THE MEASUREMENT OF INTESTINAL CALCIUM ABSORPTION BY EXTERNAL RADIOISOTOPE COUNTING: APPLICATION TO STUDY OF NEPHROLITHIASIS


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SUMMARY

1. Gastro-intestinal absorption of calcium was studied in man by the measurement of forearm radioactivity in a large-volume liquid scintillation counter following separate oral and intravenous doses of $^{47}$CaCl$_2$. From the ratio of the percentages of total radioactivity appearing in the forearm following these separate determinations the fractional absorption of calcium was estimated.

2. Changes of forearm radioactivity with time following the administration of this isotope were studied; evidence is presented that the radioactivity in the forearm at 4 h after administration of the isotope gives a valid assessment of fractional calcium absorption.

3. Fractional calcium absorption determined by this technique correlated well with the net calcium absorption as determined from stool radioactivity after oral administration of isotope.

4. In normal subjects it was shown that fractional calcium absorption measured by this technique varies inversely with the stable calcium load and that the absolute amount of calcium absorbed from given loads increases with the size of the load in the range 20–1000 mg calcium.

5. Gastro-intestinal calcium absorption was measured at various oral calcium loads in a group of fifteen patients with recurrent calcium-containing renal stones. All the patients were normocalcaemic; some had hypercalciuria. In the patients with hypercalciuria, calcium absorption, fractional and absolute, was significantly increased at all calcium loads as compared to that of patients with normal urinary calcium.

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It is concluded that hyperabsorption of calcium from the gastro-intestinal tract plays a crucial role in the aetiology of hypercalciuria, probably by causing an increase in the renal filtered calcium load.

Abnormalities of gastro-intestinal absorption of calcium are well recognized features in many disorders of calcium metabolism. The measurement of calcium absorption by full balance procedures is time-consuming and requires the accurate collection of timed specimens of both urine and faeces; thus it is subject to inherent errors. The availability of $^{47}$Ca, a $\gamma$-emitting isotope with a short half-life, led to the development of simplified procedures to measure gastro-intestinal calcium absorption based on the determination of blood radioactivity at timed intervals after oral administration of the isotope (Bhandarkar, Bluhm, MacGregor & Nordin, 1961; Jaworski, Brown, Fedoruk & Seitz, 1963; Avioli, McDonald, Singer & Henneman, 1965; Nordin, Young, Oxby & Bulusu, 1968).

A disadvantage of such procedures is the difficulty of accurate counting of the small amount of radioactivity in plasma after a reasonable oral dose of isotope. Even with low plasma concentrations of radioactivity, accretion by bone claims a large proportion of the circulating tracer.

In 1964 Lutwak & Shapiro described a technique for measuring calcium absorption in man with a large-volume liquid scintillation counter to count forearm radioactivity after an oral dose of $^{47}$Ca. The technique was expanded by Curtis, Fellows & Rich (1967), who calculated the fractional absorption of oral calcium from forearm radioactivity after oral and intravenous doses of $^{47}$Ca. They compared the accumulation of tracer in the forearm following an oral dose of $^{47}$Ca to that measured following an intravenous dose of the same isotope. In their technique, forearm radioactivity was measured 23 h after an intravenous dose of $^{47}$Ca and compared with that measured 25 h after an oral dose. The method required counting on three successive days, and a total isotope dose of 26 $\mu$Ci of $^{47}$Ca. We have modified the technique so that the radioactivity is measured on only 2 separate days, and the total dose of isotope is between 3 and 4 $\mu$Ci of $^{47}$Ca. These modifications allow repeated studies on any one patient with only a low total dose of isotope.

Early results with this method showed hyperabsorption of calcium in many patients with recurrent kidney stones (Zisman, Pak & Bartter, 1967; Wills, Pak & Bartter, 1968; Zisman, unpublished observations). A correlation between increased urinary calcium excretion and nephrolithiasis was reported by Flocks (1939), a relationship that is now well established. Excessive gastro-intestinal absorption of calcium, as reflected by a low faecal calcium, has been reported in this condition by many workers (Henneman, Benedict, Forbes, & Dudley, 1958; Harrison, 1959; Jackson & Dancaster, 1959; Parfitt, Higgins, Nassim, Collins & Hilb, 1964); this has led to the view that hyperabsorption of calcium is the primary cause of nephrolithiasis (Dent & Watson, 1965). Other workers, however, have concluded that the primary lesion is a defect in the renal tubular handling of calcium, and that the increased gastro-intestinal absorption of calcium, if it occurred, was secondary (Jackson & Dancaster, 1959; Edwards & Hodgkinson, 1965).

In the present study patients with recurrent nephrolithiasis, all of whom were normocalcaemic, some of whom were hypercalciuric, were subjected to increasing oral calcium loads. The study confirms our preliminary reports showing that gastro-intestinal absorption of calcium is
Gastro-intestinal calcium absorption

increased in those patients with hypercalciuria (Zisman et al., 1967; Wills et al., 1968). The results of all the studies indicate that the use of calcium 'trapping' by the forearm which 'integrates' radioactivity in blood over time provides a simple and reliable measurement of gastro-intestinal calcium absorption.

MATERIALS AND METHODS

Initial studies were done on seven normal subjects and four patients with disorders of calcium metabolism. The normal subjects were all volunteers hospitalized at the Clinical Center of the National Institutes of Health. The diagnoses in the patients, previously established from clinical, radiological and biochemical data, are given in Table 1.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Serum calcium (mg/100 ml)</th>
<th>Serum phosphorus (mg/100 ml)</th>
<th>Urinary calcium (mg/24 h*)</th>
<th>Duration of symptoms (years)</th>
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<td>3.2</td>
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<tr>
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</table>

* Mean of three 24-h values while the patient took a 400 mg calcium diet.
† Chronic urinary tract infection with pseudomonas aeruginosa.
‡ Chronic urinary tract infection with providence group II gram negative bacillus.

Fifteen patients who had passed calcium-containing stones on at least two separate occasions were studied. In each case the stones had been analysed by X-ray diffraction technique (performed by Louis C. Herring & Co., 15 W Underwood Avenue, Orlando, Florida), and found to be calcium phosphate or calcium oxalate mixed with calcium phosphate. All the patients were normocalcaemic on repeated testing and had normal plasma alkaline phospha-
tase activity and normal serum total protein, blood urea nitrogen and serum creatinine concentration. None of them had evidence of metabolic bone disease as assessed by radiological bone survey. During the studies they were receiving a diet containing approximately 400 mg of calcium per day, which they had been taking for at least 4 weeks: this diet excluded all dairy products, fish and fish oils. They were classified as hypercalciuric if urinary calcium excretion was above 200 mg/day while they were taking this diet. Two of the patients in the group with hypercalciuria (J.S. and E.G.) were classified as 'idiopathic' hypercalciuria, having had surgical exploration of the neck and biopsy of four normal parathyroid glands; in the other three patients in this group, all other causes of hypercalciuria had been excluded, but they had either declined surgical exploration or had not been considered for it for other reasons (A. S. and C.S. declined operation and S.D. had mild congestive cardiac failure). Initial values for all patients are given in Table 1.

All subjects were studied while they were taking a fixed metabolic diet containing each day approximately 400 mg of calcium and 800 mg of phosphorus, confined to air-conditioned areas, and restricted in their physical activities. After an overnight fast they were given 1 to 2 μCi of $^{47}$CaCl$_2$ (specific activity greater than 150 mCi per g calcium, Oak Ridge) orally in 50 ml of distilled water; stable CaCl$_2$ added as carrier varied in the different experiments, as detailed below. After an interval of 1–4 days, 1–2 μCi of $^{47}$CaCl$_2$ was given intravenously in the left arm. The right forearm was used for all counting of radioactivity. A standard solution was prepared by adding one half of the administered dose of $^{47}$CaCl$_2$ to 1 litre of 1 N HCl. All subjects remained fasting until completion of a 4-h counting period.

Radioactivity in the forearm was measured immediately before and at timed intervals after the administration of the isotope by means of a large-sample liquid scintillation counter (Armac, Packard Instrument Corp. Inc., Downers Grove, Illinois, U.S.A.). The counting time was 10 min. The discriminator settings were selected to permit counting within 8% of the $E_{max}$ of $^{47}$Ca at 1.31 MeV. The fractional deviation of counting (Andrews, 1961) for forearm radioactivity was 0.024. The patient was positioned in a chair beside, but not directly in front of, the counting chamber so that the detector was shielded from body radioactivity other than that in the forearm. During the counting procedure the patient also wore a lead apron to shield the detector further from the body. The position of the forearm was standardized during counting by having the patient grasp a bar at the inner end of the counting chamber. Immediately after the patient had been counted the 1 litre standard was counted with the same settings. The counting results were expressed as percentages of the dose administered. The ratio of the percentage of dose ‘trapped’ after oral $^{47}$Ca to the percentage of dose ‘trapped’ after intravenous $^{47}$Ca was taken to represent fractional calcium absorption. The product of the fractional calcium absorption and the stable oral calcium carrier load gave the ‘absolute’ amount of dietary calcium absorbed at that load.

To provide an estimate of the anatomical localization of the isotope in the tissues at times after administration, the following experiment was carried out. One μCi of $^{47}$Ca was injected intraperitoneally into each of a group of Sprague-Dawley rats weighing 200–300 g. The same amount of isotope was used to prepare a standard, as detailed previously. At fixed times after the administration of the isotope, the animals were killed. Immediately after death the right hind leg was disarticulated at the hip with the soft tissues intact. The soft tissues (skin, fat and muscle) were then carefully dissected from the bones. The soft tissues and bones were counted separately in the Armac scintillation counter. Percentages of total limb radioactivity in bone
and soft tissues were then calculated (Table 2). The results show that the major portion of the isotope was not localized to bone until after 48 h. The forearm radioactivity 4 h after administration of $^{47}$Ca thus probably includes a large contribution of uptake by the soft tissues.

**Table 2. Percentage distribution of total radioactivity of right hind-limb of rats**

<table>
<thead>
<tr>
<th>Time after dosage (h)</th>
<th>Total radioactivity in limb</th>
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<tr>
<td></td>
<td>Bone (%)</td>
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<tr>
<td>4</td>
<td>66</td>
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<td>24</td>
<td>73</td>
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<td>120</td>
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<td>144</td>
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**RESULTS**

*Time course of calculated fractional calcium absorption*

In three normal subjects and in four patients the change with time in the calculated fractional calcium absorption was studied by measurements of forearm radioactivity over a period of 48 h following the administration of $^{47}$Ca. After overnight fasting, all subjects were given an oral dose of 2 $\mu$Ci of $^{47}$Ca, with 180 mg of calcium as calcium chloride except in patient L.E., who was given 20 mg of calcium as calcium chloride. The subjects remained fasting until 4 h after they had received the isotope. After an interval of at least 4 days, 1-2 $\mu$Ci of $^{47}$Ca were given intravenously. The amount of radioactivity in the forearm was determined, as detailed previously, at hourly intervals for 8 h and then at 12, 24, 36 and 48 h after the dose of $^{47}$Ca. From the results fractional calcium absorption was determined for each of these times.

The fractional calcium absorption values, as calculated at 4, 12, 24, 36 and 48 h after the administration of the isotope, are given in Table 3. In all the subjects, the calculated value for fractional calcium absorption showed a rapid increase during the first 2 h and then showed only small further changes with time. As can be seen from the later values in Table 3, the 4-h value represents a ‘plateau’. From these results it appeared that later values were not different from the 4-h ones and the 4-h value was chosen for the subsequent studies detailed in Table 4.

*Effect of stable calcium carrier load on fractional calcium absorption*

Fractional calcium absorption was calculated for each load, and the 4-h values are detailed in Table 4. The results show that fractional calcium absorption as estimated by this technique varied inversely with the stable calcium carrier load.

In four normal subjects the effect of variations in the stable calcium carrier load was studied with loads ranging from 20–1000 mg of calcium as calcium chloride. After the subject had fasted overnight he was given an oral or intravenous dose of $^{47}$Ca as detailed previously. Forearm
radioactivity was counted at hourly intervals for the next 4 h. The subject remained fasting until the completion of the counting period.

In all the patients, fractional calcium absorption was inversely related to the oral calcium load (Table 4). In the patients with hypercalciuria the mean fractional calcium absorption was significantly higher at all the loads studied than that in the normocalciuric patients (Table 4). Again there was an inverse relationship between the calcium load and the fractional calcium absorption (Fig. 1).

### Absolute calcium absorption

For each subject studied the ‘absolute’ amount of dietary calcium absorbed at the given carrier load was calculated from the product of the fractional calcium absorption and the stable calcium load. The results (Table 4) show that in the normal subjects the ‘absolute’ amount of calcium absorbed increased with the carrier load.

In the patients with hypercalciuria the mean values were significantly higher over the whole range of calcium loads studied. As seen in Fig. 2, the slope of the curve relating absolute absorption to oral load was greater in the patients with hypercalciuria; in both groups absorption was higher at the 1000 mg load than at the 500 mg one, so there was no evidence that a ‘plateau’ of absorption had been reached.

### Comparison of fractional calcium absorption with net calcium absorption, determined from stool radioactivity

In six subjects studies were performed to compare the result of fractional calcium absorption, as estimated by the technique described above, with net calcium absorption, as determined from stool radioactivity. On the night before the oral dose of Ca, a carmine marker was given and all stools were collected from the time of the appearance of the carmine until all the carmine had passed (this took from 3 to 6 days). The radioactivity in the stools was measured
### Gastro-intestinal calcium absorption

#### Table 4. Fractional and absolute calcium absorption at various stable calcium carrier loads

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<th>200 mg</th>
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**Absolute calcium absorption at stable carrier loads**

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<td>113.89</td>
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</table>

**Fig. 1.** Mean fractional calcium absorption ± SEM plotted against oral calcium load for normocalciuric and hypercalciuric patients.
and the net fraction of calcium absorbed was calculated by subtracting from the dose the fraction recovered in the stool.

The results are given in Table 5, together with the 4-h values of fractional calcium absorption in the same subjects. In the case of V.L., a patient with metabolic bone disease associated with chronic renal failure and in K.H., a normal subject, the results differ by 0.07 and 0.04, respectively, and in patient M.M. with sarcoidosis, by 0.05. In the other subjects the results differed by 0.02 or less. Thus the values obtained by these two techniques showed good correlation in the subjects studied.

### DISCUSSION

The methods currently available for the measurement of gastro-intestinal calcium absorption are time-consuming and require strict metabolic regimen. Thus, they are difficult to perform.
without specialized facilities. They cannot be applied without hospitalization; accordingly they are of limited value in the screening of large groups of patients such as those with nephrolithiasis. Of the methods available, the determination of calcium absorption from the radioactivity of blood after an oral dose of isotope is relatively simple, but for various reasons, probably does not accurately reflect true calcium absorption. In our own and other groups, this method has been found to be of limited value (Bronner, Richelle, Saville, Nicholas & Cobb, 1963; Kinney, Tauxe & Dearing, 1965; Zisman, Pak & Bartter, unpublished data).

Even with an oral dose of radioactive calcium much larger than that we have used, the amount of radioactivity in a blood sample of reasonable volume is relatively small, so that the accuracy of counting is limited. Also, the total amount of calcium within the blood compartment is small with respect to the total exchangeable calcium pool, and the radioactivity of blood after an oral dose is limited not only by excretion in the urine but also by accretion into bone.

The technique described here, based on that of Curtis et al. (1967), has the advantage of simplicity; it reflects changes in the large calcium compartments, as reflected by forearm radioactivity. With the modifications we have described, the patient must be present for only a short period of time on two separate occasions, and a low total dose of isotope is given. This permits the use of the test in children, and repeated tests in any one individual without an excessive total body dose. Thus, the effects of various therapeutic procedures on calcium absorption can be assessed in a relatively short time.

Several theoretical factors should be considered in interpreting the results of this method. The overall time curve of uptake of isotope by the forearm after oral administration of $^{47}$CaCl$_2$ differs from that after intravenous administration. This results from the delay in the transport of the isotope into the blood after an oral dose, which results in delay in the uptake of isotope from blood by the forearm. After oral administration of $^{47}$CaCl$_2$ the rate of uptake of isotope into the forearm is a complex function of continually changing input into blood with varying rates of uptake by the forearm. After intravenous administration, the forearm radioactivity reflects uptake from a single 'instantaneous' input. The ratio of forearm radioactivities (oral to intravenous), therefore, does not give a precise estimate of calcium absorption. Nevertheless, our results suggest that this ratio of forearm radioactivities reflects mainly the gastro-intestinal absorption of calcium. The contribution of the different rates of uptake by the forearm (slow absorption vs. 'instantaneous' injection) might be expected to be prominent during the period of calcium absorption, but not after 24 h. In fact, the ratios of forearm activities at 4 h were essentially the same as those at 24 h (Table 3); this suggests that the ratio of forearm radioactivities at 4 h does in fact reflect calcium absorption. This conclusion is supported by the good correlation observed between fractional calcium absorption at 4 h, as calculated by this method, and that calculated from the recovery of radioactivity from stools (Table 5).

The fractional calcium absorption was inversely related to the carrier load of calcium. This suggests an increasing saturation of the sites of absorption at higher carrier loads. Thus, although the total number of calcium atoms coming into contact with the sites increases with load (and absolute absorption increases) the specific activity of the absorbate decreases with load, and the fraction of the total gut calcium absorbed decreases as the calcium load is increased.

In the patients with nephrolithiasis and normal urinary calcium, the values obtained for both fractional and absolute calcium absorption showed no significant difference from those obtained by us in a small group of normal subjects (Table 4) except at the 500 mg calcium load. The
discrepancy at that load may be attributable to the very small number (four) of normal sub-
jects. In view of the close correlation between the values obtained in these patients and those
for the normal subjects, there appeared to be no value in extending the data in normal sub-
jects, as we were primarily interested in differences between the patients with hypercalciuria
and those with normal urinary calcium. In the group of patients with hypercalciuria, two were
classified as having ‘idiopathic’ hypercalciuria following surgical exploration of the neck; we
consider that the others in this group probably fall into this classification, although neck
exploration had not been performed.

The mechanism for the increased urinary calcium excretion in patients with hypercalciuria
has not been established. Henneman et al. (1958) supported the original hypothesis of Albright,
Henneman, Benedict & Forbes (1953) that the possible sequence of events leading to ‘idiopathic
hypercalciuria was ‘pyelonephritis, tubular damage, decreased reabsorption of calcium,
hypercalciuria, tendency to hypocalcaemia, compensatory parathyroid hyperplasia, hyperphos-
phaturia, and finally hypophosphataemia’. They suggested that if such a sequence of events
could be proven the syndrome could be termed ‘primary renal tubular hypercalciuria’. The
view that the primary defect was one of tubular handling of calcium has been supported by
other workers in independent studies (Jackson & Dancaster, 1959; Edwards & Hodgkinson,
1965). Recently however, Peacock & Nordin (1968) have reported that tubular reabsorption of
calcium in patients with hypercalciuria was the same as that in subjects with normal urinary
calcium. They found that ‘tubular reabsorption of calcium’ in the patients with hypercalciuria
was the same as that in other patients with nephrolithiasis and in normal subjects, they con-
cluded that the increased urinary excretion of calcium in most cases of ‘idiopathic’ hypercalciu-
ria’ must be due to an increased filtered load of calcium’.

Calcium is excreted by the kidneys through a combination of filtration and tubular reabsorp-
tion of the diffusible fraction of the serum calcium. Normally 95–99% of the filtered calcium is
reabsorbed. Thus, a 5% increase in the glomerular filtration rate, without change in tubular
reabsorption, could cause a four- to five-fold increase in calcium excretion; similarly a 5% change in reabsorption could cause a six-fold change in urinary excretion (Kleeman, Bernstein,
Rockney, Dowling & Maxwell, 1961). Thus, a very small change in the diffusible serum calcium
fraction following gastro-intestinal absorption could, by increasing the filtered load, account
for hypercalciuria.

An abnormality in gastro-intestinal calcium absorption in patients with hypercalciuria was
first reported by Henneman et al. (1958). They studied four patients with renal stones resulting
from ‘idiopathic’ hypercalciuria on metabolic balance regimen, and found that faecal calcium
was approximately half the normal value in each of them. Other workers have reported similar
findings (Parfitt et al., 1964). The role of enhanced gastro-intestinal absorption of calcium in the
aetiolog of hypercalciuria has been questioned, however: whereas some workers have reported
that urinary calcium excretion could be lowered with a low-calcium diet (Henneman et al.,
1958; Harrison, 1959; Gill & Barter, 1961; Dent & Watson, 1965; Peacock, Hodgkinson &
Nordin, 1967), others reported that it did not change (Jackson & Dancaster, 1959; Edwards &
Hodgkinson, 1965; Phillips & Cooke, 1967). The discrepancy probably represents inadequate
restriction of the dietary calcium intake in the second group: in the case reported by Dent &
Watson (1965), the hypercalciuria was not fully controlled until the patient used distilled water
for both drinking and cooking. These authors concluded that the primary defect in hyper-
calciuria could be an inability of the intestinal mucosa to limit absorption in the usual way,
resulting in excessive calcium absorption. Cannigga, Gennari & Cesari (1965) also reported evidence for hyperabsorption in six stone-forming patients, one of whom had primary hyperparathyroidism with osteitis fibrosa cystica. But in four of the remaining five patients there was hypercalcaemia, and thus it is not possible to exclude primary hyperparathyroidism in them with certainty. The studies reported here suggest that a primary defect in the intestinal handling of calcium plays a crucial role in the increased urinary calcium excretion in patients with hypercalciuria, probably by causing small but significant increases in the diffusible serum calcium fraction, undetectable by normal assay techniques, but resulting in significant increases in the filtered calcium load.

Since it has been shown that calcium absorption is affected by the pre-existing diet, it is important to note that both groups of patients had been on a low-calcium diet for a long period of time prior to the study. With long-term balance studies Malm (1958) showed an increase in calcium absorption as an adaptation to a low-calcium intake. Similarly, it has been reported for rats that the small intestine responds facultatively to a low-calcium diet by increasing the active transport of calcium (Kimberg, Schachter & Schenken, 1961). In the patients reported here, it seems most unlikely that the pre-existing low-calcium diet accounts for the hyperabsorption in the second group, as any increase in absorption resulting from a low-calcium diet alone would presumably affect both groups.

In conclusion, the test described here, based on external radioisotope counting provides a rapid, simple method for the assessment of gastro-intestinal calcium absorption which compares favourably with methods previously described. Because a low dose of isotope is used, the test can be repeated in any one individual without need for an excessive total body dose of isotope. With this method it has been shown that hyperabsorption of calcium from the gastro-intestinal tract is an important factor in and probably a proximal cause of 'idiopathic' hypercalciuria.

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