A RAPID METHOD FOR MEASUREMENT OF FRACTIONAL INTESTINAL ABSORPTION OF CALCIUM

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A single method for fractional intestinal calcium absorption (FCaA) measurement on plasma samples is proposed. FCaA is measured within a time interval of less than 8 hr using only one isotope successively injected intravenously and ingested 2 hr later. Assuming that plasma disappearance of the intravenously administered dose is identical to that of the absorbed oral dose we have verified that: (A) the absorption of the radioactive oral dose was achieved within 4 hr, (B) the log curve of plasma radioactivity obtained after total absorption of the oral dose is a straight line parallel to that obtained after the intravenous dose. This corresponds to the "exchangeable calcium expanding pool" model.

FCaA was measured in 60 subjects. FCaA was 58.9% ± 3.7 (mean ± s.e.m.) in controls, 90.7% ± 4.7 in primary hyperparathyroidism, 79.3% ± 4.8 in idiopathic hypercalciuria, 81% in Paget's disease, and 35.8% ± 2.6 ($p < 0.001$) in chronic renal failure treated conservatively. Following hemodialysis FCaA was transiently increased to 50.6% ± 2.5 ($p < 0.001$) when performed 10–12 hr after the hemodialysis run (calcium dialysate concentration = 7 mg%).

The methods commonly used for the measurement of fractional intestinal calcium absorption (FCaA) compare the plasma decay of radiocalcium after intravenous injection and after oral administration (*1–4*). Some of them use only ^{47}Ca (*3,4*), the others ^{45}Ca and ^{47}Ca (*1,2*). The period of study lasts at least 24 hr (*5*), and more often many days elapse before getting the results (*6,7*). We propose a new method which provides the rate of FCaA with one isotope, ^{47}Ca (or ^{45}Ca), in less than 8 hr.

This method is based on the parallelism of the plasma radioactivity curves plotted as a function of time on logarithmic coordinates after intravenous injection and then ingestion of ^{47}Ca [exchangeable calcium expanding pool model (*8–10*)]. This parallelism implies that both the radiocalcium plasma diffusions after injection and after ingestion are identical. If this parallelism appears in a brief delay after oral administration and remains constant for hours, it becomes possible during the interval where the two curves are parallel to measure the FCaA.

MATERIALS AND METHODS

Sixty subjects divided into three groups were studied:

Group 1. Subjects in this group were selected to compare plasma kinetics of radiocalcium after injection and after ingestion of the isotope.

Five μCi of ^{47}Ca were injected intravenously to fasting subjects: two healthy controls and three patients with chronic renal failure (CRF) not treated by hemodialysis (plasma creatinine from 4.8 to 15 mg%). Two hours later, 10 μCi of ^{45}Ca were given orally with 100 mg $CaCl_2$. A 3-ml blood sample was taken 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 20, and 48 hr after the injection. The ^{47}Ca was counted immediately in every sample. The ^{45}Ca was counted 2 months later.

In order to verify kinetics of intravenously administered radiocalcium, three patients with CRF undergoing hemodialysis were only given an intravenous dose of ^{47}Ca and blood samples were taken from 0.5 to 12 hr.

Group 2. This group was subdivided into seven subgroups in which FCaA was measured. Subgroup A is nine voluntary healthy subjects, B is 20 patients with CRF [plasma creatinine from 3.5% to 16.8%

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TABLE 1. DIETARY INTAKE OF CALCIUM AND PHOSPHATE AND PLASMA LEVELS OF CALCIUM, PHOSPHATE, AND CREATININE IN HEALTHY CONTROLS (GROUP 2A), PATIENTS WITH CRF TREATED CONSERVATIVELY (2B) AND CRF PATIENTS UNDERGOING HEMODIALYSIS (2C)

		Daily dietary intake		Plasma levels		
Subgroups	No.	Ca (mg)	P (mg)	Ca (mg%)	P (mg%)	Creat. (mg%)
2A	9	750 ± 50	1,300 ± 50	9.37 ± 0.14	3.42 ± 0.11	1.05 ± 0.06
2B	20	400 ± 30	950 ± 50	8.40 ± 1.36	5.81 ± 1.60	10.39 ± 1.02
2C	15	670 ± 30	1,200 ± 50	9.40 ± 1.49	4.0 ± 1.31	7.73 ± 0.51

(mean 10 mg%)], and C is 15 patients with CRF on hemodialysis. Twenty to 24 hr a week of hemodialysis was performed with Kiil-type dialyzers equipped with Cuprophan membrane. The hemodialysis bath concentration of calcium was 7 mg%. Subgroup D is three patients with primary hyperparathyrodism due to an adenoma subsequently proven by surgery, E is two patients with Paget's disease, F, is three patients with idiopathic hypercalciuria, and G is one patient with CRF (plasma creatinine 4.1 mg%) and 25-OH-cholecalciferol intoxication (plasma calcium 12.7 mg%).

In Table 1 are shown the daily dietary intake of calcium and phosphate and the calcium, phosphate, and creatinine plasma levels in subgroups 2A, 2B, and 2C.

The test was performed on subjects fasting for 12 hr in whom all drug therapy was withdrawn for at least 3 weeks prior to the study. No patient had been given vitamin D except the patient of subgroup G. Fasting hemodialyzed patients were tested 10–12 hr after the end of the hemodialysis run. FCaA was measured in 9 of the 15 hemodialyzed patients before and after the hemodialysis run.

Three microcuries of ^{47}Ca were injected intravenously. A 3-ml blood sample was taken 30, 60, 90, and 120 min later. Then 15 μCi of ^{47}Ca were given orally in 30 ml of distilled water containing 100 mg of $CaCl_2$ as a carrier. Blood samples were taken 2, 4, 6, 8, and 10 hr after ingestion in ten patients, and only at the 2nd, 4th, and 6th hr in the others. Two hours after the oral administration of radiocalcium, they were allowed to break their fast.

We tested the reproducibility of the FCaA measurement by repeating the study in two controls belonging to Group 2A, three patients belonging to Group 2B, and three patients belonging to Group 2C.

In order to verify that the FCaA modifications brought about by dialysis in Group 2C were not dependent on the amount of carrier dose, we repeated the study in three Group 2C patients using 500 mg of $CaCl_2$.

Group 3. This group was introduced to explore the correlation between radiocalcium absorption as measured by fecal recovery and FCaA measurement.

The FCaA test was made on seven patients with various degrees of impaired renal function (plasma creatinine from 1 to 18 mg%) with ^{45}Ca on the protocol described above. The day after, 30 μCi of ^{47}Ca were given orally with 100 mg of $CaCl_2$ to patients who had been fasting for 12 hr. Stools of the following 8 days were collected and mixed together before being either homogenized in water or ashed. They were then counted in a gamma scintillator equipped with an 800-channel analyzer (Intertechnique).

CALCULATION

FCaA. The plasmatic curve plotted on logarithmic coordinates from the first four samples obtained after the intravenous injection is adjusted using a least square regression technique. The curve is then extrapolated. The plasma radioactivity sampled after oral administration gives the data for drawing the ingestion curve (Fig. 1).

FCaA calculation is based on values obtained 4 hr after the oral dose. It is again calculated with values obtained at the 6th hr.

The FCaA formula is:

$$FCaA = \frac{a}{b} \times \frac{B}{A} \times 100$$

where A is ingested dose, B is injected dose, a is $a' - a''$, a' is activity, on the ingestion curve at time t measured from the ingestion, and a'' is activity on the extrapolated part of the injection curve at time t, is activity on the injection curve after a time interval following the injection equal to t.

Fecal balance. The intestinal absorption of ^{47}Ca was estimated according to $^{57}Sc/^{57}Ca$ ratio in stool as described by Ogg, Pearson, and Veall (*11*).

Statistical studies. Student's t-test was used.

RESULTS

Controls. To test the validity of the method, the plasma curve obtained in patients of Group 1 was plotted on logarithmic coordinates after ^{47}Ca injec-

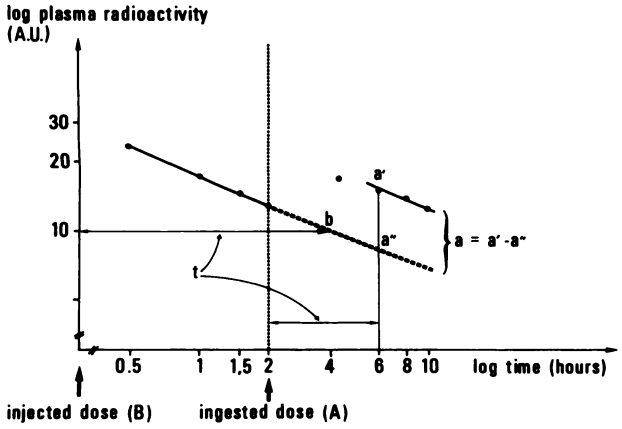

FIG. 1. Log radioactivity vs log time plasma curves. ^{47}Ca was injected at 0 time and ingested at 2 hr (for FCaA calculation see text). AU = Arbitrary Units.

tion. A straight line results, extending from 10 min to 20 hr in controls and from 30 min to 24–48 hr in patients with CRF. After the ^{45}Ca oral dose, in the same group, the plasma curve becomes linear 4 hr after ingestion. The two curves remain parallel until 20 hr after the injection (Fig. 2). In three patients with CRF undergoing hemodialysis, the plasma curve after an intravenous dose of ^{47}Ca is a straight line from 0.5 to 12 hr.

Similar results are obtained in all subjects from Groups 2 and 3 as illustrated in Table 2 for the period lasting from 4 to 6 hr. This fact allows calculation of FCaA. No significant variation of FCaA calculation at 4 hr and 6 hr has been observed.

FCaA values. FCaA of healthy controls (Group 2A) is 58.4% ± 3.6 (mean ± s.e.m.). FCaA of patients with primary hyperparathyroidism (Group 2D) are, respectively, 100, 87.5, and 84.5%. In patients with idiopathic hypercalciuria it is 86, 82, and 70%, respectively. It is 76 and 66% in the two patients with Paget's disease and 100% in the patient with 25-OH-cholecalciferol intoxication (Fig. 3).

The mean FCaA of the 20 patients with CRF (Group 2B) is 35.8% ± 2.6. It is significantly lower than the FCaA of healthy controls ($p < 0.001$). There is a significant inverse linear correlation between FCaA and the plasma creatinine level ($y = -0.13 \times +50.73$, $r = -0.55$, $p < 0.01$). In hemodialyzed patients (Group 2C), FCaA is 50.6% ± 2.5. This rate is significantly different from the rate observed in CRF patients who are not on hemodialysis ($p < 0.01$). This result is not significantly different from that obtained in healthy controls ($p < 0.05$).

The FCaA was measured in 6 of the 15 hemodialyzed patients before and 10–12 hr after the hemodialysis run. Hemodialysis significantly increases the FCaA value ($p < 0.01$) (Fig. 4).

When a carrier dose of 500 mg is used, a similar increase is noted. FCaA before hemodialysis is 20.4, 31.3, 17.4% and after hemodialysis is 22.7, 64.2, 26.2%, respectively.

Repeated studies made in eight subjects indicate that variations in FCaA calculation are in the order of ±2%.

Relation between FCaA and radiocalcium retained as measured by fecal loss. There is a significant linear correlation between these two parameters ($y = 0.93x - 4.21$, $r = 0.94$, $p < 0.001$). The mean value of radiocalcium retained as measured by fecal loss is about 7% higher than when estimated with FCaA.

DISCUSSION

The test we propose for the measurement of FCaA takes less than 8 hr to perform. This short period minimizes errors which may be induced by changes in calcium metabolic homeostasis. This fact represents an advantage over the other simplified techniques which have been proposed to measure FCaA. FCaA can be calculated after an oral dose of ^{47}Ca followed by an intravenous dose of ^{47}Ca, 1–14 days later, using serial forearm counting (*6*). The results obtained by this technique are influenced by incorporation of ^{47}Ca into bone and soft tissues.

The test we propose requires only one isotope. This is an advantage over the technique proposed by De Grazia and Rich (*1*), where FCaA can be calculated from comparative diffusion of an intravenously and an orally administered dose of ^{47}Ca and ^{45}Ca. Blood or urine sample must be counted immediately and 1.5–2 months later after near total decay of ^{47}Ca has occurred. However, simultaneous counting of both isotopes is possible but necessitates an elaborate method (*2*).

The plasma radioactivity curve, after adminis-

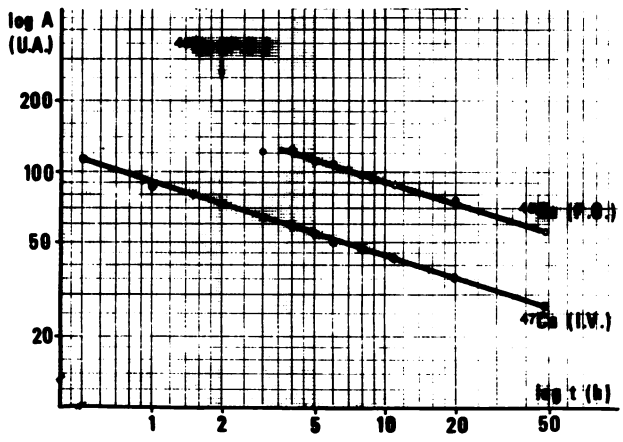

FIG. 2. Log radioactivity vs log time of ^{47}Ca injected and ^{45}Ca ingested: (patient EB, CRF, plasma creatinine 15 mg%). AU = Arbitrary Units.

TABLE 2. FCaA CALCULATED ACCORDING TO PLASMA SAMPLES TAKEN AT 4 AND 6 HR AFTER INGESTION OF RADIOCALCIUM AND EXPRESSED IN % DOSE

FCaA	No.	4 hr	6 hr	t	p
Healthy controls	9	58.4 ± 3.6	56.2 ± 3.8	0.436	0.60
CRF	20	36.0 ± 2.8	35.1 ± 2.3	0.250	0.80
CRF on hemodialysis	15	50.6 ± 2.8	50.7 ± 2.6	0.026	0.90

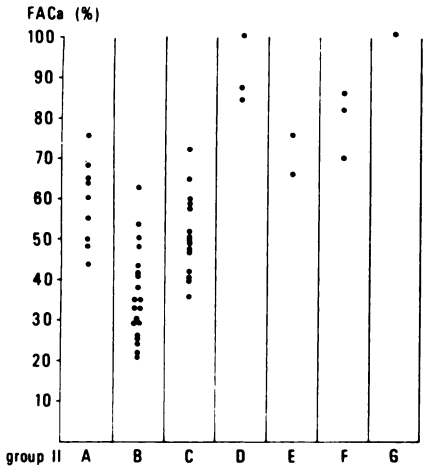

FIG. 3. FCaA values in various subgroups 2 (A = healthy controls, B = CRF, C = CRF undergoing hemodialysis, D = primary hyperparathyroidism, E = Paget's disease, F = primary hypercalciuria, G = CRF with 25-OH-cholecalciferol intoxication.

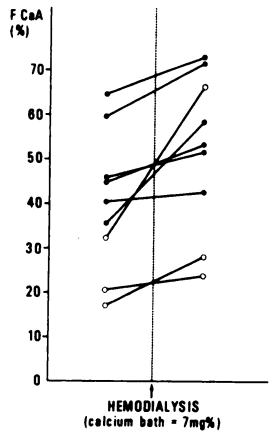

FIG. 4. FCaA before and after hemodialysis run in nine patients belonging to group 2C. Carrier dose of $CaCl_2$:100 mg (closed circle), 500 mg (open circle).

tration of the oral dose, has the same slope as the injection curve from 4 hr after ingestion, both in healthy controls and in patients. This means that, after 4 hr, an equilibrium is maintained between absorption from the gut and disappearance from the plasma. A value for FCaA may theoretically be obtained from the 4 hr-sample. FCaA measured from a 6 hr-sample showed no significant difference from that obtained from the 4 hr sample in Groups 2A, 2B, and 2C (Table 2) (insufficient numbers in Groups 2D to 2G). This hypothesis is supported by the findings of numerous authors (*6,12–14*). They have shown that net calcium absorption is achieved within 3 hr from ingestion.

FCaA varies with the carrier dose mainly related to the specific radioactivity of the ^{47}Ca ingested (*1,6*). We have chosen to perform all tests with 100 mg of $CaCl_2$. This quantity permits a good dissociation between the injection curve and the ingestion curve. It allows detection of all variations of FCaA whatever the age (*15*), the diet (*15–17*), the physicochemical state of calcium in the intestinal lumen (*18*), the biliary secretion (*19*), the parathyroid hormone secretion, or the vitamin D administration (*20,21*).

The amount of ^{47}Ca used for the test provides a bone radiation less than 1 rad. The absorbed dose has been estimated for bone retention of the whole dose without exit. This value is 30 times lower than the permissible annual maximal dose allowed for professional exposure (*22*).

The FCaA results measured according to plasma curves are lower than those obtained by fecal studies. This difference could be explained by minor losses in stool collection, delayed excretion of ^{47}Ca beyond the collection, enteropathic cycling of calcium as suggested by Birge (*6*), or by hypothetical transitory sequestration of calcium in the intestinal wall. However, the strong correlation between the values obtained with these two methods tends to validate our test.

The various rates of FCaA that we have observed in our different groups of subjects confirm the results already published. Intestinal absorption of radiocalcium in primary hyperparathyroidism, idiopathic hypercalciuria, Paget's disease, and vitamin D intoxication is significantly higher than in healthy subjects ($p < 0.001$). On the contrary, FCaA in untreated CRF is diminished ($p < 0.001$) (*23,24*), this diminution being proportional to the degree of renal failure. Our experience with hemodialyzed patients (calcium bath = 7 mg%), whether the carrier dose used is 100 or 500 mg, shows that a transient increase of FCaA occurs after each run. This result conflicts with other published data (*25–27*).

We have found no significant correlation between the FCaA variation and variations of plasma creatinine, plasma phosphates, plasma bicarbonates, and plasma protein induced by the hemodialysis run. Further studies are necessary for statistical correlation.

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