CALCIUM METABOLISM IN THE RABBIT II. TIME DISTRIBUTION OF INTRAVENOUSLY ADMINISTERED RADIOCALCIUM IN THE YOUNG RABBIT

CALCIUM METABOLISM IN THE RABBIT
II. TIME DISTRIBUTION OF INTRAVENOUSLY ADMINISTERED RADIOCALCIUM IN THE YOUNG RABBIT

By

Hiroshi ITOH and Tadashi HATANO

Department of Animal Husbandry, Faculty of Agriculture, Tohoku University, Sendai, Japan

(*Received, October 30, 1961*)

It has long been known that there is an exchange of minerals between the gastrointestinal contents and the blood. A method using ferric oxide as an inert reference substance, to determine the extent and site of this exchange was proposed in 1926 by Bergeim (1, 2). Subsequently, many studies on the experimental animal and farm animal have been reported by the use of the ferric oxide, chromic oxide and the other indicator methods.

Huang *et al.* (3) and Yoshihara and Sugisaki (4) have reported on the digestion and absorption of some kinds of nutrients at the various segments of the rabbit gastrointestinal tract, using the chromic oxide indicator method. However, there has been found no decisive evidence that the index substance added to the diet run parallel to the main stream of the digesta in each segment of the digestive tract.

Isotopic tracer make it possible to follow the distribution of a peculiar quantity of some element in the aminal body, and by comparing this distribution at various times after administration of the tracer, the nature and rate of exchange processes can be evaluated. Information on this mineral exchange by these isotopic tracer methods is of nutritional importance, and of practical use to feeding of the farm animals, particularly with regard to calcium, which is allways a limiting factor in animal nutrition.

In a previous report Itoh and Hatano (5) described the time distribution of calcium in the gastrointestinal tract of the young and mature rabbit and stated that the dry weight of digesta in the young rabbit digestive tract varied in relative distribution of the corresponding segments at the various periods after feeding, and furthermore the calcium content in dry matter showed more variation with the digesta of the stomach, small intestine and cecum playing a major role in the transfer of calcium at each period.

In this experiment, radiocalcium (Ca^{45}) has been used to label the circulating calcium in the young rabbit at various times after feeding of the white clover hay diet, and the disappearance rate of radioactive calcium from the blood and the appearance in the gastrointestinal tissues and contents and some other soft tissues, the deposition by the bone and the excretion in the urine and feces have been used to evaluate the transfer of calcium and to prove the main portions of the active secretion and absorption of this material in the digestive tract.

Materials and Methods

Twenty-two young rabbits each 50 days of age were used in this experiment. The details of the animals and the feeding management of them and the white clover hay used (No. WL8) have been previously described (5, 6).

The rabbits were divided into six groups by the time after the feeding until the slaughter. In each group, a rabbit was intravenously injected with the radiocalcium at the same time of the feeding and another one at various intervals ranging from ¼ to three hours before the slaughter to clarify the transfer of the radiocalcium during ½ to 24 hours intervals after feeding, and the interrelation between the transference rate of blood calcium and the digestive rate of the dietary calcium.

Of all rabbits used in this experiment, litter number, sex, age in days at the slaughter and body weight, and hours after feeding and administration of the isotope are indicated in Table 1.

A half milli-litre neutral solution of calcium chloride containing radiocalcium (20 μc Ca^{45}, 0.20 mg Ca per head) was injected into a marginal ear vein of each animal and they were returned to the metabolism cages until the sacrificed time, which occurred from a quarter to 24 hours after injection.

Besides the treatments of the gastrointestinal tract in the sacrificed animal which were described in the previous report (5), liver, kidney and both femurs were taken from all animals and two soft tissues were occasionally wet digested with concentrated nitric acid. Quantitative urine and fecal collections were initiated at the time of injection and continued until the animals were sacrificed and they were treated by the method described by Comar *et al.* (7). Total and radioactive calcium values in all samples of the tissues, gastrointestinal digesta, feces, urine and bones were determined from the oxalate precipitate, as described by Comar *et al.* (7).

Blood samples were withdrawn at intervals after injection from the marginal vein of the other ear of that injected with the isotope. Calcium contents of blood plasma were determined by the titration method using the sodium versene solution described by Baron and Bell (8). Total blood calcium contents of the rabbit were calculated by the plasma volume in all blood (55%) and the blood

volume per body weight (6.2%) (9).

Two rabbits were orally administered with 1 gm of ethylene diamine tetraacetic acid disodium salt (sodium versene) as an affecting substance for the calcium metabolism in the animal body and radiocalcium was injected at same time of the feeding. The transfer of radiocalcium in these rabbits were compared with that of the control.

Results and Discussion

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

The standard specific activities of the blood calcium and each tissue and content of the various segments of the digestive tract are given in Table 1, and the percent dose of Ca^{45} in the blood, total gastrointestinal tissues and contents, liver, kidney, urine, feces and femurs of each young 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 rapid rate, that is, as shown in Table 2, as early as 15 minutes after injection of the isotope the radioactivity in the blood calcium decreased to about 10 per cent of the administered value, and at the 30-minute period, it decreased to less than a half of the value at the 15-minute period. Thereafter the radioactivity of the blood calcium decreased at a slower rate until the 24-hour period.

The standard specific activities of the blood calcium in the rabbits which were sacrificed at a half or one hour after injection of the isotope, showed considerable variation at each interval after feeding as shown in Figs. 1 and 2. The variation of these values at a half hour after administration of the isotope have expressed that the more the time after feeding is short, the larger the disappearance of Ca^{45} from the blood occurred. In general, it appears that the transfer of the blood calcium considerably decrease when it spent some long time after feeding and the transfer of calcium in the digesta is stabled along the digestive tract. Furthermore, it seems that these variations may occur by the increasing of the blood calcium soon after feeding and thereafter the gradual decreasing of them.

In the cases of the one-hour period after injection of the isotope, the disappearance rate of the blood radiocalcium decreased step by step in the course of time after feeding, as shown in Fig. 2. A young rabbit No. Y15 which received the second feeding at six hours after the first feeding and sacrificed at three hours after the second showed a fairly high disappearance rate of the

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

Rabbit No.	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9	Y10	Y11	Y12	Y13	Y14	Y15	Y16	Y17	Y18	Y19	Y20	Y21*	Y22*
Litter No.	Ⅳ	Ⅲ	Ⅲ	Ⅳ	Ⅲ	Ⅰ	Ⅳ	Ⅱ	Ⅱ	Ⅰ	Ⅱ	Ⅰ	Ⅱ	Ⅰ	Ⅱ	Ⅰ	Ⅰ	Ⅰ	Ⅱ	Ⅱ	Ⅳ	Ⅳ
Sex	♂	♂	♂	♀	♀	♀	♂	♀	♀	♂	♀	♂	♀	♂	♂	♀	♀	♂	♂	♂	♂	♂
Age (day)	49	50	51	49	50	50	50	48	51	51	48	50	48	49	49	49	50	50	52	52	50	49
Body weight (g)	920	900	780	940	820	960	920	975	895	925	935	1000	915	860	1115	825	930	930	875	830	790	950
Time of sacrifice (hr post-injection)	½	½	1	1	2	½	1	1½	3	¼	½	1	3	6	1	9	1	12	1	24	3	6
Time afetr feeding (hr)	½	1	1	2	2	3	3	3	3	6	6	6	6	6	9	9	12	12	24	24	3	6
Blood calcium	5.53	7.93	2.82	4.02	2.14	10.62	3.65	2.51	1.84	21.04	12.58	4.24	0.98	0.47	2.72	0.48	4.54	0.38	5.74	0.28	1.65	0.67
Tissues																						
Stomach	0.25	1.85	0.93	1.20	0.59	2.13	1.53	1.39	0.66	5.16	4.18	2.13	0.67	0.41	1.58	0.34	2.78	0.22	3.41	0.28	0.52	0.26
Small intestine	0.79	0.97	0.49	0.99	0.40	1.45	1.02	0.52	0.39	2.85	2.14	1.37	0.42	0.37	0.89	0.26	1.91	0.27	2.15	0.19	0.36	0.26
Cecum	0.46	0.46	0.40	0.51	0.32	0.88	0.54	0.37	0.39	1.36	1.06	0.61	0.40	0.22	0.46	0.16	0.33	0.13	0.39	0.18	0.27	0.21
Colon	0.64	1.06	0.37	0.61	0.38	0.83	0.48	0.25	0.63	1.84	1.30	0.62	0.35	0.24	0.79	0.22	0.69	0.13	1.21	0.19	0.25	0.29
Rectum	1.38	0.62	0.64	0.64	0.77	1.43	0.96	1.00	0.71	2.16	1.93	0.99	0.85	0.79	0.94	0.69	0.91	0.37	1.60	0.36	0.88	0.53
Contents																						
Stomach	0.01	0.01	0.01	0.03	0.02	0.04	0.03	0.04	0.02	0.05	0.08	0.05	0.06	0.05	0.04	0.02	0.09	0.01	0.35	0.19	0.01	0.15
Small intestine	0.90	1.16	0.42	0.63	0.21	1.73	1.60	0.68	0.28	3.72	2.46	2.37	0.30	0.17	2.77	0.09	8.77	0.11	5.80	0.19	0.25	0.42
Cecum	0.65	0.20	0.28	0.34	0.49	0.05	0.17	0.56	0.35	0.01	0.09	0.13	0.16	0.18	0.15	0.02	0.04	0.13	0.06	0.22	0.01	0.17
Colon	0.50	0.67	0.35	0.43	0.21	0.25	0.30	0.33	0.42	0.07	0.28	0.18	0.31	0.20	0.24	0.18	0.10	0.09	0.50	0.22	0.39	0.16
Rectum	0.09	0.19	0.29	0.32	1.17	0.07	0.23	0.57	0.46	0.01	0.47	0.18	0.51	0.23	0.15	0.25	0.23	0.17	0.21	0.23	0.97	0.40

* These rabbits were administered with 1g of sodium versene in the clover hay diet.

radiocalcium from the blood owing to the increase of the transfer of calcium in the digestive tract and of the absorbed calcium in body by the second feeding.

The segments of the gastrointestinal tract which had the highest standard specific activity were replaced by the time after administration of the isotope. In the rabbits which were administered with the radiocalcium intravenously within one and half hours before the slaughter, except of the rabbit No. Y1 (hours after Ca^{45} administration : hours after feeding, $\frac{1}{2} : \frac{1}{2}$), the highest standard specific activity occurred in the gastric tissue, and two hours and later after injection of the isotope, the maximum values occupied the rectal tissue. It seems that the radioactivity of blood in the underlaying musculature which are adhered to the gastric tissue affects this result. This fact is evidenced by the following; the radioactivity of the gastric tissue decreases at a rate similar to that of the blood calcium, on the other hand, the decrease of radiocalcium in the rectal tissue is slower, and two hours and later period the specific activity of the former tissue is lower than that of the latter. In general, the highest specific activity among all portions of the gastrointestinal tissues with the exception of the gastric tissue was observed in the small intestinal tissue during an earlier period after administration of the radiocalcium and in the rectal tissue at the later period.

2. *Standard specific activity of the gastrointestinal contents*

The time variations of the standard specific activities of the intravenously administered radiocalcium were much larger in the contents than that of the tissues in comparable regions and at comparable intervals.

The standard specific activities of the gastric contents showed the lowest values on an approximate constant level and that of the other contents varied at a considerable larger rate at each time after both the administrations of the isotope and the diet, and these variations were large in the small intestine and small in the cecum, in general. It is evident that the appearance of Ca^{45} in the digesta of all digestive tract during a shorter period of time after intravenous administration of the isotope are interpreted as an indication of more or less secretion of calcium into the gut of the young rabbit.

The isotopic contents of the stomach digesta show the smallest values with the exception of a few cases, however this existence is interpreted by the entrance of the Ca^{45} labeled saliva and the secretion of the gastric juice contained some quantity of the isotope. It is considered that the radiocalcium in the stomach flows down to the lower segment (duodenum) with the calcium in digesta (feed) and does not remain there for a long time. The standard specific activities of the gastric contents in the rabbits sacrificed at 24 hours after feeding showed exceedingly high values as compared with the other rabbits. It appears that these facts are caused by the lowest calcium contents

in the stomach at this period and these calcium remain there in the insoluble or unavailable forms.

Within two hours after injection of the isotope, the highest specific activities of the digesta were found in the small intestine, and they were 15 to 120 times as high as that of the stomach, and two hours and later period, three to 15 times that of the stomach except of Y 20 rabbit (24 : 24), although lower than that of the rectum digasta at comparable times. It is an obvious fact, therefore, that a much larger amount of the endogenous (radioactive) calcium is excreted in the small intestine as compared with the other segments. These results are in agreement with the result of Wallace *et al.* (10) who indicated that the rat administered with radiocalcium intramuscularly have excreted the largest amounts of them in the small intestine.

In the rabbits which spent many hours after feeding and were injected with radiocalcium at one hour before the slaughter, the specific activity of the small intestinal content in No. Y15 (1 : 9) and 19 (1 : 24) rabbits was slightly higher than that of the blood calcium and in rabbit No. 17 (1 : 12) two times the value of the specific activity of the blood calcium were observed in the small intestine digesta, and these facts expressed that the more secretion of the small intestinal digestive juice containing the endogenous calcium might be expect when a larger quantity of the undigestible content containing a less amount of calcium pass through this segment.

The intestinal absorption of calcium in the intact organism and isolated intestinal loops have been reported by several investigators. Schachter and Rosen (11) have indicated that there is a mechanism in the proximal small intestine of the young rabbits for actively transporting calcium against a concentration gradient *in vitro* from the mucosal to the serosal surface by the use of the everted gut-sacs.

The active secretion of calcium in the small intestine was proved from the present data using the intravenously administered radiocalcium. It is considered, therefore, that the small intestine transport calcium more actively and the calcium secretory functions are more active than the calcium absorptive functions owing to the higher calcium concentration per dry matter of the small intestinal content than that of the stomach at comparable times after feeding, as previously reported by Itoh and Hatano (5).

During a shorter period of time following tracer administration in the groups of sacrificed time ranging from three to 24 hours after feeding, the specific activity of the cecal content showed the lowest values owing to the large amount of the stable calcium already there, and it seems that the existence of the radioactivity at this period proves the slight secretion of calcium in the cecum. Afterward, these values of the cecal content increased and varied with the inflow of the digesta from the upper portions, especially small intestine

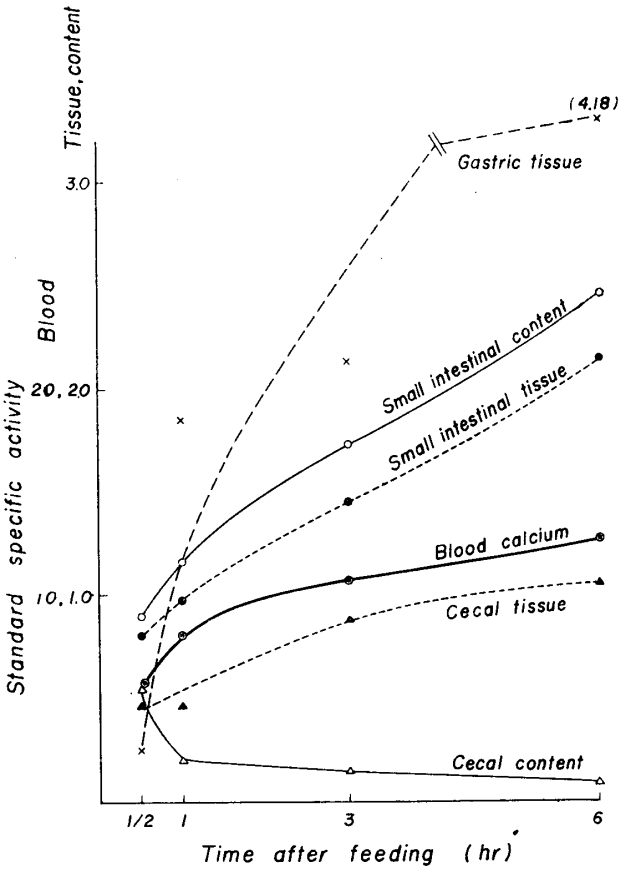

Fig. 1. Variations at various times after feeding of the standard specific activity of the blood calcium and some gastrointestinal tissues and contents in young rabbits at half an hour following radiocalcium administration

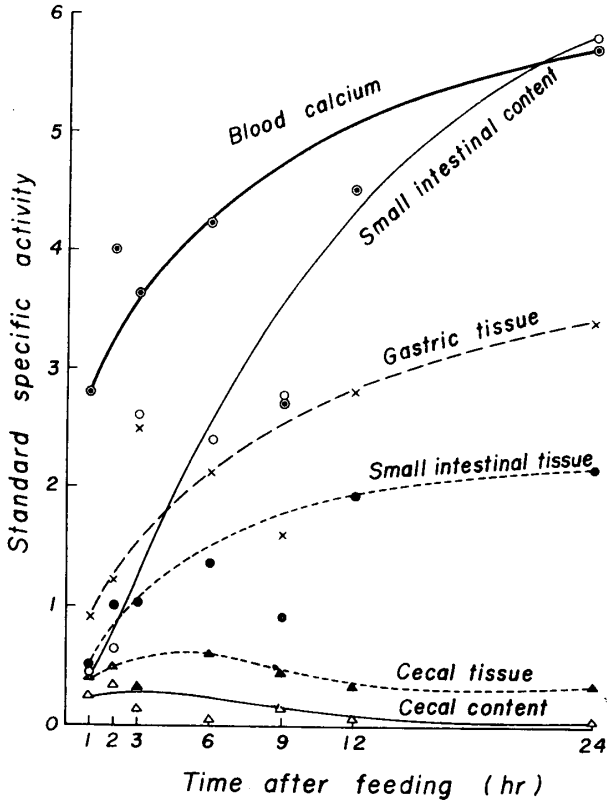

Fig. 2. Variations at various times after feeding of the standard specific activity of the blood calcium and some gastrointestinal tissues and contents in young rabbits at one hour following radiocalcium administration

which had the active secretory function of the endogenous (radioactive) calcium. The facts that the gradual increase of the radioactivity in the cecal content mainly occurs by the inflow of the small intestinal content, are interpreted by the increasing of them after the time a considerable larger amount of the radiocalcium are secreted in the small intestine, and on the other hand, by the rapid decreasing of the radioactivity in the blood calcium at this period. On the contrary, in the rabbits sacrificed at a short period after feeding, the specific activity of the cecum digesta showed the values similar to the small intestine, indicating the rapid inflow of both dietary and endogenous (radioactive) calcium in the cecum with the less digesta.

As shown in the time variation of the specific activity of the blood calcium, the time distribution of the radioactivity in the gastrointestinal tissues and contents varied over various periods of time after feeding although it required definite intervals of time after injection of the isotope. It is considered, therefore, that the digestive degrees of the contents in various segments at

each period of time after feeding affect the calcium metabolism, that is, the transfer of the intravenously administered radiocalcium.

The variations of the specific activities in the gastric tissue, and tissues and contents of the small intestine and cecum at various times after feeding in the two groups of rabbits, that is, one group is administered with radiocalcium at 30 minutes before the slaughter and the other one is dosed at one hour before, are shown in Figs. 1 and 2, respectively. The former group was observed at the half- to six-hour period after feeding and the latter group was at one to 24 hours. The trends of the variations in that of comparable segments in both groups were almost the same.

The specific activities of the gastric tissue and the small intestinal tissue and content at the same hour period following tracer administration increased in the course of time after feeding, and in the cecum a less variation was observed.

Possibly to consider the following reasons on the former observation, that is, the specific activities of the blood calcium are maintained at the comparatively higher value in the course of time after feeding, and that of the small intestinal contents are led to the higher values by the decrease of the calcium concentration per dry matter of the gastric and small intestinal contents at comparable times as described in a previous report (5), even when the radioactivity which entered with saliva and secreted in the stomach and small intestine with the digestive juice was decreased.

The approximately constant level of the specific activity in the cecum digesta during the definite intervals of time after administration of the isotope was kept by the increasing accumulation of the dietary calcium in the course of time after feeding, in spite of the increasing specific activity of the small intestine digesta at comparable times and the inflow of these higher radioactivities into the cecum. Furthermore, it is supposed that the absorption of the endogenous calcium are higher than that of the dietary calcium which contained a comparatively larger amount of the indigestible calcium.

As described in our previous report (5), the calcium concentration per dry matter of both cecum and colon digesta showed the similar values at comparable times after feeding. It seems, therefore, that the absorption and secretion of calcium equilibrate in the colon, if all digesta of the small intestine reach to the colon passing through the cecum. However, the specific activity of the colon digesta is higher than that of the cecum at comparable times, indicating the more active secretion of calcium in the colon. And it is considered that the dietary calcium are absorbed at the colon with a similar rate of the endogenous calcium secreted at the upper segments, mainly the small intestine.

It is suggested that the calcium is deformed to the more absorbable one by the digestive process in the cecum, and thereafter those are absorbed at

the colon which have the more suitable function on the calcium absorption.

It is considered that the higher specific activity of the colon digesta as compared with the cecum digesta during a short period of time following tracer administration is caused by the direct effluence of the small intestinal contents into the colon.

In our previous report (5), it was indicated that the calcium concentration per dry matter of the rectum digesta were higher than that of the feces in comparable forms of the hard and soft type and the active absorption of calcium may have occurred in the rectum.

The variations of the specific activity in the rectum digesta are shown as: During a short period of time after administration of the isotope, comparatively higher values are observed in the rectum as compared with the other segments and it shows that the secretion of the endogenous calcium may occur in this portion, and furthermore the maintenance of a higher specific activity during the later period show the higher resorption of the endogenous calcium in comparison with the dietary one, and this fact may express that the main part of calcium in this digesta remains in the indigestible form and some of them are unavailable. And an increasing radioactivity of the contents toward the anterior portions of the digestive tract indicates the digesta which have a higher specific activity transfer to the lower segments gradually.

At two hours and later period of time after the injection, the highest specific activity of the digesta was found in the rectum. Possibly this was a consequence of the higher absorption of the dietary (stable) calcium as compared with the resorption of the endogenous calcium mainly secreted in the upper segments or indicative of the higher secretion of radiocalcium in the rectum.

By the fact that these two contrary arguments relate with each other, it is supposed that the selectivity of the calcium absorption may be varied in some conditions (the hard and soft types) of the rectum digesta at various periods after feeding and either activity of the calcium absorption and secretion might be superior to one another at definite periods of time.

As is suggested in a report by Smith *et al.* (12) on the transfer of phosphate in the gastrointestinal tract of swine (a simple-stomach animal), the apparent selective resorption of endogenous calcium observed in this experiment may result from a functional deficiency of some digestive systems with simple stomachs.

The variations of the specific activity of the digesta in comparable segments of the digestive tract at each period after injection of the isotope in the young rabbits which were sacrificed at three hours and later after feeding were summarized as follows: In the gastric content the slightly higher values were observed during the earlier period and thereafter the constant low levels were indicated, and the highest values of the small intestine at the earliest time

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

Rabbit No.	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9
Time of sacrifice (hr post-injection)	$\frac{1}{2}$	$\frac{1}{2}$	1	1	2	$\frac{1}{2}$	1	$1\frac{1}{2}$	3
Time after feeding (hr)	$\frac{1}{2}$	1	1	2	2	3	3	3	3
Blood calcium	3.62	3.16	1.78	1.51	0.90	2.49	1.48	1.04	0.63
Total gastrointestinal tissues	1.74	1.74	1.55	0.89	0.87	2.02	1.16	0.79	0.46
Total gastrointestinal contents	3.66	5.21	8.09	6.17	9.33	5.33	6.32	7.24	8.98
Liver	0.79	0.89	0.82	0.43	0.26	0.78	0.41	0.34	0.23
Kidney	0.44	0.85	0.36	0.42	0.24	0.75	0.40	0.26	0.19
Total urine	6.56	0.12	2.58	18.22	5.08	7.70	3.17	4.88	9.73
(standard specific activity)	(1.13)	(0.14)	(2.54)	(3.80)	(0.66)	(1.78)	(1.98)	(2.01)	(3.54)
Total feces	—	—	—	—	—	—	—	—	0.008
TOTAL	16.81	11.99	15.18	27.64	16.68	19.07	12.94	14.55	20.23
Femurs	4.26	3.78	4.83	3.92	5.62	3.49	4.97	4.23	4.09
(standard specific activity)	(0.084)	(0.092)	(0.130)	(0.078)	(0.140)	(0.077)	(0.092)	(0.101)	(0.110)

Rabbit No.	Y10	Y11	Y12	Y13	Y14	Y15	Y16	Y17	Y18	Y19	Y20	Y21*	Y22*
Time of sacrifice (hr post-injection)	$\frac{1}{4}$	$\frac{1}{2}$	1	3	6	1	9	1	12	1	24	3	6
Time after feeding (hr)	6	6	6	6	6	9	9	12	12	24	24	3	6
Blood calcium	10.39	4.18	1.57	0.45	0.23	1.37	0.19	1.53	0.15	1.93	0.10	0.56	0.33
Total gastrointestinal tissues	3.37	1.80	1.31	0.47	0.41	1.05	0.30	1.53	0.24	1.49	0.18	0.84	0.54
Total gastrointestinal contents	3.65	3.45	5.94	4.32	8.24	4.96	1.89	5.54	4.88	4.27	7.13	13.96	7.71
Liver	2.71	0.88	0.36	0.18	0.01	0.58	0.03	0.65	0.05	1.27	0.04	0.14	0.06
Kidney	1.10	0.70	0.36	0.17	0.07	0.32	0.04	0.46	0.03	0.42	0.02	0.18	0.06
Total urine	0.71	2.32	7.20	12.10	5.72	2.67	19.22	0.27	23.58	1.94	3.04	15.11	8.10
(standard specific activity)	(1.31)	(0.79)	(2.91)	(3.90)	(3.16)	(1.15)	(4.11)	(1.66)	(4.50)	(0.004)	(2.69)	(0.75)	(5.02)
Total feces	—	0.02	—	4.18	6.56**	—	7.62**	—	5.82**	—	6.16**	1.65	3.02
TOTAL	21.93	13.35	16.74	21.87	21.24	10.95	29.29	9.98	34.75	11.32	26.67	32.44	19.82
Femurs	2.53	3.63	3.62	4.43	4.39	3.67	3.55	4.06	3.46	3.02	3.68	3.65	4.75
(standard specific activity)	(0.062)	(0.090)	(0.085)	(0.113)	(0.113)	(0.081)	(0.092)	(0.091)	(0.082)	(0.079)	(0.100)	(0.091)	(0.101)

* These rabbits were administered with 1g of sodium versene.
** These values were shown in Fig. 4 graphically.

decreased rapidly during a short time after then according to the disappearance of the blood radiocalcium. In the cecum, colon and rectum, these values reached to the peak at the one- and three-hour intervals after dose of the isotope, and six hours and later after injection it equilibrated at about one third values of each peak. These data will be graphically expressed in another paper (13) as compared to the results of the mature rabbits.

Thomas *et al.* (14) have reported that the blood disappearance rates of the intravenously administered radiocalcium and fecal rate of calcium excretion can be increased by administering 1250-3750 mg of sodium versene by stomach tube in the rabbit.

The specific activity of the gastrointestinal tissues in the rabbits (Y 21 and Y 22) which were dosed orally with one gm of the sodium versene added to the clover hay diet and sacrificed at three or six hours after administration of the isotope and feeding were similar to that in the tissue of the corresponding segments in the normal rabbits Y9 (3 : 3) and Y14 (6 : 6), however in that of the digesta, the cecum of Y21 rabbit showed the lowest value and the rectum was two times of that of the control, and Y22 rabbit showed the higher value in the small intestine. It is considered, therefore, that the quantity of total secreted calcium in the digestive tract significantly had no affect by the ingestion of sodium versene, but it affects the increasing transfer of the dietary calcium to the lower parts of the tract and the decreasing absorption at the lower segments.

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

The distribution (percent of the dose) of the intravenously administered radiocalcium in the blood, the tissues and contents of all digestive tract, liver, kidney, urine, feces and femurs (bone) are given in Table 2. In general, these values of the blood calcium, total gastrointestinal tissues, liver and kidney decreased in the course of time after injection of the radiocalcium, and on the contrary, the total digesta increased gradually.

As shown in Table 2 and Fig. 3, the specific activity of the femur varied with interest at each period following tracer administration. The rapid uptake of radiocalcium by the bones occurred immediately after the intravenous administration and it continued during the first few hours. At the only 15-minute interval after injection of the isotope, 2.5 percent of the radioactivity (standard specific activity 0.06) was deposited in a femur, and at the half- or one-hour period after injection, the specific activity showed the values ranging from 0.077 to 0.092 at all times after feeding except of rabbit No. Y3 (1 : 1), and these values increased gradually until the six-hour period with the exception of No. Y5 (2 : 2). And at the nine- and 12-hour period the specific activity decreased until the level of the one-hour period, indicating the elution of radiocalcium from the bone during the earlier period after the deposition.

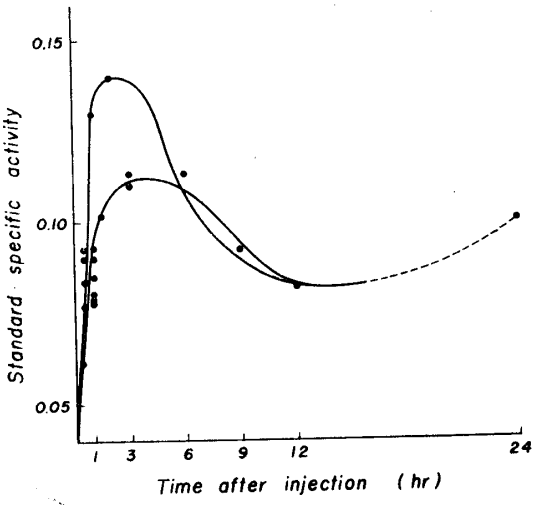

Fig. 3. Standard specific activity of femur in young rabbits at various times after intravenous injection of radiocalcium

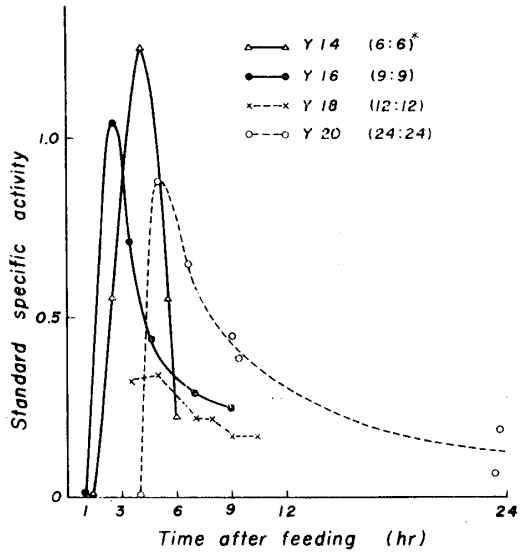

Fig. 4. Standard specific activity of feces of four young rabbits at various times after intravenous administration of radiocalcium and feeding of the white clover hay diet
* (hr after injection of Ca^{45} : hr after feeding)

The rabbits which were sacrificed at one or two hours after administration of the isotope, that is Y3 (1 : 1) and Y5 (2 : 2) showed the highest specific activity of the femur, being 0.13 and 0.14, respectively.

In general, the bone uptake rate of the intravenously administered radiocalcium in this experiment was slightly lower than the data of Thomas *et al.* (15).

Urinary excretion of calcium in the rabbit was more active than that of the other animals, and when the rabbit was fed with the white clover hay diet of high calcium content and a higher calcium-phosphorus ratio, calcium was excreted in large quantities and precipitated as calcium carbonate in the high alkaline urine.

Although urinary excretion of the radiocalcium had no connection with the time after administration of the isotope, the highest specific activity was observed in urine excreted during the 2.5- to five-hour period after the injection, and was higher than that of the blood at comparable times occasionally. And the individual differences were observed in these values as same as shown in the radioactivities of liver and kidney.

It is considered that the specific activity of urine is affected by the quantity of the blood radiocalcium at the period when the renal function is more active.

There is a very interesting relation between the bone uptake and the urinary excretion of the intravenously administered radiocalcium.

In the rabbits which urinary excreted large amounts of the radiocalcium, Y4 (1 : 2), Y16 (9 : 9) and Y18 (12 : 12), the comparatively lower bone uptake rate was observed. Especially, rabbit Y4 excreted about 18 percent of the injected isotope in the urine within the one-hour period of time, and showed the lower specific activity (0.078) of the femur as compared with the rabbbits Y3 (1 : 1) and Y5 (2 : 2), which had similar experimental conditions of Y4, having 0.13 and 0.14 of the value, respectively. It is considered, therefore, that when a large quantity of the urinary calcium is excreted, bone uptake of the intravenously administered radiocalcium decrease.

However, it is assumed that the lower specific activities of the femur in the Y16 and Y18 rabbit are caused by the elution of the once deposited radiocalcium and some parts of these liberated calcium are excreted in the urine.

On the basis of these observations two curves of the bone uptake rate were observed at the period ranging from one to six hours after injection of the isotope in Fig. 3.

Some quantity of the radiocalcium was found in the feces excreted after only five minutes following tracer administration intravenously. Possibly this was a consequence of the rectal secretion of calcium. Time variations of the standard specific activities of the feces in rabbits Y14 (6 : 6), Y16 (9 : 9), Y18 (12 : 12) and Y20 (24 : 24) which were administered radiocalcium at the same time of the feeding were represented in Fig. 4. Although the highest specific activities of the feces differed in each rabbit, these values were observed at the period ranging from 2.5 to five hours after intravenous injection of the isotope and afterward it decreased gradually.

Summary

Twenty-two young rabbits each 50 days of age 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 to prove the main portions of the active absorption and secretion of this material in the digestive tract.

1. The distribution in the rabbit of intravenously injected radiocalcium at various times after its administration indicates a very large and rapid transfer of blood calcium to the gastrointestinal tissues and contents, and the blood disappearance rate decreases in the course of time after the feeding even at the same interval following tracer administration.

2. All segments of the digestive tract of the young rabbit participate in

the excretion of calcium with the small intestine playing a major role in this capacity, and the excretion of calcium in this segment exceeds the absorption. It is assumed that the calcium metabolism are affected by the digestive activity in the upper segments of the intestinal tract at various times following feed ingestion, owing to the observation of the various specific activities of radiocalcium at various times after feeding even at the same following tracer administration.

3. It appears that the resorption of the endogenous calcium are higher than the absorption of the dietary calcium in the cecum. It seems that, in the colon, the dietary calcium are absorbed at a rate similar to the resorption of the endogenous calcium, and the direct effluence of the small intestinal contents into the colon without the passing through the cecum occurs occasionally. It is supposed that the selectivity of the calcium absorption may vary under the some conditions of the rectum digesta at various periods after feeding.

4. The rapid uptake of radiocalcium by the bone is observed immediately after injection of the isotope and the elution of calcium from the bone may occur at the earlier period after the deposition. Considerably large amounts of calcium are excreted in the urine and an interesting relationship is recognized between the bone uptake and urinary excretion of the intravenously administered radiocalcium.

Acknowledgement : This work has been supported by a Grant in Aid for Fundamental Scientific Research from the Ministry of Education. The authors wish to thank Messrs. S. Kurihara and H. Kumada for their technical assistance.

References

1) Bergeim, O. (1926). J. Biol. Chem., **70**, 47.
2) Bergeim, O. (1926). Ibid., **70**, 51.
3) Huang, T.C., *et al.* (1954). J. Nutrition, **54**, 621.
4) Yoshihara, I. and A. Sugisaki (1960). Jap. J. Zootech. Sci., **31**, 9. (in Japanese)
5) Itoh, H. and T. Hatano (1960). Tohoku J. Agr. Res., **11**, 341.
6) Itoh, H. and T. Hatano (1960). Ibid., **11**, 133.
7) Comar, C.L., *et al.* (1951). Nucleonics, **8**, 19.
8) Baron, D.N. and J.L. Bell (1957). Clinica Chimica Acta, **2**, 327.
9) Dukes, H.H. (1955). The physiology of domestic animals, 7th Ed. Comstock Publishing Assoc., N.Y.
10) Wallace, H.D., *et al.* (1951). J. Nutrition, **43**, 469.
11) Schachter, D. and S.M. Rosen (1959). Am. J. Physiol., **196**, 357.
12) Smith, A.H., *et al.* (1955). J. Nutrition, **57**, 497.
13) Itoh, H. and T. Hatano (1961). Tohoku J. Agr. Res., **12**, 341.
14) Thomas, R.O., *et al.* (1954). Am. J. Physiol., **176**, 381.
15) Thomas, R.O., *et al.* (1952). Ibid., **169**, 568.