In the region of neutrality, and 2 pH units on either side of it, a substantial variance occurs at the lowest irradiation level which gradually lessens as the dose is increased. To this portion of the curve are attributable the imidazole groups of the protein. It is evident that the low molecular weight fraction (F-II) requires acid/-base milliequivalents of constant value in this region whereas the high molecular weight fraction initially (0.37 Megarads) binds 0.12 milliequivalents of base; this requirement gradually diminishes as the dose of ionizing radiation is increased.

In 1:20 solute:solvent ratio during the ionization process, a given gelatin fraction shows entirely different charge structure from that when irradiation occurs at 1:100 ratio. The decrease in volume requirements of acid and base indicates a reduced availability of titratable amino and carboxyl groups.

In subsequent reports we shall present the results of further studies showing experimental evidence supporting our belief that the two fractions not only differed from each other but behaved differently under changing irradiation stress.

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Kinetics of Steroid Effects on Ca^{47} Dynamics in Dogs with the Analog Computer I

By EDWARD R. GARRETT†, RICHARD L. JOHNSTON, and ELLIOTT J. COLLINS

Intravenous administration of the short-lived isotope Ca^{47} in dogs was followed by the measurement of Ca^{47} in blood, feces, and urine during a control phase, a steroid regimen (2 mg./Kg./day), and a final recovery, post-steroid phase. Methods and procedures were developed to analyze the data by a combination of digital and analog computer techniques. In all dogs and in all phases the specific rate constants of a polyexponential fit for the distribution of Ca^{47} in all hypothetical compartments under all the experimental conditions studied were similar. The cumulative fecal and urinary Ca^{47} eliminations were fitted to a first order curve as a function of time by the analog computer. The most striking effect of steroid therapy in the young dog used as his own control is the increase in first order Ca^{47} elimination within the first 200 hours to $1\frac{1}{2}$ to 2 times the control values. After cessation of steroid therapy, the per cent of the total intravenous dose eliminated decreased and approached the amount of the control period within these 200 hours. The residual Ca^{47} is tied up in the bone and is slowly released at an apparently linear rate over longer periods of time. The rate of release between 200 and 700 hours is twice as great with steroid regimen as without for both control and recovery phases.

RADIOCALCIUM and other bone-seeking isotopes have been used in kinetic analyses of changes in blood and excretion of injected radioisotope (1–4). The radioisotope, Ca^{47}, has recently become available and presents certain advantages over previous radiocalcium studies in that it is a gamma emitter of short half-life of 4.56 days and an animal can be used as his own control in short-term sequential studies of the effect of a therapeutic agent or experimental condition on the kinetics of radiocalcium distribution, metabolism, and excretion.

The advantages of this isotope have permitted us to initiate an investigation of the osteoporotic effects of adrenal steroid regimen and its potential reversibility. Preliminary studies have demonstrated that this phenomenon is common to all the adrenal steroids in varying degree (5, 6) but one was chosen for the detailed study presented in this paper.

This paper presents a detailed quantitative analysis of our first studies on the effects of adrenal steroids on the distribution and kinetics of intravenously administered Ca^{47}. The digital- and analog-computer techniques described herein greatly facilitated the quantification of the huge masses of data it was necessary to obtain in such studies.

Special emphasis was also placed on the initial disappearance curves of radiocalcium from the blood, studies of which have not received too much attention previously (7).

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The technical assistance of Vernon F. Baker, D. J. Weber, and R. S. Schwikert is gratefully acknowledged. Without the technical knowledge and collaboration of Clayton D. Alway, the analog computer would not have been applicable. The operation of the digital computer by E. Markovich was greatly appreciated.
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METHODS

Treatment of Animals.—Three beagle dogs and one mongrel were used in these experiments. The animals were conditioned to the metabolism cages and laboratory routine prior to experimentation. Pertinent data for dogs 0 through 3, given as initial age in months, body weight in Kg. and sex are for dog 0: 7, 9.30, M; dog 1: 7, 7.50, M; dog 2: 9, 6.25, F; and dog 3: mongrel of indeterminate age, 9.20, F. The methods and procedures of intravenous administration of Ca^{47} and of the determination of radioactivity in blood serum, urine, and feces have been detailed elsewhere (5).

The studies were conducted in five sequential phases. Ten days after the initial administration and study of the distribution of 50 μc. of Ca^{47} (control, study *A*), 2 mg./Kg./day of 6α-methylprednisolone were given orally. Two weeks later, Ca^{47} was again administered and the distribution studied (steroid, study *B*). Five weeks after initiating steroid therapy, Ca^{47} was again administered and the distribution studied (steroid, study *C*). Steroid therapy was reduced at the rate of 1 mg./day for 52 days and was halted 112 days after its inception. The radioisotope Ca^{47} was administered and the distribution studied at 40 days (recovery, study *D*) and at 117 days (recovery, study *E*) after cessation of steroid therapy.

The dogs were sampled for blood after intravenous administration of Ca^{47} at 2, 4, 6, 8, 10, and 15 minutes; then at 15-minute intervals to 120 minutes; at 30-minute intervals to 4 hours; at hourly intervals to 9 hours; at 12, 24, 30, 36, and 48 hours; and at subsequent 24-hour intervals for 10 days.

The urine was collected at 4, 8, 12, 24, 30, 36, and 48 hours and at subsequent daily intervals until the study was terminated. Catheterization was used throughout the first 48 hours. The feces were collected at the end of 12 hours and at subsequent daily intervals.

In the studies biopsies were performed on a long bone and costal bone 48 hours after radioisotope administration, each time from a different limb. Antibiotics, tetracycline phosphate and novobiocin, were administered for 8 days after surgery. The site of the biopsy was replicated as closely as possible. However, the data were so variable from dog to dog and without any consistent pattern among the sequence of studies *A* through *E* that they are not worth reporting. Similar experiences have been encountered from biopsies of bone after radioisotope administration by Rich (8).

Treatment of Data.—The actual counts of Ca^{47} in blood, feces, and urine were converted into the % of the initial count of the dose of Ca^{47} given to each dog. This necessitated the counting of a standard aliquot of Ca^{47} in solution simultaneously with the samples of the studies to correct the values for the natural radioactive decay of Ca^{47}. The errors in counting were computed by statistical methods considering the time of counting and the magnitude of the count for the tissue, the standard, and the background (9).

The blood serum data reported consisted of the time of blood withdrawal, the total radiation count, c for v ml. of serum counted for t minutes. Alternately, a background count c_B, was taken for t_B minutes. A fraction of the Ca^{47} solution used for injection was conserved as a standard and was counted alternately with all samples, a count of c_S for t_S minutes. The initial total Ca^{47} dose, D in c.p.m. after correction for background, was also counted alternately with the standard, c_{S_o} minutes, at the time of intravenous administration of the dose. The Burroughs *E*-102 digital computer was programed to calculate and read out the Ca^{47} content of blood serum at time t, $Ca_B{}^{47}$, per ml. of serum as the % of the initial total dose as

$$Ca_B{}^{47} \text{ (per ml. serum, \% of total dose)} = 100\, q(c/tv - c_B/t_B)/D \quad \text{(Eq. 1)}$$

where

$$q = (c_{S_o}/t_o - c_{B_o}/t_o)/(c_S/t_S - c_B/t_B) = S_o/S \quad \text{(Eq. 2)}$$

The error E due to counting per ml. serum as the % of the initial total dose can be estimated from

$$E = Ca_B{}^{47}\sigma_{qc}/qc \quad \text{(Eq. 3)}$$

where the counting error of D is ignored since D is a constant for all serum samples. The fractional error in the count after correction for the normal radioactive decay of Ca^{47} can be estimated (10) as

$$\sigma_{qc}/qc = \sqrt{\sigma_c{}^2/c^2 + \sigma_q{}^2/q^2} \quad \text{(Eq. 4)}$$

where

$$\sigma_c = K\sqrt{c/t^2 + c_B/t_B{}^2} \quad \text{(Eq. 5)}$$

and

$$\sigma_q = q\sqrt{\sigma_{S_o}{}^2/S_o{}^2 + \sigma_S{}^2/S^2} \quad \text{(Eq. 6)}$$

$$\sigma_S = K\sqrt{c_S/t_S{}^2 + c_B/t_B{}^2} \quad \text{(Eq. 7)}$$

$$\sigma_{S_o} = K\sqrt{c_{S_o}/t_{S_o}{}^2 + c_B/t_B{}^2} \quad \text{(Eq. 8)}$$

where the K values are given in the literature (9).

When 9.158×10^6 c.p.m. of Ca^{47} were intravenously administered with a standard count at the time of injection of $S_o = 3891$ for $t_{S_o} = 1$ minute with a background count of $c_B = 1070$ for $t_B = 30$ minutes, typical data for a blood serum sample taken 2 minutes later are: $c = 6625$, $t = 1$ minute, $v = 1$ ml., $c_B/t_B = 36$ c.p.m., $c_S = 3868$ for $t_S = 1$ minute and thus $q = 1.006263$, and $Ca_B{}^{47} = 0.0724\%$ of initial radioactive dose per ml. of serum. With a K of 1.96 for 95% limits (9) $\sigma_S = 122.4647$, $\sigma_{S_o} = 122.28968$, $\sigma_c = 159.9653$, then $\sigma_q = 0.045305$, $\sigma_{qc} = 340.42131$, $\sigma_{qc}/qc = 0.051064$, and the error E due to counting variability is $0.0724 \pm 0.0037\%$.

The urine and feces data reported were similar to that of the blood except that the volume of urine collected V was included with the v ml. of urine counted c for t minutes; and the weight of feces collected W was included with w g of feces counted c for t minutes. The methods of calculation for Ca^{47} excreted by feces and urine were similar to those outlined for the blood serum except that the fecal and urine excretion of Ca^{47} was calculated in terms of the % of the total dose

$$P = 100q(c/t - c_B/t)W/wD \quad \text{(Eq. 9)}$$

where W/w of the feces becomes V/v in the case of urine.

The cumulative % excretion of injected dose at any time is summed, $\sum_{i=1}^{n} P_i$, and the error E in the accumulated ΣP_i is estimated from

$$E(\Sigma P_i) = \sqrt{\sum_{i=1}^{n} \sigma_i^2} \qquad \text{(Eq. 10)}$$

where a σ_i for a particular i^{th} urine or fecal sample is equivalent to the previously described σ_{qc}.

For a typical urine sample taken 4 hours after intravenous injection of Ca^{47}, $c = 5467$ for $v = 4.0$ ml. counted for $t = 5$ minutes with a background count of $c_B = 1385$ for 30 minutes and a standard count of $c_S = 10773$ for $t_S = 1$ minute. The total volume of urine, $V = 5.5$ ml. The correction factor calculated out to $q = 1.06704$ so that the % of the initial dose of Ca^{47} excreted in this urine sample was $P = 0.02685\%$. Since this was the first urine sample, $\Sigma P_i = P_1$. The error computations were $\sigma_S = 203.4489$, $\sigma_{S_0} = 210.1275$, $\sigma_q = 0.028165$, $\sigma_c = 29.08595$, $\sigma_{qc} = 43.6772$, so that the fractional error of the count was $\sigma_{qc}/qc = 0.03744$. The error in % of the original dose excreted in the urine was $0.0268 \pm 0.0010\%$.

For a typical fecal sample taken 24 hours after intravenous injection of Ca^{47}, $c = 879$ for $w = 2.8437$ g counted for $t = 1.0$ minute with a background count of $c_B/t_B = 33$ c.p.m. The total weight of the feces was $W = 730$ g. The correction factor calculated out to $q = 1.318133$ so that the % of the initial dose of Ca^{47} excreted in this fecal sample was $P = 4.8356$. The error computations were $\sigma_S = 208.2955$, $\sigma_{S_0} = 238.7188$, $\sigma_q = 0.03240$, $\sigma_c = 59.1907$, $\sigma_{qc} = 83.0112$ so that the fractional error of the corrected count was $\sigma_{qc}/qc = 0.07165$. The error E in % of the original dose excreted in this fecal sample was 4.84 ± 0.351.

Analog Computer Programing and Operation for Serum Data.—The Ca_B^{47} content of a ml. of the blood serum expressed as the % of the total radioactive dose is a function of time and can be described by an additive series of exponentials (1, 7, 11–13)

$$Ca_B^{47} = \sum_{i=1}^{n} A_i e^{-k_i t} \qquad \text{(Eq. 11)}$$

The final exponential of the series, $e^{-k_n t}$, can be obtained by a direct analog computer fitting, *i.e.*, by determining the best potentiometer setting, k_n, and best initial voltage A_n to fit the terminal data of a plot of Ca_B^{47} against time by the discharge of a capacitor (14, 15).

An equivalent procedure which gives the same information as to k_n and A_n is to plot the logarithm of Ca_B^{47} against time and determine the k_n from the slope ($= k_n/2.303$) and the A_n from the intercept (log A_n at $t = 0$) of the semilogarithmic plot for the terminal data of the blood serum Ca^{47} as a function of time. Typical plots are given in Fig. 1 for the data obtained in excess of 1,000 minutes after Ca^{47} administration.

The values plotted as log Ca_B^{47} between 1,500 and 7,000 minutes (Fig. 1) approximate linearity and provide a good estimate of a first order decrease of radioisotope for the elimination of Ca^{47} from the blood characterized by an exponential. The data beyond 7,000 minutes were frequently higher than could be expected on the premise that this was the true terminal exponential. However, these aberrant values would have little effect on the summation of exponential expressions

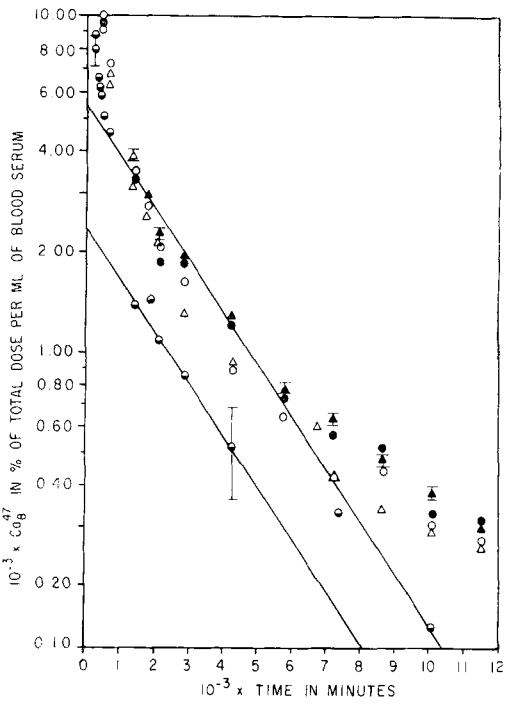

Fig. 1.—Typical semi-logarithmic plots of Ca_B^{47} in blood, % of total dose per ml. of serum, against time for that portion of total curve assigned to the final exponential. The data are for dog 0, studies A, ◐; B, ⊙; C, ●; D, △; and E, ▲. The horizontal lines above and below some of the data indicate the 95% confidence limits due to counting error.

$$\sum_{i=1}^{n-1} A_i e^{-k_i t} = Ca_B^{47} - A_n e^{-k_n t} \qquad \text{(Eq. 12)}$$

where Ca_B^{47} was defined in Eq. 11.

Both the analog computer and graphical techniques described gave similar values of k_n and A_n.

The analog computer program for determining the sum of exponentials is given in Fig. 2. The data calculated from Eq. 12 were fitted by the read out on the Mosely model 14 recorder summed from the necessary exponential functions so as to fully describe the overall decrease of Ca^{47} with time. The rapid decrease of Ca^{47} content of blood serum in the first 15 minutes was independent of the slower decreases over the remaining first thousand minutes. This is fitted on the analog computer with the best A_1 and k_1 value. Subsequently, an A_2 and k_2 value was attempted to account for the remaining data. It was found that two more exponentials were necessary to fully characterize the data so that the data have been plotted as $Ca_B^{47} - A_4 e^{-k_4 t}$ and is equal to $A_1 e^{-k_1 t} + A_2 e^{-k_2 t} + A_3 e^{-k_3 t}$. The $A_2 + A_3$ values and k_2 and k_3 values of the potentiometers were chosen to completely fit the data.

Thus the expression

$$Ca_B^{47} = A_1 e^{-k_1 t} + A_2 e^{-k_2 t} + A_3 e^{-k_3 t} + A_4 e^{-k_4 t} \qquad \text{(Eq. 13)}$$

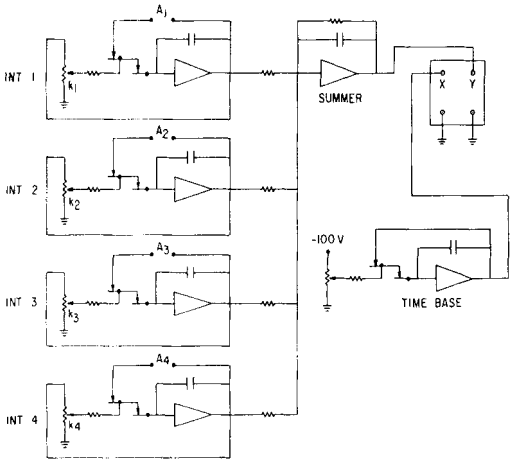

Fig. 2.—Analog computer program for fitting the sum of exponentials to plots of Ca_B^{47} in blood, % of total dose per ml. of serum, against time.

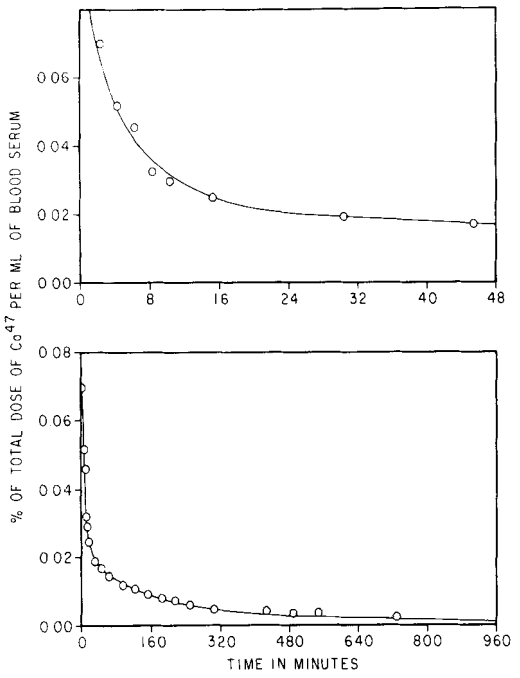

Fig. 3.—Examples of goodness of fit of analog computation of summed polyexponentials to the early phases of the plots of Ca_B^{47} in blood, % of total dose of Ca^{47} per ml. of blood serum against time, dog O, study *E*.

and the determined A_i and k_i values accounted for all the Ca_B^{47} data as a function of time with the possible exception of amounts in the order of thousandths of the original dose in the blood in excess of 7,000 minutes after administration of the dose. The drawn lines in Figs. 3 and 4 are typical examples of the goodness of fit obtained from the program of Fig. 2 and plotted out on the recorder. The A_i and k_i data so obtained for all studies on all 4 dogs are given in Table I.

TABLE I.—TABULATION OF CONSTANTS[a] FOR ADDITIVE SERIES OF EXPONENTIAL $A_i e^{-k_i t}$, CHARACTERIZING THE DECREASE OF Ca^{47} FROM THE BLOOD AFTER INTRAVENOUS ADMINISTRATION[b]

Study[c]	Dog O				Dog 1[d]		Dog 1			Dog 2		
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
A_1	0.0203	0.0380	0.0642	0.0527	0.0613	0.0108	0.0958	0.0646	0.0772	0.0766	0.0561	0.0416	0.0133	0.0432	0.0400	0.0363	0.0633
A_2	0.0114	0.0191	0.0188	0.0158	0.0186	0.0232	0.0284	0.0190	0.0200	0.0260	0.0268	0.0193	0.0071	0.0199	0.0197	0.0143	0.0302
A_3	0.0090	0.0119	0.0130	0.0109	0.0141	0.0106	0.0214	0.0192	0.0298	0.0244	0.0158	0.0191	0.0060	0.0176	0.0191	0.0123	0.0167
A_4	0.0024	0.0045	0.0055	0.0049	0.0053	0.0038	0.0060	0.0067	0.0086	0.0076	0.0108	0.0089	0.0063	0.0080	0.0068	0.0113	0.0087
$\Sigma_i A_i$	0.0431	0.0735	0.1015	0.0845	0.0993	0.0484	0.1516	0.1095	0.1356	0.1546	0.1095	0.0889	0.0827	0.0887	0.0856	0.0742	0.1189
$\Sigma_i A_i - A_1$	0.0288	0.0255	0.0373	0.0316	0.0380	0.0376	0.0558	0.0449	0.0584	0.0580	0.0534	0.0473	0.0194	0.0455	0.0456	0.0379	0.0556
$10^3 k_1$	4.48	4.17	4.17	2.75	4.64	4.19	6.37	3.30	4.20	4.11	2.94	4.39	3.49	4.02	4.04	2.80	4.53
$10^4 k_2$	1.37	1.38	2.47	2.21	5.56	2.10	3.62	3.40	3.42	3.77	3.11	6.70	3.27	3.37	3.02	3.10	2.91
$10^5 k_3$	3.24	3.19	3.19	3.15	4.41	2.96	3.25	3.23	3.22	3.22	2.48	4.05	5.90	2.74	2.63	3.07	3.50
$10^6 k_4$	5.90	5.45	5.45	6.06	6.30	5.90	5.50	5.74	5.90	5.45	5.90	5.19	5.90	5.90	5.90	5.50	5.06

[a] A_i are in % of initial total dose of Ca^{47}/ml. of serum and k_i are in sec.$^{-1}$. [b] Study *A* was the control study. Studies *B* and *C* were the steroid administration studies. Studies *D* and *E* were the recovery studies, *i.e.*, after cessation of steroid administration. [c] The injected doses of Ca^{47} were, in c.p.m., 5.92×10^6 for *A*, 9.16×10^6 for *B*, 15.2×10^6 for *C*, 5.72×10^6 for *D*, and 8.62×10^6 for *E*. The only exception was *A*, Dog 3, *viz.*, 2.96×10^6 c.p.m. [d] Dog deceased after this run.

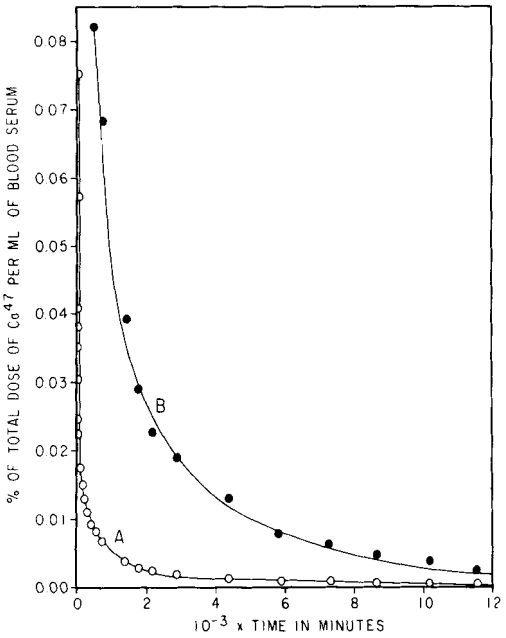

Fig. 4.—Examples of goodness of fit of analog computation of summed polyexponentials to the later phases of the plots of Ca_B^{47} in blood, % of total dose of Ca^{47} per ml. of blood serum against time, dog O, study *E*. Curve B is a ten-fold magnification, and the ordinate values should be divided by 10.

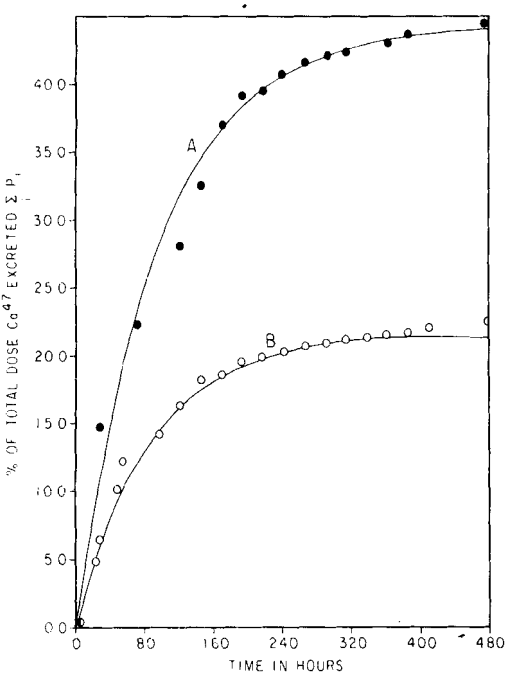

Fig. 5.—Examples of goodness of fit of analog computation of pseudo first-order fecal excretion with time, $\Sigma P_i = P_\infty (1 - e^{-kt})$, of cumulative Ca^{47}. Curve A is for dog 2, study *E*; Curve B is for dog O, study *E*.

Feces and Urine Data.—The Ca^{47} appearing in the urine and feces is cumulatively plotted as ΣP_i against time as in the typical curves of Figs. 5 and 6 for feces and is reasonably consistent with the expression

$$\Sigma P_i = P_\infty(1 - e^{-kt}) \qquad \text{(Eq. 14)}$$

where P_∞ is the total % of the total dose of Ca^{47} appearing in the feces (or urine) over the entire period on the postulate of first-order elimination as defined in Eq. 14.

The Ca^{47} data from feces (or urine) were fitted to this expression on the assumption that none had been eliminated at zero time. It was observed that the first-order fit was not strictly true, that an increase in the ΣP_i, percentage of total Ca^{47} dose excreted in urine and feces, occurs for a longer period of time than that interval defined by the blood studies, that fecal and urinary elimination is not strictly first-order in that a slight positive deviation from the first-order asymptote occurs with time, Fig. 6. Also, a short lag period before the initial fecal elimination of Ca^{47} is to be expected due to gastrointestinal holdup and was indicated in several of the studies. The urinary elimination mirrors the fecal elimination and a set of typical cumulative curves for all studies on one dog is given in Fig. 7. The drawn lines are typical examples of the goodness of analog computer fitting to the first-order expression of Eq. 14.

RESULTS

Blood serum.—The A_i and k_i values for the sum of exponentials in Eq. 13 that describe the Ca_B^{47} content of blood serum as a function of time are given in Table I for the various dogs and studies.

Typical analog computer fittings of the data from which these values were derived are given in Figs. 3 and 4. In general, the fit was very good by a four-factor exponential except perhaps for the very tail end of the curve in excess of 7,000 minutes where the data maintained a level above zero. The counting error is included in the size of the data circles.

Inspection of Table I shows that there is no great difference in the k_i values for any of the four exponentials of Eq. 13 whether the dog is under control (study *A*), under steroid regimen (studies *B* and *C*), or in recovery from steroid regimen (studies *D* and *E*). All the stated k_i values for any given factor are within the error of the fit regardless of the experimental condition of the animal.

The major difference within dogs and among the studies under the various experimental conditions was in the magnitude of the A_i values or the total amount of Ca^{47} transferred from the blood as characterized by the four exponentials.

This is most readily and graphically demonstrated in the example plotted in Fig. 8 where the log Ca_B^{47} *vs.* time for all studies in a given dog are parallel (*i.e.*, the k_i are similar) but the curves are displaced along the ordinate (*i.e.*, the amounts of Ca_B^{47} appearing in the blood vary with the experimental condition of the animal.).

This can be observed with the $\Sigma_i A_i$ values given in Table I and indicates that animals under steroid therapy (studies *B* and *C*) have higher Ca^{47} contents

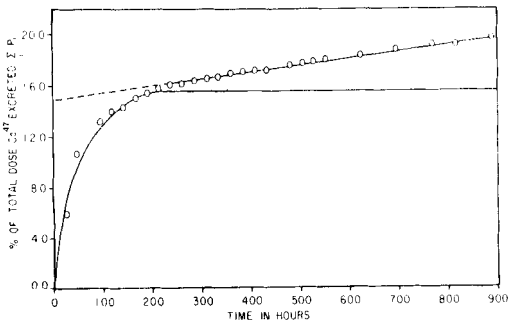

Fig. 6.—Example of zero order deviation $\Sigma P_i = mt + b$, from the analog computer fitted pseudo first-order asymptote P_∞, of the cumulative fecal excretion with time, dog O, study *D*.

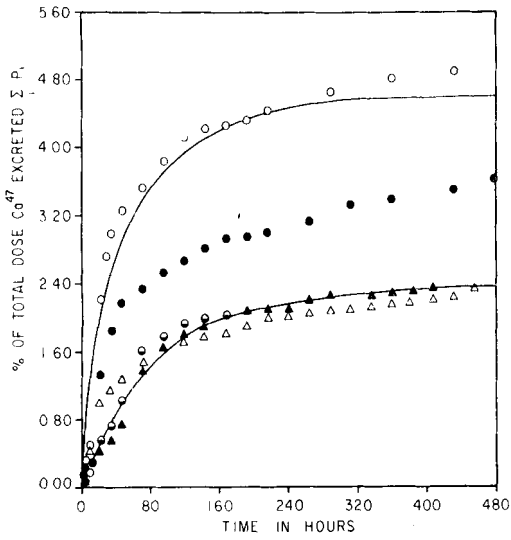

Fig. 7.—Typical examples of cumulative urinary and fecal excretion of Ca^{47} with time for a dog under different experimental conditions with typical analog computer fits. The urinary Ca^{47} excretion of dog 2; study *A*, ◐; *B*, ⊙; *C*, ●; *D*, ◬; and *E*, ▲.

in the blood than control animals (study *A*) or on recovery from steroid (studies *D* and *E*). The only study that provides an exception from this latter case is study *E* of dog 3.

With all dogs the A_1 and A_2 values of the control (study *A*) were less than that value for the steroid regimen (studies *B* and *C*).

Feces and urine.—The P_∞ and k values in accordance with the first order cumulative excretion of Ca^{47} as in Eq. 14 are given in Table II as fitted to the plots of total Ca^{47} excreted in feces (or urine) *vs.* time by the analog computer with the curve forced through the origin. Typical plots of such fits are given in Figs. 5 and 6 for feces and in Fig. 7 for urine. Since the data are cumulative, large deviations may be expected from a smooth curve with the initial fecal Ca^{47} contents. The good fit is well demonstrated for the duration of time covered by the blood studies. The counting error is included in the circles representing the data.

Over longer periods of study the fecal and urinary elimination of Ca^{47} deviates from first order, and this deviation over the theoretical asymptote, P_∞, can be approximated by a straight line of slight slope (Fig. 6)

$$\Sigma P_i = mt + b \qquad \text{(Eq. 15)}$$

where the slope m in % of total dose excreted per hour and intercept b are also given in Table II.

Inspection of the fecal and urinary elimination curves for all studies and dogs clearly showed that the amount of Ca^{47} appearing in both urine and feces for dogs on steroid regimen is greater than for these dogs during their control period as is demonstrated in the typical plots of Fig. 7. In addition, during the period of recovery from the steroid regimen, significantly less Ca^{47} appeared in the feces and urine.

The rate constants k among runs for a particular dog and among dogs show no significant correlation with the treatments and no highly significant variation outside of the experimental error of their estimates. Thus the k_u for urinary elimination is *ca.* 5×10^{-6} sec.$^{-1}$ ($t_{1/2}$ = 39 hours), and the k_f for fecal elimination is *ca.* 3×10^{-6} sec.$^{-1}$($t_{1/2}$ = 64 hours). The possible gastrointestinal lag in these latter cases may give erroneously low values for k_f.

The deviations defined in Eq. 15 of Ca^{47} data ΣP_i in the urine and feces from the first-order accumulative asymptote P_∞ defined in Eq. 14 are significant with respect to the counting errors. Unfortunately, fecal and urinary data were not accumulated beyond 170 hours for the control run and no deviation from first-order elimination of Ca^{47} could be calculated. Inspection of the data, however, indicates that in the first recovery study *D* on feces, the slopes m are less than for the steroid studies *B* and *C*. For both steroid recovery studies *D* and *E*, on urine, the slopes m are less than for the steroid studies *B* and *C*.

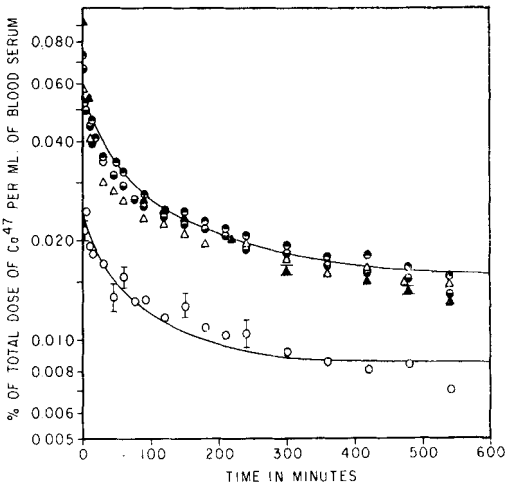

Fig. 8.—Typical examples of Ca_B^{47} blood content with time for the early phases of a dog under different experimental conditions with typical analog computer fits, dog 3, study *A*, ⊙; *B*, ◓; *C*, ◒; *D*, ◬; and *E*, ▲. The horizontal lines above and below some of the data indicate the 95% confidence limits due to counting error.

TABLE II.—CONSTANTS FOR CHARACTERIZATION OF ACCUMULATION OF Ca^{47} IN DOG FECES AND URINE FROM 1,[a] $\Sigma P_i = P_\infty (1 - e^{-kt})$ AND 2[b], $\Sigma P_i = mt + b$

Dog	Study	Feces				Urine			
		P_∞	10^6k	m	b	P_∞	10^6k	m	b
0	A	18.5	2.60	...[d]	...	1.10	5.98	...[d]	...
	B	30.0	4.37	0.0202[e]	24.6	1.14	4.11	0.0015[e]	0.76
	C	33.9	3.28	0.0257[e]	28.8	1.62	4.73	0.0012[e]	1.30
	D	15.5	5.43	0.0054[f]	14.9	0.397	4.32	0.00116[e]	0.21
	E	21.6	3.19	0.108[e]	17.5	0.845	1.92	0.00097[e]	0.45
1	A	21.0	2.97	...[d]	...	1.026	8.38	...[d]	...
	B[c]	31.9	2.56	0.0207[e]	22.5	3.33	5.61	0.00338[e]	2.64
2	A	35.0	3.70	...[d]	...	2.06	5.83	...[d]	...
	B	41.5	5.06	0.0225[e]	36.3	4.40	5.91	0.00162[e]	3.67
	C	35.75	3.30	0.167[e]	30.0	3.09	5.01	0.00182[e]	5.30
	D	27.25	2.06	0.0056[e]	20.2	1.86	5.79	0.00138[e]	1.73
	E	44.4	2.89	0.0170[e]	37.1	2.335	3.18	0.00069[e]	0.61
3	A	55.9	2.66	...[d]	...	2.80	6.09	...[d]	...
	B	61.0	4.62	0.0183[e]	52.7	4.14	4.91	0.00258[e]	3.85
	C	60.9	3.82	0.0173[e]	51.9	5.77	5.12	0.00237[e]	2.52
	D	25.0	1.46	0.0041[f]	21.1	2.025	5.20	0.00135[e]	1.67
	E	30.4	4.14	0.0163[e]	25.6	0.876	2.54	0.00112[e]	1.88

[a] Where ΣP_i is expressed in terms of % of total dose and k in sec.$^{-1}$. [b] This is the later deviation from the first-order accumulation of fecal and urinary Ca^{47}, *i.e.*, from P_∞ where m is in % of total dose/hour and t is in hours. [c] Dog deceased after this run. [d] No values obtained beyond 170 hours after Ca^{47} administration. [e] *Ca.* 200 to *ca.* 450 hours. [f] *Ca.* 370 to *ca.* 900 hours.

DISCUSSION

Blood serum.—The disappearance of labeled Ca^{47} from the blood of the dog after intravenous administration can be represented by a four-factor linear homogeneous equation of exponentials as in Eq. 13 in agreement with the observations of Thomas, *et al.* (2), for the rabbit and Bronner, *et al.* (4), for man.

This mechanistically implies that the initial 100% of the Ca^{47} administered distributes itself into four compartments as

$$\sum_1^4 A_i = A_1 + A_2 + A_3 + A_4 \quad \text{(Eq. 16)}$$

and that a certain fraction, *i.e.*, $A_1/\Sigma_i A_i$, $A_2/\Sigma_i A_i$, etc., leaves the blood into these compartments with respective rate constants k_1, k_2, k_3, and k_4. This also implies that the losses of Ca^{47} into each of these compartments are independent processes. This is not a physiologically true model since the transfer of Ca^{47} from blood into the tissue, although it may simulate a first-order process, actually may be a series of equilibria, or one transfer in a sequence of transfers, and the k_i and A_i may be interdependent.

However, the summation of exponentials of Eq. 13 may serve as a first approximation of a physiological model (2). The major contribution to the first exponential may be the relatively fast diffusion or transfer of Ca^{47} into extravascular spaces, some soft tissues, and the gastrointestinal tract. The major contribution to the second and third exponentials may be the slower diffusion or transfer into other depots. One such may be a metabolic pool, *e.g.*, chelation and/or complexation with bone surface. A simultaneous contribution may be the apparently irreversible incorporation of radioisotope into bone, *i.e.*, Ca^{47} covered by subsequent calcium deposition and unavailable for ready redistribution among blood and tissues during the interval studied. The major contribution to the fourth exponential may be the fecal and urinary elimination of radioisotope where the first-order accumulative rates are of similar magnitude as the fourth exponential. The positive deviations from first-order eliminations and the fourth exponential characterizing the blood curves may be due to resorption of radioisotope from bone. It must be emphasized that these cited assignments are oversimplified possibilities.

From the A_i data of Table I, the apparent volume of a particular compartment in terms of equivalent ml. of serum may be estimated by $1/A_i$ where $1/\Sigma A_i$ is an estimate of the total ml. of serum in the dog. The effect of steroid regimen is to increase the Ca^{47} content of the blood, a phenomenon which is reversed on cessation of such therapy. This may indicate that steroid therapy affects the instantaneous diffusion of Ca^{47} from the blood to some easily accessible other compartment characterized by A_1 and k_1 and/or the blood volume is significantly decreased by steroid therapy. There are too many variables extant in this study to permit a definite assignment of cause. This phenomenon will be further evaluated in future studies.

Urine and feces.—If it is postulated that the Ca^{47} is either in the feces, urine, or some "deep" compartment that may be "bone" at 200–400 hours after administration, then the difference of the measured amounts excreted from the total dose could give the % of the original dose in the "bone." Steroid regimen decreased the incorporation of calcium into the "bone" and with cessation of steroid regimen provided a "rebound" effect, an increased incorporation of blood calcium into "bone." On these premises, the gross effect of adrenal steroid administration is to induce a reversible osteoporosis. The procedures described herein can provide a quantitative measure.

The deviations of the cumulative Ca^{47} excreted in the urine and feces from first-order asymptotes were significant with respect to counting errors. It is naive to assume that the slow diffusion of Ca^{47} through the "bone" from the sites of "accretion"

to the sites of "resorption," the subsequent reentry into the blood and the final elimination into the urine and feces is a simple constant rate that is only apparent after the first-order accumulation of the greater portion of Ca^{47} excreted in feces and urine. However, it may be practical to use the slope of this straight line as a possible measure of "resorption" of calcium from "bone." The quantification of these slopes well indicates a decrease in the rates of "resorption" from bone on cessation of steroid therapy, a possible physiological "rebound" from steroid therapy. The data were insufficient to contrast steroid regimen with the control period.

The male beagles showed greater incorporation of calcium into the "deep" compartment than the slightly older female. The older mongrel showed the least such incorporation but the greatest "rebound."

SUMMARY

Methods and procedures have been developed to utilize the analog computation of a summation of four polyexponentials to describe the Ca^{47} content of blood as a function of time after intravenous administration. Quantitative measures of determining calcium incorporation into a "deep" compartment that may be synonymous with "bone" have been described. The exponential constants for the distribution and elimination of Ca^{47} for all dogs and under all experimental conditions studied, *i.e.*, control, steroid, and recovery periods, were similar.

The short half-life of Ca^{47} of 4.56 days permitted a sequence of studies of experimental effects on calcium dynamics as followed by the study of the distribution and excretion of Ca^{47} with each dog used as his own control.

The most pronounced effect of steroid regimen in dogs is the increased amount of Ca^{47} accumulated in the feces over the control period. A similar effect is observed with the Ca^{47} accumulated in the urine although the percentage of the total administered dose excreted in the urine (2–6%) is very much less than that excreted in the feces (20–60%).

A possible, but less well defined effect of steroid regimen, is the decrease in apparent blood volume from the control period as based on radioisotopic measurement of Ca^{47}-labeled blood. It is also well indicated that the rate of Ca^{47} return from the "deep" compartment is accelerated by steroid therapy. In the classical nomenclature, it may be that bone "accretion" is inhibited and bone "resorption" accelerated by steroid therapy to induce the reversible osteoporosis of Cushing's syndrome.

A "rebound" effect is also noted in that on cessation of steroid therapy, the dog utilizes more Ca^{47} and the rate of return from the "deep" compartment is lessened.

The possible utilization of more effective osteoporotic agents as measured by these techniques should be of great interest in the removal of bone-seeking radioisotopes produced from radiation or fall-out contamination. Also, these methods are applicable to the recognition and quantification of nonosteoporotic anti-inflammatory agents, and therapeutic agents that may prevent or treat bone diseases.

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