CLXXI. THE EFFECT OF BODY STORES ON THE EFFICIENCY OF CALCIUM UTILIZATION

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MANY Ca and P balance experiments with animals and man have been carried out during the last 35 years. The resulting data are rather difficult to interpret because of much variation from unknown causes. It occurred to the writer that possibly the degree of Ca "saturation" of the tissues was a factor influencing the efficiency with which an individual utilized his dietary Ca.

It has been shown by Fairbanks & Mitchell [1936] that a low Ca content of the body brought about by subsistence on a diet poor in this element predisposes to a subsequent high retention of Ca when liberal amounts are furnished in the diet. In their experiment, the Ca content of the diet was 1.25% in the experimental period which followed a preliminary period on a low Ca diet. The 1.25% Ca level is so much in excess of the Ca requirement for maximum storage, however, that the animals were able to replenish their depleted Ca stores even with a low percentage retention. On the basis of the intake and balance figures given in their paper, the writer has calculated the average percentage retention for the animals that had been on the low diet in a previous period: this was found to be only 24%. Hence, this experiment does not elucidate the question as to whether an individual becomes more efficient in the utilization of the available dietary Ca in times of stress.

In order to test this point it is essential that the Ca furnished in the diet in a test period, following a preparatory period on a low Ca diet, be below the requirement for maximum storage. It was felt that the point was of sufficient importance in connexion with the determination of the Ca requirements of man and animals to justify experimental enquiry.

FIRST EXPERIMENT

This experiment was conducted according to the paired feeding method. The food intake of a pair was restricted to the amount consumed by the member eating the less.

Six young hooded male rats were selected and paired, the two members of a pair being of the same litter and as nearly alike as possible. Four of the animals were 33 and two were 30 days old when they were started on the balance experiment.

A basal diet of the following composition was fed:

Yeast	...	...	5
Casein	...	...	5
Wheat gluten	...	...	15
Corn starch	...	...	60
Butter fat	...	...	12
Salt mixture	...	...	3

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The salt mixture was De Loureiro's [1931] with calcium phosphate omitted as adapted by Henry & Kon [1937], and was made up from B.D.H. "Analar" reagents.

This basal diet contained 0.10 % Ca and 0.19 % P; its P content was adjusted to 0.4 % by addition of K_2HPO_4 . In the preliminary period the Ca was fed at two levels, namely 0.15 and 0.8 %, one member of a pair being fed the 0.15 % Ca diet, the other the 0.8 % Ca diet. The Ca content was regulated by addition of $CaCO_3$. In all cases the odd number rats received the low Ca diet in the preliminary period.

The diets were fed in four 7-day balance periods. The animals were kept in Hopkins metabolism cages made of stainless steel. At the end of each 7-day collection period the cages, beakers and funnels were rinsed in 5 % HNO_3 and the washings added to the urine. The urine was evaporated to dryness and ashed before being analysed.

The 4 weeks' preliminary period was followed by a 5 weeks' test period during which the Ca content of the diet was adjusted to the 0.4 % level for both members of a pair. At the end of the test period the animals were killed, the intestinal tract was removed and the carcass analysed for Ca and P.

Results

The food intake and gain in live weight are given in Table I. The average daily gain in live weight in the preliminary period was 1.5 g. for the animals on the low Ca diet and 1.7 g. for those on the high Ca diet; in the test period the live weight gain was almost the same in both groups, averaging 2.2 g. daily. The relatively low gain in the preliminary period was due to lack of appetite in the animals on the low Ca diet. There was not a single instance of refusal of food in the animals on the high Ca diet. On the low Ca diet, on the other hand, refusals occurred frequently, although the attempt was made not to feed more than the amount which would be eaten within 24 hr.

The food consumption improved in the test period and naturally a better growth was obtained. The figures for growth indicate a better food utilization on the high Ca diet, which was to be expected in the light of the studies by Toepfer & Sherman [1936].

Table I. *Food consumption and live weight gain (g.) in first exp.*

	Pair I		Pair II		Pair III	
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
Initial live wt.	39	36	48	44	40	41
Final live wt.	153	150	167	169	165	175
Carcass wt.	133	128	145	147	143	153
Av. daily gain in preliminary period	1.3	1.5	1.4	1.6	1.8	2.0
Av. daily gain in test period	2.2	2.1	2.3	2.3	2.2	2.2
Daily food consumption in preliminary period	5.1	5.1	4.8	4.8	6.0	6.0
Daily food consumption in test period	8.7	8.7	9.2	9.2	9.3	9.3

The average Ca and P balances for the four weekly preliminary periods are given in Table II. On an average the animals on the low Ca intake stored 191 mg. Ca and 179 mg. P during the 4 weeks as compared with an average storage of 570 mg. Ca and 293 mg. P by their pair mates on the high Ca diet.

Table II. *Mean storage of Ca and P in first exp.*

		Ca					P				
		In-			Stored		In-			Stored	
Rat		take	Faeces	Urine	mg.	%	take	Faeces	Urine	mg.	%
no.		mg.	mg.	mg.			mg.	mg.	mg.		
Weekly av. in pre-liminary period	1	53.2	2.5	1.7	49.0	92.1	142.0	12.9	86.9	42.2	29.7
	2	284.0	128.7	20.6	134.7	47.4	142.0	59.1	13.2	69.7	49.1
	3	49.9	12.6	1.4	35.9	71.9	133.0	14.2	85.6	33.2	25.0
	4	266.0	119.0	22.0	125.0	47.0	133.0	53.2	17.8	62.0	46.6
	5	63.0	3.2	1.4	58.4	92.7	168.0	13.2	96.0	58.8	35.0
	6	336.0	148.0	20.3	167.7	49.9	168.0	61.2	19.1	87.7	52.2
Weekly av. in test period	1	244.8	50.4	7.8	186.6	76.2	244.8	38.1	87.5	119.2	48.7
	2	244.8	116.6	8.4	119.8	48.9	244.8	71.5	88.3	85.0	34.7
	3	256.8	63.7	8.6	184.5	71.8	256.8	46.5	89.7	120.6	47.0
	4	256.8	137.2	21.3	98.3	38.3	256.8	74.6	83.9	98.3	38.3
	5	259.2	68.5	7.5	183.2	70.7	259.2	45.7	100.0	113.5	43.8
	6	259.2	109.2	8.1	141.9	54.7	259.2	65.5	94.4	99.3	38.3

At the start of the experiment two male rats similar to the experimental animals were analysed for Ca and P and found to contain 0.52 % Ca and 0.40 % P. In Table III will be found calculations of the Ca and P contents of the experimental animals at the end of the preliminary period, based on the assumption that these animals had the same initial Ca and P percentages as those of the two animals analysed. The average Ca and P contents of the rats on the low Ca diet worked out at 411 and 348 mg. respectively. The corresponding figures for the animals on the high Ca diet were 779 mg. Ca and 454 mg. P. The rats on the high Ca diet thus contained 90 % more Ca and 30 % more P than their pair mates on the low Ca diet, but in spite of the low Ca content of these latter animals, they seemed to be in excellent condition. The only difference between the pair mates which could be detected by the eye was a difference in the incisor teeth. The incisors of the depleted animals were opaque with white chalky patches. The incisors of the replenished animals were transparent with a uniform brownish lustre.

The balance data for the test period are given in Table II. The table shows that the depleted animals made up for previous shortage by a more efficient utilization of the Ca and P in the food. The depleted animals stored on an average

Table III. *Ca and P stored and Ca and P content of the carcasses at the end of first exp.*

Rat no.	1		2		3		4		5		6	
	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P
Initial content, mg.	203	156	187	144	250	192	229	176	208	160	213	164
Stored preliminary period, 28 days, mg.	196	169	539	279	143	133	500	248	234	235	671	351
Stored test period, 35 days, mg.	933	596	599	425	923	603	492	491	916	567	710	496
Total stored, mg.	1332	921	1325	848	1316	928	1221	915	1358	962	1594	1011
Found in carcass, mg.	1334	899	1292	842	1365	906	1370	906	1370	906	1538	1000
Difference, mg.	-2	+22	+33	+6	-49	+22	-149	+9	-12	+56	+56	+11
Difference %	0.1	2.4	2.5	0.7	3.6	2.3	10.9	1.0	0.9	6.3	3.6	1.1
Ca and P as % of net wt.	1.00	0.68	1.01	0.66	0.94	0.58	0.93	0.62	0.96	0.63	1.01	0.65
Ca/P ratio of minerals stored in preliminary period	1.2		1.9		1.1		2.0		1.0		1.9	
Ca/P ratio of minerals stored in test period	1.6		1.4		1.5		1.0		1.6		1.4	
Ca/P ratio of minerals in carcass	1.5		1.6		1.5		1.5		1.5		1.5	

924 mg. Ca and 589 mg. P during the test period as compared with 600 mg. Ca and 471 mg. P stored by their pair mates. During the entire experiment the storage was nearly equal in the pair mates. The average figures for the depleted animals are 1115 mg. Ca and 768 mg. P as compared with 1170 mg. Ca and 763 mg. P in the replenished ones.

The Ca and P contents of the animals as calculated from the balance figures showed good agreement with the Ca and P found by analyses of the carcasses, as will be seen in Table III. The Ca data for number 4 furnished an exception to this rule and showed a deviation of 10.9%. The deviation for the other animals ranged from 0.1 to 6.3%. In the preliminary period the animals on the high Ca intake lost a large percentage of the Ca in the faeces. The average for the three animals was approximately 45%. The animals on the low Ca intake excreted only very small amounts of Ca in the faeces; two of the animals excreted about 5%, the third about 25% of the food Ca in the faeces. In the amount of Ca lost in the urine there was also a marked difference between the pair mates. In the preliminary period the loss was from 6.1 to 8.3% of the intake for the animals on the high Ca intake. On the low Ca intake the corresponding figures were from 2.3 to 3.2%. Two of the animals on the low intake stored approximately 92% of the food Ca, the third stored 72%. On the high Ca diet the storage was from 47 to 50%.

The P excretion also showed a striking difference between the pair mates. On the low Ca diet the P was excreted mainly in the urine, on the high Ca diet mainly in the faeces. In the urine the averages were from 57 to 64% on the low Ca diet and only from 9 to 13% on the high Ca diet. In the faeces the excretion of P ranged from 36 to 42% on the high Ca diet and only from 8 to 11% on the diet low in Ca. The difference in P storage was about 20% in favour of the animals on the high Ca intake.

In the following 5 weeks' test period when the pair mates were fed the same diet in equal amounts, the depleted animals utilized the dietary Ca more efficiently than did their replenished pair mates. This was mainly brought about by better absorption from the intestine. The difference in the urinary Ca was comparatively slight, but this difference was in favour of the depleted animals too. The replenished animals lost from 42 to 53% of the Ca in their faeces as compared with 21 to 26% in the case of the depleted ones.

The P excretion in the urine was nearly equal for the pair mates. In the faeces on the other hand there was a difference of about 10% in favour of the depleted animals.

SECOND EXPERIMENT

This experiment was conducted in much the same way as the first one, the only differences being shorter balance periods and a lower Ca content of the

Table IV. *Food consumption and live weight gain (g.) in second exp.*

	Pair I		Pair II		Pair III	
	No. 7	No. 8	No. 9	No. 10	No. 11	No. 12
Initial live wt.	50	33	52	53	51	43
Final live wt.	146	153	150	159	159	161
Carcass wt.	135	142	138	147	147	148
Av. daily gain in preliminary period	2.0	2.7	1.8	2.0	2.0	2.5
Av. daily gain in test period	2.5	3.0	2.9	3.0	3.1	3.1
Av. daily food consumption	7.0	7.0	6.9	6.9	7.6	7.6
Av. daily food consumption in preliminary period						
Av. daily food consumption in test period	10.4	10.4	10.2	10.2	11.0	11.0

ration during the test period. The preliminary period and the test period each lasted for only 3 weeks and the Ca level in the test period was adjusted to 0.25 %. A carmine marker was administered at the end of the preliminary period in order to effect a better separation between the faeces from the two periods.

The six male rats selected for this experiment were 33 days of age at the start. The food intake and gain in live weight as shown in Table IV were higher than in the previous experiment. The animals in this experiment gained only slightly less in 6 weeks than the animals in the first experiment did in 9 weeks.

Results

The balance data are given in Table V. The average storage for the preliminary period was 212 mg. Ca and 154 mg. P on the low Ca diet. On the high Ca diet the average storage was 545 mg. Ca and 280 mg. P. Assuming that these rats had the same initial percentages of Ca and P as the two rats analysed in the first experiment, the difference in Ca and P contents of the rats on the high and the low Ca diets at the end of the preliminary period was found to be less than in the first experiment. The calculated figures show that the animals on the high Ca diet contained 60 % more Ca and 25 % more P than did their pair mates on the low Ca diet. An examination of the incisor teeth did not reveal such a striking difference as that found in the first experiment, although the incisors of the depleted animals were definitely poorer than those of the replenished ones.

Especially in the first week of the test period the depleted animals proved to be more efficient in the utilization of dietary Ca. The total Ca excretion in the faeces and the urine was more than three times as high in the replenished animals as in their depleted pair mates. The difference in the Ca excretion between the pair mates in the preceding period, however, was so large that if only a small amount of faeces from the diet of the preliminary period had been collected at the beginning of the test period, the Ca contained herein would easily account for the higher Ca content of the faeces from the replenished rats in the first week of the test period. The use of a marker does not entirely dispose of this possibility since the marker does not effect a perfect separation. The P excretion, however, strongly indicated that no excreta from the previous period had been collected in the test period. In the week prior to the change in diet the P excreted in the faeces was 5-6 times larger in rats on the high than in those on the low Ca diet. In the urine the reverse condition held true. On the low Ca diet 3-4 times more

Table V. *Mean storage of Ca and P in second exp.*

	Rat no.	Ca						P					
		Intake mg.	Faeces mg.	Urine mg.	Stored			Intake mg.	Faeces mg.	Urine mg.	Stored		
					mg.	%					mg.	%	
Weekly av. in preliminary period	7	74.0	3.7	1.3	69.0	93.2		197.3	16.9	131.1	49.3	25.0	
	8	394.6	178.7	42.9	173.0	43.8		197.3	80.4	19.9	97.0	49.2	
	9	72.5	3.1	1.6	67.8	93.5		193.3	16.2	127.9	49.2	25.5	
	10	386.6	190.4	27.2	169.0	43.7		193.3	78.8	35.3	79.2	41.0	
	11	79.5	2.7	1.4	75.4	94.8		212.0	17.8	138.7	55.5	26.2	
	12	424.0	185.0	36.0	203.0	47.9		212.0	81.2	27.6	103.2	48.7	
Weekly av. in test period	7	182.5	14.0	2.6	165.9	90.9		292.0	26.7	140.0	125.3	42.9	
	8	182.5	22.5	6.6	153.4	84.1		292.0	26.9	134.8	130.3	44.6	
	9	179.2	10.8	2.6	165.8	92.5		286.6	24.7	126.9	135.0	47.1	
	10	179.2	25.2	9.2	144.8	80.8		286.6	30.3	136.2	120.1	41.9	
	11	191.6	10.7	3.4	177.5	92.6		306.6	25.7	145.7	135.2	44.1	
	12	191.6	20.0	7.1	164.5	85.9		306.6	28.6	143.1	134.9	44.0	

P was excreted in the urine than on the high Ca diet. During the first week of the test period the pair mates excreted nearly equal amounts of Ca and P in the faeces and urine.

In the following 2 weeks the depleted animals stored only slightly more Ca than did their pair mates. The difference was mainly brought about by a smaller Ca loss in the faeces though the urinary Ca excretion also favoured the depleted animals. In P metabolism there was no significant difference between the pair mates.

The agreement between the Ca and P contents of the rats calculated on the basis of balance data and the Ca and P contents found by analyses of the carcasses was very close (Table VI). The maximum deviation was only 2.5% for Ca and 4.0% for P.

Table VI. *Ca and P stored and Ca and P contents of the carcasses at the end of the second exp.*

Rat no.	...	7		8		9		10		11		12	
		Ca	P	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P
Initial content, mg.		260	200	172	132	270	208	276	212	265	204	224	172
Stored preliminary period, 21 days, mg.		207	148	519	291	203	148	507	238	226	167	609	310
Stored test period, 21 days, mg.		498	376	460	391	497	405	434	360	532	406	493	405
Total stored, mg.		965	724	1151	814	970	761	1217	810	1023	777	1326	887
Found in carcass, mg.		990	712	1166	807	963	732	1198	801	1011	759	1300	891
Difference, mg.		-25	+12	-15	+7	+7	+29	+19	+9	+12	+18	+26	-4
Difference %		2.5	1.7	1.3	0.9	0.7	4.0	1.6	1.1	1.2	2.4	2.0	0.4
Ca and P as % of net wt.		0.73	0.53	0.82	0.57	0.70	0.53	0.82	0.54	0.69	0.52	0.88	0.60
Ca/P ratio of minerals stored in preliminary period		1.4		1.8		1.4		2.1		1.4		2.0	
Ca/P ratio of minerals stored in test period		1.3		1.2		1.2		1.2		1.3		1.2	
Ca/P ratio of minerals in carcass		1.4		1.4		1.3		1.5		1.3		1.5	

During the preliminary period on the low Ca diet the animals stored on an average 212 mg. Ca and 154 mg. P. The corresponding figures for their pair mates were 545 mg. Ca and 280 mg. P. In the test period the average storage was 509 mg. Ca and 396 mg. P for the depleted animals, as compared with 462 mg. Ca and 385 mg. P for their replenished pair mates.

In the test period the depleted animals stored 92% of the Ca and 44.7% of the P. The corresponding figures for their pair mates were 83.6 and 43.5. The replenished animals excreted about 6% more Ca in the faeces and about 2.5% more Ca in the urine than did their pair mates. It must be kept in mind, however, that the difference between the pair mates decreased after the first week of the test period.

DISCUSSION

Data reported in the literature indicate that 0.4% Ca in the diet is inadequate for maximum storage when growth is normal. The rate of growth obtained in these experiments, however, was below normal and the possibility that the replenished animals were able to keep their tissues "saturated" with Ca must be considered.

The analyses of the carcasses support the view that the animals in the first experiment did keep their tissues "saturated" with Ca on the 0.4% Ca level. The balance data on the other hand show a much smaller Ca content of the live

weight increase in the test period than in the preliminary period on the 0.8% Ca level. Calculated on the basis of the balance data and gain in live weight the live weight increase contained 1.19% Ca and 0.61% P in the preliminary period. In the test period on 0.4% Ca the live weight increase contained 0.78% Ca and 0.61% P. The high Ca figure for the preliminary period is of course partly brought about by the low initial Ca content of the animals, but even when allowance is made for the increase in Ca percentage due to increase in age, there is still a difference left between the two periods which may be explained as resulting from the inadequacy of the Ca level of the diet in the test period.

In the second experiment the Ca level was so far below the requirement that the replenished animals were rapidly depleted of Ca, which undoubtedly contributed to improved utilization as the test period advanced. The carcasses of the depleted animals contained 0.71% Ca as compared with 0.84% in the replenished animals. The corresponding figures for the first experiment were 0.97 and 0.98.

From the findings of the two experiments it seems justifiable to draw the conclusion that the degree of Ca "saturation" has an effect on the efficiency of Ca utilization. The efficiency is greater with depleted than with replenished stores. This must be taken into consideration when interpreting mineral balances in animals and man. Equilibrium or positive balance is not necessarily proof of adequacy of intake. It might be in part a reflexion of the level of previous mineral nutrition. In other words, an intake which will barely maintain equilibrium with depleted stores might not suffice to maintain equilibrium when the stores are filled. In this connexion one might naturally ask if it is desirable to keep the Ca stores filled. Our present knowledge is not sufficient to answer this question, but it does seem likely that Ca stores may be considerably reduced and Ca utilization consequently improved without any harmful effect to the individual.

Under the conditions of this experiment, the P excretion in the faeces was determined to a considerable extent by the amount of Ca present. In the preliminary period of both experiments, on the high Ca diet about 40% of the ingested P was lost in the faeces and 8-10% in the urine. The corresponding figures for the low Ca diet are 8-10% in the faeces and 60-70% in the urine. McGowan [1933] showed with rabbits that a shift in the P excretion from the urine to the faeces occurred after addition of CaCO_3 to the diet but that the retention was better with CaCO_3 than with dicalcium hydrogen phosphate or steamed bone flour. He claimed that the neutralization of gastric HCl by CaCO_3 or other basic salts was the cause of the lowered Ca and P absorption from the intestine. In the test periods of the present experiment it was not the neutralization of gastric HCl which caused an increased loss of P in the faeces. The pair mates were fed the same diet but none the less the replenished animals lost more Ca and P in the faeces than did their depleted pair mates. This fact seems best explained on the assumption that the large amount of Ca present in the faeces of the replenished animals due to reduced absorption interfered with the absorption of P by reason of the formation of relatively insoluble calcium phosphates. The experiment does not, however, exclude the possibility that the neutralization of the HCl of the stomach did play a part in the high P excretion in the faeces of the animals on the high Ca diet in the preliminary period.

SUMMARY

1. The degree of Ca "saturation" of the tissues affects the efficiency of Ca utilization when Ca is furnished at levels below the requirement for maximum retention.

2. Under the conditions of these experiments the P excretion in the faeces tends to run parallel with the Ca excretion.

3. Lack of Ca in the diet affects the appetite adversely.

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