# STUDIES IN CALCIUM METABOLISM. THE FATE OF INTRAVENOUSLY INJECTED RADIOCALCIUM IN HUMAN BEINGS [1, 2, 3]

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In the course of studies on the effect of phytates on calcium uptake in man (1) it became necessary to determine the extent to which endogenous calcium in the feces might contribute to the calcium balance. Although this point has been studied by several investigators (2–5), there is considerable disagreement on the significance of the fecal route for calcium excretion in man. Thus Malm (6) has observed that different individuals may exhibit pronounced variations in the fecal calcium excretion and has suggested that extended observations be made on each proposed test subject. Since this approach is not practical in many cases, it seemed desirable to establish the significance of the endogenous calcium output in the feces under our experimental conditions.[5]

The method chosen for investigation was to inject radiocalcium ($Ca^{45}$) intravenously and to determine its concentration in the serum, urine and feces during several days. Such studies have been carried out in animals (7–10), but few such data are available for man (11–13).

## MATERIAL, DESIGN AND METHODS

*Subjects.* Nine adolescent boys, institutionalized for mental inadequacy, but otherwise normal,[6] served in the first study (Experiments A and B). In a later experiment (Experiment C), one individual (No. 57) was selected for intensive study. He was a spastic, but neither clinical nor laboratory examinations revealed any abnormalities in his mineral metabolism. The boys ranged in age from 10.9 to 15.7 years, with mean and median ages of 12.6 years. Their height ranged from 124 to 160 cm. with mean and median heights of 142 cm. Their weight ranged from 29.8 to 44.5 kgm., with a mean weight of 35.6 kgm., and a median weight of 36.0 kgm. The mental age of the boys averaged 7.2 years, (median: 7.2 years) and ranged from 5.8 to 9.2 years.

The young male adult in Experiment C was 21 years old, weighed 51.4 kgm. and had a mental age of 10.0 years.

*Design.* The subjects (Experiments A and B) were divided into two groups. One group (I.V.) was given the $Ca^{45}$ intravenously, while the other group (P.O.) received $Ca^{45}$ by mouth. One month later, the P.O. group received the intravenous injection, while the I.V. group received the isotope orally.[7] This was done to minimize the effect of periodic or seasonal variations (6, 14) and to use each individual as his own control. Preliminary tests had shown that one month after the administration of 0.7 $\mu c$ $Ca^{45}$, the level of $Ca^{45}$ in the blood and in the excreta was no longer measurable. Two years later (Experiment C), one individual was studied intensively

[1] A portion of this paper was presented at the XIX Physiological Congress, Montreal, Canada, August 31–September 4, 1953. This article is based in part on a dissertation presented by one of us (FB) in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Food Technology, Massachusetts Institute of Technology (1952).

[2] This investigation was supported by grants of the Quaker Oats Company, The National Institutes of Health (NIH Contract A-81 and A-81[C]) and the Atomic Energy Commission (AEC Contract AT[30-1]952).

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[5] When Dr. F. X. Aylward was a guest of the Massachusetts Institute of Technology, his friendly interest in our studies provided some of the original stimulus for establishing this point.

[6] All of the subjects were under medical care and observation during their usually indefinite institutionalization. They were selected on the basis of clinical examination. Roentgenographs of the chest, knees, hands, skull and spine of six subjects (Nos. 10, 11, 13, 14, 15, 16) were evaluated by an experienced roentgenologist, who concluded that they did not deviate from the normal range with respect to calcification.

[7] The results on the study with ingested $Ca^{45}$ will be presented elsewhere.

with a larger dose of intravenously administered $Ca^{45}$ under conditions similar to those which prevailed in Experiments A and B.

*Dietary regime.* All individuals ate the regular institutional diet, which was shown by analysis to meet the Recommended Dietary Allowance of the National Research Council (15) for subjects of this type. Beginning two weeks preceding, and continuing through the experiment, all subjects received daily an additional glass (240 ml.) of milk and one multi-vitamin tablet.[8] On the day of the experiment, the subjects ate a special test breakfast consisting of 180 gm. of cooked oatmeal (approximately 35 gm. rolled oats, 8.3 per cent moisture), and of 240 ml. whole milk. The total calcium and phosphorus contents of the breakfasts were, respectively, 329 and 370 mgm. in Experiment A, 294 and 295 mgm. in Experiment B, and 295 and 303 mgm. in Experiment C.

*Radiocalcium.*[9] The $Ca^{45}$ was diluted in 0.85 per cent sodium chloride solution in a rubber-capped vial which was sterilized by autoclaving at 20 psi. for 20 min. Three milliliters of the solution were withdrawn from the vial into sterile 5 ml. glass syringes and injected into the antecubital vein of the subject approximately 30 minutes following the test breakfast. The dose given was 0.70 microcuries ($152 \times 10^3$ counts/minute) in Experiment A, 0.74 microcuries ($159 \times 10^3$ c/m) in Experiment B, and 2.02 microcuries ($583 \times 10^3$ c/m) in Experiment C.

The accuracy of the dose measured in this way was tested by "injecting" a dose into a volumetric flask with the aid of a syringe and measuring the radioactivity of a suitably diluted aliquot. The quantity of $Ca^{45}$ dispensed was found to be identical (within five per cent) to that calculated.

*Serum and specimen collections*

1. *Serum.* To reduce the number of venipunctures to a minimum, schedules of staggered collections of serum on the first day were worked out for the individuals in Experiments A and B. Thereafter, the sera were collected once daily for four days.

The serum samples in Experiment C were obtained nine times on day 1, once daily for four more days, then once weekly, and finally once monthly.

2. *Urine and feces.* Twenty-four-hour specimens of urine and feces were collected in glass jars daily for five days in Experiments A and B. In Experiment C, the collection period was extended beyond the first five days as follows: 48-hour specimens of urine and of feces were obtained on days 8–9, 15–16, 22–23, 29–30 and 58–59 following injection.

The urine was preserved by refrigeration and by the addition of glacial acetic acid, 2 per cent by volume. Feces were preserved under toluene.

*Methods of analysis for $Ca^{40}$ and $Ca^{45}$.* The procedures employed have been described previously (1). In the analysis for $Ca^{45}$ content, suitable standards were employed in all counting runs and the decay factors determined experimentally. All counting data were corrected for decay from the time of injection and are reported in terms of total activity (per cent of injected $Ca^{45}$) or in terms of specific activity (per cent of injected $Ca^{45}$ per milligram calcium).

The error in replicate calcium determinations was usually held to 3 per cent standard deviation (S.D.); when samples were ashed before isolation of the calcium oxalate, the total error approximated 5 per cent S.D.

Counting errors varied according to experiment and tissue and were commonly held below 10 per cent S.D.

## RESULTS AND DISCUSSION

### *Serum*

The results of the measurements of the specific activity of the serum in Experiments A, B and C are presented in Figure 1. The values plotted here are the experimental values after adjustment to the mean body weight of the boys of Experiments A and B. For example, 56 minutes following the injection, the observed specific activity of subject No. 16 was 0.050 per cent of the injected $Ca^{45}$ per milligram serum calcium. However, his body weight was 1.1 times that of the group average of 35.6 kgm. Since he had a proportionately larger serum calcium reservoir, the observed specific activity value was 1/10 lower than it would have been if this boy had been smaller. Consequently the adjusted specific activity value was 0.055 per cent per mgm. Ca.

Because of the schedule of staggered venipunctures on Day 1 (Experiments A and B) the resulting plot is a composite of individual curves and not strictly comparable to the curve obtained on only one individual in Experiment C. At the same time it explains the comparatively wide scatter of the values of Experiments A and B.

As can be seen from Figure 1, the specific activity of the serum decreases monotonically as a function of time and shows no apparent tendency

[8] Vi-Penta® Perles were kindly furnished by Hoffman-LaRoche Inc., and were guaranteed to contain: vitamin A, 5000 U.S.P. units; vitamin $B_1$, 3 mgm.; vitamin $B_2$, 3 mgm.; vitamin $B_6$, 1 mgm.; panthenol, 3 mgm.; niacinamide, 20 mgm.; vitamin C, 75 mgm.; vitamin D, 1000 U.S.P. units; vitamin E acetate, 1 mgm.

[9] Obtained from the Oak Ridge Installation of the Atomic Energy Commission. Authorization for use of tracer doses of $Ca^{45}$ in mentally disturbed patients was granted through the Sub-committee on Human Applications.

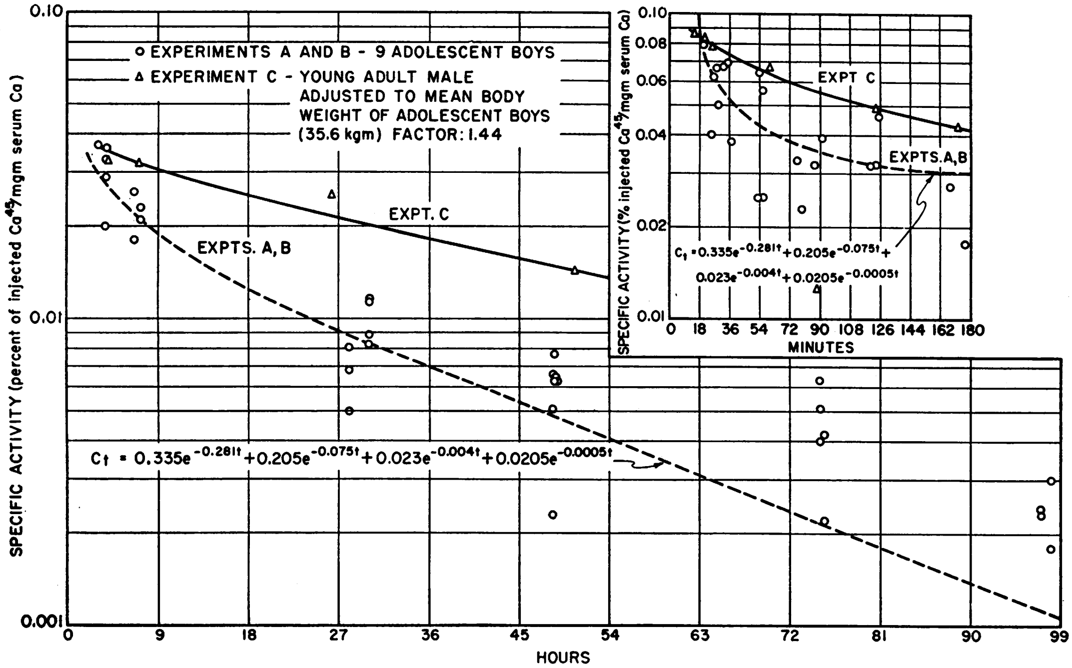


FIG. 1. DISAPPEARANCE OF INJECTED CALCIUM[45] FROM THE SERUM

Zero time points and zero level points not drawn, but included in derivation of analytical expression which is plotted.

to level off at a constant or equilibrium value.[10] It seemed of interest to derive an analytical expression for the best curve that could be drawn through the experimental points obtained in Experiments A and B. This was done by fitting a weighted sum of exponentials to the data by means of successive isolation.[11] The following expression was obtained:

$$C_t = 0.335e^{-0.281t} + 0.205e^{-0.075t} + 0.0234e^{-0.004t} + 0.0205e^{-0.0005t} \quad (1)$$

where

$C_t$ = per cent of injected $Ca^{45}$ per milligram serum calcium

and

t = minutes after injection

[10] It might be argued that the relatively small quantity of isotope given resulted in erroneously low values as time progressed and that the true values drop off more gradually than shown by the curve in Figure 1. In Figure 3, the very low values were omitted in deriving the group average. As can be seen, there is no indication that the curve reaches an equilibrium value. A similar indication of this is shown in the curves for Experiment C (Figure 4).

[11] Our thanks are due to Dr. J. G. Bryan of the Department of Mathematics, Massachusetts Institute of Technology, for having worked out the method and for having derived the equation for our use.

Equation 1 applies to a period of up to four days (6000 minutes) following the injection. Half of the observed experimental points fall within 30 per cent of the values predicted by Equation 1. The curve described by the equation is plotted in Figure 1.

In devising the analytical expression, it was necessary to include calculated zero-time values (total injected $Ca^{45}$/total serum calcium) in order to anchor down the curve. Inclusion of these values may be criticized because:

(a) Instantaneous mixing of the injected $Ca^{45}$ is assumed, yet mixing is probably not complete for at least 30 seconds (16) and a significant fraction of the injected portion may have disappeared by the time mixing was completed. (See also the curve for Experiment C, Figure 1.)

(b) The lack of data for the time interval 0 to 20 minutes in Experiments A and B makes extrapolation hazardous, as more than one exponential may be involved in that span of time.

If data had been obtained for a longer period than 6000 minutes, it is likely that terms would have to be added to Equation 1 in order to express correctly the events depicted by the experimental observations. On theoretical grounds, an expression with no less than five terms is required, if the terms are thought of as describing events in different compartments of the body (at least two compartments in the skeletal systems (17), and one each in the intestinal tract, the serum, and the soft tissues). In the present state of knowledge, however, the terms of Equation 1 ought not to be considered as denoting body compartments, but only as the mathematical representation of experimental observations (10).

Equation 2 can be used to calculate the rate at which the serum calcium leaves the blood (disappearance rate). This rate is defined as the derivative of Equation 1 at time $t = 0$, divided by $C_t$ at time $t = 0$ (*cf.* 18). Calculated for Equation 1 at $t = 0$, the disappearance rate for calcium from the serum is 19.3 per cent per minute.

Minder and Gordonoff (7) obtained disappearance rate curves on rabbits, and a value of 18.2 per cent per minute can be calculated from the expression they reported. However, Thomas, Litovitz, Rubin, and Geschickter (8), who also studied rabbits, reported much higher values, namely $88 \pm 36$ per cent per minute for old rabbits and $72 \pm 12$ per cent per minute for young rabbits. The difference between the values for the young and old rabbits was not found to be statistically significant, in contrast to observations on age differences in cattle (10). In the latter species, disappearance rates varied from 102 per cent per min. for calves 10 days old to 26 per cent per min. for cattle 13 years old.

Armstrong, Johnson, Singer, Lienke, and Premer (9), who have conducted similar studies on dogs, report a disappearance rate of 52 per cent per min. with a range of 30 to 60 per cent per minute in six animals.

The experimental limitations involved in determining the early portion of the disappearance curve also limit the interpretations which can be placed on the observed disappearance rate. For this reason, the numerical value reported here should be considered as correct only with respect to order of magnitude.

Figure 1 shows that on a comparable basis a larger fraction of the injected $Ca^{45}$ was detected in the blood of the young adult than in the blood of the boys. Though one individual is not necessarily representative of a group of young adults, the data obtained on this adult subject do not conflict with the observations of Hansard, Comar, and Davis (10) who reported a slower rate of disappearance of injected $Ca^{45}$ in older cattle as compared with young calves.

*Urine data*

The data in Tables I and II, as well as in Figure 2,[12] appear to suggest that the adolescent boys of Experiments A and B on the whole retained calcium much better than the young adult in Experiment C. However, the output of urinary $Ca^{45}$ by these boys varied from 1.5 to 11.2 per cent of the dose, as shown below:

| Subject No. | | % $Ca^{45}$ in urine in 5 days | Age (years) |
|---|---|---|---|
| 10 | | 1.47 | 11.2 |
| 11 | | 5.91 | 12.6 |
| 12 | | 2.78 | 12.4 |
| 13 | | 6.38 | 13.1 |
| 14 | | 4.82 | 10.9 |
| 15 | | 1.64 | 15.7 |
| 16 | | 6.84 | 13.1 |
| 17 | | 11.20 | 12.7 |
| 18 | | 4.46 | 11.6 |
| | Mean | 5.06 | 12.6 |
| 57 | | 13.1 | 21.0 |

There was therefore little correlation between chronological age and the urinary calcium output. The extent to which these differences indicate differences in physiological age is problematical.

Figures 3 and 4 indicate the relationship between the specific activity of the urine and the serum. As would be expected, the specific activity values of urine and serum fall on essentially similar curves. Maurer and Zimmer (19) have already described a clinical test where the specific activity of the urine is taken as a measure of the specific activity of the blood.

Blau, Spencer, Swernov, and Laszlo (12) have also reported that the specific activities of serum and urine were equal. However, their data, obtained on two adults, indicate a simple exponential decline with a half-time of eight days. Figures 3 and 4 indicate that no simple exponential decline was observed in our subjects. Furthermore, since the specific activities of urine and serum are alike, Equation 1 with its four terms will also fit the urine data of the boys.

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[12] For the purpose of the graphs shown in Figure 2, the values obtained on the 48-hour samples of urine collected in Experiment C were suitably adjusted to represent 24-hour values.

TABLE I

*$Ca^{40}$ and $Ca^{45}$ levels in the urine and feces of nine boys following a single intravenous injection of $Ca^{45}$*

| | | | Urine | | | Feces | | |
|---|---|---|---|---|---|---|---|---|
| Expt. | Subject | Day | Calcium *mgm.* | Per cent of injected $Ca^{45}$/mgm. Ca | Per cent of injected $Ca^{45}$ | Calcium *mgm.* | Per cent of injected $Ca^{45}$/mgm. Ca | Per cent of injected $Ca^{45}$ |
| B | 10 | 1 | 44 | 0.0157 | 0.69 | 28 | <0.0007* | <0.02* |
| | | 2 | 28 | 0.0078 | 0.21 | 1,064 | 0† | 0† |
| | | 3 | 37 | 0.0060 | 0.23 | 1,082 | 0.0022 | 2.40 |
| | | 4 | 54 | 0.0051 | 0.28 | ‡ | | |
| | | 5 | 34 | 0.0023 | 0.08 | ‡ | | |
| | 11 | 1 | 169 | 0.0210 | 3.55 | 1,009 | <0.013 | <0.1 |
| | | 2 | 138 | 0.0079 | 1.09 | 116 | 0 | 0 |
| | | 3 | 146 | 0.0041 | 0.59 | 662 | <0.0012 | <0.8 |
| | | 4 | 152 | 0.0035 | 0.53 | | | |
| | | 5 | 50 | 0.0030 | 0.15 | | | |
| | 12 | 1 | 64 | 0.0204 | 1.30 | 798 | <0.0008 | <0.06 |
| | | 2 | 66 | 0.0076 | 0.50 | 373 | 0 | 0 |
| | | 3 | 96 | 0.0052 | 0.50 | 1,306 | <0.0002 | <0.03 |
| | | 4 | 64 | 0.0038 | 0.24 | | | |
| | | 5 | 23 | 0.0102 | 0.23 | | | |
| | 13 | 1 | 167 | 0.0188 | 3.12 | 1,190 | 0 | 0 |
| | | 2 | 198 | 0.0074 | 1.48 | 822 | 0 | 0 |
| | | 3 | 266 | 0.0048 | 1.28 | 32 | <0.0021 | <0.07 |
| | | 4 | 83 | 0.0036 | 0.30 | | | |
| | | 5 | 121 | 0.0022 | 0.20 | | | |
| A | 14 | 1 | 151 | 0.0200 | 3.02 | 203 | 0 | 0 |
| | | 2 | 93 | 0.0146 | 1.36 | 2,011 | 0.0023 | 2.24 |
| | | 3 | 93 | 0.0047 | 0.44 | 432 | 0.0050 | 2.11 |
| | | 4 | sample lost | | | 1,059 | <0.0014 | <1.5 |
| | | 5 | sample lost | | | 618 | <0.0016 | <1.0 |
| | 15 | 1 | 71 | 0.0156 | 1.11 | 604 | 0 | 0 |
| | | 2 | 62 | 0.0043 | 0.27 | 1,728 | <0.0006 | <1.0 |
| | | 3 | 61 | 0† | 0† | 993 | <0.0024 | <2.3 |
| | | 4 | 56 | 0.0029 | 0.16 | 1,074 | 0 | 0 |
| | | 5 | 80 | 0.0013 | 0.10 | 119 | 0 | 0 |
| | 16 | 1 | 167 | 0.0216 | 3.76 | 901 | <0.0003 | <0.2 |
| | | 2 | 247 | 0.0059 | 1.65 | 1,111 | 0.0022 | 2.48 |
| | | 3 | 175 | 0.0033 | 0.59 | 1,745 | 0 | 0 |
| | | 4 | 163 | 0.0035 | 0.57 | 1,463 | 0 | 0 |
| | | 5 | 126 | 0.0023 | 0.27 | 198 | 0 | 0 |
| | 17 | 1 | 337 | 0.0204 | 6.88 | — | — | — |
| | | 2 | 279 | 0.0082 | 2.31 | 386 | 0 | <0.02 |
| | | 3 | 269 | 0.0065 | 1.02 | 1,802 | 0.0019 | 3.44 |
| | | 4 | 204 | 0.0034 | 0.69 | — | — | — |
| | | 5 | 193 | 0.0029 | 0.27 | 930 | 0 | 0 |
| | 18 | 1 | 138 | 0.0169 | 2.33 | 16 | 0 | 0 |
| | | 2 | 139 | 0.0064 | 0.89 | 1,047 | 0.0053 | 1.40 |
| | | 3 | 171 | 0.0042 | 0.72 | 509 | 0.0022 | 1.11 |
| | | 4 | 104 | 0.0028 | 0.34 | 1,550 | 0 | 0 |
| | | 5 | 116 | 0.0016 | 0.18 | 314 | 0 | 0 |

* The results preceded by the "less than" (<) sign represent counting data which did not differ from background on the 5 per cent significance level, but where all replicate measurements gave counts above background. They are minimum counting values, representing the probable maximum output of $Ca^{45}$ by an individual on that day.

† "0" values represent counting data which did not differ significantly from background.

‡ Because the $Ca^{45}$ content of the feces of subjects No. 10–13 was so very low, analyses were not carried out on the fecal samples of Experiment B which had been collected on Days 4 and 5.

TABLE II

*$Ca^{40}$ and $Ca^{45}$ excretion in the urine and feces of a young adult male following a single intravenous injection of $Ca^{45}$ on the morning of Day 1 (Experiment C)*

| Day | Urine | | | Feces | | |
|---|---|---|---|---|---|---|
| | Ca *mgm.* | Per cent of injected $Ca^{45}$/mgm. Ca | Per cent of injected $Ca^{45}$ | Ca *mgm.* | Per cent of injected $Ca^{45}$/mgm. Ca | Per cent of injected $Ca^{45}$ |
| 1 | 133 | 0.262 | 3.49 | 552 | 0.00006 | 0.31 |
| 2 | 262 | 0.152 | 4.01 | 2,510 | 0.0012 | 2.90 |
| 3 | 225 | 0.086 | 1.94 | 1,100 | 0.00067 | 0.72 |
| 4 | 236 | 0.089 | 2.10 | 853 | 0.0019 | 1.64 |
| 5 | 278 | 0.058 | 1.60 | 1,190 | 0.00097 | 1.30 |
| 8–9 | 549 | 0.031 | 1.69 | 2,460 | 0.00042 | 1.01 |
| 15–16 | 524 | 0.012 | 0.60 | 2,110 | 0.00017 | 0.33 |
| 22–23 | 431 | 0.009 | 0.37 | 2,820 | 0.00013 | 0.34 |
| 29–30 | 457 | 0.006 | 0.25 | 1,980 | 0.00008 | 0.17 |
| 57–58 | 533 | 0.002 | 0.12 | * | 0.00010 | 0.14 |

* Repeated analyses gave inconsistent results. Specific activity calculated on basis of each aliquot's actual Ca content.

*Feces data*

Tables I and II and Figure 5 [13] appear to indicate that the boys in Experiments A and B excreted less $Ca^{45}$ in their feces on the average than the young adult of Experiment C. In view of the reports of Thomas, Litovitz, Rubin, and Geschickter (8) and of Hansard, Comar, and Davis (10), this difference can possibly be interpreted as reflecting higher retention of $Ca^{45}$ by the boys, as compared with the older adult. Only studies with groups of young male adults will make it possible to extend this observation.

The peak $Ca^{45}$ output in the feces occurred on

[13] For the purposes of the graphs shown in Figure 5, the values obtained on the 48-hour samples of feces obtained in Experiment C were suitably adjusted to represent 24-hour values. For this purpose use was made of records kept by the attendant to indicate time and date of defecation on the part of the experimental subject.

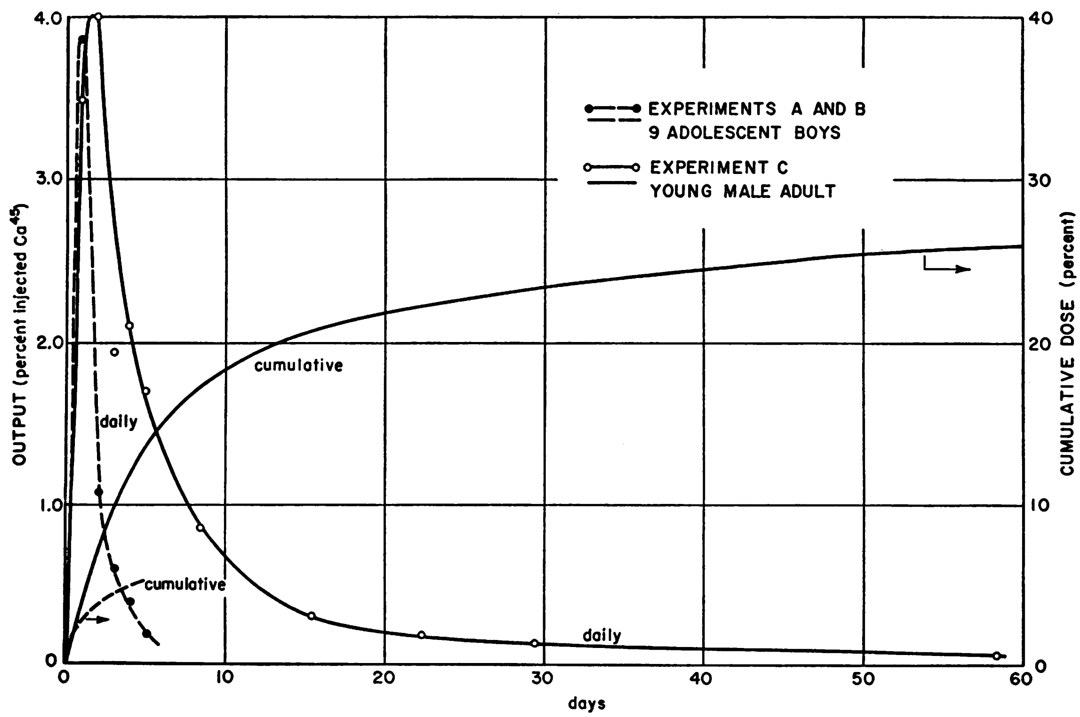


FIG. 2. EXCRETION OF CALCIUM[45] IN THE URINE

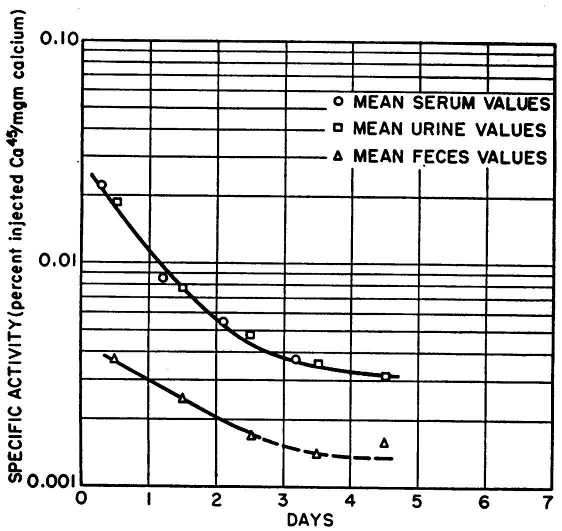


FIG. 3. MEAN SPECIFIC ACTIVITY OF $Ca^{45}$ IN SERUM, URINE AND FECES OF NINE ADOLESCENT BOYS (EXPERIMENTS A AND B)

Counts not differing significantly from background ("0" values, Table I) excluded.

the second or third day, in agreement with the results of Bellin and Laszlo (11).

*Combined excretion data*

The data in Tables I and II indicate that the total quantity of injected $Ca^{45}$ excreted during the first few days is quite small. In no case did the combined output of isotope exceed 7 per cent of the injected $Ca^{45}$ during any one day (Tables I and II), or 15 per cent during the first five days (Table II). The average combined excretion of the boys in five days was only 8 per cent (Figure 6).

Clearly these observations also apply to the quantity of absorbed $Ca^{45}$ which these subjects might reëxcrete in a comparable period following a test meal containing $Ca^{45}$. If, for example, one of the boys had eaten two microcuries of $Ca^{45}$ (suitably diluted with ordinary calcium) and if a total of one microcurie had been recovered in the stool at the end of five days, it would be possible to conclude that perhaps (1 + 0.1) microcuries had been absorbed. We have in fact observed similar relationships (1). It may therefore be said that the quantity of calcium which is absorbed and then immediately reëxcreted is quite small; perhaps it may even be neglected in a first approximation because of inherent experimental error (*cf.* [12]).

These considerations suggest that nearly all of the calcium which enters the body at a given moment is at first retained (presumably largely in the skeleton [20]), and that equilibrium is maintained by excreting calcium which had entered the

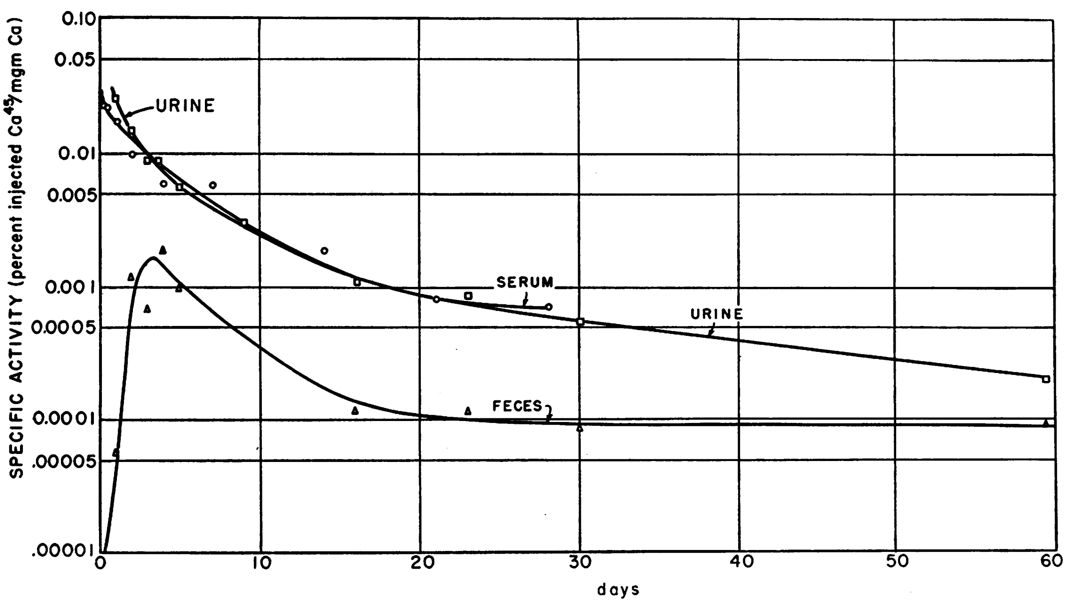


FIG. 4. SPECIFIC ACTIVITY OF CALCIUM IN SERUM, URINE AND FECES OF YOUNG ADULT MALE (EXPERIMENT C)

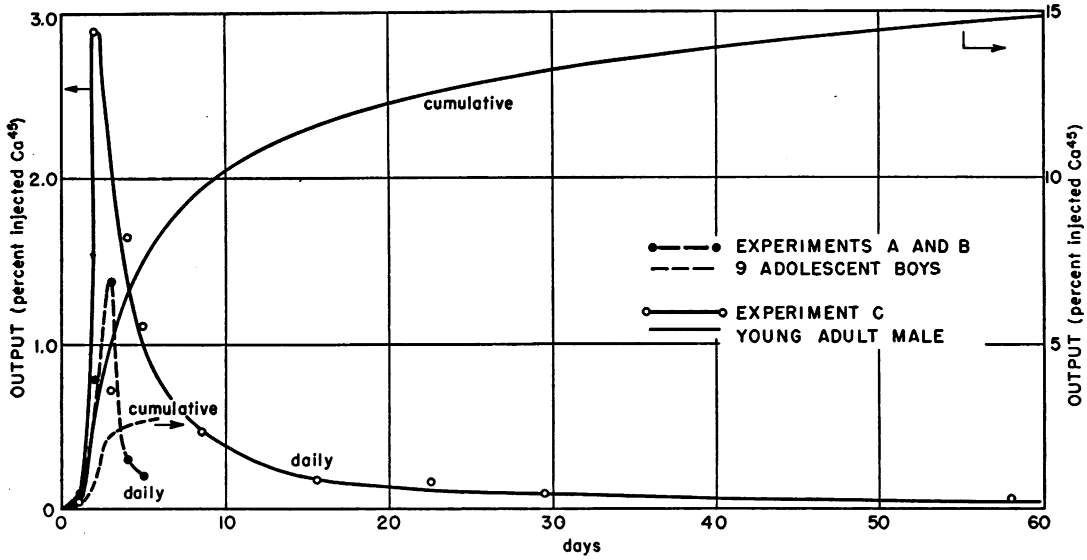


FIG. 5. FECAL OUTPUT OF INJECTED CALCIUM[45]

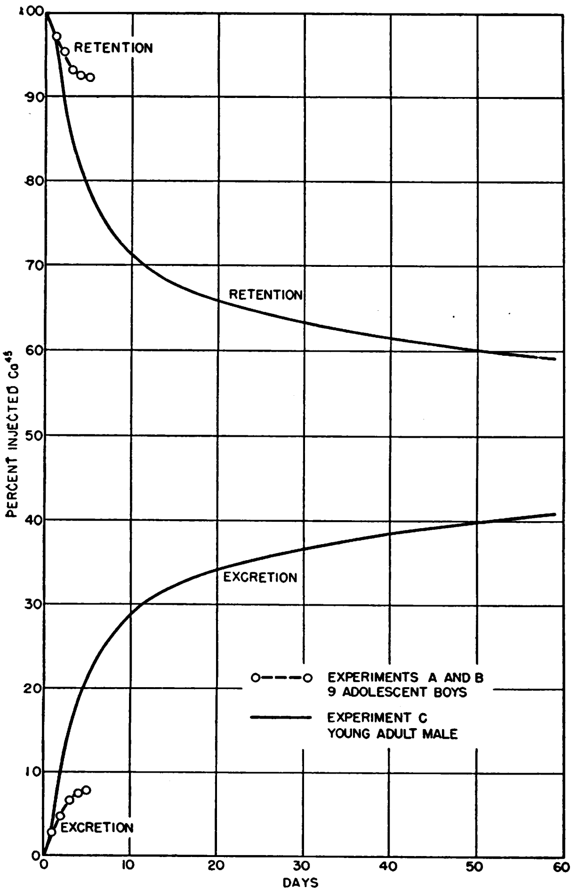


FIG. 6. TOTAL CUMULATIVE EXCRETION AND RETENTION OF INTRAVENOUSLY ADMINISTERED CALCIUM[45]

body previously. This speculation becomes more plausible if the retention of injected $Ca^{45}$ is relatively long. Singer and Armstrong (21) have reported this to be the case for rats.

Figure 6 shows the excretion and retention of $Ca^{45}$ in our subjects. As can be seen, more than half of the injected $Ca^{45}$ was still in the body of subject No. 57 (Experiment C) at the end of two months. A plot of this retention curve on semilogarithmic coordinates (Figure 7) indicates that the "biological half-life" of $Ca^{45}$ approximates

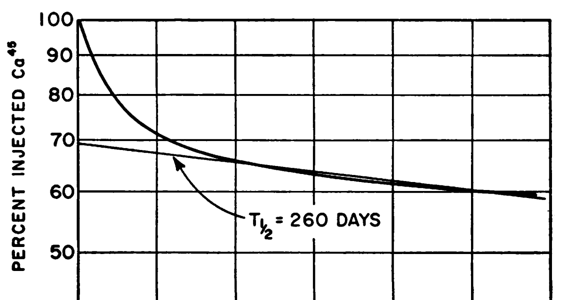


FIG. 7. COMBINED RETENTION (INJECTED $Ca^{45}$–URINARY $Ca^{45}$–FECAL $Ca^{45}$) OF RADIOCALCIUM BY YOUNG ADULT

Best straight line between 18th and 59th day is: $\hat{y} = 69.04 - 0.1784x$, where x = days. Thus, zero day = 69.04 and 59th day = 58.51; $58.51 = 69.04e^{-59a}$, where a = 0.00266; thus, $T_{1/2} = \frac{0.693}{0.00266}$ or 261 days.

260 days during the period 18 to 59 days following the injection.

*Ratio of urinary to fecal $Ca^{45}$*

Species differences appear to affect the manner in which "endogenous calcium" is partitioned between urine and feces. Evidently rats excrete eight to twenty times more endogenous calcium in the feces than in the urine under normal conditions (21–23). This is also shown by the data of L'Heureux, Tweedy, and Zorn (24), when they injected minimal quantities of inert calcium with their dose. In cows (10, 25, 26) and in dogs (9, 27) the feces also appears to be the more important route of elimination for endogenous calcium. Human beings, on the other hand, excrete more endogenous calcium in the urine than in the feces. In all of our subjects the urinary fecal ratio of injected $Ca^{45}$ (and therefore also of endogenous calcium) exceeded unity (Tables I and II). This ratio was plotted for subject No. 57, with the cumulative output curves (Figures 2 and 5) as a basis. It remained relatively stable at approximately 1.65 (range: 1.4 to 2.0) during the period of 17 to 59 days following the injection.

The urinary-fecal ratio of injected $Ca^{45}$ can be used to predict urinary calcium output. If it is assumed that all of the injected $Ca^{45}$ will in time be recovered in the urine and feces, then it can be calculated that $\frac{100 \times 1.65}{1 + 1.65}$ or 62.3 per cent of the injected $Ca^{45}$ will eventually have been excreted via the urine. This suggests that a comparable fraction of the absorbed dietary calcium is excreted in the urine. These subjects absorbed approximately 40 to 50 per cent of the one gram of calcium fed in their diets.[14] Therefore, the urinary calcium output of the young adult ought to have approximated $0.45 \times 1 \times 0.62$ or 0.28 gm. His average output was actually 0.24 gm. (calculated from the data of Table II).

Undoubtedly, many factors affect the partition of endogenous calcium between the urinary and the fecal routes. Thus Steggerda and Mitchell (4) were able to increase materially the calcium output in the stool of human subjects by the administration of citrate ion. Yet it is noteworthy that the ratio of urinary to fecal $Ca^{45}$ output also exceeded unity in the subjects studied by Geissberger (13), by Bellin and Laszlo (11) and by Blau, Spencer, Swernov, and Laszlo (12).

*Endogenous calcium in feces*

In an injection experiment all of the $Ca^{45}$ in the feces is of endogenous origin; as a result, the following relationship exists:

$$S.A._s = \frac{A_f}{E_f} \quad \text{and} \quad E_f = \frac{A_f}{SA_s} \qquad (2)$$

where

$S.A._s$ = specific activity of serum at time t

$A_f$ = the total activity recovered in the feces at time t

$E_f$ = the total quantity of stable endogenous calcium in the feces at time t

With the aid of Equation 2 and of the data in Figure 1 and in Table II, it can be calculated that under the conditions of Experiment C, endogenous calcium constituted approximately 15 per cent of the average daily fecal output of 1.2 gm. Ca. This is somewhat less than one-half of the total (*i.e.*, urinary plus fecal) endogenous calcium output. It is in general agreement with the data of Blau, Spencer, Swernov, and Laszlo (12).[15]

Several investigators (3, 5) have been unable to observe any effect on fecal calcium output as the result of massive intravenous injections of inert calcium ($Ca^{40}$). As a result it has often been asserted that the feces do not represent an important avenue for the excretion of endogenous calcium. Evidence from this communication, as well as results of animal experiments (22–26, 29), leave no room to doubt that endogenous calcium finds its way into the gastrointestinal tract and that some of it is excreted in the feces.

The increase in the fecal calcium excretion of an adult human being following an intravenous injection of as much as 500 mgm. inert Ca in a

[14] On the basis of studies to be published. Similar relationships are also reported by Blau, Spencer, Swernov, and Laszlo (12).

[15] While this article was in press, Brine and Johnston (28) published calculations based on studies which have appeared in the literature to the effect that when the calcium intake is zero, the probable calcium output in the feces of adult man approximates 75 mgm. This quantity may be considered the endogenous calcium output and is reasonable confirmation of the studies reported here.

day would probably amount to no more than 50 mgm., or five to ten per cent of his daily fecal output (on the assumption that the calcium is injected under physiological conditions). Clearly this increase would be difficult to detect and experiments in which inert calcium is injected intravenously therefore tend to lead to negative results.

## SUMMARY AND CONCLUSIONS

1. The fate of intravenously injected radiocalcium ($Ca^{45}$) was studied in nine boys and one young adult, all of whom had been institutionalized for mental inadequacy.

2. The rate at which the injected $Ca^{45}$ disappeared from the blood of the nine boys was determined. Observations made over a period of five days, together with assumed zero-time values, were expressed by the equation:

$$C_t = 0.335e^{-0.281t} + 0.205e^{-0.075t} + 0.0234e^{-0.004t} + 0.0205e^{-0.0005t} \quad (1)$$

where

$C_t$ = specific activity of the serum (per cent dose per mgm. Ca)

and

$t$ = time (minutes)

This equation yielded a disappearance rate for the serum calcium of approximately 19 per cent per minute.

3. The average quantity of injected $Ca^{45}$ excreted in the urine and the feces by the nine boys did not exceed 8 per cent by the end of five days. The young adult excreted a total of 20 per cent during this period, and, by the end of sixty days, he had excreted a total of approximately 40 per cent of the injected dose.

4. In all individuals studied the urine constituted the more important route of $Ca^{45}$ elimination and, on the average, contained 1.5 to 2 times the quantity of $Ca^{45}$ excreted in the feces.

5. The quantity of injected $Ca^{45}$ excreted in the feces during several days was small. This suggests that only a minor fraction of the calcium absorbed on any one day is reëxcreted promptly and that the major portion is retained for some time. The "biological half-life" of $Ca^{45}$ in one young adult was calculated to approximate 260 days, during the period of 18 to 59 days following the injection of the isotope.

6. Because injected $Ca^{45}$ was recovered in the feces it would appear that the feces serve as one route for the elimination of endogenous calcium. Data obtained on one young adult over a period of two months indicate that approximately 15 per cent of his average daily fecal output of 1.2 gm. Ca was endogenous in origin.

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