	Total faecal phosphatase	Phosphatase per g. of faeces
1	1	182	14·4	3	1	1061	24·2
	5	149	15·0		2	1178	27·4
	6	186	15·4		3	1433	29·9
	7	165	15·6		4	1230	29·0
	8	239	22·0		5	1286	27·8
	9	292	24·6		6	1074	24·8
	10	200	18·5				
Mean		202	17·9	Mean		1210	27·7
2	1	637	28·5	4	1	1598	23·4
	2	1033	33·7		2	1589	22·3
	3	1010	31·5		3	1436	20·8
	4	676	24·4		4	1423	22·0
	5	870	29·0		5	1513	22·2
	6	535	22·9		6	1306	19·3
Mean		794	28·3	Mean		1478	21·7

Discussion

It is felt that the preliminary nature of the observations of the effect of large quantities of crude fibre in the diet on phosphatase excretion in the faeces should be emphasized. At the present time an exact interpretation of the results is not possible because neither the individual contribution of the different secreting organs nor the rates of destruction of the secreted enzyme within the lumen of the intestine during the different periods were assessed. Nevertheless certain conclusions can profitably be drawn.

While it is possible that there was a proportional increase of all secretions, amounting to about 700 % in period 4 as compared with period 1, it is more probable that there were greater increases in the secretions of certain organs and smaller, or even no, increases in the case of others. Also, there is no information regarding the possible exhaustion, or semi-exhaustion, of the enzyme within an organ suddenly called upon to increase secretion. In such a case the phosphatase value of the faeces would be, by underestimation, an imperfect guide to measurement of increased secretory activity. Although there is no clear evidence that bulk of ingesta has any marked effect in increasing the speed of propulsion of material through the intestinal tract, if this does occur the faecal phosphatase value would overestimate increased secretory activity owing to reduced opportunity for enzyme destruction.

In the present investigation an objection might be raised to comparing period 1 with period 4 on the ground that this involves the assumption that results found for the fibre-free period represent the normal state of affairs and can therefore be accepted as basic in subsequent discussion. But even if this period be discarded and period 2, with the 10 % fibre intake, be accepted as normal, period 4 still exhibits an increase of 100 % in faecal phosphatase excretion. If, however, the balance data for Ca and N can be taken as a guide there is no evidence of dysfunction during period 1. Moreover, beyond the occasional appearance of moist boli, defecation was normal.

The main source of the increases in faecal phosphatase excretion is probably the wall of the intestine, since the greater bulk calls for more lubrication during propulsion. In this connexion the distribution of phosphatase within the faecal boli was examined and it was found that the concentration was greater in the pellicle than in the centre. This difference could only have arisen after the boli had been formed and was therefore attributable to the secretory activity of the large intestine. During the periods when 10 and 20 % of fibre were added to the diet the quantity of phosphatase per g. of faeces fell within the same range for both periods and the average values were closely similar. In period 4, which represents with an intake of 30 % of fibre about the limit that the rat will tolerate, the excretion per g. of faeces fell. This coincides with the concentration of dietary fibre which Westerlund [1938, 2] found increased the faecal Ca output in the rat when fed on a very low Ca diet. The phosphatase data suggest that a 30 % level of fibre reduces the efficiency of the intestine in relation to the bulk of ingesta although the total activity is further increased (from the period 2 level). This impairment of efficiency is also revealed in Westerlund's experiment, where Ca intake was very low. As is discussed below this does not occur with diets of normal Ca content.

Several workers have studied the influence of dietary fibre on Ca retention. Bloom [1930] found that the addition of 8 % of fibre to a diet of normal Ca content had no effect upon Ca retention of rats, and this was confirmed by Adolph *et al.* [1938] who, however, used a low Ca diet. Ascham [1930–31] considered that bulk may increase faecal Ca losses in dogs but felt that her results might be inconclusive. Conversely, Westerlund [1938, 1, 2] reported that the incorporation of paper pulp or acetone-extracted, electrodialysed straw flour beyond the level of 20 % increased the faecal elimination of Ca by rats subsisting on a diet extremely low in Ca and P. It was considered that the increased bulk stimulated secretory activities within the intestine and that an increased rate of transit of the ingesta through the intestine prevented complete reabsorption of the Ca of the secretions, and the conclusion was drawn that Ca requirement was increased. As stated above, there is no good evidence that increased bulk promotes more rapid propulsion of ingesta, and Westerlund states that he had previously failed to establish any relation between Ca absorption and crude fibre consumption in dairy cows. But, as is discussed below, faecal Ca studies and balance data cannot measure total absorptive processes; only net values can be derived. Morgan [1934], studying adult human subjects consuming coarsely ground cellulose added to a standard diet, found that Ca balances tended to become negative during the high fibre period. Sjollema [1923] observed that increased fibre consumption resulted in negative Ca balances in the rabbit where balances previously had been positive. It is generally accepted that the rabbit is an unsatisfactory animal for Ca balance studies owing to the erratic nature of the results yielded. This peculiarity of the rabbit is well shown by the wide variations in blood Ca frequently observed and by the observation of Cowell [1937] that the rabbit excretes considerable quantities of Ca through the colon. This last characteristic is the opposite of that found by Nicolaysen [1934] and Christiansen [1936] for dogs and by McCance & Widdowson [1939] for man.

The Ca balance results of the present study agree with those of Bloom [1930] and of Adolph *et al.* [1938]. But the results can only be adequately interpreted when examined in conjunction with the phosphatase values. Although it was reasonable to expect that increased bulk of ingesta would induce greater secretory activity within the alimentary tract, it was not expected that such increase would be sevenfold. It is remarkable that such an increase was not associated with a detrimental effect upon the Ca balance. In this respect it is of importance to consider the observation of Nicolaysen [1934] that the output of Ca in the digestive juices of adult man amounts to about 1 g. daily, that is to say, about twice the daily Ca requirement. (This observation is similar to that of Watson [1933] who observed that the salivary P, mainly inorganic, of sheep can amount to as much as 7·7 g. daily, which is several times the daily requirement for growth.) That reabsorption of this secreted Ca is conducted with an extraordinary degree of efficiency is made clear by considering the phosphatase increases. The enormous increase in secretory activity which must have been associated with the increases observed in faecal phosphatase occasioned no losses of Ca from the organism. It is probably correct to assume that the increase in the volume of the secretions would cause some diminution in the concentration of Ca in the secretions, but it is unlikely that the reduction would be severe, since the electrolyte pattern of the secretions bears a relation to that of the blood (as has been shown by Watson for salivary P and blood P in sheep) and there is no record of increased bulk of ingesta reducing blood Ca.

The efficiency with which the secreted Ca is reabsorbed is probably the outcome of the Ca being in ionized form, or at least in forms readily ionizable under conditions existing within the intestine.

Mitchell [1923–24] found that N excretion was increased by 42 % when rats receiving a N-free, low fibre diet had access to filter paper and his 'metabolic N' correction is generally accepted in certain types of protein metabolism studies.

Whiteacre *et al.* [1929–30] considered that high fibre diets reduced the N balances in human subjects but, unfortunately, no analyses of the diets for crude fibre were performed. Morgan [1934] reported that an increased fibre intake had a slight detrimental effect on N retention in man. In the case of the rabbit, Sjollema [1923] failed to find any effect of crude fibre on N retention.

The generally observed detrimental effects on N retention of increased fibre intake were found in the present study. The highest retention was observed during the period of lowest fibre intake and the lowest retention occurred during the period of highest fibre intake. During period 3 a higher retention was observed than in period 2; this may have arisen owing to the shortness of the experimental periods and the lack of accustoming intervals between periods. The percentage of N intake excreted in the faeces for the 0, 10, 20 and 30 % fibre periods were: 10·6, 12·3, 15·0 and 14·4. In this case again an unexpectedly high value occurs in period 3, resulting from the aberrant values found for urinary and faecal N on the first day of this period when, apparently, there was poor absorption of N. If the faecal N value for this day is omitted in calculating the percentage of dietary N lost in the faeces the series becomes: 10·6, 12·3, 14·0 and 14·4. Since the faecal N originates only in part from the dietary N, an attempt is made in Table 5 to recalculate the data in such a manner as to reveal any connexion existing between the increases in faecal N excretion consequent on increased fibre intakes and increased phosphate excretions. It is realized that several debatable assumptions are involved in this approach but, in the absence of values for the different forms of N in the faeces, a first approximation is attained.

Table 5. *Relation of 'N-cost' of dietary fibre to the excretion of phosphatase in faeces*

Period	Faecal N mg.	N originating from intake mg.	'N-cost' mg.	Phosphatase increase	Phosphatase increase / 'N-cost'
1	307	—	—	—	—
2	335	288	47	592	13
3	342	259	83	1008	12
4	324	240	84	1276	15

In column 2 are recorded the faecal N averages from Table 3 (except in the case of period 3, where the aberrant value for the first day has been excluded from the calculation of the average). In column 3 is recorded the quantity of N in the faeces that is calculated to have arisen from and on account of the basal diet intake, the computation being based on the period 1 finding that the faecal N is equal to 10·6 % of N intake when no fibre is added to the diet. As stated above, Mitchell found that the 'metabolic-N' bore a close relation to the quantity of diet consumed; this was confirmed by Adolph & Wu [1934], who demonstrated in addition that increased bulk of ingesta did not interfere with corrected protein digestibility unless the material passed through the intestine at a more rapid rate than usual. Neither Adolph & Wu nor ourselves found any evidence of increased rate of transit when filter paper was fed to rats. In column 4 are given the differences between the values in columns 2 and 3; this fraction is considered to be the portion of the faecal N which was lost to the animal as a result of the quantity of fibre ingested and is referred to as the 'N-cost' of the fraction. In column 5 are given the increases in phosphatase excretion over the basal period 1 level. Finally, in the last column are presented the ratios Phosphatase increase:-'N-cost'.

The agreement between the ratios suggests that there is a relation between phosphatase excretion and increased N excretion on higher fibre intakes. Theoretically, it seems probable that the extra N eliminated is partly in the form of epithelial fragments and partly mucoprotein. It is to be expected that such contributions of these forms of N as occur in the lower regions of the small intestine will escape hydrolysis and absorption. It will certainly be true of those originating in the large intestine.

From the results obtained it is indicated that studies of phosphatase excretion may be valuable aids in investigations of secretory activities of the intestinal tract and this probably applies also in the case of other enzymes. In questions of pathological changes of the tract the estimation might be of value. Concerning the latter, the test might be of use in studies of cases of hypersensitivity of the intestinal tract to fibre in human subjects.

Summary

Increasing the fibre content of the diet of rats to levels as high as 30 % had no influence on Ca retention but reduced the N balance.

Large increases were observed in faecal phosphatase excretion when the fibre intake was raised and this was thought to indicate increased secretory activity within the intestines.

The absence of any depressing effect of large increases in the intestinal secretions on the Ca balance was considered to indicate that the reabsorption of secreted Ca is highly efficient.

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