XLVII. STUDIES UPON THE MODE OF ACTION OF VITAMIN D

IV. THE ABSORPTION OF CALCIUM CHLORIDE, XYLOSE AND SODIUM SULPHATE FROM ISOLATED LOOPS OF THE SMALL INTESTINE AND OF CALCIUM CHLORIDE FROM THE ABDOMINAL CAVITY IN THE RAT

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(*Received 9 December 1936*)

Previous experiments [Nicolaysen, 1937] indicate that the action of vitamin D in the rat's gut is confined to a direct action on the absorption of Ca. The object of the experiments to be described below was to contribute further to knowledge about the nature of the action of vitamin D in the gut.

Experimental

The "rachitic" rats used in these experiments were fed on a vitamin D-deficient ration for at least 6 weeks before they were used for experiment. They were thus all in a late stage of vitamin D deficiency. The control rats were kept on the same diet, but with the addition of 50 I.U. vitamin D daily (as calciferol).

Preparation of isolated loop

Before the beginning of the experiment all the rats were kept without food overnight. The isolation of the loop was performed under ether narcosis. The mesenterium of the first 10–15 cm. of jejunum in rats weighing 70–120 g. is so short that measurement of the length of the loop is rather uncertain. A 30 cm. loop was isolated in each rat, starting about 15 cm. below the pylorus, and the lumen was rinsed out with 0·9 % NaCl solution. The remaining fluid was pressed gently out between two fingers. The distal end was tied off, 3 ml. of the solution to be tested were injected into the lumen with a 3 ml. pipette and the proximal opening tied off. The loop was then replaced in the abdomen, the wall closed and the narcosis discontinued. After definite intervals the rats were killed by decapitation and the loops removed for analyses.

The pipette used for the injection into the loops delivered with an accuracy of ±0·2 %. To judge from the variations in the lengths and the weights of the emptied loops after removal, an error of as much as 10 % due to differences in the measurement of the length of the loop may occur. The method, which has been extensively used by other workers [Verzar & Laszt, 1935], might at first sight appear unphysiological, but the condition of the rats at the end of the experiment was quite good, and the isolated loop looked quite normal. Only with the highest concentrations were the loops slightly distended at the end of the experiment.

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Absorption of calcium from isolated loops

The solutions used for injections into the loops were neutral calcium chloride solutions. Calcium gluconate was tried, but even hypotonic solutions sometimes seemed to irritate the gut, causing a considerable secretion; and inconsistent results followed. In preliminary experiments it was observed that it was impossible to rinse all the remaining Ca out of the lumen at the end of the experiment. Rinsed and emptied loops were found to contain up to 0·6 mg. Ca per g. of loop, as compared with 0·08–0·12 mg. Ca per g. of loop observed in 10 experiments in which no Ca was injected. The average value in these 10 blank experiments was 0·1 mg. Ca per g. loop. In the actual experiments therefore the following procedure was adopted to allow for the Ca remaining in the lumen. The loops were rinsed out with distilled water, pressed out and weighed. The loops together with the rinsed-out content were ashed with nitric and perchloric acids. The ash solutions were transferred to volumetric flasks and aliquot parts analysed for Ca by the oxidimetric method. The weight of the loop (1·4–1·7 g.) × 0·1 mg. was then subtracted from the value found for unabsorbed Ca. The error resulting from this procedure is negligible.

In case it might be thought that precipitation of Ca would affect the rate of absorption, experiments were done to decide this point. When 14·7 mg. Ca were injected, about 75 % of the remaining Ca was found in the filtrate derived from the content plus the washing water.

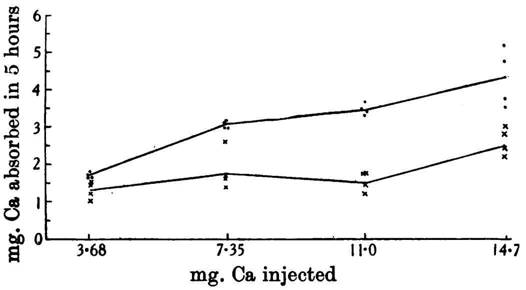

Fig. 1. Absorption of Ca from isolated loops of the jejunum. 3 ml. $CaCl_2$ injected into 30 cm. loops.

• Normal rats. × Vitamin D-deficient rats.

Results. The results of the experiments with Ca are given in Fig. 1. Groups of 4 normal and 4 "rachitic" rats were used to test each concentration. This number of animals is naturally insufficient to cover the possible range of variability. The results here presented are consistent enough, however, to justify the conclusion that the same difference is observed with regard to Ca absorption between normal and vitamin D-deficient rats, as was previously recorded in metabolism experiments [Nicolaysen, 1937].

The shape of the smoothed curves indicates that there is a definite relationship between the rate of absorption and the concentration of the injected Ca solution. This is in agreement with previous observation [Nicolaysen, 1937] that up to a certain limit the absorption of Ca depends upon the amount ingested. From these results it would seem possible that the absorption of Ca is due to diffusion, and that the reduced absorptive rate in vitamin D-deficient rats may therefore be due to a reduced rate of diffusion or a reduced permeability. On the other hand, the possibility of a chemical reaction of some kind cannot be ruled out.

The absorption of calcium from the abdominal cavity

If the decreased rate of absorption of Ca in vitamin D deficiency is due to a decreased permeability, there is a possibility that all cells in the body are affected. If this is so one should be able to demonstrate a decreased rate of absorption of Ca from the abdominal cavity, where there would seem to be no possibility of a chemical reaction being involved in the absorption of Ca. To test this possibility the following experiments were performed.

Experimental procedure. The injection of the $CaCl_2$ solution into the abdominal cavity was performed under an ether narcosis so light that the animals were on their legs again immediately after the injection was finished. After intervals of 15, 30, 60 and 180 min. the animals were killed by decapitation. The skin was removed, and with a small incision the abdominal cavity was opened over a large funnel inserted into a Kjeldahl flask. The remaining fluid ran out. The abdominal wall was then opened with a large cross-section, whereupon eventration followed. The wall and the viscera were rinsed repeatedly with distilled water. The whole sample was ashed with nitric and perchloric acids, the ash solutions transferred to volumetric flasks and analyses carried out on aliquot parts.

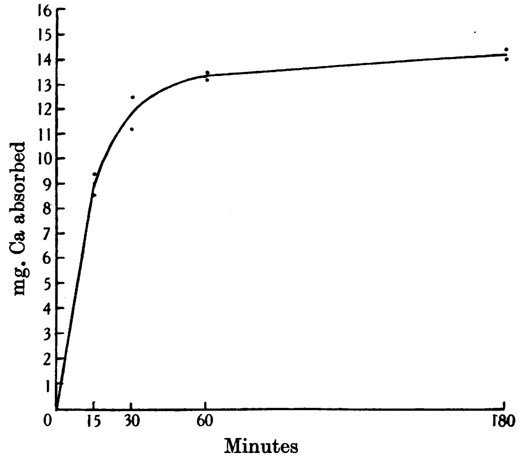

Fig. 2. Absorption of Ca from the abdominal cavity. 15·3 mg. Ca as $CaCl_2$ injected in 5 ml. water.
• gives maxima and minima.

Groups of 12 normal and 12 vitamin D-deficient rats were used for these experiments, i.e. 3 normal and 3 "rachitic" rats at each interval. Fig. 2 gives the results for the normal rats only, as there was no difference between normal and vitamin D-deficient rats. The curve is constructed through the average values. The maxima and minima are marked for every interval. The only conclusion which is permissible from these experiments is that this possible action of vitamin D on the permeability of the cells in the body is not a universal one.

The absorption of xylose and of sodium sulphate from isolated loops

Verzar & Laszt [1935] have found the same type of absorption curves for sodium sulphate and for the sugars xylose, sorbose and mannose from isolated loops as that reported here for Ca. As a demonstration of a reduced rate of absorption in vitamin D deficiency of substances other than Ca would make it appear probable that vitamin D exerts its effect on the permeability of the absorptive epithelium of the gut, the absorption of xylose and of sodium sulphate was studied. Another reason for choosing this type of substance is that the output of dry faeces per g. food intake is not increased in vitamin D-deficient rats [Innes & Nicolaysen, 1937], which indicates that the ability to absorb the main food constituents other than Ca is not primarily affected in vitamin D-deficient rats.

Xylose. The technique was that described in the opening portion of the paper. The xylose remaining in the loops at the end of the experiment was rinsed out with distilled water. After filtration the solutions were made up to a definite volume in volumetric flasks, and analyses performed on aliquot parts. Hawkins [1929] has shown that xylose and glucose g. for g. reduce the same amount of ferricyanide. The analyses were therefore performed by the Hagedorn-Jensen method.

Fig. 3 gives the results obtained in the vitamin D-deficient rats only, as identical results were obtained in normal rats.

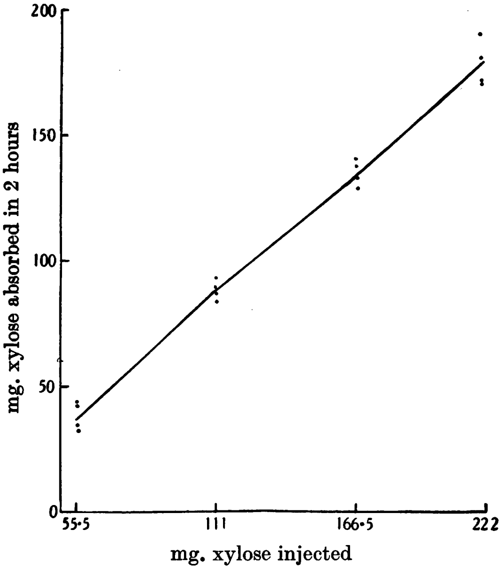

Fig. 3.

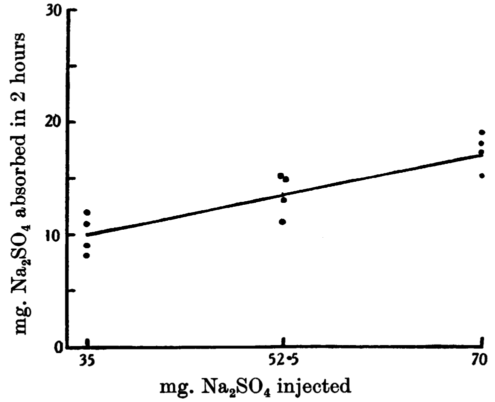

Fig. 4.

Fig. 3. Absorption of xylose from isolated loops of the jejunum. 3 ml. solution injected into 30 cm. loops.

Fig. 4. Absorption of Na_2SO_4 from isolated loops of the jejunum. 3 ml. Na_2SO_4 solution injected.

Sodium sulphate. After filtration of the contents of the loops together with the washing water, the sulphate was determined by precipitation with $BaCl_2$ and weighing as $BaSO_4$. Identical results were obtained in both groups of rats. Fig. 4 gives the results obtained in the rachitic rats only.

Discussion

The experiments in which the absorption of Ca was studied from the abdominal cavity and xylose and Na_2SO_4 from isolated loops fail to elucidate further the mode of action of vitamin D on the absorptive epithelium of the gut. They contribute, however, to knowledge about the localization of the point of attack of the vitamin and about the specificity of the action.

The absorption curves for xylose and Na_2SO_4 given by Verzar & Laszt [1935] are identical with those presented here, and the absorption curve for Ca is of the same type. Verzar considers that these "physical" curves prove that the absorption is a diffusion process. This argument may be satisfactory for a substance like xylose which ordinarily takes no part in intermediary metabolism. It seems, however, that more evidence is needed before the same final statement can be made about the mode of absorption of an ordinary food constituent like Ca. If in fact it is true of Ca, the consequence would then be that vitamin D influences the permeability of the absorptive epithelium of the gut.

Slowness of absorption of Ca. It is of interest to compare the rates of absorption from the gut of the three substances studied. In Fig. 5 a comparison has been made of the percentage absorptions in 2 hours of calculated isotonic solutions. The figure illustrates well the extreme slowness with which $CaCl_2$ is absorbed, the percentage absorption being only half that of the slowly absorbed Na_2SO_4. The rate of absorption of Ca in the experiments with 14·7 mg. Ca (see Fig. 1) is 0·9 mg. per hour. The whole length of the small intestine in these rats is about 70 cm. The rate of absorption from the lower part of the small intestine is the same as from the upper part, as the rate of absorption was found to be doubled in some experiments in which the whole of the small intestine was used. The rate of absorption of Ca from isolated small intestine is calculated to be about 2 mg. per hour. This figure corresponds well with the maximum absorption found in metabolism experiments (42 mg. per day) [Nicolaysen, 1937].

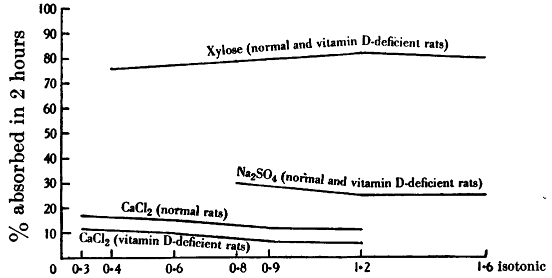

Fig. 5. Percentage absorptions of calculated isotonic solutions of $CaCl_2$, Na_2SO_4 and xylose from isolated loops of the jejunum.

Vitamin D and intestinal p*H*. Zucker & Matzner [1923] discovered that the intestinal content and the faeces are less acid in vitamin D-deficient than in normal rats. It has since been regarded as a possibility that vitamin D influences the secretion into the digestive tract of a substance making the content more acid. Assuming that ions other than Ca are absorbed at a normal rate, a relative preponderance of cations will be the consequence of the reduced absorption of Ca in vitamin D-deficient animals. This would explain the reduced acidity in the intestinal content. Although the absorption of all ions which are normally present in the intestinal content has not been investigated, there is considerable reason to believe that the rate of absorption of these ions is in fact normal. The Steenbock-Black diet is rich in NaCl (1 %), and the faeces of vitamin D-deficient rats contain no more water than those excreted by normal rats. This demonstrates indirectly that the NaCl is absorbed as well by the rachitic as it is by the normal rats, and that other ions presumably are not present in any excess in the faeces of rachitic rats. The effect of vitamin D deficiency on the absorption of phosphate from isolated loops will be investigated, but from the metabolism experiments [Nicolaysen, 1937] one would not expect the rate to be diminished.

The reduced rate of absorption of Ca in vitamin D deficiency seems to explain the chief symptoms of rickets. An increased amount of Ca in the gut will precipitate more phosphate than normally, so that a relative Ca and P starvation follows. This again may explain the reduced concentration of Ca and P in the blood and the faulty bone formation which follows.

SUMMARY

The rate of absorption of Ca from isolated loops increases with increasing concentration both in normal and vitamin D-deficient rats, but the rate in vitamin D-deficient rats is considerably lower than in normal rats.

The rates of absorption of Ca from the abdominal cavity and of xylose and sodium sulphate from isolated loops are identical in normal and vitamin D-deficient rats.

My thanks are due to the Medical Research Council and to Dr L. J. Harris for their kind hospitality during this work. I am indebted to Mr G. G. Grindley for his skilled assistance with the operations.

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