CALCIUM METABOLISM IN THE RABBIT III. TIME DISTRIBUTION OF INTRAVENOUSLY ADMINISTERED RADIOCALCIUM IN THE MATURE RABBIT

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By

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In the studies on the time distribution of calcium in the gastrointestinal tract of the rabbit by Itoh and Hatano (1), on the excretion of Ca^{45} into the gastrointestinal tract of the rat by Wallace *et al.* (2) and on the transfer of phosphate in the digestive tract of the swine by Smith *et al.* (3), these investigators have reported that some differences in the metabolism of the corresponding materials exist between the young and mature animals.

In this experiment, the estimations of the blood disappearance rates, the distributions in the gastrointestinal tissues and contents, the bone uptake rates and the urinary and fecal excretions of the intravenously administered radiocalcium in the mature rabbit at various times after feeding of the white clover hay diet were used to evaluate the transfer of calcium and these results were compared with the data of the young rabbit described in our previous report (4).

Materials and Methods

Thirteen mature rabbits were used in this experiment. The details of the animals and the feeding management of them and the white clover hays used have previously been reported (1, 5).

It is known that the active transfer of calcium in the digestive tract of the mature rabbit occurs at the later period after feeding as compared with that of the young rabbit. They were divided into four groups with regard to the intervals after feeding, at the three-, six-, 12- and 24-hour periods, and injected with radiocalcium intravenously at various times before the slaughter.

The sex, body weight, hours of post-injection of radiocalcium and hours after feeding of the mature rabbit used in this experiment are shown in Table 1.

Table 1. Standard specific activity of tissues and contents of the gastrointestinal tract of the mature rabbit
Results expressed as: (% dose of Ca^{45}/mg Ca) × 10

Rabbit No.	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12	M13
Sex	♀	♂	♂	♂	♀	♂	♀	♀	♀	♂	♀	♂	♀
Time of sacrifice (hr post-injection)	½	1	3	⅙	⅓	½	1	3	6	1	12	1	24
Time after feeding (hr)	3	3	3	6	6	6	6	6	6	12	12	24	24
Body weight (kg)	1.86	1.85	1.89	1.86	1.96	2.15	1.85	2.15	1.82	2.00	1.80	2.05	1.80
Blood calcium	4.88	2.89	1.16	8.54	7.05	4.39	3.66	1.38	0.60	3.30	0.47	4.14	0.21
Tissues													
Stomach	2.34	1.76	0.52	5.22	3.80	2.40	1.25	0.44	0.41	2.09	0.31	2.24	0.15
Duodenum	1.22	0.84	0.48	2.83	3.22	1.43	0.82	0.41	0.90	0.97	0.20	0.57	0.13
Small intestine	1.24	0.61	0.45	1.38	1.40	0.84	0.75	0.70	0.37	0.82	0.29	1.31	0.10
Cecum	0.66	0.46	0.23	0.83	0.71	0.62	0.26	0.26	0.23	0.57	0.27	0.76	0.11
Colon	1.04	1.13	0.49	1.69	1.10	0.66	0.53	0.56	0.23	1.44	0.33	0.95	0.08
Rectum	1.10	1.02	0.51	2.17	0.82	1.12	0.38	0.56	0.38	1.08	0.48	0.86	0.17
Contents													
Stomach	0.003	0.17	0.15	0.07	0.01	0.01	0.16	0.14	0.16	0.03	0.07	0.12	0.004
Duodenum	0.82	0.53	0.19	2.28	1.15	2.43	0.58	0.41	0.21	0.35	0.13	1.24	0.03
Small intestine	0.41	0.41	0.36	0.63	0.28	0.83	0.31	0.23	0.13	0.61	0.16	2.29	0.02
Cecum	0.04	0.09	0.12	0.04	0.01	0.04	0.03	0.10	0.21	0.01	0.21	0.04	0.09
Colon	0.14	0.17	0.14	0,18	0.07	0.04	0.04	0.13	0.19	0.43	0.25	0.44	0.08
Rectum	0.03	0.02	0.09	0.04	0.03	0.05	0.07	0.10	0.24	0.01	0.27	0.09	0.24

Thirty to 40 μc of Ca^{45} (0.3-0.5 mg Ca) as $CaCl_2$ in 0.5 *ml* of neutral solution per head were administered, and the method of injection and treatment of each sample was similar to that used in the young rabbit (4).

The small intestine was divided into two parts and the upper one-fourth of it was represented as the duodenum.

Total and radioactive calcium in each sample were determined from the oxalate precipitate, as described by Comar *et al.* (6).

Results and Discussion

The results of the chemical and radiological analyses of the various samples were used to calculate their standard specific activities, and expressed as ten folds of percent of the dose of Ca^{45} per mg calcium.

The standard specific activities of the blood and each tissue and content of the digestive tract at various times after injection of the isotope are indicated in Table 1 and the percent of the dose of Ca^{45} in the blood, total gastrointestinal tissues and contents, liver, kidney, urine, feces and femur of each mature rabbit are shown in Table 2.

1. Standard specific activity of the blood calcium and the gastrointestinal tissues

Intravenously administered radiocalcium disappeared from the blood at a very large and rapid rate in the mature rabbit and this rate was slightly slower than that observed in the young rabbit being the same as the data of Thomas *et al.* (7).

In the mature rabbit, these values at one hour following tracer administration varied at various times after feeding but the decreasing level in the course of time after feeding as shown in the young rabbit was not observed.

The highest specific activity in the gastrointestinal tissues occurred in the stomach (containing the adhered underlaying musculature) within one hour after administration of the isotope and in the small intestine during three to six hours and thereafter in the rectum.

In comparison with the specific activities of the duodenal tissue and the posterior parts (ileum and jejunum) of the small intestinal tissue, the former tissue was higher than the latter one during an early period of time after feeding, and an inversive relationship was observed in the later period occasionally.

2. Standard specific activity of the gastrointestinal contents

The time variations of the standard specific activities of the contents in the various segments of the digestive tract following tracer administration showed the peculiar type in each segment (Table 1 and Fig. 1).

Within three hours after administration of the isotope the highest specific activity occurred in the duodenum, followed by the lower small intestine.

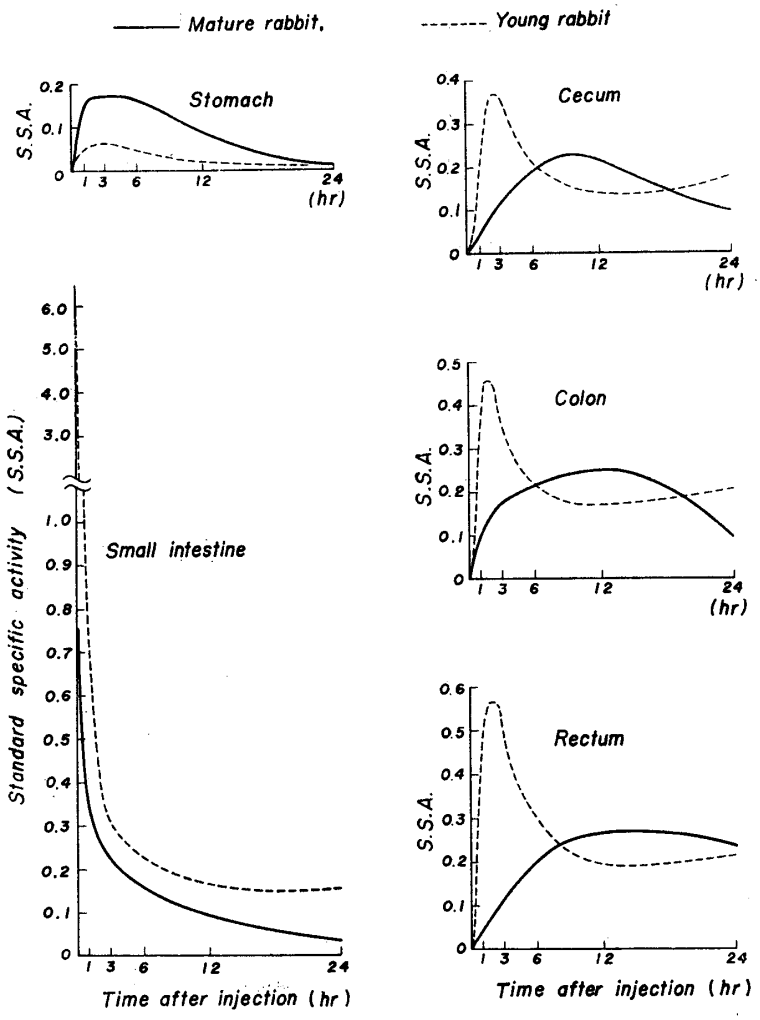

Fig. 1. Standard specific activity of each gastrointestinal content of mature and young rabbits at various times after intravenous injection of the radiocalcium

Twelve hours and later after feeding the higher specific activity was observed in the lower small intestinal contents as compared to the duodenum as shown in the tissues of both segments.

Possibly this was a consequence of a more active secretion of the digestive juice containing the endogenous (radioactive) calcium at the duodenum as compared with the posterior parts of the small intestine during about the six-hour interval after feeding or indicative of the higher secretion of the small intestinal juice supplying the digestive process in the duodenum exist some dietary materials remaining undigested or otherwise unavailable at the later period (unpublished data). On the other hand, it is considered that an increasing specific activity of the posterior small intestinal contents toward the later period after feeding are caused by the decreasing resorption of the endogenous (radioactive) calcium secreted in the duodenum, at the lower parts of the small intestine.

The variations of the standard specific activities at various times following tracer administration in each part of the gastrointestinal digesta of the mature rabbits at six hours and later period after feeding and the young rabbits at three hours and later are outlined in Fig. 1.

The time variation of the specific activity of the content in each segment of the gastrointestinal tract showed considerable differences between the mature and young rabbit.

The calcium concentration of the stomach digesta in the mature rabbit at three hours after feeding showed the value similar to the diet and the transport of the stomach digesta to the lower segment in these animals were smaller in quantity and slower in stream as compared with the young rabbit as described in a previous report (1). The specific activities of the stomach digesta in the mature rabbit at each period after administration of the isotope were considerably higher than in the young rabbit, generally.

It suggests that the secretion of the endogenous (radioactive) calcium by saliva or gastric juice in the mature rabbit is higher than in the young animal. In fact, the analytical result of the mature rabbit saliva showed the higher radiocalcium concentration within one hour period following tracer administration in comparison with the blood at comparable times, and was slightly higher than that of the young rabbit.

The specific activities of the stomach digesta in the mature rabbit increased rapidly until one hour after administration of the radiocalcium and during the one- to three-hour period there appeared to be an approximate equilibration and it decreased until the 24-hour period gradually and showed the value similar as value observed in the young rabbit. These facts may result from the longer staying and the slower effluence of the gastric contents, above described.

In general, it is considered that the stomach of the young rabbit occupied the existence of the precursory organ having acid secreting function for the small intestine, on the other hand, the mature rabbit have a stomach playing a major role in the accumulation of calcium in the ingested feedstuff.

The specific activities of the small intestinal content during a short period of time after administration of the isotope showed the values similar to that found in the blood calcium and the appreciable higher values as compared to the other segments at the corresponding intervals. Thereafter these values decreased gradually, similar to the blood disappearance rate of calcium.

The radioactivities in the small intestine digesta of the young rabbit were higher than that observed in the mature rabbit at comparable times following tracer administration and these relations were opposed to that found in the blood radioactivity.

Possibly these facts are caused by the lower secretion of endogenous (radioactive) calcium in the mature rabbit small intestine and the higher

calcium concentration of digesta in this segment as compared to the young rabbit.

The time variations of the specific activities in the cecum, colon and rectum digesta of the mature rabbits showed appreciably different figures as compared with that found in the young animals, however each figure of these three segments was approximately the same in each group.

In the mature rabbit, the specific activities of the cecal contents increased during six hours interval after administration of the isotope, and followed by the constant values until the 12-hour period and afterward it decreased gradually. The appreciable quantities of isotope were present in the colon during a shorter period of time and the peak of the concentration occurred at about 12 hours after injection in both colon and rectum digesta.

It is considered, therefore, that these differences are mainly caused by the time lag at each segment of the large intestine on the transfer of the endogenous (radioactive) calcium which are secreted in the small intestine, although these observations may be affected by the degrees of the calcium metabolism in each segment.

The maximum specific activity of the cecal contents were slightly lower than that of the other two portions of the large intestine and they increased lower down, and this may result from the larger quantity of the existing calcium in the cecum digesta and differences of the nature and rate of calcium metabolism in the colon and rectum.

In the young rabbit, the specific activities of digesta in three segments of the large intestine reached the peak ranging from one to three hours after injection of the radiocalcium and the approximately constant level of the specific activity which occupied one third value of the peak at each segment were maintained at six hours and later period, and at this period the highest values were observed in the mature rabbit.

It is evident that the calcium, that is, the dietary one and radiocalcium injected intravenously are transferred along the intestinal tract of the young rabbit with a much greater extent than in the mature rabbit. It is considered that the transfer of the digesta having the highest specific activity in the digestive tract of the young rabbit occurs as fast the six- to nine-hour intervals as before the time which was observed in the mature rabbit. This marked difference in specific activity at the same period is probably due to the greater intestinal motility in the younger rabbit labeled calcium secreted in the upper portions of the tract reaching the lower intestine sooner in the young animal than in the older.

It is possible to consider that in both age groups, the highest specific activity of the colonic content reaches at the earlier period after injection of the isotope than that observed in the cecum digesta, indicating the direct

effluence of the small intestinal contents into the colon.

In the mature rabbit, no observation was found on the definite variations in the course of time after feeding on the standard specific activity of the tissues and contents of the upper digestive tract at one hour after injection of the isotope.

It is considered, therefore, that the absorption process and transfer of calcium in each segment of the digestive tract of the mature rabbit continued with a considerably constant rate at various periods after feeding.

3. Distribution of the radioactivity in the blood, total gastrointestinal tissues and contents, liver, kidney, urine, feces and bones

The distribution of the intravenously administered radiocalcium in the blood, tissues and contents of all digestive tract, liver, kidney, urine, feces and femurs of the mature rabbit are shown in Table 2.

These values of the total digesta (except of a few cases) and femurs increased in the course of time after the administration of radiocalcium, and in the other samples it decreased the same as observed in the young rabbit, generally.

The larger quantities of the radioactivity in the gastrointestinal contents of the mature rabbit at a later period of time after the injection in comparison with that of the younger one indicate a greater accumulation of the digesta in the digestive tract of the older.

The feces which had the highest specific activity were excreted at the five- to 10-hour intervals following tracer administration in the mature rabbit, and they appeared at the period of two holds of the times after feeding that observed in the younger animals.

The urinary excretion of the radiocalcium in the mature rabbit was a considerable high value and it showed the individual differences as observed in the younger one.

The rapid bone uptake of the comparatively higher quantities of radiocalcium occurred immediately after the intravenous administration of isotope, and total bone uptake of the radiocalcium at three hour and later after injection of the isotope in the mature rabbit were higher than that of the younger animal. However, the specific activities of the femur in the mature rabbit were lower than that of the young rabbit and they reached to the equilibrium at six hours and later period and these values did not exceed 0.035 and about a half of the younger animals.

No evident results were observed on the elution of the newly deposited bone radiocalcium which was discussed in the young rabbit of a previous report (4).

Table 2. Distribution of radiocalcium in the mature rabbit
(percent of dose of Ca^{45})

Rabbit No.	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12	M13
Time of sacrifice (hr post-injection)	$\frac{1}{2}$	1	3	$\frac{1}{6}$	$\frac{1}{3}$	$\frac{1}{2}$	1	3	6	1	12	1	24
Time after feeding (hr)	3	3	3	6	6	6	6	6	6	12	12	24	24
Blood calcium	3.37	2.62	1.02	9.58	8.52	5.55	4.93	1.95	0.63	3.53	0.37	5.31	0.20
Total gastrointestinal tissues	1.60	1.38	0.58	3.70	3.87	2.73	1.69	0.89	0.61	2.36	0.48	1.84	0.27
Total gastrointestinal contents	2.36	10.24	8.66	5.59	2.74	4.20	5.41	8.75	11.63	3.00	6.69	3.52	4.62
Liver	0.21	0.27	0.14	1.37	0.38	0.20	0.14	0.27	0.09	0.43	0.15	0.33	0.02
Kidney	0.59	0.59	0.21	1.71	2.10	1.10	0.63	0.32	0.11	1.01	0.11	0.91	0.02
Total urine	8.36	4.79	5.61	2.47	4.85	0.08	4.07	4.16	23.64	10.04	20.34	0.06	23.17
(standard specific activity)	(2.34)	(0.52)	(1.89)	(0.74)	(0.39)	(0.08)	(0.80)	(2.21)	(1.44)	(0.19)	(0.35)	(0.01)	(0.66)
Total feces	—	—	—	—	—	—	—	0.12	3.63	—	9.64	—	8.13
Total	16.49	19.89	16.22	24.42	22.46	13.86	16.87	16.46	40.34	20.37	37.78	11.97	36.43
Femurs	1.76	3.11	4.41	1.29	1.67	2.28	2.78	4.63	5.24	3.03	5.62	3.19	5.80
(standard specific activity)	(0.010)	(0.016)	(0.025)	(0.007)	(0.010)	(0.011)	(0.016)	(0.024)	(0.030)	(0.016)	(0.035)	(0.018)	(0.034)

Summary

Thirteen mature rabbits were used in this experiment, and sacrificed at various times after the intravenous administration of the radiocalcium and the feeding of the white clover hay diet. The blood disappearance rate of radiocalcium, the distribution in the gastrointestinal tissues and contents, the bone uptake rate and the excretory rate in urine and feces were used to evaluate the transfer of calcium, and these results were compared to that of the young rabbit previously described (4).

1. The intravenously administered radiocalcium disappeared from the blood of the mature rabbit with a very large and rapid rate just as in the younger one. All segments of the digestive tract of the mature rabbit participate in the excretion of calcium with the small intestine playing a major role in this capacity. It is assumed that the superiority of the calcium absorption and excretion at the duodenum and lower small intestine is exchanged at various times after feeding.

2. The accumulation of calcium in the stomach and cecum of the mature rabbit are greater than that of the younger animals, and the transfer of them along the digestive tract of the former are slower than the latter. The time variations of the specific activities in the cecum, colon and rectum digesta of the mature rabbit are approximately the same in each segment, however, the trends of them differ from that of the young rabbit.

3. The bone uptake rates of the radiocalcium in the mature rabbit are considerably lower than that in the younger one and concerning the elution of calcium from the bone there was found no evidence in the older.

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