Intestinal absorption of calcium in rats given diets containing casein or amino acid mixture: the role of casein phosphopeptides

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1. In an attempt to investigate calcium absorption in the rat during the postprandial period, with the least alteration of the physical environment, the undisturbed small intestine was ligated *in situ* 2·5 or 3·0 h after ingestion of a diet containing 200 g casein /kg or an equivalent amino acid mixture, or 925 g casein/kg. Estimation of Ca absorption was made by comparing the amount of soluble ^{40}Ca or ^{45}Ca in the contents of segments from the rats receiving ^{45}Ca by intubation 30 min after withdrawal of food, ligated after a further 30 min, then killed after 0 or 30 min.

2. Under conditions such that the estimated amount of a marker, polyethylene glycol, in segments ligated in a defined position was little changed in rats killed 30 min apart, the difference in the amount of soluble ^{40}Ca was much higher in the rats fed on the basal diet containing 200 g casein/kg than in other groups.

3. This specific effect on Ca absorption, particularly in the distal portion of the small intestine, could be seen also after ^{45}Ca was directly injected into ligated segments *in situ*. The amount of ^{45}Ca in the portal blood 15 min after injection of the label was also highest in the rats given the basal diet.

4. The results were in agreement with our previous findings that the formation and accumulation of casein phosphopeptides causes an increase in the amount of soluble Ca in the distal small intestine.

In studies of protein digestion, Lee *et al.* (1980) found that in the rats receiving a diet containing casein, phosphopeptides (CPP) were formed in the small intestinal lumen as digestion progressed, enabling the amount of soluble calcium to be increased, presumably due to the formation of a soluble complex of CPP with Ca ions. These processes may inhibit the formation and precipitation of insoluble Ca salts in the distal small intestine.

The present work was designed to confirm that the bulk of soluble Ca formed in the presence of CPP may be highly available for intestinal absorption.

It is generally accepted that Ca absorption occurs mainly in the distal portion of the small intestine, where passive Ca transport takes place with a relatively normal level of dietary Ca (Wasserman & Taylor, 1976).

The results presented here provide evidence that dietary casein stimulates Ca absorption in the distal portion of the ligated small intestine in which there are digests containing CPP, soluble Ca and phosphate.

MATERIALS AND METHODS

In an attempt to measure Ca absorption, under conditions in which CPP were formed within the lumen during proteolytic digestion, ^{45}Ca was administered by stomach tube (Expt 1) or directly injected into the ligated loop (Expt 2) shortly after the ingestion of the food.

In both experiments, Ca absorption was monitored by comparing the amount of soluble ^{40}Ca or ^{45}Ca in the segments of the rats killed at 0 and 30 min after ligation under anaesthesia.

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Table 1. *Composition of experimental diets (g/kg)*

Dietary regimen ... / Ingredients	Basal (C_{20})	Amino acid mixture (AA)	Basal without starch and fat (C)
Casein	200	(200)†	925
Maize starch	680	680	—
Soya-bean oil	45	45	—
Cellulose powder	5	5	5
Mineral mixture‡	40	40	40
Vitamin mixture§	8	8	8
Choline chloride	2	2	2
Polyethylene glycol	20	20	20

† The composition of the amino acid mixture, which simulated that of casein, was as given by Ahrens *et al.* (1966).

‡ The mineral mixture based on the pattern of Rogers & Harper (1965) contained the following (g/kg mixture): $CaCO_3$ 292·9, $CaHPO_4 . 2H_2O$ 4·3, KH_2PO_4 343·1, NaCl 250·6, $MgSO_4 . 7H_2O$ 99·8, ferric citrate hexahydrate 6·23, $CuSO_4 . 5H_2O$ 1·56, $MnSO_4 . H_2O$ 1·21, $ZnCl_2$ 0·2, KI 0·005, $(NH_4)_6Mo_7O_{24} . 4H_2O$ 0·025.

§ Vitamin mixture was prepared according to the (US) National Academy of Sciences (1972) with starch to adjust the total weight.

Animals and diets

Male Wistar rats (Shizuoka Jikken Co-op. Hamamatsu, Japan) weighing 120–130 g were used in all experiments. They were individually housed in wire-bottom stainless-steel cages with free access to distilled water. All forty-eight rats were trained to consume a diet containing 200 g casein/kg (basal diet) within 1·5 h each day. The compositions of the experimental diets are shown in Table 1. Details of the feeding experiments have been described fully in a previous paper (Lee *et al.* 1980). Polyethylene glycol (PEG) was added to all diets as a non-absorbed indicator.

Expt 1. The effect of dietary casein on Ca absorption from ligated segments after an oral dose of ^{45}Ca

After the rats were meal-fed the basal diet (diet C_{20}) for 10 d, twenty-four rats were randomly divided into three groups of eight rats each. During the last 2 d a diet containing an amino acid mixture which simulated that of casein was fed to the second group (diet AA). A casein diet without carbohydrate and fat (diet C) was given to the third group of rats on the final day of the experiment.

On the last day of the experiment, 30 min after withdrawal of food, the rats were given a solution of ^{45}Ca (3 μCi ^{45}Ca in 0·5 ml of a solution containing 50 μg calcium chloride) through the inserted stomach tube. After a further 30 min the animals were anaesthetized by means of an intraperitoneal injection of pentobarbital (50 mg/kg body-weight). A central longitudinal incision was made into the abdominal wall and the small intestine was exposed.

The biliary duct was ligated twice with a silk suture, and then the small intestine was ligated at three sites, i.e. immediately below the pylorus, in the middle of the small intestine, and at the ileo–caecal junction. The ligated loops were then put back into the abdominal cavity. The entire procedure took less than 1 min. As the 0 min control, four rats in each group were killed immediately after ligation. The remaining rats were killed after 30 min under anaesthesia.

A sample (1 ml) of portal blood was withdrawn and then the upper and lower intestinal contents were flushed out with ice-cold saline (9 g sodium chloride/l). The blood and the intestinal washings were centrifuged to obtain the plasma and the soluble and insoluble Ca samples respectively. The samples were stored at −20° until analysed.

Absorption of Ca was estimated as the difference between the contents from the rats killed 0 and 30 min after ligation, i.e. 30 and 60 min after the oral dose of ^{45}Ca.

Expt 2. The effect of dietary casein on Ca absorption after direct injection of ^{45}Ca into the ligated small intestine

In Expt 1, while the food passed through the entire small intestine, changes in the transit rate as well as incomplete mixing of added ^{45}Ca, if they occurred, might lead to erroneous results. Therefore, in the second experiment, ^{45}Ca was injected directly into the ligated segments from the remaining twenty-four rats.

The procedures were similar to those in Expt 1, except that pentobarbital was injected 1 h after food withdrawal and that ^{45}Ca was injected directly into the ligated lower ileum and not given as an oral dose. An ileal loop was made by ligation at two points, i.e. 100 mm away from the ileo–caecal junction and 150–200 mm away further from the first point.

After preliminary determination of ^{40}Ca content, each 0·5 ml portion of the solution (37°, pH 7·0), containing 5 μg Ca and known but different amounts of ^{45}Ca, was injected into the loop with a fine needle. This procedure was necessary to equalize the specific activity among all groups.

To ensure uniform distribution of radioactivity, the loop was gently twisted by hand. The loop was then replaced inside the cavity as described previously.

Absorption of Ca was estimated as the amount disappearing during 30 min, based on the determinations from rats killed after 0 and 30 min. Radioactivities in the portal blood were measured at 0 and 15 min after the injection of ^{45}Ca.

Analyses

The lumen contents were centrifuged and the supernatant fraction and precipitate were separately digested by wet-ashing with a perchloric acid–nitric acid mixture. ^{40}Ca was determined by the o-cresolphthalein complexone method, or by atomic absorption spectroscopy as previously described (Lee *et al.* 1980).

Portions of the digest were neutralized with 2 M-sodium hydroxide, then a 0·5 ml portion was mixed with 150 ml NT-scintillator (PPO 4 g, toluene 700 ml, nonyl phenyl poly-ethoxyethanol (Nonion NS210; Nissan Kagaku Co., Japan; 300 ml). After being dissolved, the mixture was shaken after the addition of 2 ml of 2 M-hydrochloric acid. The procedures followed were those of Kawakami & Shimura (1974).

Radioactivities were determined in a liquid-scintillation counter (Aloka LSC 651, Japan) using the channel for ^{14}C and corrected against an external standard. The significance of differences was examined using an unpaired Student's *t* test, and errors were represented as mean values with their standard errors.

RESULTS

Expt 1. Observation of Ca absorption in vivo from ligated loops containing food digests after oral dose of ^{45}Ca

The small intestine was ligated during the period when gastrointestinal digestion occurred vigorously. This procedure minimized disturbance of the physiological environment in the lumen contents, particularly anaerobicity which would have been overlooked in absorption studies.

Table 2 shows that the amount of PEG was localized in the distal half of the gut and declined in the order: diet C_{20} > diet AA > diet C. This seemed to simply reflect the difference in the transit rate of bolus, and in the amount of food intake (g/kg body-weight: diet C_{20} 47, diet AA 25, diet C 25). Similarly, soluble Ca was largely localized in the distal half, which agreed with our previous results (Lee *et al.* 1980).

Table 2. *Expt 1. The amount of polyethylene glycol, soluble ^{40}Ca and insoluble ^{40}Ca in the small-intestine contents of rats killed 2·5 h (0 min after ligation) or 3·0 h (30 min after ligation) after ingestion of a diet† containing 200 g casein/kg (C_{20}), amino acid mixture (AA) or casein alone (C)*

(Mean values with their standard errors for four rats)

Dietary regimen ...	Basal (C_{20})		Amino acid (AA)		Casein alone (C)	
Small-intestine contents (mg)	Mean	SE	Mean	SE	Mean	SE
Upper portion‡						
Polyethylene glycol						
0 min	2·2	0·4[a]	0·8	0·1[b]	1·0	0·1[b]
30 min	1·8	0·2[a]	0·6	0·1[b]	1·7	0·4[a]
Soluble ^{40}Ca						
0 min	0·38	0·04[a]	0·14	0·02[b]	0·10	0·02[b]
30 min	0·14	0·01[a]	0·07	0·00[b]	0·06	0·01[b]
Difference	0·24	0·04**	0·07	0·02**	0·04	0·02
Insoluble ^{40}Ca						
0 min	0·02	0·00	0·02	0·00	0·02	0·00
30 min	0·02	0·00	0·01	0·00	0·02	0·00
Difference	0·00	0·00	0·01	0·00**	0·00	0·00
Lower portion§						
Polyethylene glycol						
0 min	22·0	1·4[a]	15·7	2·7[a]	8·2	0·6[b]
30 min	20·8	1·5[a]	15·6	1·7[a]	9·8	0·8[b]
Soluble ^{40}Ca						
0 min	2·37	0·13[a]	1·07	0·09[b]	0·48	0·08[c]
30 min	1·37	0·18[a]	0·97	0·09[b]	0·43	0·08[c]
Difference	0·64	0·22**	0·10	0·13	0·05	0·11
Insoluble ^{40}Ca						
0 min	1·02	0·02[a]	0·87	0·12[a]	0·32	0·04[b]
30 min	1·07	0·17[a]	0·87	0·12[a]	0·30	0·04[b]
Difference	−0·05	0·17	0·00	0·17	0·02	0·06

Difference between 0 min and 30 min was significant: $^{**}P < 0·01$.

[a, b, c] Values within the same horizontal row with different superscript letters were significantly different ($P < 0·05$).

† For details of diets, see Table 1.

‡ Upper half of the small intestine.

§ Lower half of the small intestine.

There was no significant difference between the amount of PEG at 0 and 30 min after ligation (Table 2).

The amount of soluble ^{40}Ca in the lower portion at 0 min was much higher with diet C_{20} with a Ca:PEG value of 0·108, whereas in the groups given diets AA and C the corresponding values were 0·068 and 0·059 respectively; after 30 min the values were 0·065, 0·062 and 0·044 for diets C_{20}, AA and C respectively. Thus, marked 'net absorption' of Ca could be seen only with diet C_{20}.

Table 3 shows that radioactivity disappearing from ligated loops during 30 min, which had been estimated by comparing the rats killed under anaesthesia 30 min apart, was much higher in the basal group (diet C_{20}) than in those receiving the amino acid mixture (diet AA) or casein alone (diet C). However, the amount disappearing during 30 min was not significant even with diet C_{20}. In addition, these differences either in the upper portion or in the lower portion of the small intestine as a percentage of the 0 min value were not necessarily proportional to those observed for the total amount of ^{40}Ca, which is shown

Table 3. *Expt 1. Total radioactivities (counts/min ($\times 10^{-4}$) per mg calcium) and the estimation of ^{45}Ca absorption obtained by subtracting the amount of ^{45}Ca in the ligated intestinal loops 60 min (30 min sample) after an oral dose of ^{45}Ca from the value after 30 min (0 min sample)*

(Mean values with their standard errors for four rats. The rats were administered ^{45}Ca ($8{\cdot}45 \times 10^6$ counts/min per 0·5 ml) through the inserted stomach tube)

Dietary regimen† ...	Basal (C_{20})		Amino acid (AA)		Casein alone (C)	
Small-intestine contents	Mean	SE	Mean	SE	Mean	SE
Upper portion‡						
Soluble ^{45}Ca						
0 min	48·5	9·1	36·1	13·9	39·4	13·7
30 min	11·8	3·9	17·8	4·8	18·9	4·0
Difference	36·7	9·9**	18·2	14·8	20·5	14·3
Insoluble ^{45}Ca						
0 min	2·0	$0{\cdot}3^a$	3·3	$0{\cdot}3^b$	0·7	$0{\cdot}2^c$
30 min	1·6	$0{\cdot}7^a$	3·1	$0{\cdot}5^a$	0·5	$0{\cdot}1^b$
Difference	0·4	0·8	0·2	0·6	0·2	0·2
Lower portion§						
Soluble ^{45}Ca						
0 min	111·0	$26{\cdot}4^{ab}$	125·8	$20{\cdot}9^a$	60·8	$12{\cdot}0^b$
30 min	57·1	$3{\cdot}9^a$	111·0	$12{\cdot}6^b$	39·4	$4{\cdot}0^c$
Difference	53·9	26·7	14·8	24·4	21·4	12·5
Insoluble ^{45}Ca						
0 min	18·3	$4{\cdot}0^a$	19·5	$2{\cdot}9^a$	3·0	$0{\cdot}6^b$
30 min	22·8	$6{\cdot}1^a$	31·2	$4{\cdot}7^a$	3·3	$0{\cdot}7^b$
Difference	−4·5	7·3	−11·7	5·5	−0·3	0·9

Difference between 0 min and 30 min was significant: $^{**}P < 0{\cdot}01$.

a, b, c Values within the same horizontal row with different superscript letters were significantly different ($P < 0{\cdot}05$).

† For details of diets, see Table 1.

‡ Upper half of the small intestine.

§ Lower half of the small intestine.

in Table 2. A change in the radioactivity following the conversion of soluble Ca into the insoluble form might be one of the reasons. This may be attributed to the gradual formation of insoluble ^{45}Ca at a higher pH in the lower portion in relation to the progress of the digestion process.

The specific activities of Ca, calculated from the values in Tables 2 and 3, were variable depending on the kind of diet and the segment, but were not significantly altered with time of sampling (Table 4).

A marked reduction in specific activities was found in the lower portion irrespective of diet or of the time interval after ligation, suggesting that ^{40}Ca and ^{45}Ca might not be lost from the lumen to the plasma in a similar manner, or that a significant but variable level of secretion of ^{40}Ca might occur in this portion. The latter seemed to be a possible reason for the disagreements between the results from ^{40}Ca and ^{45}Ca studies.

As shown in Fig. 1, the concentration of Ca in the portal plasma was not different among the groups. The specific activities of the intestinal contents were the least with diet C_{20} (Table 4), which reflected the higher amount of soluble ^{40}Ca (Table 2) as previously reported (Lee *et al.* 1979).

Assuming that the specific activity of the lumen contents directly affects that of the portal plasma, at least in a short period after ingestion of the radioisotope, then the higher the ratio plasma:lumen content becomes the greater the absorption of Ca. Fig. 1 shows that

Table 4. *Expt 1. Specific radioactivities (counts/min ($\times 10^{-4}$) per mg calcium) calculated from the amount of soluble ^{40}Ca (Table 2) and of soluble ^{45}Ca (Table 3), in the small-intestine contents of rats killed 30 min (0 min sample) or 60 min (30 min sample) after a dose of ^{45}Ca to rats given diets C_{20}, AA or C*†

(Mean values with their standard errors for four rats)

Dietary regimen ...	Basal (C_{20})		Amino acid (AA)		Casein alone (C)	
Small-intestine contents	Mean	SE	Mean	SE	Mean	SE
Upper portion						
Soluble fraction						
0 min	127·3	18·3[a]	266·2	96·9[ab]	363·9	73·4[b]
30 min	80·4	21·3[a]	261·9	58·7[b]	341·4	61·8[b]
Insoluble fraction						
0 min	88·2	1·7[a]	201·0	28·2[b]	31·5	4·6[c]
30 min	61·0	16·4[a]	256·6	27·5[b]	20·4	3·8[a]
Lower portion						
Soluble fraction						
0 min	47·3	12·1[a]	119·6	22·3[b]	123·3	11·8[b]
30 min	32·0	6·2[a]	113·6	3·8[b]	95·6	11·9[b]
Insoluble fraction						
0 min	18·0	3·4[a]	22·9	2·8[a]	9·5	2·0[b]
30 min	20·0	2·9[a]	36·1	2·8[b]	10·7	1·6[c]

[a, b, c] Values within the same horizontal row with different superscript letters were significantly different ($P < 0·05$).

† For details of diets, see Table 1.

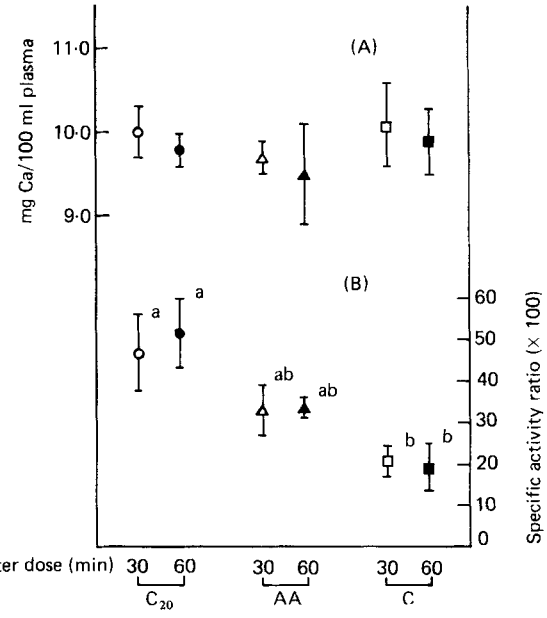

Fig. 1. Expt 1. (A) Calcium concentration (mg/100 ml) of plasma and (B) specific activity ratio of plasma (counts/min per 100 ml); total intestinal contents (counts/min per mg soluble Ca) 30 min (○) or 60 min (●) after an oral dose of ^{45}Ca. Points are mean values with their standard errors represented by vertical bars. [a, b] Values having different letters were significantly different from each other ($P < 0·05$).

Table 5. *Expt 2. The estimation of* in situ *Ca absorption by substracting the amount of* ^{40}Ca *and* ^{45}Ca *after 30 min from that at 0 min after the injection of different quantities of the radioisotope into ligated loops*

(Mean values with their standard errors for four rats. Initial specific activites in the intestinal contents with diets C_{20}, AA and C were (counts/min ($\times 10^3$) per mg Ca) 438, 425 and 435 respectively)

Dietary regimen ...	Basal (C_{20})		Amino acid (AA)		Casein alone (C)	
Small-intestine contents	Mean	SE	Mean	SE	Mean	SE
Soluble ^{40}Ca (mg)						
0 min	1·44	0·15[a]	0·70	0·12[b]	0·65	0·05[b]
30 min	1·05	0·09[a]	0·61	0·07[b]	0·56	0·06[b]
Difference	0·39	0·17**	0·09	0·14	0·09	0·08
Insoluble ^{40}Ca (mg)						
0 min	0·31	0·08	0·32	0·03	0·26	0·04
30 min	0·41	0·08	0·34	0·03	0·26	0·02
Difference	−0·10	0·11	−0·02	0·04	0·00	0·03
Soluble ^{45}Ca (counts/min ($\times 10^{-3}$))						
0 min	576	19[a]	268	5[b]	292	3[b]
30 min	476	25[a]	243	5[b]	268	10[b]
Difference	100	31*	25	7*	24	10
Insoluble ^{45}Ca (counts/min ($\times 10^{-3}$))						
0 min	44	13[a]	21	1[b]	10	1[b]
30 min	58	17[a]	21	3[b]	11	1[c]
Difference	−14	21	0	3	−1	1

Differences between the values at 0 min and after 30 min within the same dietary group were significant: * $P < 0·05$, ** $P < 0·01$.

[a, b, c] Values within the same horizontal row with different superscript letters were significantly different ($P < 0·05$).

the specific activity ratio plasma:lumen contents was higher, though not significantly, for diet C_{20} than for diet AA, and was significantly higher for diet C_{20} than for diet C. The criteria for comparison of the specific activities of the plasma with those of the intestinal contents were further investigated in Expt 2, where known but different amounts of ^{45}Ca were injected into the segments to equalize the specific activities of the contents.

Although the decrease in ^{40}Ca or ^{45}Ca during 30 min in the upper intestine from rats given diet C_{20} was the largest, as in the lower portion, we did not attempt to evaluate this as an enhancement in absorption. In the upper intestine there seems to be a rapid transit of digests, which inevitably reduces the lumen Ca level. Moreover, in this portion active transport may be a predominant process, independent of Ca concentration.

Expt 2. Observation of Ca absorption in situ *from ligated loops directly injected with* ^{45}Ca

To further confirm the specific effect of dietary casein in increasing Ca absorption in the lower portion of the small intestine, ^{45}Ca was directly injected into non-flushed ligated ileum. As shown in Table 5, the difference in the amounts of soluble ^{45}Ca and ^{40}Ca between the animals killed 30 min apart was higher with diet C_{20} than in groups given other diets. Furthermore, the radioactivity in the portal blood 15 min after injection was much higher in the rats fed on the basal diet (diet C_{20}) than in those in other groups (Table 6).

From these results we concluded that dietary casein can increase Ca absorption, at least in the distal portion of the small intestine, when it is present in an optimal amount for normal nutrition.

Table 6. *Expt 2. The concentration of ^{40}Ca, total radioactivities and specific activities in the portal venous blood 15 min after injection of different amounts of ^{45}Ca into ligated ileum to correct initial specific activities of the contents*

(Mean values with their standard errors for four rats. Blood was withdrawn from the portal vein of the same animal 0 min and 15 min after the injection of ^{45}Ca)

Dietary regimen ...	Basal (C_{20})		Amino acid (AA)		Casein alone (C)	
	Mean	SE	Mean	SE	Mean	SE
Plasma Ca (mg/100 ml)						
0 min	10·3	1·7	10·3	0·8	10·2	0·7
15 min	10·2	0·4	10·1	0·7	10·9	0·3
Total radioactivity (counts/min ($\times 10^{-3}$) per 100 ml)						
0 min	65	12	42	2	43	3
15 min	261	27[a]	158	9[b]	166	26[b]
Specific radioactivity (counts/min ($\times 10^{-3}$) per mg Ca)						
0 min	6·4	0·7[a]	4·1	0·2[b]	4·2	0·1[b]
15 min	25·8	2·8[a]	15·8	1·2[b]	15·2	2·2[b]
Specific activity ratio, plasma:ileal contents ($\times 100$)†						
0 min	1·6	0·1[a]	1·1	0·2[ab]	0·9	0·1[b]
15 min	5·9	0·1[a]	3·7	0·3[b]	3·2	0·6[b]

[a, b] Values within the same horizontal row with different superscript letters were significantly different ($P < 0.05$).

† (Plasma specific activity: initial specific activity of soluble Ca in the ligated ileal contents) × 100 (see Table 3).

DISCUSSION

Previously, we showed that there was a close relationship between the amounts of soluble Ca and CPP in the intestinal lumen of rats receiving a diet containing casein.

These results, together with the present findings, strongly support the concept originally proposed by Mellander (1950, 1963) that CPP stimulates intestinal Ca absorption by its nature as a sequestor for Ca salts. However, feeding casein did not always give rise to the formation of CPP, since the diet containing higher levels of casein (diet C) was associated with the formation of a low level of CPP and also of soluble Ca (Lee *et al.* 1980). This seemed to be due to complete proteolysis during digestion. The present finding that feeding casein alone failed to increase Ca absorption supports these causal relationships.

Of the amount of Ca which disappeared during 30 min in vivo as well as *in situ*, most seemed to be localized in the distal portion of the small intestine. The amount of ^{45}Ca disappearing from the distal half of the intestine during 30 min was calculated to be approximately 23% of the dose when a feed of 4·0 g of the diet containing 20 g PEG/kg and 200 g casein/kg was followed by a ^{45}Ca injection of $8{\cdot}45 \times 10^6$ counts/min (Expt 1). In Expt 1, where ^{45}Ca was orally administered, the calculated specific activities were quite different among dietary groups, and between two segments (Table 4).

This seemed to be due rather to different extents of dilution of the label, and also to a different contribution of ^{40}Ca and ^{45}Ca to lumen Ca absorption, than to non-homogeneity of the label on administration of ^{45}Ca.

In Expt 2, when known but different amounts of ^{45}Ca were injected directly into the ligated ileum, in order to attain uniform specific activities among the rats having different

amounts of lumen-soluble ^{40}Ca, the amounts of Ca lost during 30 min in the dietary groups C_{20}, AA and C as a percentage of initial value for ^{45}Ca were 15, 7·8 and 7·5, and for ^{40}Ca were 27, 13 and 14, respectively.

Compared with the results from Expt 1, the reduction in ^{45}Ca was much lower with all diets, which could not be well explained. Nevertheless, marked enhancement of Ca absorption was observed with diet C_{20} throughout the present studies irrespective of ^{45}Ca dosing methods.

In meal-fed rats, the stomachs were filled with a large amount of diet even several hours after food intake, during which time the amount of CPP formed might remain almost unaltered (Naito *et al.* 1972).

Similarly, ^{45}Ca absorption as well as the formation of soluble Ca may occur continuously during the process of digestion.

The prevailing view is that Ca absorption in the distal portion of the small intestine occurs mainly by passive transport, in which a concentration gradient of Ca between the lumen content and the blood is apparent. Moreover, there is increasing evidence that passive transport is predominant over the active process, at least when there is an adequate supply of Ca (Wasserman & Taylor 1976). Marcus & Lengemann (1962) reported, using a non-absorptive indicator in vivo, that ^{45}Ca absorptions in the rat ileum were 89 and 62% with a liquid and solid diet respectively, and that absorption was by passive transport.

Wasserman & Taylor (1976) assumed that the lumen concentration of Ca necessary for passive transport to occur was 6·7 mM. When rats were given diets containing 200 g casein/kg, similar to those in the present experiments, we found (Y. S. Lee, T. Noguchi & H. Naito, unpublished results) that the concentration of soluble Ca in the lumen was 23 mM, which far exceeds the value quoted by Wasserman & Taylor (1976). Hence passive diffusion of Ca from the lumen to the blood would have been possible under the present experimental conditions.

It has been well documented that Ca is efficiently utilized from milk and a variety of milk products. Several substances, including lactose, have been extensively investigated as possible factors stimulating Ca absorption.

CPP, macropeptides formed from α_{s1}- and β-caseins by proteolysis with trypsin, have a high affinity for Ca ions so as to inhibit formation of insoluble Ca salts (Mellander, 1963).

Naito *et al.* (1972) and Naito & Suzuki (1974) confirmed the formation of CPP in the small-intestine contents of rats given a diet containing casein.

When various Ca salts are taken into the stomach most of them should be dissociated into their respective ions by the strong acidity, and these transferred into the small intestine. In the proximal lumen, ions may still be dissociable, but may gradually become insoluble as the pH becomes alkaline in the distal part of the lumen. When CPP are present, they may preferentially interact with Ca ions to prevent the precipitation of Ca salts.

However, the extent of the CPP-mediated stimulation of Ca absorption in over-all Ca utilization is not known. Forbes *et al.* (1979) observed that the regression of dietary Ca content *v.* amount of femur Ca was not altered in the rats receiving casein and soya-bean diets for 4 weeks, suggesting that the difference in the quality of dietary protein does not necessarily alter over-all Ca utilization.

Y. S. Lee, H. Naito & T. Noguchi (unpublished results) found that addition of lactose had little effect on the CPP-mediated enhancement of Ca absorption, but the accumulation of Ca in femurs during 48 h was higher in the lactose-supplemented rats than in those without lactose. Therefore, the present results showing the increase in lumen Ca absorption in the presence of CPP might explain only part of the over-all utilization of Ca. On the other hand, Mykänen & Wasserman (1980) recently suggested that tryptic casein hydrolysate fractions had enhanced total utilization of Ca in chicks.

The significance of Ca transport in the distal part of the small intestine, controlled by diet and the digestion process, should be considered in the nutrition of milk and other dairy products.

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