CXII. THE EXCRETION OF CALCIUM BY THE LARGE INTESTINE OF THE RABBIT

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It is still disputed whether calcium is excreted by the large intestine. The balance of opinion seemed until recently to have favoured the view that elimination of Ca through the wall of the colon does occur, and that at least in some species of animals it can play an important part in the regulation of Ca metabolism. Three years ago, however, Nicolaysen [1934] published results of experiments on dogs which led him to conclude that Ca is not excreted by the colon. Welch *et al.* [1936] and Johnson [1937] have lately concluded from observations on human beings with isolated colons that the wall of the human colon eliminated little or no Ca. In the light of such evidence Leitch [1937] stated in a recent review: "it has been shown...that excretion of calcium into the gut does not occur". A useful account of the earlier work on this subject will be found in the paper by Nicolaysen [1934], and it is not proposed to cover this ground again. The purpose of the present paper is to show that excretion of Ca into the large intestine does take place in the rabbit.

The experiments about to be described arose from observations on rabbits in which severe skeletal decalcification followed the production of chronic renal insufficiency by repeated injections of uranium nitrate. When Ca balances were carried out on such animals it was found that the amount of Ca lost from the body during a given period often exceeded the amount of Ca excreted in the urine (Table I).

Table I. *Ca balances on rabbits with damaged kidneys*

No. of rabbit	Period of exp. days	mg. Ca			
		Food	Faeces	Urine	Balance
156	12	814	1252	24	- 462
156	10	550	873	19	- 342
160	10	927	1056	36	- 165
182	10	607	926	49	- 368

The only possible interpretation of such results seemed to be that Ca mobilized from the skeleton was being excreted into some part of the alimentary tract and eliminated with the faeces.

From time to time individual pellets from these animals were analysed for Ca and the chance observation was made that the concentration of Ca in pellets passed at any given time varied inversely with the size of the pellets; e.g. a pellet of dry wt. 0.126 g. contained 9.3 mg. Ca per g., while one weighing 0.075 g. contained 11.1 mg. Ca per g. A possible explanation for such differences was that Ca was being excreted into the formed pellets so that the greater the relative surface of the pellets in contact with the wall of the colon, the greater would be the final concentration of Ca. It was therefore decided to determine the Ca

contents of individual pellets and of samples of unformed faecal material taken from different positions in the intestine of normal rabbits to see whether evidence could be obtained that Ca was really being added to the faecal contents in some portion of the colon.

DETAILS OF EXPERIMENTS

Rabbits were kept on diets of more or less constant composition for varying periods before they were killed. Samples of the faecal contents were removed from various points along the intestine, weighed, dried in a vacuum desiccator and weighed again.

A few points must here be noted regarding the structure and functions of different parts of the rabbit's large intestine. The caecum usually contains pasty material with a water content of 70–75%. Its contents pass down to an obviously sacculated portion of colon which extends for some 6–10 in., at the lower end of which cylinders of faecal material begin to be formed, the water content remaining unchanged. Below this portion the colon loses its sacculations and for some 4–6 in. becomes a smooth thick-walled tube which will be referred to in this paper as the thick-walled colon. In this part of the colon the characteristic rounded pellets are moulded before any appreciable absorption of water has taken place. Lower down, the colon maintains its smooth external appearance, but the wall is thinner and during the whole of its course of 3 ft. or more water absorption takes place from the pellets, so that in the region of the anus these contain as a rule some 45% of water. It was found that the comparatively short thick-walled colon, and to a less extent the lower part of the sacculated colon, were responsible for any excretion of Ca which occurred in the large intestine.

For the analyses the faecal material was ashed dry in quartz dishes, usually with the help of a drop of HNO_3 . The ash was dissolved in 2 ml. of 1% HCl and made up to a known volume. Ca was precipitated from an aliquot portion with 4% ammonium oxalate. The precipitate was washed with NH_4OH and titrated with KMnO_4 as in Clark & Collip's [1925] procedure for estimating serum Ca. Total P was estimated in some of the samples by the method of Neumann [1902].

The criterion used for deciding that excretion of Ca had occurred was the finding of a greater concentration of Ca in dried faecal material taken from the lower portions of the large intestine than in that taken from the upper portions. Such changes in Ca concentration must obviously be interpreted with caution. The possible effects of water exchanges and of the exchange of solid matter other than Ca between the faeces and the wall of the colon must be taken into account. These will be considered in the discussion of the results.

RESULTS

In the earlier experiments the Ca content of faecal samples was determined in material taken from points along the whole length of the large intestine. Fig. 1 represents in diagrammatic form a result obtained in a rabbit kept previously on a diet of rather low Ca content.

Numerous similar observations were made, in none of which was there any constant indication of an increase in the concentration of Ca below the lower end of the thick-walled colon. In later experiments, therefore, the concentration of Ca was determined as a rule only in samples taken from the lower end of the ileum, the middle of the caecum, the middle of the sacculated colon, the lower end of the thick-walled colon and the lower end of the rectum, near the anus.

Table II gives the results obtained from rabbits kept on a diet of whole oats and bran with 20 g. cabbage a day.

There is in each case a slight increase in the concentration of Ca from samples near the anus when compared with that in samples from the caecum or sacculated colon, though the increase is too small to be accepted as proof that

Fig. 1. Diagram illustrating the parts of the rabbit's colon referred to in the text and showing typical concentrations of Ca and water in faecal material from a rabbit previously kept on a rather low Ca diet.

Table II. *Ca concentrations in faecal samples from rabbits on a mainly cereal diet*

No. of rabbit	mg. Ca per g. dried matter				
	Ileum	Caecum	Sacculated colon	Thick-walled colon	Anus
195	—	2.6	2.8	3.2	3.3
202	2.9	5.1	5.8	6.9	7.1
227	1.9	5.1	5.6	6.1	6.4
191	—	4.8	5.6	6.5	7.4

any significant excretion had taken place. The experiment was repeated with rabbits which had been given a diet containing 80 % soy flour with cereals and 20 g. cabbage a day (Table III).

Table III. *Ca concentrations in faecal samples from rabbits on a diet containing soy flour*

No. of rabbit	mg. Ca per g. dried matter				
	Ileum	Caecum	Sacculated colon	Thick-walled colon	Anus
192	—	6.1	9.2	10.5	12.2
205	8.9	5.9	8.9	12.5	12.7

These figures suggest that Ca may have been added to the faecal material as it passed from caecum to anus. If such an addition were to represent a true excretion it might be expected that it would be most easily demonstrated when there was the greatest amount of Ca in the body to be eliminated. It was possible, for example, that excretion by this route might be influenced by the height of the serum Ca, which is notoriously variable in the case of the rabbit. It was decided to investigate the effect of a meal of cabbage on the concentration of Ca in faecal material at different levels in the intestine, for after such a meal the serum Ca reaches levels of 16–20 mg. per 100 ml., or even more. Only the outer green leaves containing high concentrations of Ca [Cowell, 1932] were given (Table IV).

Table IV. *Concentrations of Ca in faecal samples after a meal of cabbage*

No. of rabbit	Hours after meal	Serum Ca mg./100 ml.	mg. Ca per g. dried matter				
			Ileum	Caecum	Sac-culated colon	Thick-walled colon	Anus
N. 3	1	18.5	22.5	5.0	3.2	5.5	6.5
N. 2	1	16.7	39.0	12.7	17.4	11.7	11.1
N. 1	2	21.0	—	9.3	11.9	14.7	10.0
N. 4	2	16.3	9.5	10.1	10.2	14.1	6.3
207	3	17.7	7.7	9.9	9.5	13.3	13.5
219	3	16.9	9.3	4.6	5.3	7.0	3.0
206	5	19.0	9.6	9.8	12.2	15.5	8.6
N. 5*	5	13.4	7.0	9.8	18.4	21.8	10.5
N. 6†	24	19.8	15.0	24.6	23.0	46.2	23.9
N. 7†	24	23.0	20.9	33.0	39.3	48.6	45.6

* N. 5 was under urethane during the latter part of this period.

† N. 6 and N. 7 were given as much cabbage as they would eat during the whole 24 hr.

One hour after the meal of cabbage there was little addition of Ca to the faecal contents in N. 3. The sharp rise in the concentration of Ca in the sacculated colon of N. 2 may have been due to the admixture of material from the ileum, though even when material from this latter situation has a high Ca concentration it is rare for the concentration in the upper part of the sacculated colon to be appreciably higher than that in the caecum, as would be expected if the contents of the ileum normally passed directly down into the sacculated colon before the absorption of its excess of Ca had taken place. Two hours after the meal there was a definite increase in the concentration of Ca in the material from the thick-walled colon in both animals, with no increase in the material from the sacculated colon in N. 4 and a slight increase in material from this region in N. 1. The serum Ca was at about the same level 2 hr. after the beginning of the meal as 1 hr. after. At 3 hr. there was evidence of increased concentration of Ca in both animals, most marked in material from the thick-walled colon. Rabbit N. 5 had had its colon incised under anaesthesia and the exceptionally large increase in the concentration of Ca in the region of the sacculated colon may have been due to stagnation of faecal contents here. The great difference in the concentrations of Ca near the anus in rabbits N. 6 and N. 7 was almost certainly due to differences in the rates of evacuation of pellets from the rectum.

The effect of the previous diet on the response to a meal of green cabbage was examined in two rabbits (Table V).

Table V. *Effect of previous diet on Ca concentrations in faecal samples after a meal of cabbage*

No. of rabbit	Previous diet	Hours after meal	Serum Ca mg./100 ml.	mg. Ca per g. dried matter				
				Ileum	Caecum	Sac-culated colon	Thick-walled colon	Anus
220	Low Ca 3 weeks	3½	16.3	11.7	5.6	4.7	9.3	4.4
217	High Ca 11 days	3½	20.4	17.2	18.7	18.2	32.6	23.6

The low Ca diet consisted of equal parts by weight of whole oats and bran with 20 g. aetiolated cabbage. The high Ca diet contained the same cereal mixture with 150 g. green cabbage containing 350–500 mg. Ca per 100 g. In each

case there was a considerable increase in the concentration of Ca in material from the thick-walled colon, though the levels in the caecum and sacculated colon were very different in the two animals.

The pellets taken from the thick-walled colon in which they are moulded have a smooth, moist surface and appear almost as if they are coated with a pellicle. If this outer shell is removed and analysed, it is found to contain a considerably higher concentration of Ca than the central portion, as in the following examples (Table VI).

Table VI. *Concentrations of Ca in the outer and inner portions of faecal pellets*

No. of rabbit	Hours after cabbage	mg. Ca per g. dried material			
		Sacculated colon	Thick-walled colon whole pellet	Thick-walled colon outer shell	Thick-walled colon inner portion
206	5	11.5	15.5	16.7; 18.9	11.8; 11.5
207	3	9.5	13.2	16.8; 18.4	9.9; 9.5

In both cases Ca appears to have been added to the faecal material as it passed from the sacculated to the thick-walled colon. Duplicate estimations of the Ca in the outer shells did not agree very well on account of the difficulty in separating the same thickness of covering from different pellets, but in every case they gave higher concentrations of Ca than the inner portions. The fact that the inner portions contained in both cases practically the same concentration of Ca as the contents of the sacculated colon suggests that Ca excreted by the wall of the colon is deposited in an insoluble form on the surface of the pellets. It can readily be shown that dilute alkali extracts practically none of the Ca from faecal pellets, whereas dilute acids extract it readily. In some of the animals examined, appreciable quantities of a clear fluid were found in the lumen of the thick-walled colon. This fluid was found to have an alkaline reaction, its pH, measured without precautions to avoid loss of CO₂, being 8.5-9. The Ca contents of two samples of this fluid were 8 and 11 mg. per 100 ml. respectively.

The concentration of total P was followed in the faecal material from different situations in the intestine in some of the rabbits. In general, there was either a slight continuous fall from caecum to anus or the maintenance of a fairly constant level, as illustrated in Table VII.

Table VII. *Concentrations of total P in faecal samples*

No. of rabbit	Previous diet	mg. P per g. dried matter				
		Ileum	Caecum	Sacculated colon	Thick-walled colon	Anus
195	Low Ca	—	13.5	9.9	4.9	3.7
202	Cereal + 50 g. cabbage	8.7	20.2	21.9	18.2	17.0
205	Soy flour (high P)	12.1	22.3	22.8	23.9	21.7
207	Cabbage meal 3 hr. before	9.2	19.3	—	17.3	16.2
N. 1	Cabbage meal 2 hr. before	—	17.8	—	17.3	—
N. 3	Cabbage meal 1 hr. before	11.6	12.3	—	11.3	8.2

Estimations of total P in the outer shell and inner portion of individual pellets gave the same kind of results as had been obtained for Ca, suggesting that insoluble calcium phosphate had been deposited on the surface of the

pellets. There seems to be no reason why absorption of soluble phosphate should not proceed simultaneously with the deposition of insoluble calcium phosphate on the surface of the pellet.

DISCUSSION

The evidence just presented indicates that under certain conditions the concentration of Ca per g. of dried faecal matter increases as the faeces pass along the upper part of the large intestine of the rabbit. Before this can be accepted as proof that actual excretion of Ca has taken place, possible changes in the concentration of other faecal constituents must be considered which might account for an apparent increase in Ca concentration without there having been any actual addition of Ca to the faeces. Changes in the water content of the faeces need not be seriously considered. The absorption of water does not take place to any significant extent until that part of the colon has been reached in which as a rule there is no further apparent increase in the concentration of Ca. Moreover, Ca determinations have always been made on dried material. The absorption of faecal matter other than water might produce the observed increase in the concentration of Ca, but this is extremely unlikely for the following reasons. In some of the experiments the concentration of Ca in pellets from the thick-walled colon was twice as great as that in material from the sacculated colon. It would be necessary to assume that one half by weight of the dry faecal matter discarded by the caecum disappeared in passing along the few inches of colon between these points in order to explain the doubling of the concentration of Ca along these lines. The apparent increase in the concentration of Ca occurs most noticeably in the thick-walled colon, often after the faeces have already been moulded into pellets. The fact that these freshly moulded pellets have a smooth, glazed surface makes it most improbable that any considerable part of the faecal material is absorbed after this stage of pellet formation has been reached. In some of the experiments there was no increase in the apparent concentration of Ca. If any considerable absorption of solid faecal material took place in the upper part of the colon in these cases, it would have to be assumed that a corresponding degree of absorption of Ca occurred in the same situation to account for the maintenance of a steady concentration of Ca. This seems most unlikely in view of the insolubility of faecal Ca at, or on the alkaline side of, neutrality. For these reasons it is believed that the apparent increases in the concentration of Ca in the upper part of the colon do in fact represent actual additions of Ca to the faeces, and if this belief is justified there seems to be no alternative but to admit that Ca can be excreted by the large intestine of the rabbit.

The magnitude of any increases which have been observed in the concentration of Ca in the faecal material presumably depended on the length of time it remained in the particular section of the colon which was excreting Ca, as well as on the activity of the excretory process. No information was obtained in these experiments concerning the rate of movement of the faeces along the colon. Varying rates of movement probably account for many of the irregularities found in the distribution of Ca in pellets taken from different situations in the colon. In general there was a distinct tendency for the concentration of Ca to remain fairly constant in pellets taken from any point below the thick-walled colon, provided that the rabbit had been on the same diet for several days previously. This seems to indicate a certain degree of uniformity both in the rate of Ca excretion and in the rate of forward movement of the faecal matter in the

upper part of the colon. An attack of diarrhoea was found to produce large fluctuations in the concentration of Ca along the whole length of the colon.

Little indication is given in these experiments of the physiological importance of the process of Ca excretion by the colon. It is not suggested that in the normal rabbit the colon is the preferential route for the elimination of Ca. A few hours after a meal of green cabbage the bladder of a rabbit may be distended with urine from which a copious precipitate of Ca salts, probably largely bicarbonate, has already separated. For example, a rabbit on a cereal diet without cabbage passed urine containing 7.8–12 mg. Ca per 100 ml., while its faeces at this time contained from 2.5 to 4.5 mg. Ca per g. of dried matter. It was then given a daily supplement of 150 g. of green cabbage containing some 700 mg. Ca. The Ca concentrations in dried pellets passed during the next 4 days were 4.6, 14.1, 11.8 and 8.2 mg. per g., while the concentrations in corresponding samples of urine were 142, 188, 309 and 410 mg. per 100 ml. On the third day 340 mg. were eliminated in the urine. If in this rabbit Ca excretion by the colon had gone on at a similar rate to that observed in other rabbits given similar amounts of cabbage, it can be calculated that an abnormally large bulk of faeces would have to be passed to bring the excretion of Ca by the colon up to the level of the urinary excretion. On the other hand, the experiments reported in Table I on rabbits with damaged kidneys showed that under abnormal conditions excretion of Ca by way of the faeces could exceed the urinary excretion, though there was no proof in these animals that the Ca lost from the body was not derived from unabsorbed secretions arising in the upper part of the alimentary canal, as Nicolaysen [1934] found to be the case in dogs.

The mechanisms controlling the excretion of Ca by the colon remain obscure. Reference to Table IV will show that there was no very close relation between the level of the serum Ca and the increase in the concentration of Ca in the faeces taken from the thick-walled colon. In one animal the serum Ca was raised to 20 mg. per 100 ml. without producing any increase in the concentration of Ca in the contents of the colon. As a rule the excretion of Ca by the colon has been most apparent when the dietary supply of Ca has been greatest. Some indication of the state of saturation of the normal rabbit with Ca seems to be obtainable from a determination of the Ca concentration of the contents of the caecum. This may be brought by a purely cereal diet below the level of 3 mg. Ca per g. of dried matter, and under such conditions excretion of Ca by the colon has been negligible. Excretion of Ca by the colon has always been demonstrated so far in normal rabbits which have received a liberal supply of green cabbage. Attempts have been made to throw further light on the problem by examining the effect of vitamin D, parathormone, thyroxine, thyroidectomy and parenteral administration of salts of Ca and P on excretion of Ca by the colon. The results of these experiments will be reported in a future communication.

SUMMARY

1. The concentration of Ca in dried faecal pellets taken from a specialized portion of the colon where the pellets are moulded is under certain conditions greater than its concentration in dried material from the caecum and upper part of the sacculated colon.
2. Higher concentrations of both Ca and P are found in the outer shells of faecal pellets than in the inner portions when a rabbit has received a mixed diet containing plenty of Ca.

3. Reasons are given for interpreting these observations as evidence that Ca can be excreted by the upper part of the rabbit's colon.

4. The physiological significance of this excretion of Ca by the colon is briefly discussed.

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