Experiments on the Practicability of Increasing Calcium Absorption with Protein Derivatives

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(Received 10 February 1944)

Amino-acids increase the solubility of salts formed by divalent cations such as Mg^{++} and Ca^{++} (Lehmann & Pollak, 1942a). This effect has been suggested as a possible mechanism for the solubility changes of calcium *in vivo*. Thus, breakdown of amino-acids in the body should lead to a precipitation of calcium salts. It was possible to show, *in vitro*, a precipitation of calcium from a solution containing $CaHPO_4 \cdot 2H_2O$ and amino-acids, when the latter were deaminated by the addition of amino-acid oxidase (Lehmann, 1942). On the other hand, calcium salts in the food should become more available by the addition of amino-acids or protein to the diet (Lehmann & Pollak, 1942b). Sparingly soluble calcium salts should become more soluble in the presence of amino-acids, and the absorption of soluble calcium salts should be raised, since the latter are certain, once they are administered, to form sparingly soluble salts such as carbonates and phosphates. Experiments on man showed these suggestions to be justified. McCance, Widdowson & Lehmann (1942) demonstrated that by increasing the daily protein intake from about 60–160 g., the calcium absorption was improved considerably. The present work was undertaken to obtain more data on this protein effect, with a view to its possible use in calcium therapy.

EXPERIMENTAL

Preparation used. A calcium preparation, which might possibly be useful for calcium therapy, must fulfil a variety of conditions. It must not be too bulky and its ingredients must not be too expensive. It has never been satisfactorily proved that prolonged overfeeding with calcium is dangerous. However, the possibility has to be kept in mind, and a preparation which is likely to be absorbed should preferably not contain excessive amounts of the mineral. This consideration does not apply to many of the calcium preparations usually employed, as it can reasonably be supposed that little of the calcium they contain is utilized.

300 mg. of calcium were aimed at as a daily dose for a human being. As a cheap source of amino-acids, peptone was chosen, while glutamic acid was added to improve the taste. The following powder was prepared:

$CaHPO_4 \cdot 2H_2O$	100 mg.
Peptone	2000 mg.
Glutamic acid	200 mg.
Lactose	550 mg.
Gum acacia	150 mg.

If four of these powders were taken three times daily, and were fully absorbed, then 279 mg. of calcium would be available above the amount absorbed from the daily food.

A control powder was prepared containing no peptone or glutamic acid. This powder contained 100 mg. of $CaHPO_4 \cdot 2H_2O$, 2750 mg. of lactose and 150 mg. of gum acacia. It has recently been shown by Roberts & Christman (1942) that a single dose of lactose has no effect on the calcium absorption in the rat. The peptone contained traces of calcium and the control powder was adjusted, to allow for this, by the addition of an equivalent amount of $CaHPO_4 \cdot 2H_2O$ (0.3–0.6 mg. calcium/g. peptone).

Mode of testing. The following course was followed to test the effectiveness of the peptone powders. The urinary calcium was measured after the oral intake of 12 control powders and also after the intake of 12 peptone powders (the daily dose). The powders were washed down with water. Members of the hospital staff were used to test various experimental procedures and, when a standard type of experiment was arrived at, the serial investigations were done on mental patients. First, 12 control powders and 750 ml. of distilled water were given after a period of 14 hr. during which time no drink or food had been taken; the urine was collected during the following 5 hr. and the urine calcium determined after ashing. Then, after an interval of at least 3 days and not more than 5 days, the experiment was repeated, but with 12 of the powders containing peptone and glutamic acid. An increased urinary calcium in the second test was taken to indicate an increase in intestinal absorption. McCance & Widdowson (1939) have shown that the human kidney is exceedingly sensitive to variations in the level of calcium in the blood. When the blood calcium is raised suddenly by an intravenous injection of calcium, almost the whole of the injected mineral is excreted through the kidney. It is to be expected that during the more gradual parenteral absorption the excretory stimulus will be smaller, and that a smaller proportion only will leak through the kidneys. This is borne out by the results of McCance *et al.* (1942). These results showed that the increase in urinary calcium brought about by addition of protein to the diet is only a part of the total increase in the calcium absorbed. In the same investigation (McCance *et al.* 1942) urea was given in a control experiment to exclude a possible increase of urinary calcium after peptone intake, which might result from any secretory stimulus by the urea formed from the peptone.

RESULTS

Urinary calcium. Table 1 shows the results obtained in nineteen experiments done on eighteen patients. In fourteen out of the eighteen subjects the urinary calcium was higher after the intake of

Table 1. *Urinary calcium excretion in human beings (mg.) during the five hours after 750 ml. of distilled water had been drunk*

No.	Sex	Body wt. (kg.)	Diagnosis	Urinary calcium (mg.)		
				After 12 control powders	After 12 peptone powders	Difference
1	L.M.	F.	Hypomania	281.0	320.0	+ 39.0
2	M.T.	F.	Schizophrenia	84.0	244.0	+ 160.0
3	T.B.	M.	Dementia	28.2	22.4	- 5.8
4	F.L.	F.	Reactive depression	270.0	294.0	+ 24.0
5	M.C.	F.	Oligophrenia	475.0	492.0	+ 17.0
6	G.M.	F.	Schizophrenia	13.7	48.7	+ 35.0
7	J.M.	F.	Mental deficiency	140.0	61.5	- 78.5
8	E.H.	F.	Melancholia	25.5	356.0	+ 330.5
9	W.K.	F.	Schizophrenia	93.6	107.0	+ 13.4
10	L.P.	M.	Secondary dementia	44.0	86.6	+ 42.6
11	R.N.	F.	Hypomania	32.4	79.6	+ 47.2
12	T.G.	F.	Schizophrenia	1.9	38.2	+ 36.3
13	D.P.	F.	Melancholia	34.2	60.0	+ 25.8
14	C.K.	F.	Mania	131.0	108.9	- 22.1
14	C.K.	F.	Mania	96.0	83.4	- 12.6
15	E.W.	F.	Hysteria	1.7	3.43	+ 1.73
16	A.B.	F.	Presenile dementia	41.2	38.8	- 2.4
17	L.C.	F.	Melancholia	44.3	50.0	+ 5.7
18	M.S.	F.	Mental deficiency	40.6	52.0	+ 11.4

peptone powders than after the intake of the control powders. It will be seen that one subject (14 C.K.) was used twice. On both occasions, which were actually over a month apart, similar results were obtained. This was no accidental coincidence, for unequivocal results were obtained on a number of occasions when the same subject was used repeatedly. These experiments are not included in the present paper, as they were not done according to the standard procedure. They were obtained during preliminary tests which were carried out in order to establish a suitable experimental routine for the nursing staff and the subjects.

As the increase in urinary calcium after peptone powders can be expected to be only a portion of the mineral which was actually absorbed, and as it must be supposed that absorption goes on for a period longer than 5 hr., it seems reasonable to conclude that the calcium in the peptone powders was much more available than that contained in the control powders. While these experiments were in progress, it became necessary for outside reasons to bring them to an end within a limited time. It was obvious that, in order to obtain results of a

statistical value, of above 95 % probable significance, a much larger number of tests had to be done than the time allotted to us allowed. We included therefore, in the second half of the series, a third test. No powders were given to the subjects, who drank only 750 ml. of distilled water. The fasting period and the collection of urine were arranged exactly as in the other tests. The water blank experiment was performed not less than 2 days and not more than 5 days before the test with the lactose control powders. The urinary calcium (mg.) in these eight cases were:

Water blank	Lactose control powders	Peptone powders
28 ± 25	47 ± 41	57 ± 31

It will be seen that again the urinary calcium is higher after ingestion of peptone powders than after that of lactose control powders. As pointed out above, many more cases would be needed to obtain a result of above 95 % probable significance. However, the eight cases are sufficient to show another statistical difference between the results obtained with peptone and control powders. The

Table 2. *Urinary calcium excretion in human beings (mg.) during the five hours after 750 ml. of distilled water had been drunk*

No.	Sex	Body wt. (kg.)	Diagnosis	Urinary calcium (mg.)		
				After 12 control powders	After 12 ' $\frac{1}{2}$ peptone powders'	Difference
19	M.O.	F.	Melancholia	47.6	122.0	+ 74.4
20	M.P.	F.	Melancholia	4.0	1.1	- 2.9
21	L.P.	M.	Secondary dementia	44.0	68.0	+ 24.0
22	T.B.	M.	Dementia	12.4	26.7	+ 14.3

difference between the urinary calcium after the water blank tests, and the urinary calcium after the tests with the peptone powders, shows a probable significance of above 95%. This is not the case for the difference between the water blank tests and the lactose control tests.

Table 2 shows four experiments in which the water blank tests and the experiments with the lactose control powders were done as usual. The peptone powders, however, contained only one-half the amount of peptone and glutamic acid; they were made up to full weight with lactose ('½ peptone powders'). It will be seen that an increase in urinary calcium was also found when the protein derivatives were diminished by one-half.

Serum calcium. The serum calcium of all subjects was determined before the tests. It was normal (with one exception) and varied between 9.8 and 10.6 mg./100 ml. In the case of four subjects it was measured immediately before the peptone powders were taken, and for five hours afterwards at half-hourly intervals; no rise in serum calcium was observed. This was to be expected from the work of McCance & Widdowson (1939) on the regulatory mechanism of the kidney, which exerts its function by preventing the calcium serum level from rising above normal. Thus any calcium filtering from the intestine into the blood stream after the ingestion of the powders would not be allowed to accumulate. Provided the serum calcium level was normal initially, the excess calcium would either be deposited in the tissues or excreted by the kidneys. One of our subjects (L.P.), however, was found to have a hypocalcaemia, the serum calcium being 8 mg./100 ml. In his case the serum calcium rose to normal after the ingestion of the peptone powders, but not when '½ peptone powders' or lactose control powders were given (Fig. 1). This patient was

treated with four peptone powders, three times daily, for 17 days. His serum calcium was determined during this period in the morning while he

Fig. 1. Serum calcium, mg./100 ml., in an hypocalcaemic subject (L.P. ♂), after ingestion of 750 ml. of distilled water and 279 mg. calcium as $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$. I, 12 lactose control powders; II, 12 '½ peptone powders'; III, 12 peptone powders.

was still fasting. It rose on the first day to 10 mg./100 ml. and remained at this level throughout. When the treatment was stopped, the serum calcium fell slowly and was, after 3 weeks, back at 8 mg./100 ml.

SUMMARY

A calcium peptone powder has been devised which appears suitable for increasing calcium absorption in man.

We should like to thank Dr R. Strom-Olsen, Medical Superintendent of Runwell Hospital, for giving us the opportunity to carry out these investigations. We were helped with materials by Organon Laboratories Ltd. One of us (H. L.) was supported by a research grant from the Ella Sachs Plotz Foundation.

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The Metabolism of Lysine

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(Received 27 January 1944)

In spite of the considerable amount of work which has been done on the biochemistry of lysine, the intermediary metabolism of this important amino-acid still remains obscure. The work recorded here

does not give a clear-cut answer to this problem, but the observations reported may, we believe, be helpful in arriving at an ultimate solution.

There is little doubt that at least a part of the