CALCIUM METABOLISM IN THE RABBIT I. TIME DISTRIBUTION OF CALCIUM IN THE GASTROINTESTINAL TRACT OF YOUNG AND MATURE RABBIT

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I. TIME DISTRIBUTION OF CALCIUM IN THE GASTROINTESTINAL TRACT OF YOUNG AND MATURE RABBIT

By

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The nutrition of the rabbit has not been as well studied as has that of the rat and mouse. It has been known that the calcium requirements of the rabbit, although as yet quantitatively undetermined, are relatively high (1). Wooley and Mickelsen (2) reported that the addition of extra amounts of calcium carbonate to a purified diet increased growth and decreased mortality in three- to four-week-old rabbit. There are only few studies on the metabolism of calcium, especially the transfer of calcium in the digestive tract of the rabbit, and little is known concerning the portion of the gastrointestinal tract playing a major role in capacity of the absorption and excretion of calcium. Thomas *et al.* (3, 4) have reported on the dynamics of calcium metabolism in the normal young and adult rabbits by the determination and correlation of blood disappearance rates, urinary excretion rates, and bone uptake rates of radioactive calcium after the intravenous injection.

However, for many years there has been much controversy in the literature concerning the absorptive and excretory role of the gastrointestinal tract as it is related to calcium metabolism. Some workers have found no evidence of an active excretion, while others have accepted the view that the large intestine in particular has an important function in regulating the blood level of certain minerals. Findings with the various species have not been in good agreement, indicating a possible species difference in this regard. Dukes (5) suggested that the small intestine is doubtless of great importance as an organ of absorption in herbivores as well, and the large intestine of all herbivores must be well adapted for absorption. This would seem to be especially true in the mono-gastric herbivores, where such extensive digestive changes are reserved for the large intestine.

Henry and Kon (6) found no active excretion of calcium into the large intestine of rat. Adolph and Liang (7) suggested that apparently in the rat the intestinal tract does not excrete calcium in amounts which are related to the metabolic calcium level. Bergeim (8) stated that there was a marked excretion of calcium into the lower bowel of rachitic rats, while in normal animals there appeared to be an approximate balance between excretion and absorption. However, this work revealed no proof that excretion actually took place. Wallace *et al.* (9) suggested that all segments of the intestinal tract of young and mature rats participate in the excretion of calcium with the small intestine playing a major role in this capacity, which is contrary to the view that the large intestine predominates in this function. Huang *et al.* (10) stated that two thirds of the digestible material was absorbed from the lower digestive tract in the rabbit, as the results of the studies on the states of the digestion and absorption of the nutrients by the use of the chromic oxide indicator method. Furthermore, Yoshihara and Sugisaki (11) observed the same results on the absorption in the large intestine of the rabbit by the use of chromic oxide and a marked absorption of phosphorus was confirmed in the large intestine, especially in colon and rectum. Kametaka (12) reported that the main portion of nutrient absorption is the large intestine, in the rabbit using MnO_2 as an indicator substance.

Interesting work by Cowell (13) in which individual fecal pellets of rabbits were analyzed provides good evidence that in this species the colon excretes important quantities of calcium. Herndon and Hove (14) suggested that the ash and calcium utilization were approximately the same in both normal and cecectomized rabbits. Alexander and Chowdhury (15) found the evidence that the lactic acid was produced by microbial activity in the rabbit's stomach and appeared in part to be absorbed through the gastric epithelium.

In the rabbit, the green herbages occupy a main part of the feeding stuffs, although the range of them in common use is much wider. Particularly, the legume forages are used in the feeding of the rabbit, because of the several superiorities they have, that is, they are the highest in protein and calcium and suit the rabbit's taste, over all other green forages. The chemical composition, especially calcium content of the legume forages included the several white clover hays, which were used in this experiment were reported previously (16).

In the experiments reported here, the time distribution of water and calcium in the gastrointestinal tissues and contents of the young and mature rabbit which were fed on the white clover hay, and the calcium in it occupies the only source of the diet, have been determined to evaluate the absorption and excretion of water and calcium in the digestive tract.

Materials and Methods

Animals used in this experiment are 25 young Japanese White rabbits and 17 mature rabbits of the same kind. The mean body weight of these young and mature rabbits are 900 and 1900 g, respectively.

Twentyfive young rabbits were selected from the four litters (27 animals) and three of them were born from the same mother at the intervals of about four months. The average body weight and numbers of the littermates of the four litters are presented in Table 1.

Table 1. Body weight of young rabbit in four litters.

Litter No.	Number of rabbits		Average body weight (g)
	Male	Female	
I	4	3	919±47
II*	3	4	934±88
III*	3	3	846±34
IV*	4	3	900±50
Total	14	13	902±68

* They were born from the same mother.

Each littermate was placed with their mother which were fed on mainly white clover (white clover hay in winter season) added with some amounts of wheat bran, barley and soybean oil meal and supplemented with small amounts of NaCl and cod liver oil for five weeks. After then, the diet of the young rabbits were changed to the white clover hay from the mixed ration above, gradually. They were fed on only the white clover hay for five days before the experimental day. The mature rabbits were fed on the mixed ration described above, during about a month and the white clover hay for about one week before the experimental time.

During a period of five days before the experiment, the young rabbits received 20 g of the white clover hay with 50 *ml* of water at 10:00 a.m. and 4:00 p.m.. The mature rabbits received 60 g of the white clover hay with 120 *ml* of water at 10:00 a.m. daily. The white clover hays used in this experiment are No. WL 8 white clover hay and No. R3 Ca^{45} labeled white clover hay for the young rabbit and No. L3, W4 and No. R2 labeled hays for the mature rabbits, previously described (16).

Young rabbits were divided into six groups of the intervals after the first feeding; that is, within two,** at three, six, nine, 12 and 24 hours respectively, and mature rabbits in four groups; at three, six, 12 and 24 hours.

** Within two hours intervals, it contains two rabbits at the 30-minute period, three animals at one hour and two at the two-hour period.

Numbers of animals and their mean body weight in each group of young and mature rabbit are presented in Table 2.

Table 2. Body weights of young and mature rabbits used.

Time since feeding (hr)	Number of rabbits	Average body weight (g)
	Young Rabbit	
Within 2*	7	870± 53
3	5	922± 42
6	6	917± 40
9	3	947±120
12	2	930± 0
24	2	852± 23
Total	25	904± 66
	Mature Rabbit	
3	4	1877± 50
6	7	1949±127
12	3	1883± 69
24	3	1890±109
Total	17	1910± 89

* Two rabbits at half an hour, three animals at one hour and two at the two-hour period.

Each experimental animal was placed in the individual wire metabolism cage over a urine-feces separator (25×22×25 cm for young rabbit, 60×50×50 cm for mature rabbit) on the day before the experiment. After definite intervals of time since the administration of the white clover hay diet, rabbits were sacrificed by air-injection from the marginal ear vein after the withdrawing of blood by the cardiac puncture. The entire gastrointestinal tract was removed and carefully cleaned of adhering connective tissue, and was then divided into the desired segments. Samples were taken of the tissue and contents of stomach, small intestine, cecum, colon and rectum. In the case of the mature rabbit, the upper one-fourth of the small intestine representing the duodenum. However, in this report the data of both segments were put together again and represented as the small intestine. The contents of each segment were carefully removed into the porcelain evaporating dish or petri dish. After the tissues of each segment were washed with distilled water, they were put on the blotting paper to drain, and weighed. All samples were dried at 85°~95°C for about 24 hr and then ashed in an electric furnace at 600°C. Ash was dissolved in 2*N* HCl solution and made up to volume. Total calcium contents were determined from the oxalate precipitate, as described by Comar *et al.* (17).

Results and Discussion

1. Chemical analyses of the gastrointestinal tissues

The results of the chemical analyses of the gastrointestinal tissues are summarized in Table 3 as the means and their standard deviations for each time group of young and mature rabbits.

Table 3. Dry and raw weight, dry matter and total calcium in gastrointestinal tissues of young and mature rabbits.

	Stomach	Small intestine	Cecum	Colon	Rectum
	Dry weight (g)				
Young rabbit	2.4±0.2*	4.1±0.4	2.1±0.3	0.9±0.1	0.7±0.1
Mature rabbit	4.6±0.7	5.8±1.4	3.9±0.8	1.9±0.3	1.5±0.3
	Raw weight (g/kg body weight)				
Young rabbit	14.8	32.4	20.4	7.2	5.6
Mature rabbit	13.5	18.3	13.8	6.8	5.0
Time after feeding (hr)	Dry matter (%)				
	Young rabbit				
Within 2**	17.6±1.6	15.9±3.7	11.7±2.2	12.7±1.2	13.9±2.2
3	17.6±0.9	13.5±0.9	12.0±0.3	12.5±1.0	13.9±2.6
6	18.0±1.6	14.5±0.7	11.5±0.9	14.2±0.8	14.2±2.4
9	18.0±0.1	14.8±1.3	10.6±0.8	13.9±1.0	14.9±0.4
12	17.7±0.2	14.0±0.5	12.5±0.3	14.6±0.5	12.1±1.0
24	16.1±0.4	12.8±0.1	11.7±1.4	13.7±0.5	13.0±2.5
	Mature rabbit				
3	18.5±0.6	15.1±0.2	12.9±0.8	14.5±2.3	13.0±0.7
6	18.2±0.8	17.2±1.6	15.1±1.3	14.6±1.3	15.6±0.9
12	17.9±0.5	16.4±1.7	15.4±0.7	15.4±0.3	16.1±1.6
24	17.4±1.5	15.4±2.5	16.1±0.4	15.2±2.0	17.7±0.8
	Calcium concentration (mg/g dry matter)				
	Young rabbit				
Within 2	1.3±0.2	1.4±0.2	2.0±0.3	2.4±1.1	3.2±1.4
3	1.1±0.2	1.1±0.1	1.6±0.4	2.6±1.5	2.3±0.4
6	0.8±0.1	1.3±0.8	1.5±0.4	1.7±0.6	2.0±0.2
9	0.8±0.1	0.9±0.1	1.3±0.4	1.8±0.7	2.1±0.5
12	0.7±0.04	0.9±0.03	2.0±0.6	1.6±0.1	2.1±0.1
24	0.8±0.02	0.9±0.04	1.4±0.2	1.2±0.02	1.6±0.03
	Mature rabbit				
3	0.7±0.01	0.6±0.2	1.4±0.2	1.2±0.2	2.0±0.3
6	0.7±0.2	0.8±0.1	1.5±0.3	1.3±0.3	1.8±0.6
12	0.6±0.1	1.0±0.2	1.5±0.1	1.5±0.1	1.7±0.6
24	0.7±0.2	0.9±0.3	1.2±0.2	1.3±0.2	1.6±0.3

* Mean±standard deviation.

** Two rabbits at half an hour, three animals at one hour and two at the two-hour period.

As the dry weight in the tissue of each segment of the digestive tracts

in both age groups were shown to be unchanged at the various intervals after feeding, the mean values of all experimental animals in both age groups was expressed. The tissue of the small intestine showed the largest dry weight, followed by stomach and cecum, being smallest in colon and rectum, in both age groups. The dry weight of all tissues except the small intestine in the mature rabbit showed about two times that of the young animal, but the small intestine of the former animal was slightly larger than that of the latter one. Furthermore, in the raw weight of tissue of the stomach, colon and rectum per kg of the body weight, there were no differences between the young and mature rabbit, and that of the small intestine and cecum in the young rabbit were higher than in the aged animal. It seemed, therefore, that the increase of tissue-weight of the small intestine and cecum are out of proportion to the increase in body weight.

The dry matter percentage of the gastrointestinal tissues in the young rabbit are slightly lower than that of the mature animal, generally. It appears, therefore, that the dry matter content of the rabbit gastrointestinal tissue increases with the increasing age, and it is similar to that observed in swine by Smith *et al.* (18). As the sample of the stomach contained the musculature, it showed the highest content of the dry matter, followed by the small intestine. The water content of the stomach and small intestine slightly increased, when the digesta in both tracts decreased at 24 hours after feeding. The dry matter of the cecum showed the lowest value of all tissues and some progressive changes in these values with colon and rectum were observed on account of the water content of the digesta at various intervals after feeding. The dry matter content of the gastric and small intestinal tissues in the young rabbit are similar to that of swine (mono-gastric animal) which is shown by Smith *et al.*, but in tissues posterior to the cecum these values are lower than that of swine. The water content of the tissue in the rabbit increases lower down and on the contrary decreases in the swine. The dry matter of the mature rabbit gastrointestinal tissue decrease lower down in the anterior portion to the cecum.

A remarkable trend of the variation in the dry matter of each tissue except the small intestine at various intervals after feeding were observed in the mature rabbit compared to the younger animals. That is, in the stomach, the dry matter decreased in the course of time after feeding and in the digestive tract posterior to the cecum it showed an increase of the dry matter. It is supposed, therefore, that the rise in the water content in the tissue of all segments of the digestive tract was accompanied by a decrease of the contents of digesta in them and vice versa.

The calcium concentration of the young rabbit gastrointestinal tissues are generally greater than that observed in the mature animal. And the calcium

content of the upper tissues are lower than that of the lower tissues. However, it is impossible to account whether these facts are due to the difference of the distribution of the capillary in the tissues, or to the calcium concentration in the tissues and blood. In the young rabbit, the calcium concentration of the intestinal tissue except the cecum slightly decreased in process of time after feeding and especially the colon and rectum of the animals sacrificed during a short period of time contained about two times of calcium contents in the animal at 24 hours after feeding. It is supposed, therefore, that the calcium concentration of tissues are affected with the variation of calcium contents in digesta of each segment of the gastrointestinal tract at each interval after feeding. The calcium concentration in the tissue of the small intestine and colon in the mature rabbit slightly increased and of the rectum decreased until 12 hours after feeding.

2. Chemical analyses of digesta in the gastrointestinal tract

The results of the dry weight and total calcium contents of the digesta in each segment of the gastrointestinal tract are presented in Table 4.

A) Dry weight of the gastrointestinal contents: Dry weight of the digesta in all portions of the tract of the mature rabbit are higher than that of the young animal generally, but in the lower portion the similar and lower dry weight were observed in the mature at some intervals after feeding.

In the gastrointestinal contents, the highest dry weight occurred in the cecum in the young rabbit at all times after feeding, but in the mature animal the higher dry weight of the stomach were observed more than the cecum. It seems that the transfer of the stomach digesta to the posterior tract in the young animal are higher than that of the mature. The content of the stomach decrease rapidly in the course of time after feeding in both age rabbits. In the young rabbit, the more amount of digesta transferred from three to six, and nine to 12 hours after feeding. At 24 hours after feeding, about one fifth of the dry weight of the gastric content at full time was contained, but these values are lower than the data by Carmichael *et al.* (19) which show that even after withholding food for 24 hours it remained half full.

The dry weight of the small intestinal content in the young rabbit showed a comparative constant value at any time except of the twelve-hour interval after feeding but some changes were observed in the mature animal.

The similar results were also observed in the cecal contents, and the more ingesta were stored at about six hours after feeding in both age rabbits.

Although in the young rabbit the constant dry weight of the colonic contents except of the lower amount of the digesta at 24 hours after feeding were observed and these values were varied in the aged rabbit.

In the rectal content in both age groups, their dry weight varied at various intervals after feeding.

In general, of the gastrointestinal tract of the rabbit, the stomach and cecum acts as the accumulation of the digesta, and it seems that although the transference of the digesta from these stores occurs in approximately

Table 4. Dry weight and total calcium in gastrointestinal contents of young and mature rabbits.

Time after feeding (hr)	Stomach	Small intestine	Cecum	Colon	Rectum
	Dry weight (g)				
	Young rabbit				
Within 2*	9.5±1.4**	1.3±0.4	15.5±1.6	2.2±0.4	2.9±0.7
3	9.0±0.3	1.6±0.4	15.6±2.2	2.3±0.4	3.9±1.0
6	5.2±1.1	1.3±0.4	18.6±4.3	2.2±0.7	2.3±1.0
9	5.6±1.8	1.3±0.6	17.8±1.9	2.3±0.4	2.7±1.0
12	2.9±0.7	0.7±0.1	18.9±0.8	2.3±0.5	2.0±0.6
24	2.2±0.3	1.1±0.1	17.1±0.3	1.3±0.5	1.5±0.2
	Mature rabbit				
3	29.6±1.1	1.6±0.1	13.8±0.6	1.6±0.1	3.6±1.1
6	22.5±6.8	3.6±1.5	28.0±8.4	3.9±1.1	4.9±1.9
12	16.3±6.2	1.9±0.1	19.6±6.8	1.6±0.4	1.2±0.3
24	6.3±1.7	2.5±0.7	12.2±5.6	2.3±1.1	5.5±2.8
	Total calcium contents (mg)				
	Young rabbit				
Within 2*	82.6	17.6	226.0	28.7	34.2
3	39.9	15.1	186.3	31.3	51.2
6	16.2	8.8	309.2	40.2	43.8
9	22.3	10.7	249.7	33.7	46.6
12	7.3	2.9	364.4	44.7	43.5
24	2.1	2.2	304.7	22.6	26.1
	Mature rabbit				
3	400.6	22.3	192.8	17.3	50.1
6	179.6	27.2	367.3	50.7	74.2
12	93.0	14.3	304.0	25.1	24.3
24	61.7	17.4	137.2	27.9	37.1

* Two rabbits at half an hour, three animals at one hour and two at the two-hour period.

** Mean±standard deviation.

constant quantity and rate in the fifty days old rabbit, it occurs at a comparatively irregular rate in the mature animal.

B) Total calcium content of digesta in the gastrointestinal tract: The total calcium contents of the digesta in various segments of the gastrointestinal tract varied with the same trends of the variation in the dry weight, and the more changes were observed at the times involved after feeding. Calcium content of the stomach digesta decreased in the highest rate in the course of time after feeding, particularly, this rate of the young rabbit was larger than that of the mature one.

In spite of the similar dry weight of the stomach digesta were observed

in the young rabbit within three hours after feeding, the calcium contents in the rabbit at the three-hour interval after feeding decreased to a only half value of that within two hours. In the mature rabbit, the similar results were observed during the three-hour to six-hour period after feeding.

Possibly this was a consequence of the effluence of calcium from the stomach to small intestine, which was transformed to the soluble one by the gastric juice at the earlier times after the ingestion of the white clover hay diet. It seems that the fact that thereafter the decreasing the effluence of calcium from the stomach digesta to the posterior tract occurs, is an indicative of the remaining of the comparative insoluble of otherwise unavailable parts of calcium in the hay. In the young rabbit at the twenty-four-hour period, although the dry weight of the stomach digesta have remained about one fourth of the rabbit sacrificed soon after feeding, the calcium content of the stomach digesta in the former rabbit is only 2 mg and it occupies only about a fortieth part of the latter rabbit. It is supposed, therefore, that calcium in the stomach digesta transfer to the lower tract at a very large and rapid rate and then the absorption and excretion will take place.

The total calcium content of the small intestine digesta in the young rabbit, nine hours after feeding are higher than at the six-hour period owing to the second feeding (at six hours after the first feeding). At 24 hours after feeding, only 2 mg of calcium are contained in the small intestinal content, and it is same as the value of the stomach digesta in these animals. In the mature rabbit, some changes of the calcium contents were observed owing to the variations of the dry matter at comparable times. The time variation of the total calcium content in the digesta posterior to cecum in both age rabbits are similar to that of the dry matter in comparable regions and at comparable times. It is assumed, that the absorption of calcium from the digesta and the retention of the absorbed calcium in the body (stores in the bone, excretion from kidney and reexcretion to the digestive tract) reaches to the highest quantity after about the three-hour period.

The results of the percentage of dry matter and calcium concentration on dry basis of the white clover hay ingested, the gastrointestinal contents and feces in the young and mature rabbits which were sacrificed at various intervals after feeding are represented in Figures 1 and 2.

C) *Dry matter percent of the diet, gastrointestinal contents and feces*: In both age groups, the highest dry matter were observed in the rectal contents and feces and followed by the diet and the cecum, colon and stomach digesta, being lowest in the small intestine.

The dry matter of the diet fed to the young rabbit are higher than that of the mature animal. In comparison of the digesta in the various segments of the digestive tract, the higher dry matter were observed in the stomach

and small intestine of the mature rabbit compared to the young animal, and the values obtained in the posterior tract to the cecum except the rectal content at the three- and twenty-four-hour periods after feeding in the mature

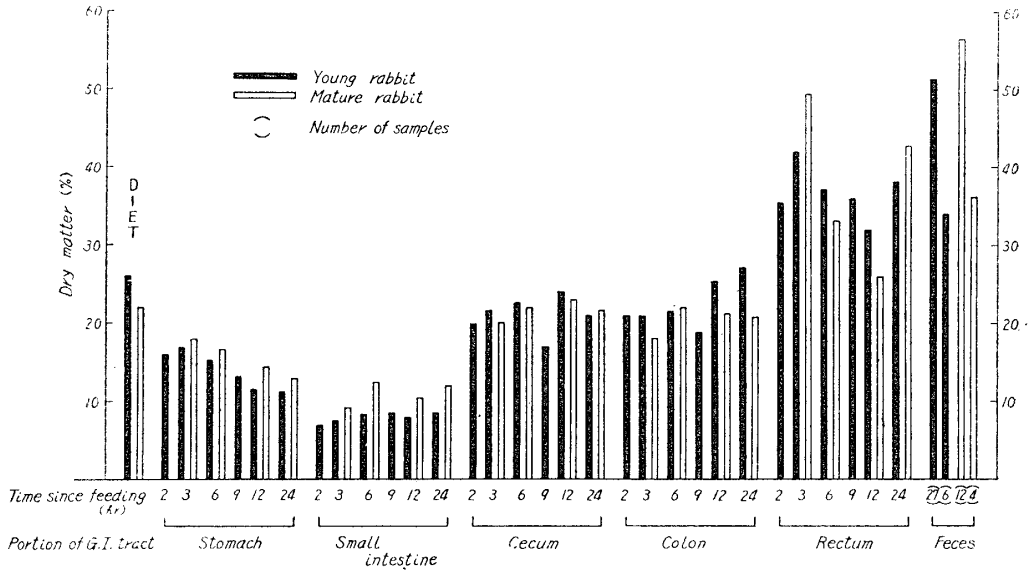

Fig. 1. Dry matter of the diet, gastrointestinal contents and feces of young and mature rabbits.

rabbit were lower than that of the young animal, generally. Therefore, the addition (secretion) of water to the stomach and small intestine digesta and the loss (absorption) of water from the cecal, colonic and rectal contents in the young rabbit are higher than that of the adult animal, thus it is recognized that the function of the absorption and excretion of water in the gastrointestinal tract of the younger rabbit are more active than in the aged animal.

The variations in the dry matter of the gastrointestinal contents at all intervals after feeding are as follows. The dry matter of the gastric contents of the young rabbit decreased to about 16 percent at three hours after the ingestion of the white clover hay diet which have about 26 percent of the dry matter, and thereafter it decreased to 12 percent gradually. The differences in the dry matter between the diet and stomach digesta of the mature rabbit are not so much in the case of the young. These smaller amounts of the dry matter in the stomach digesta may result from the entry of saliva and the secretion of the gastric juice. And it is supposed that the gradual increase of the water content are caused by the continuous secretion of the gastric juice at all times the digesta exist in the stomach after the effluence of the soluble calcium into the posterior tract during a short period of time.

Within the three-hour interval, the dry matter of the small intestinal content shows 7 to 9 percent and are a half of the stomach at the same time, in both age groups. These facts are the result from the secretion of the

digestive juice at the small intestine. Afterward, the dry matter of them slightly increased gradually. Then possibly the decreasing difference between the dry matter of the stomach and small intestine digesta of the corresponding times was a consequence of the decreasing secretion of the digestive (small intestinal) juice in course of time after feeding. This trend is much in the mature animal. Then it is possible to consider the other reasons on this fact, that is, the more amounts of the undigested content enter the small intestine after the larger quantity of the available materials in the stomach digesta flow out to the lower tract at the earlier intervals after feeding, and furthermore involving a difference in the quantity or composition of the digestive secretion.

The dry matter of the cecum digesta are even high, being three times the value of the small intestine at each interval after feeding, and it reaches to the peak at about the twelve-hour period. Then the active absorption of water must occur in the cecum and when this organ was filled with the digesta these digestive function increase, and it appears that the absorption activity of the available nutrients are increased at this period too.

Although the dry matter of the colon digesta are almost similar to that of the cecum, the colon may have a quite different function on the metabolism of water. If it is considered that all small intestinal contents enter the colon after passing through the cecum, the same water content of the digesta in both segments may result from the equilibrium of absorption and excretion of water in the colon. It seems, therefore, that the finding of the highest dry matter in the colon digesta of the young rabbit at the twenty-four-hour period is due to a peak of the dry matter in the cecum at that period in the colonic content rather than the absorption of water in the colon which increases at a later time. However it will be necessary to discuss the changes in the compositions of the colonic content by the absorption of the digestible nutrient and the excretion of the endogenous materials and the existence of the preceding substance of hard and soft feces. It appears that the effects of the second feeding at six hours after the first one are represented the higher water contents of the both segments at the nine-hour period.

The highest dry matter of digesta and the individual variations of them were observed in the rectum at all intervals after feeding. These variations are confirmed with existence of whether the hard feces which have higher dry matter or the soft feces have lower dry matter.

In general, the functions of the absorption and excretion of water in all segments of the gastrointestinal tract in the young rabbit is more active than that of the aged animal.

In the dry matter of feces in both age groups, considerable variations were observed in the individuals and at the intervals after feeding, and the

results obtained from the hard and soft feces were represented in Fig. 1. The water content of the hard feces are lower than that of the rectum digesta in both age animals. It is clear, therefore, that the water is actively absorbed at the distal portion of the rectum too.

D) Calcium concentration of the diet, gastrointestinal contents and feces: The results of the calcium concentration on dry basis of the diet, gastrointestinal contents and feces in the young and mature rabbit at all intervals after feeding are represented in Fig. 2.

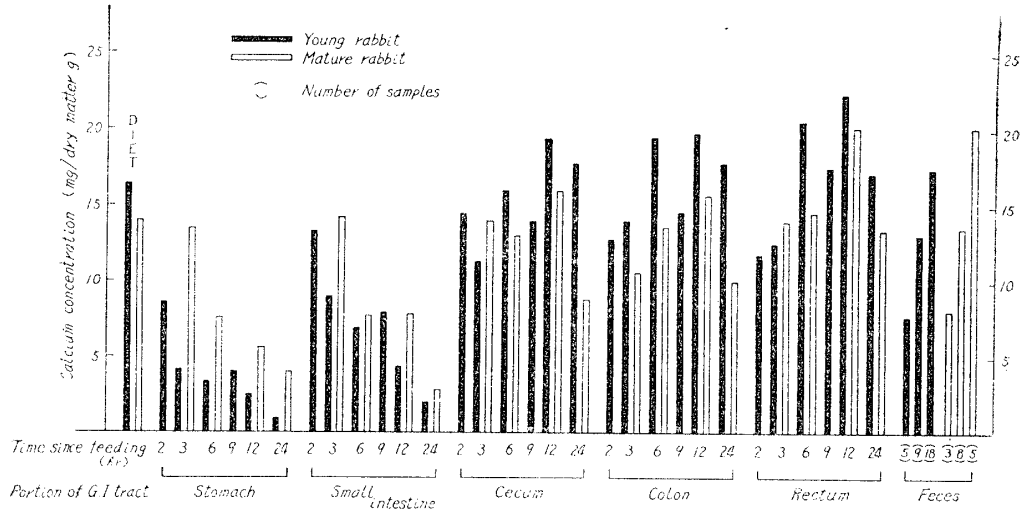

Fig. 2. Calcium concentration of the diet, gastrointestinal contents and feces of young and mature rabbits.

In general, the tendencies in the changes of calcium concentration in all gastrointestinal contents except the small intestine are similar to that of the dry matter observed in comparable regions. It decreases in the stomach and small intestine in process of time and increases in the posterior portions to the cecum until 12 hours after feeding.

In the stomach digesta at the three-hour interval, the largest differences of the calcium concentration were observed between the young and mature rabbit.

In the young rabbit, only one fourth of the calcium concentration in the white clover hay diet ingested (16 mg) exists in the gastric content at three hours after feeding, and on the contrary, the mature rabbit stomach digesta contains the same amount of the calcium concentration in the diet (14 mg) at this time. It is considered, therefore, that the loss in calcium contents from the stomach digesta of the young rabbit are even high as compared to that of the mature animal, and this fact may result from the other reasons with the exception of that the large amounts of the soluble calcium are produced by the gastric juice at the earlier period after the ingestion of diet flow out to the posterior portion of the digestive tract. Furthermore, in the young

rabbit within two hours after feeding, about a half amount of calcium in the diet are contained in the stomach digesta, and the other reasons on the disappearence of calcium from the stomach digesta must be considered.

About a half of the calcium concentration in the diet and the three-hour stomach digesta are contained in the mature rabbit gastric content at six hours after feeding. It is suggested from the fact that the effluence of calcium from the gastric content occurs at a higher rate and at early times after feeding in the young rabbit are the result from the larger secretion of the gastric juice and the stronger digestion activity of them as compared with that of the mature animal. Furthermore, the same calcium concentration are observed in the diet and the three-hour stomach digesta of the mature rabbit, and it seems that the calcium concentration of saliva may be higher than in the young animal. Thomas *et al.* (3) have reported that a large amount of the dietary calcium was deposited in the bones at a higher rate in the young rabbit and on the contrary, in the adult rabbit the depositing rate of blood calcium in the bones are not so high and rapid. Thus it is assumed that the comparatively higher amount of calcium was secreted in the saliva.

It is very interest to discuss the other reasons on the effluence of calcium from the gastric content, that is, the direct absorption of calcium through the gastric epithelium take place possibly in the rabbit. However, it is impossible to prove these facts from the general knowledge previously reported and from the results in this experiment.

Alexander and Chowdhury (15) have reported very interesting results, that is, since the stomach epithelium secretes an acid juice it did not seem likely that the interior of the organ would be a suitable microbial fermentation chamber, nevertheless, it has been found that lactic acid is produced at such a low pH, and the lactic acid produced in the stomach appeared in part to be absorbed through the gastric epithelium, and in the first part of the small intestine. Therefore, if the rapid absorption of the lactate is clearly shown, it is sufficiently considered the possibility on the absorption of calcium through the gastric epithelium as Ca-lactate.

After three hours interval in the young rabbit and six hours in the mature animal, the calcium concentration of the stomach digesta slightly decreased gradually owing to the lower effluence of calcium by the remaining of the undigested and unavailable calcium.

The calcium concentration of the small intestine digesta decreased in the course of time after feeding in both age groups. At all times after feeding, the calcium concentration of the small intestine digesta are higher than that of the stomach digesta in the young rabbit, but almost the same values were observed in both segments of the mature animals. It is assumed that some excretion of calcium may occurs in the small intestine. However, at the

same intervals, the increase of the calcium concentration in the small intestine digesta compare to the gastric content are interpreted as an indication of a greater absorption of the other digestible materials from this organ as also shown in the cecum.

Wallace *et al.* (9) have reported that the small intestine of young and mature rats play a major role in the excretion of calcium, which is contrary to the view that the large intestine predominates in this function. If these facts are certain in the rabbit too, it seems that in the small intestine of the younger rabbit, the excretion of calcium are superior to the absorption of them and both functions have equilibrated in the aged animal.

Within three hours after feeding, the calcium concentration of the cecum digesta in the both age groups are almost similar to the small intestine digesta. Subsequent to the three-hour period a striking difference of the calcium content existed between the small intestine and cecum digesta, and these differences were larger in the younger rabbit than in the aged one. It is considered, therefore, that the calcium concentration of the cecum digesta are affected on the calcium content in the upper portion of the gastrointestinal tract until the three-hour period after feeding. When a large amount of the digesta entered this organ and the active digestion and absorption occurred after the six-hour period, it must be considered that the excretion of calcium occurs in the cecum at this later period.

However, the reports of Huang *et al.* (10), Yoshihara and Sugisaki (11) and Kametaka (12) have indicated that the large intestine of the rabbit participates in the absorption of water and digestible materials with the colon and rectum playing a major role in this capacity, thus it is impossible to recognize that calcium is excreted in the large intestine particularly. The apparent increase of the calcium concentration in the cecum digesta result from the accumulation of entering calcium from the small intestine and the existence of calcium in the concentrated state owing to the correlatively higher absorption of the other nutrients in comparison with the lower absorption of calcium. Afterwards, the absorption of water and the digestible nutrient included calcium in the large intestine have continued until the defecation.

In general, that the young rabbits have larger amount of calcium in the digesta posterior to the cecum compared with the mature animal which have shown the correlative absorption of the other nutrient to calcium to be very high when a large part of the digesta exists in the cecum.

The calcium concentration of the young rabbit gastrointestinal contents at the nine-hour interval after feeding showed the higher value in the stomach and small intestine and the lower amount in the large intestine as compared to that of the rabbit at the six- and 12-hour intervals owing to the second

feeding at six hours after the first.

In the calcium concentration of the feces in both age groups, some variations were observed in the individuals and at various times which were excreted as in the case of the dry matter, and the calcium concentration of the hard and soft feces also varied in times which were excreted. Then 33 feces of the young rabbit and 16 samples of the mature animal were divided into three groups, respectively, by the concentration of calcium.

In general, although the higher calcium content of the rectum digesta were observed in the young rabbit as compared with the mature animal, the fecal calcium content of the former rabbit were lower than that of the latter. It appears that the active absorption of calcium occurs in the rectum of the rabbit. And it is more active in the young rabbit than in the aged animal in spite of the absorption of water being slightly lower in the young animal as compared to the aged one.

Summary

The distribution of water and calcium in 25 young rabbit and 17 mature rabbit gastrointestinal tissues and contents were determined at various intervals after the feeding of the white clover hay diet which contains a high amount of calcium to evaluate the transfer of water and calcium in the digestive tract.

1. The dry weight of the rabbit small intestinal tissue did not increase in proportion to the increase of the body weight. The water content of the gastrointestinal tissues slightly decreases with increasing age. The calcium content in the gastrointestinal tissues were affected by the changes of that of the digesta in various segments.

2. A very large and rapid transfer of the stomach digesta to the lower tract were observed in the young rabbit as compared to that of the aged animals. The dry weight of the small intestine digesta in the young rabbit were preserved at a comparatively constant value. At six hours after feeding, the dry weight of the cecum digesta reached to the maximum content. In general, the stomach and cecum are the chief storing organs of digesta in the rabbit, and the digesta in these portions flowed out to the lower tract at a constant rate and quantity in the young rabbit, but in the mature animal at a irregular and slower rate.

3. The highest water content were observed in digesta of the small intestine and the lowest one were in rectum. It seems that the absorption of water in the cecum are more active than the excretion and these functions in the colon are approximately equilibrated. In general, the function of the absorption and excretion of water in the gastrointestinal tract of the young rabbit are more active than that of the mature animal.

4. The effluence of calcium from the gastric contents in the young rabbit shows a larger amount and occurs at the earlier intervals after feeding as compared with that of the other digestible nutrients and the transfer of calcium in the mature animal. It appears that calcium may absorb from the gastric epithelium and excrete in the small intestine but these facts are not clarified from the data of this experiment directly. Although a large amount of calcium must be absorbed in the cecum, it is supposed that the other digestible nutrients will absorbed more actively, correlatively. It is assumed that the absorption of calcium exceed the excretion of them, in the colon and rectum.

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