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

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

The effect of adrenal steroids on the distribution of intravenously administered Ca^{47} has been studied further by use of an analog computer. A distributive model, consistent with the literature, has been programmed on the computer and has been shown to be in agreement with the fecal and blood serum levels of Ca^{47}. Adrenal steroid effects can be assigned to a decrease in the calcium capacity of the metabolic pool with respect to the calcium capacity of the blood and to enhanced transfer of calcium to the gastrointestinal tract. This distributive model has been correlated with the empirical equations computer-derived for fitting the Ca^{47} data. Blood levels and fecal excretion of Ca^{47} are enhanced by steroid and depressed on recovery. It has been definitely proved that when the quasi equilibria of radioactive calcium among blood, soft tissues, metabolic pool, and contents of the gastrointestinal tract have been achieved, the relatively slow fecal elimination of Ca^{47} at an apparently linear rate is doubled by the steroid administered and relapses to the control values on recovery. The phenomena are consistent with the hypothesis that adrenal steroids not only inhibit the entry of endogenous calcium into the bone and enhance its secretion into the gastrointestinal tract but also promote the resorption of calcium from bone.

THE PREVIOUS PAPER in this series (1) considered the kinetics of adrenal steroid effects in four dogs and fitted the obtained values of intravenously administered Ca^{47} in blood, feces, and urine as functions of time with the analog computer.

It had been observed that steroid regimen increased the amount of Ca^{47} accumulated in the feces and urine with respect to the amounts of a prior control or presteroid period, and that this increase was reversed subsequently on cessation of steroid dosage (1, 2). It had also been observed that Ca^{47} blood levels after intravenous administration of Ca^{47} were generally higher during the steroid regimen than during the prior control period (1, 2).

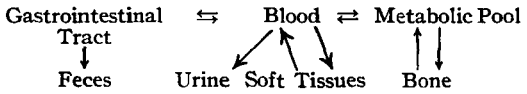
On recovery from steroid administration, a decreased rate in the resorption of Ca^{47} (possibly from the bone) was reflected in the long term fecal and urinary excretion of the radioisotope (1). Unfortunately, long term fecal and urinary excretion studies of Ca^{47} had not been conducted during the prior control periods.

The dogs in these previous studies (1) were relatively unmatched, were treated with antibiotics, and were biopsied. This chemotherapy and possible surgical shock may have had an indeterminate effect on the studies and their results.

In light of these circumstances it was believed that further detailed investigation was warranted to eliminate possible perturbing influences such as antibiotics or surgery, to take full advantage of the knowledge that prolonged studies of Ca^{47} fecal excretion may give insight into the effects of adrenal steroids on the resorption of calcium from bone, to use animals as "matched" as possible to evaluate the consistency of effects in control, steroid, and recovery phases, and to study induced osteoporosis in the fast growth period of the immature dog.

This paper reports on these investigations and utilizes the analog computer to quantify the blood levels, fecal, and urinary amounts of Ca^{47} as functions of time in accord with the method previously established (1). In addition, analog computer methods and techniques are used to fit a more realistic model for the compartmental transferences of Ca^{47}.

Such a model, variously modified, has been considered by several authors (3–7):



(Eq. 1)

It must be well understood that curve or data fitting with time can be accomplished not only by the model of Eq. 1 but also by kinetically equivalent models (3). For example, the transfer of calcium from the blood to the metabolic pool as mediated through the soft tissues may also fit the data. The amount of Ca^{47} excreted in the urine by the dog is small and would make a

Received September 13, 1962, from the College of Pharmacy, University of Florida, Gainesville.
Accepted for publication December 17, 1962.
This investigation was done at the Research Division, The Upjohn Co., Kalamazoo, Mich.
The technical assistance of D. J. Weber and V. F. Baker is gratefully acknowledged. The analog computer would not have been possible without the technical knowledge and collaboration of Clayton D. Alway.
† Present address: The Schering Corp., Bloomfield, N. J.

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

Study[c]	Dog 1 A	Dog 1 B	Dog 1 C	Dog 2 A	Dog 2 B	Dog 2 C
A_1	0.0404	0.0350	0.0298	0.0356	0.0266	0.0405
A_2	0.0177	0.0158	0.0143	0.0175	0.0097	0.0124
A_3	0.0073	0.0094	0.0088	0.0073	0.0108	0.0109
A_4	0.0019	0.0029	0.0031	0.0017	0.0032	0.0030
$\Sigma_i A_i$	0.0673	0.0631	0.0560	0.0621	0.0503	0.0668
$\Sigma_i A_i - A_1$	0.0269	0.0281	0.0262	0.0265	0.0237	0.0263
$10^3 k_1$	3.77	2.00	3.10	2.68	2.88	3.49
$10^4 k_2$	2.77	1.63	1.82	2.04	3.13	1.90
$10^5 k_3$	3.50	2.12	2.34	3.50	2.66	2.73
$10^6 k_4$	5.06	4.06	4.75	5.06	4.49	4.83
Study[c]	**Dog 3 A**	**Dog 3 B**	**Dog 3 C**	**Dog 4 A**	**Dog 4 B**	**Dog 4 C**
A_1	0.0530	0.0415	0.0395	0.0530	0.0595	0.0450
A_2	0.0199	0.0151	0.0200	0.0199	0.0200	0.0200
A_3	0.0079	0.0186	0.0100	0.0087	0.0100	0.0054
A_4	0.0021	0.0031	0.0032	0.0022	0.0038	0.0035
$\Sigma_i A_i$	0.0829	0.0783	0.0727	0.0838	0.0933	0.0739
$\Sigma_i A_i - A_1$	0.0299	0.0368	0.0332	0.0308	0.0338	0.0289
$10^3 k_1$	3.36	2.07	5.78	3.37	2.61	2.22
$10^4 k_2$	2.30	1.89	2.32	1.96	1.40	0.97
$10^5 k_3$	3.46	4.97	2.56	3.47	1.50	1.62
$10^6 k_4$	5.17	4.34	4.71	5.06	4.75	6.59

[a] A_i are in per cent of initial total dose of Ca^{47}/ml. of serum and k_i are in sec.$^{-1}$ [b] Study A was the control study; Study B was during 2 mg./Kg./day steroid administration; the recovery Study C was conducted after cessation of steroid administration. [c] The injected doses of Ca^{47} were in c.p.m.–11.01 × 10^6 for A, 8.11 × 10^6 for B, and 14.3 × 10^6 for C.

negligible contribution to the overall balance of compartments and rates.

Reversible first-order expressions for Ca^{47} transfer between gastrointestinal tract and blood, between metabolic pool and blood, and between metabolic pool and bone are gross oversimplifications of complex physiological phenomena. However, such a model as Eq. 1 is most certainly a closer fit to reality than a linear equation of exponentials (1, 8–13).

The apparent first-order rate constants, k_i, which can be derived from analog computer fitting of the Ca^{47} data have an alternative interpretation as r/Ca_i, the fractional amount of calcium Ca_i renewed or turned over in a unit time where r is the turnover rate of calcium, amount/unit time, renewed in the compartment (see *Appendix*) (14, 15).

EXPERIMENTAL AND METHODS

Treatment of Animals.—Four beagles were used in these experiments. The animals were conditioned to the metabolism cages and laboratory routine prior to experimentation. Pertinent data for Dogs 1–4 given as initial age in days and sex are for Dog 1, 227 (F); Dog 2, 222 (F); Dog 3, 213 (M); Dog 4, 213 (M). The methods and procedures of intra-

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[c]	Feces P_∞	Feces 10^6k	Feces m	Feces $\%Ca^{47}_{Bone}$[d]	Feces 10^4R[e]	Feces b	Urine P_∞	Urine 10^6k	Urine m	Urine 10^6R[e]	Urine b
1	A	9.6	3.12	0.0129	90	1.43	6.30	0.41[f]	5.88	0.00065	7.22	0.303
	B	33.2	3.05	0.0141	66.1	2.13	28.2	0.70	6.24	0.00115	17.4	0.535
	C	21.0	3.65	0.0088	78.5	1.12	17.6	0.44	9.52	0.00031	3.94	0.392
2	A	9.6	4.46	0.0136	89.9	1.51	6.90	0.48[f]	6.50	0.00048	5.34	0.373
	B	32.6	4.7	0.0155	66.5	2.33	30.3	0.84	8.71	0.00113	16.9	0.700
	C	25.1	5.76	0.0101	74.3	1.35	23.4	0.52	10.89	0.00034	4.57	0.456
3	A	10.8	3.88	0.0114	88	1.30	8.30	1.22[f]	3.52	0.00105	11.9	0.96
	B	29.6	4.27	0.0203	69.1	2.93	24.8	1.33	5.1	0.00137	19.8	1.06
	C	17.0	5.78	0.0092	82.5	1.11	15.7	0.54	6.77	0.00039	4.72	0.44
4	A	11.2	2.94	0.0116	87.9	1.32	7.95	0.91[f]	5.20	0.00073	8.3	0.77
	B	29.0	4.8	0.0230	69.8	3.29	24.6	1.19	4.8	0.00100	14.3	0.93
	C	16.5	3.79	0.0097	82.3	1.17	13.5	1.16	5.97	0.00041	4.98	1.03

[a] Where ΣP_i is expressed in terms of per cent total dose and k in sec.$^{-1}$ [b] This is the deviation from the first-order accumulation of Ca^{47}, *i.e.*, P_∞ where m is in per cent total dose/hour and t is in hours. [c] The injected doses of Ca^{47} were in c.p.m.–11.01 × 10^6 for Study A, 8.11 × 10^6 for Study B, 14.3 × 10^6 for Study C, for all dogs. [d] This column relates to per cent of the total dose as Ca^{47} remaining in bone at *ca.* 200 hours and as can be estimated from 100 $-P_\infty$(urine) $- P_\infty$(feces). [e] $R = m/\%\ Ca^{47}_{Bone}$ is an estimate of the resorption rate adjusted for the Ca^{47} content of the bone where $\%\ Ca^{47}_{Bone}$ is defined in *footnote f*. [f] An anomalously high count was obtained for the first urine samples during the control phases that did not appear for other phases or for the same control phase for other studies. Plots of Ca^{47} accumulated in urine for runs A *vs.* time showed an intercept value at zero time. A technological bias is implied. *Ignoring of this first value of the urine for* the control phase, runs A, brought the urine data into the same pattern as the fecal data. These first urine Ca^{47} values in control phase A which were ignored were (in terms of per cent of total Ca_0^{47} dose at zero time): Dog 1, 0.22%; Dog 2, 0.38%; Dog 3, 0.65%; Dog 4, 0.28%.

TABLE III.—ESTIMATED SECOND LINEAR RATE,[a] R' OF RESORPTION OF Ca^{47} FROM BONE DURING RECOVERY PHASE, STUDY C, AFTER CESSATION OF STEROID ADMINISTRATION

Dog	m'[a]	b'	$10^4R'$	t', Hrs.
1	0.0146	22.5	1.89	800
2	0.0117	30.5	1.67	690
3	0.0127	22.5	1.69	760
4	0.0115	20.5	1.45	750

[a] $R' = m'/\%\ Ca^{47}_{Bone}$ is an estimate of the second linear Ca^{47} fecal excretion rate with time after t' hours adjusted for the $\%\ Ca^{47}_{Bone}$ content presumed to be in bone. The $\%\ Ca^{47}_{Bone}$ value is derived from $100 - b'$ at t' hours, *i.e.*, the per cent of the original Ca^{47} dose not excreted in the feces at that time where $b' = \Sigma P_i$ at $t = t'$. The slope, m', is in per cent of total dose/hour of the cumulative Ca^{47} (per cent of original Ca^{47} dose), *i.e.*, excreted in the feces, *i.e.*, ΣP_i after $t = t'$ and $\Sigma P_i = m'(t - t') + b'$. At t', *ca.* 700 hours, a sharp discontinuity in the linear fecal excretion of Ca^{47} observed after 200 hours did occur and these data describe the properties of this new linear relation of the cumulative Ca^{47} excretion, ΣP_i, in the feces with time.

venous administration of Ca^{47} and of the determination of radioactivity in blood serum, urine, and feces have been detailed elsewhere (2).

The studies were conducted in three sequential phases. At the ages cited above 50 μc. of Ca^{47} were administered (control, Study *A*), and the Ca^{47} distribution was studied with respect to time. At "Day 20" after the initial administration of Ca^{47}, 2 mg./Kg./day of a typical corticoid, 6-α-methylprednisolone was administered orally and Ca^{47} was again intravenously injected at "Day 41" and its distribution studied with time (steroid, Study *B*). At "Day 61," the steroid regimen was reduced for each dog at the rate of 1 mg./day so that at "Day 81," steroid administration was ceased. At "Day 131," Ca^{47} was again administered and distribution studied with time (recovery, Study *C*).

The times of blood sampling and urine and feces collection after Ca^{47} administration were as previously given (1), except the feces and urine collections were prolonged for 25 days after Ca^{47} administration during the control and steroid studies and for 58 days during the recovery studies. No antibiotics were administered or biopsies were performed.

Treatment of Data.—The procedures used for calculating and treating the data from the blood serum, urine, and feces were given in detail in the previous publication (1). Sufficient sample was taken and time of counting used so that all counting error was less than 2%. As previously discussed, values were corrected for normal decay from the data obtained by the simultaneous counting of an aliquot of the initial Ca^{47} solution used for injection.

Curve Fitting and the Analog Computer.—The disappearance of labeled Ca^{47} from the blood after intravenous administration can be represented by a four-factor linear homogeneous equation of exponentials (1, 8–13)

$$Ca_B{}^{47} = 100\ Ca^{47}/Ca_0{}^{47} = \Sigma_i A_i e^{-k_i t} = A_1 e^{-k_1 t} + A_2 e^{-k_2 t} + A_3 e^{-k_3 t} + A_4 e^{-k_4 t} \quad \text{(Eq. 2)}$$

where Ca^{47} is the amount of Ca^{47} per ml. of blood

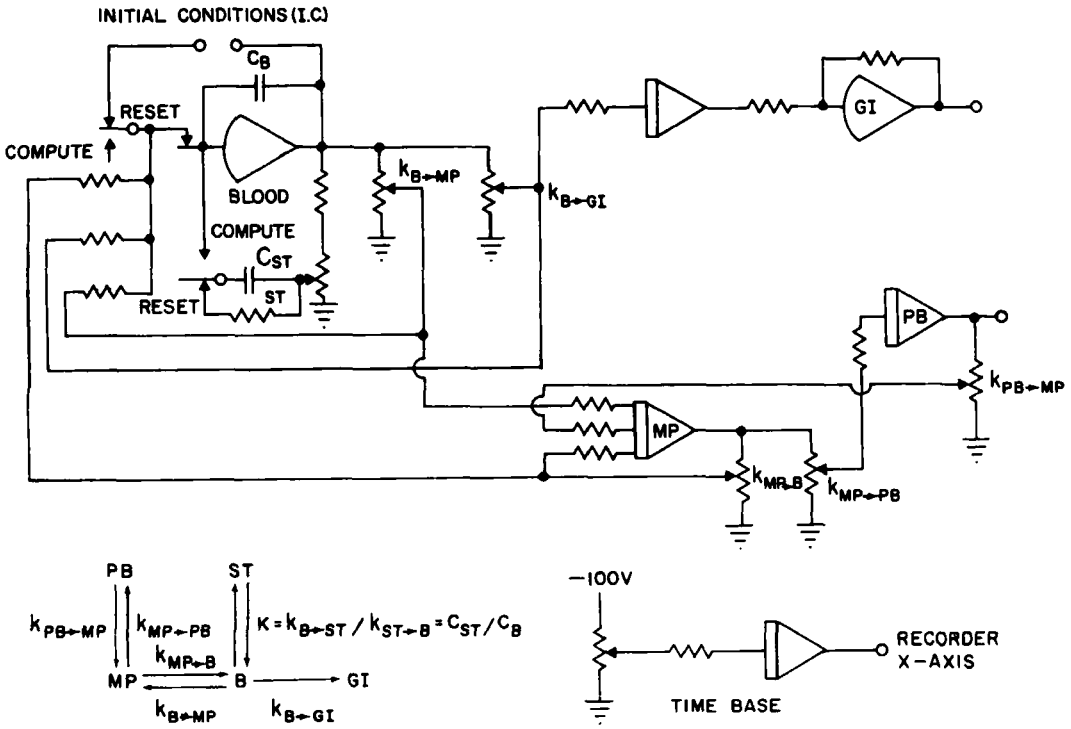


Fig. 1.—The electronic dog. Schemata for the programming of the analog computer for Ca^{47} data in the blood and feces up to 240 hours. The k values represent the respective potentiometers that are varied to obtain the rate constants, the sequence of their respective subscripts represent the directions of transfer of the Ca^{47}. The symbols, *B* (blood), *ST* (soft tissues), *MP* (metabolic pool), *PB* (bone), and *GI* (gastrointestinal tract) represent the various possible depots for Ca^{47} in the animal. The C_{ST} and C_B represent the capacitors for the soft tissue-blood equilibrium.

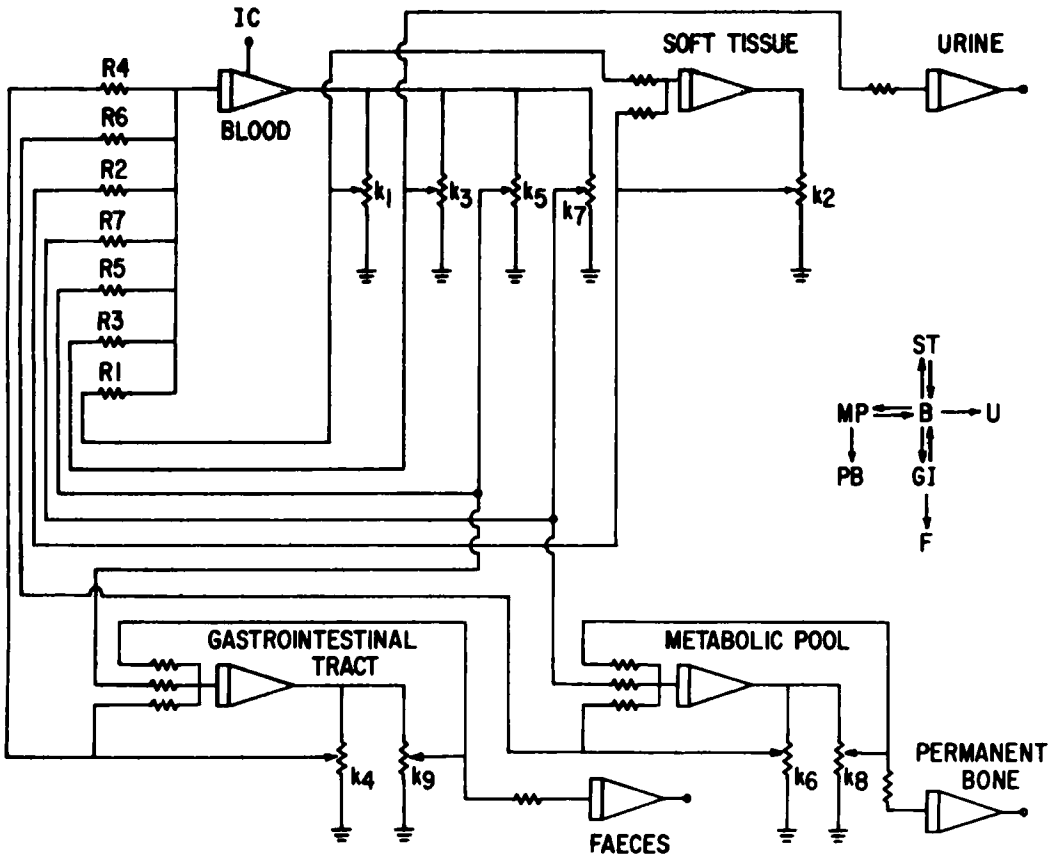


Fig. 2.—The electronic dog. A more complete schemata for the programming of the analog computer and used in part to fit Ca^{47} data up to 11 hours.

serum at any time, t, and Ca_0^{47} is the initial dose, so that Ca_B^{47} is the per cent of total dose administered/ml. of serum.

The significance and limitations of this expression as a reflection of reality and the methods of determination of A_i and k_i by analog and digital computer methods have been previously described (1).

The A_i and k_i values for these studies of four dogs during a control phase A, a steroid regimen, B, and a recovery phase, C, are given in Table I.

The parameters of the curve fitting of the fecal and urinary data by the analog computer are given in Tables II and III in accord with the methods and developments previously given (1).

The Electronic Dog and the Analog Computer

Programming of the Chosen Model for Ca^{47} Distribution.—The basic philosophy of evaluating the consistency of a physiological model with experimental data by means of simulation with an analog computer has been given previously (14). The analog computer setup as finally programmed for all data in excess of 700 minutes is given in Fig. 1. The output voltage of each integrator represents the time variable amount (concentration times a volume factor) of Ca^{47} distributed to a particular portion of the dog's anatomy on the basis of the model chosen, *i.e.*, blood (B), gastrointestinal tract (GI), metabolic pool (MP), and permanent bone (PB).

The integrators are connected by rate setting potentiometers in a conventional manner to simulate the distribution of Ca^{47} on the basis of the chosen model.

The amount of the dosage is represented by the initial condition voltage (IC) applied to the blood

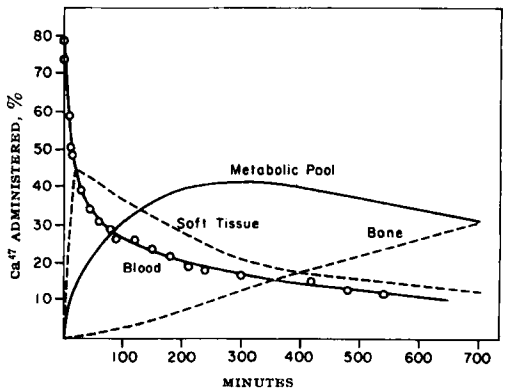


Fig. 3.—Ca^{47} as per cent of intravenously administered dose in the various depots of Dog 1, steroid phase B *vs.* time up to 700 minutes on the basis of a good fit to the plotted blood serum data. The other plots are the "read-out" of the analog computer in accordance with the program of Fig. 2.

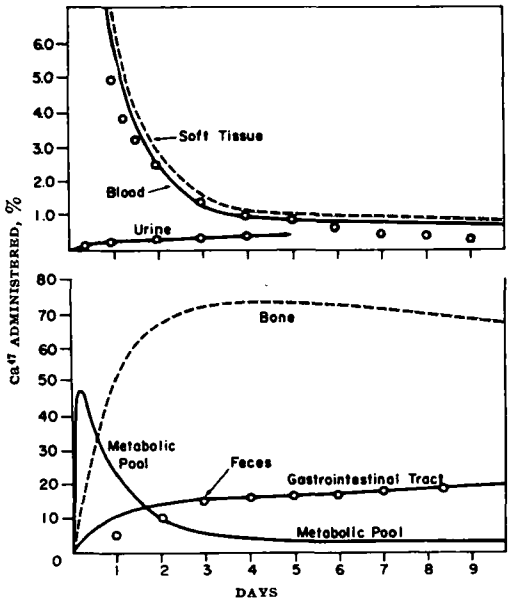


Fig. 4.—Ca^{47} as per cent of intravenously administered dose in the various depots of Dog 1, steroid phase *B vs.* time up to 9 days on the basis of a good fit to the plotted blood serum data and the latter values in the feces. The other plots are the "read-out" of the analog computer in accordance with the program of Fig. 1. A portion of the urine data is plotted for comparison.

(*B*) integrator since the method of administration of Ca^{47} was intravenous.

An additional integrator generates a voltage proportional to time as required by the X-Y recorder used, Fig. 1. The time scale was chosen so that for the 700-minute curves of Figs. 3 and 5, 1 minute of machine time equaled 10^3 minutes of real time; for the 10-day curve of Figs. 4 and 6, 1 minute of machine time equaled 10^4 minutes of real time.

Originally, the more elegant computer program given in Fig. 2 was used in fitting the data. However, drift or leakage resulted over the entire time interval of 10 days with the large number of integrators and with apparent rate constants which varied over four powers of ten. A more expensive computer comprised of precision components (resistors and capacitors) would have been able to simulate without drift. However, with the equipment at hand it was necessary to improvise.

The rate of decrease of Ca^{47} in the blood can be fitted to a linear sum of exponentials and the k_1 value of the first of these, $A_1e^{-k_1t}$, representative of the rapid equilibration between blood and soft tissues, is relatively of high magnitude. The residual exponentials contribute little to Ca^{47} decrease in the blood while the first exponential is operative. Conversely, the equilibration between blood and soft tissue at times in excess of 100 minutes can be considered as instantaneous with respect to the other distributive factors that are rate determining in the loss of Ca^{47} from the blood.

Thus a valid simplification of the program of Fig. 2 in excess of 100 minutes is the removal of the integrator representing the soft tissue (*ST*) and substitution of equilibrating capacitors as in Fig. 1 to account for this rapid equilibration of blood Ca^{47} with soft tissue. The model used can be considered as liquid instantaneously seeking the same level in joined compartments of capacities proportional to the apparent compartmental volumes of blood and soft tissue, respectively, for Ca^{47} or, of course, for calcium since the contents of the compartments would have the same specific activities.

$$Ca_B{}^{47}/Ca_B = Ca_{ST}{}^{47}/Ca_{ST}$$

The integrator representative of Ca^{47} in the feces in Fig. 2 is also absent in Fig. 1 for technological operation of this particular computer system. The Ca^{47} eliminated from the blood by this route is only

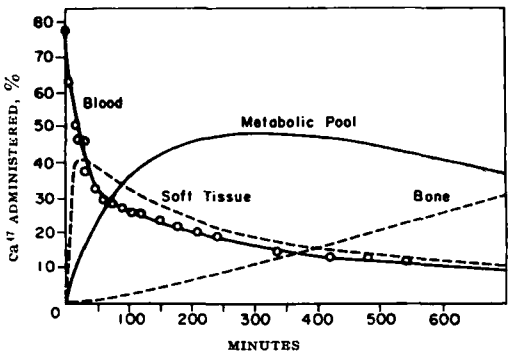


Fig. 5.—Ca^{47} as per cent of intravenously administered dose in the various depots of Dog 1, recovery phase *C vs.* time up to 700 minutes on the basis of a good fit to the plotted blood serum data. The other plots are the "read-out" of the analog computer in accordance with the program of Fig. 2.

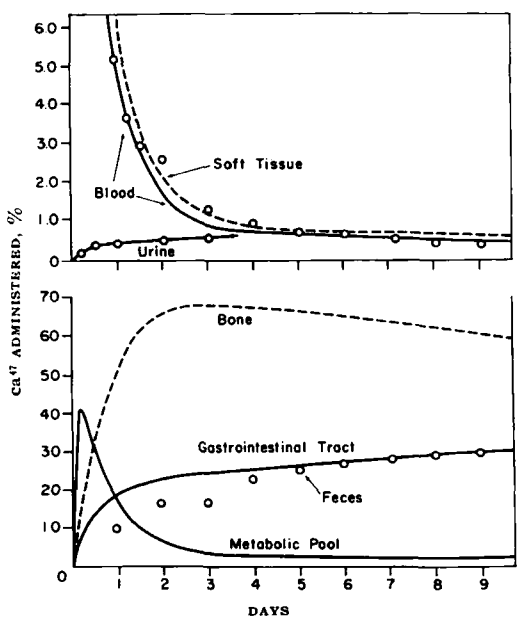


Fig. 6.—Ca^{47} as per cent of intravenously administered dose in the various depots of Dog 1, recovery phase *C vs.* time up to 9 days on the basis of a good fit to the plotted blood serum data and the latter values in the feces. The other plots are the "read-out" of the analog computer in accordance with the program of Fig. 1. A portion of the urine is plotted for comparison.

TABLE IV.—RATE CONSTANTS[a] OF *In Vivo* TRANSFERENCES OF Ca^{47} IN THE DOG AS DERIVED FROM THE FITTING OF BLOOD SERUM AND FECAL Ca^{47} DATA *vs.* TIME BY THE ANALOG COMPUTER

Dog	Study[b]	$10^{2}k_{B\to ST}$	$10^{2}k_{ST\to B}$	$10^{4}k_{B\to MP}$	$10^{5}k_{MP\to B}$	$10^{6}k_{MP\to PB}$	$10^{7}k_{PB\to MP}$	$10^{5}k_{B\to GI}$
1	A	2.26	1.50	3.52	4.77	2.18	1.98	0.67
	B	1.11	0.89	1.74	5.09	2.27	1.19	1.73
	C	1.65	1.45	1.94	4.73	1.78	1.11	1.01
2	A	1.55	1.15	3.33	4.18	2.11	1.48	0.76
	B	1.52	1.36	0.92	2.52	2.47	2.18	1.27
	C	2.11	1.37	1.63	2.92	1.17	0.91	1.33
3	A	2.15	1.21	2.63	2.22	1.67	1.92	0.91
	B	1.10	0.97	1.52	2.68	1.39	0.87	1.75
	C	3.14	2.64	2.71	5.00	1.84	1.32	1.09
4	A	2.13	1.24	3.38	3.34	2.06	1.67	0.95
	B	1.67	0.95	1.88	3.35	1.67	1.11	1.91
	C	1.35	0.87	1.50	2.66	2.00	0.98	0.93

[a] k_i in sec.$^{-1}$ (See model in Fig. 1.) [b] Study A was the control study; Study B was during 2 mg./Kg./day steroid administration; the recovery Study C was conducted after cessation of steroid administration.

experimentally manifested in fecal elimination. However, the effect on Ca^{47} levels in the blood resulting from the transfer of Ca^{47} into the gastrointestinal fluid most certainly occurs relatively quick in comparison to the lag in the determination of Ca^{47} in the feces.

Also apparent first-order fecal eliminations of Ca^{47} would obviously serve as a gross oversimplification of the complicated processes of absorption into the gastrointestinal tract, including binding with fecal matter and subsequent mechanical displacement down the colon.

The computer used was a modified Heath analog computer and the recorders were Mosely model 4.

Fitting the Model to the Analog Computer.—The blood Ca^{47} for the first 700 minutes (real time, Figs. 3 and 5) where computer drift is not operative is fitted by the computer program of Fig. 2 with the processes from the gastrointestinal tract to the feces omitted. The program was revised for the 10-day scale (Figs. 4 and 6) in accordance with Fig. 1. No discontinuities in the fit were observed.

The equilibration constant K and the rate constants $k_{B\to ST}$ and $k_{ST\to B}$ were derived from the original polyexponential fit. The k_1 of the first exponential (Table I) was considered equal to the sum of the forward and reverse rate constants of the model

$$k_1 = k_{B\to ST} + k_{ST\to B} \qquad \text{(Eq. 3)}$$

The apparent soft tissue (ST) compartment size A_1 is also derived from the first exponential of the polyexponential fit. Thus the equilibrium constant, K, for Ca^{47} distributed between the soft tissue and the blood would be

$$K = A_1/(\Sigma A_i - A_1) = k_{B\to ST}/k_{ST\to B} = C_{ST}/C_B \qquad \text{(Eq. 4)}$$

where ΣA_i is the extrapolated value of the Ca_B^{47} content of the blood at zero time (of intravenous administration).

From the data ΣA_i, A_i and k (given in Table I and Eqs. 3 and 4), $k_{B\to ST}$ and $k_{ST\to B}$ can be derived. These values are given in Table IV and were used in fitting the first 700 minutes of Ca_B^{47} data (Figs. 3 and 5). They were also used to calculate the ratio of the two capacitors (Eq. 4), C_{ST} and C_B, representing blood and soft tissue compartmental size, respectively, as used in the program of Fig. 1 and in the curve fitting of Figs. 4 and 6.

After the insertion of the appropriate values for the capacitors and resistors representative of $k_{B\to ST}$ and $k_{ST\to B}$, the capacitors and resistors associated with the rate constants $k_{B\to MP}$ and $k_{MP\to B}$ representative of the blood $\rightleftarrows$ metabolic pool equilibrium were then adjusted until a reasonable fit for the data over the first 100–150 minutes (Figs. 3 and 5) were obtained. A condition for this initial fit was that the constant, k_2, for the second exponential of the polyexponential fit, $\Sigma_i A_i e^{-k_i t}$ can be defined

$$k_2 = k_{B\to MP} + k_{MP\to B} \qquad \text{(Eq. 5)}$$

The 10-day scale (Figs. 4 and 6) was mounted on the recorder and the machine-time/real-time ratio adjusted accordingly. The extrapolated value of Ca_B^{47} at zero time, $(Ca_B^{47})_0$, *i.e.*, the per cent of total administered Ca^{47} per ml. of serum at the time of intravenous administration, was considered equivalent to 100% of the administered radioisotope. Thus, 100 $Ca_B^{47}/(Ca_B^{47})_0$ could be considered equivalent to the per cent of Ca^{47} intravenously administered that was in the blood at any time. The Ca^{47} fecal content was plotted cumulatively as ΣP_i on this same ordinate as per cent of Ca^{47} administered.

The program of Fig. 1 was then used to obtain appropriate $k_{B\to GI}$ and $k_{MP\to PB}$ values by adjustment of resistors and capacitors. The $k_{B\to GI}$ value was initially chosen so as to fit the Ca_B^{47} data and to be consistent with the P_∞ asymptote approached by the cumulative ΣP_i values of the feces at *ca.* 4–5 days, on the assumption of a first-order fecal elimination of Ca^{47}. The $k_{MP\to PB}$ value was concomitantly chosen to fit the Ca_B^{47} data and account for the Ca^{47} not in the blood or feces.

Subsequently, a $k_{PB\to MP}$ value was chosen to fit the positive linear deviation of Ca^{47} fecal excretion, ΣP_i, from the apparent asymptote, P_∞. Minor readjustments were made to the $k_{B\to GI}$ and $k_{MP\to PB}$ constants when necessary.

The 700-minute (Figs. 3 and 5) and 10-day (Figs. 4 and 6) scales were alternately reinserted in the recorder and since all of these rate constants were interacting, fine adjustments in their values were made to assure consistency with the Ca_B^{47} data of the blood, the amounts excreted in the feces, gastrointestinal tract, GI, (in excess of 5 days), and the amounts of Ca^{47} that would have to be in the permanent bone, PB, and metabolic pool, MP, to account for the stoichiometry.

For ease of fitting, the several per cents of the

administered Ca^{47} that appeared in the urine were ignored (Figs. 4 and 6) since their overall contribution was not of great significance.

The rate constants so determined are given in Table IV. They were calculated from the relation

$$k(\text{sec.}^{-1}) = P/RC \times (\text{machine time})/(\text{real time}) \quad \text{(Eq. 6)}$$

where P is the potentiometer setting, R is the resistance in megohms, and C is the capacitance in microfarads.

The output of each compartment was graphed on the recorder as a function of time after the intravenous administration of Ca^{47}. Typical plots are given in Figs. 3–6.

RESULTS

Empirical Equations Fitted to the Ca^{47} Content of the Blood.—The parameters of the four-factor linear homogeneous equation of exponentials, $\Sigma_i A_i e^{-k_i t}$, given in Eq. 2 and representative of the disappearance of Ca^{47} from the blood of the four dogs during a control phase, A, a steroid regimen, B, and a recovery phase, C, are given in Table I.

As previously noted (1), no specific or consistent patterns appear in the k_i values that can be ascribed to the effect of steroid regimen or steroid recovery.

The major difference within dogs appears in the magnitude of the A_i values, the total amount of the Ca^{47} transferred from the blood to the four hypothetical compartments. This is most readily demonstrated in the plots of $Ca_B{}^{47}$ (as per cent of the total administered dose per ml. of serum) in the blood *versus* time for the four dogs. In general, the curves were similar but displaced along the ordinate. There is a significantly higher concentration of $Ca_B{}^{47}$ in the blood for the steroid phase than for the control phase in all cases.

The $Ca_B{}^{47}$, before the A_4 value of the fourth exponential becomes the determining factor (<750 minutes) and after the fast decrease in $Ca_B{}^{47}$ due to the first exponential (>30 minutes) has been expended, is lower in the recovery phase than in the steroid phase. However, the $Ca_B{}^{47}$ did not reach the lower levels attained with the dog as his own control nor was it as significantly different from the $Ca_B{}^{47}$ values for the steroid phase. The curves of $Ca_B{}^{47}$ against time are similar to those given in the figures of the prior paper in this series (1, 2). Figure 1a of the latter reference (2) is representative of the data.

It has been previously shown (1) that the A_1 (in three out of four dogs), A_2 (in four out of four dogs), and A_3 and A_4 (in three out of three dogs) were all lower for the control period than for the steroid regimen studies. With minor exceptions, these A_1, A_2, and A_3 values decreased for the recovery periods. There were no highly significant differences between the A_4 values of steroid phases and the subsequent recovery A_4 values.

The present study confirmed a good part of these observations (Table I). The major differences in the A_i values that account for the displacement of the Ca^{47} in blood serum curves are in the A_3 and A_4 values. In four dogs out of four the steroid regimen phases have higher A_3 and A_4 values than the control.

The slight decrease in $Ca_B{}^{47}$ values (30 minutes > t < 750 minutes) from steroid to recovery phase may be accounted for by the lessened value of A_3 (for three out of four dogs) (Table I). There were no significant differences in A_4 values between steroid and recovery phase.

The A_1 and A_2 data did not permit conclusions regarding differences among phases of treatments.

Empirical Equations for Ca^{47} Content of the Feces and Urine.—The appearance of Ca^{47} in the feces and urine has been represented by apparent first-order expressions (1)

$$\Sigma P_i = \text{Accumulated } Ca^{47} \text{ in feces (or urine)} = P_\infty(1 - e^{-kt}) \quad \text{(Eq. 7)}$$

where P_∞ is the total per cent of the total dose that should appear in the feces (or urine) over the entire period on the postulate of first-order kinetics. The two pertinent parameters are P_∞ and k, and are given in Table II for all dogs and phases as best fitted by the analog computer (1). Again, as in the previous study (1), the amount of Ca^{47}, *i.e.*, P_∞ appearing in the urine and feces by an assumption of first-order kinetics is greatly enhanced by a steroid regimen, Run B over Run A. (See Fig. 7 for Dog 3.)

In fact, the increase in fecal elimination for these studies on steroid regimen is even more pronounced than previously (1), primarily due to the lower P_∞ values for the dogs in the control phases of the first studies. The P_∞ values for dogs of similar age during the steroid regimen were similar, *ca.* 30% of the total Ca^{47} administered.

The "rebound" effect or decrease in P_∞ for the recovery phase (Table II) was definitely present (Fig. 7) but not as pronounced in the present studies since the P_∞ values for the control phases were so much lower. However, P_∞'s for the recovery phases were of the same magnitude in both sets of studies.

The apparent rate constant, k, for the fecal and urinary elimination among runs for particular dogs did not significantly correlate with the conditions of the run.

As was also demonstrated in other papers of this series (1, 2), the fecal (and urinary) elimination of Ca^{47} deviates from first order by an amount of radioisotope excreted in excess of an expected asymptote,

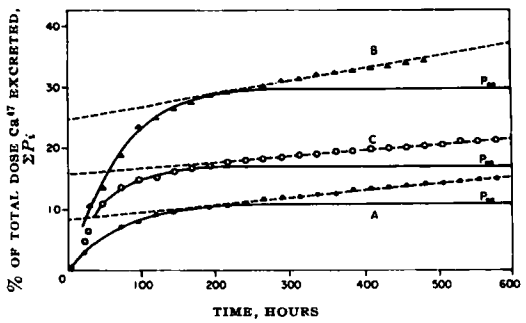


Fig. 7.—Typical example of cumulative fecal excretion of Ca^{47} with time over 600 hours. The data is for Dog 3; A is control study, B is steroid study, and C is recovery study. The solid curves represent the pseudo first-order fecal excretion with time, $\Sigma P_i = P_\infty(1 - e^{-kt})$. The dashed lines represent the apparent linear deviation from the first-order asymptote P_∞, *i.e.*, $\Sigma P_i = mt + b$.

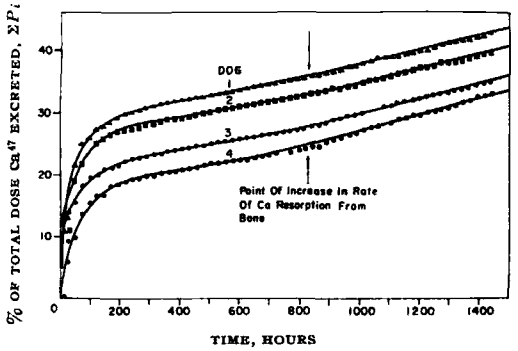


Fig. 8.—Cumulative fecal excretion of Ca^{47} with time for the recovery phases of the dogs over 1500 hours. For reasons of clarity, the data are plotted as Y for Dog 1, Y-4.8% for Dogs 2 and 3, and Y-12% for Dog 4.

i.e., P_∞. This deviation can be approximated by a straight line of slight slope

$$\Sigma P_i = mt + b, \quad t > 200 \text{ hr.} \qquad \text{(Eq. 8)}$$

The slope, m, is the per cent of the total Ca^{47} dose excreted per hour and b is the intercept. The m and b values are given in Table II for both urinary and fecal elimination. Typical plots of this linear deviation are given for the fecal excretion of Dog 3 in Fig. 7.

Since the m values may serve as estimates of the possible rates of reabsorption of Ca^{47} from bone, these values should be adjusted to account for any bias introduced by greater amounts of radioisotope in bone. A reasonable adjustment can be made by dividing m by per cent of the dose of administered Ca^{47} in the bone. This per cent (of the total dose) of Ca^{47} in the bone can be estimated from the difference between 100% and the sum of the apparent first-order asymptotes of Ca^{47} excretion in urine and feces reached at *ca.* 200 hours. Thus

$$R = m/[100 - P_\infty(\text{urine}) - P_\infty(\text{feces})] \qquad \text{(Eq. 9)}$$

where the apparent resorption rate R is the Ca^{47} available for excretion into the feces per unit of Ca^{47} in the bone. This value may be a satisfactory measure of the calcium resorption from bone.

The R data of Table II show that the Ca^{47} reabsorption rate is almost double that of control for all cases of steroid administration and relapses on recovery to at least the rate of the control period and probably less. This phenomenon is also mirrored by the urine data.

Subsequent to this linear deviation from the expected first-order asymptote of Ca^{47} excretion, a further increase in bone resorption rate was observed when the fecal collections were carried out in excess of 700 hours during the recovery phases. The plots are given in Fig. 8.

This new enhancement of resorption rate can be characterized by the linear expression

$$\Sigma P_i = m'(t - t') + b' \qquad \text{(Eq. 10)}$$

where m' represents the new rate and b' is the value of Ca^{47} accumulated in the feces at time $t = t'$, *ca.* 700–800 hours, which represents the time of transition from the old to the new resorption rate.

Again, the m' for the various dogs can be adjusted for Ca^{47} content in the bone, dividing m' by the per cent of total Ca^{47} dose in the bone. The value $100 - b'$ is considered a good estimate of this latter value. Thus

$$R' = m'/(100 - b') \qquad \text{(Eq. 11)}$$

where the R' is the estimated second linear rate of Ca^{47} available for excretion into the feces per unit of Ca^{47} in the bone. This value may be a satisfactory measure of the calcium resorption from bone 700 hours after Ca^{47} administration and during the recovery phase. The m', b', t', and R' values are given in Table III.

Extended studies of fecal elimination during the recovery phase showed that the estimated rates of Ca^{47} resorption subsequently increased by *ca.* 20–40% over the rates of resorption during the 200–700 hour period post-Ca^{47} injection.

Rates of Distribution of Intravenously Administered Ca^{47} as Determined by the Analog Computer.—The apparent first-order rate constants for the compartmental model in Fig. 1 are listed in Table IV. There are no consistent effects of steroid administration and subsequent recovery on the apparent first-order rate constants for the transfer of Ca^{47} from the blood, B, to the soft tissue, ST, *i.e.*, $k_{B\to ST}$ and $k_{ST\to B}$. The relative apparent volume (14) of the soft tissue, V_{ST}, available for Ca^{47} to the blood serum apparent volume, V_B

$$V_{ST}/V_B = k_{B\to ST}/k_{ST\to B} = Ca_{ST}/Ca_B \qquad \text{(Eq. 12)}$$

is of the order 1.2–1.7 (average 1.4) among dogs. This may also be interpreted as the ratios of the calcium contents of both compartments. (See *Appendix.*)

A satisfactory estimate of half the time it would take to reach equilibrium between the blood and soft tissue is 4 minutes, a value which is similar to that previously determined (1).

A definitive decrease in the rate constant assigned to the transfer of Ca^{47} from the blood to the metabolic pool, $k_{B\to MP}$, is observed for steroid over the control period. In three out of four dogs an increase in the rate of return of Ca^{47} from the metabolic pool to the blood, $k_{MP\to B}$, was observed after cessation of steroid.

The ratio of these rates, $k_{B\to MP}/k_{MP\to B}$, may be more indicative of the steroid effect on Ca^{47} distribution. In the control phase the ratio (average 9.4) is twice the value of the ratio while steroid is administered (average 4.6) which is almost equivalent to the value of the ratio during the recovery phase (average 5.2).

An alternate method of appraising this data is by examining the apparent relative volumes or calcium contents of the compartments (14–16). From this perspective, the capacity of the metabolic pool for Ca^{47} and calcium with respect to blood serum may be considered as halved on steroid administration. This relative decrease in the capacity of the metabolic pool remained after cessation of steroid in these studies.

With the model used the major source of the differences in $Ca_B{}^{47}$ values with time within a dog can be attributed to the blood-metabolic pool equilibrium and the blood-gastrointestinal tract transfer, $k_{B\to GI}$.

The values assigned this latter rate constant almost double during steroid phase with respect to the control. On recovery from steroid, they decidedly tend to revert to the control levels.

The introduction of a $k_{PB \rightarrow MP}$ rate constant to account for the return of Ca^{47} from the bone to the metabolic pool is obviously an oversimplification of a complex process. It is significant, however, that the apparent linear increase in Ca^{47} excretion after 5 days (Figs. 4 and 6) can be accounted for by this assumption that Ca^{47} is resorbed from the bone.

DISCUSSION

Most Significant Steroid Effects.—The results of these detailed studies of intravenously administered Ca^{47} in young Beagles support and extend those previously published (1, 2). The most significant effects of steroid radiocalcium metabolism include the increased amounts (more than twice control) of Ca^{47} accumulated in the feces (P_∞ in the Table II) and the increased rate of apparent resorption (*ca.* twice control) of Ca^{47} from a "deep" compartment that may be assigned to bone (R in Table II). Both of these effects are decidedly reversed on the cessation of steroid administration.

Explanation and Correlation of Curve-Fitting.—The fitting of the data by the analog computer (Fig. 1) permits an explanation of these phenomena. It also permits a correlation of the parameters of the model with the sum of exponentials, Eq. 2, fitted to the Ca_B^{47} decrease with time.

The first exponential, Table I, and the $k_{B \rightarrow ST}$ and $k_{ST \rightarrow B}$ (Table IV) values are synonomous in their significance, the presumed fast transfer of Ca^{47} into extravascular spaces and soft tissues. The second exponential (Table I), $A_2e^{-k_2t}$, is largely representative of a slower transfer to the compartment represented as the metabolic pool (Figs. 1, 3, 5). This compartment could be assigned to the reasonably reversible chelation and/or complexation of calcium with bone or matrix.

The analog computer fitting of this model, the ratio $k_{B \rightarrow MP}/k_{MP \rightarrow B} = Ca_{MP}/Ca_B$ (Table IV), which decreases with respect to control on steroid administration, may explain the apparent increase in Ca^{47} blood levels during steroid, *i.e.*, the relative increase in A_3 and A_4 values (Table I). The relative constancy of this ratio between steroid and recovery studies would also explain the fact that the polyexponential A_3 and A_4 values do not appreciably differ between steroid and recovery (Table I).

The model fitting shows that the inhibition of transfer of Ca^{47} from the blood to the metabolic pool, $k_{B \rightarrow MP}$, by a presently unknown mechanism of steroid action, is a plausible explanation for steroid induced osteoporosis. If this metabolic pool represents the sites from which the radioisotope is incorporated into a deep compartment such as bone by the assigned rate constant, $k_{MP \rightarrow PB}$ (Table IV), there does not have to be significant effects of steroid on this latter transfer. This was indicated by the lack of a significant variation of $k_{MP \rightarrow PB}$ with treatment within the dogs. The decrease of Ca^{47} in bone on steroid-induced osteoporosis is a ready explanation on the premise that the amount of calcium in the metabolic compartment necessary for bone incorporation is lessened.

This hypothesis would be consistent with the postulates that an effect of the adrenal steroids is to inhibit the synthesis of protein matrix (17, 18). Thus, less sites would be available for a comparatively reversible binding of calcium with bone precursor as represented by the values for the ratio, $k_{B \rightarrow MP}/k_{MP \rightarrow B}$, as modified by steroid (Table IV).

The recovery period studies again demonstrate reversal of steroid effects in that an increase in the $k_{B \rightarrow MP}$ value is observed on cessation of steroid.

The model used and as fitted (Fig. 1) showed a marked increase with steroid in the apparent first-order rate constant, $k_{B \rightarrow GI}$, for the transfer of Ca^{47} across the intestinal wall or by biliary or gastric secretion as crudely estimated from the fecal excretion data. As has been stated previously (2), this is representative of the excretion of endogenous calcium and is explained by a steroid mechanism that would increase the rate of calcium transfer from the blood to the intestine. The possibility of decreased calcium binding by protein, thereby increasing the amounts of diffusible calcium has already been cited (2, 19) and is consistent with this data. An alternative explanation (2) is the inhibition of calcium return *via* gastrointestinal absorption which is also consistent with recent reports (20).

The analog computer plottings (Figs. 4 and 6) even with an oversimplified first-order process for Ca^{47} transfer from bone to metabolic pool, show that the positive deviation from first-order fecal and urinary eliminations (Table II, Figs. 7 and 8) can be accounted for by a resorption of radioisotope from bone, at least for the data up to 10 days (Figs. 4 and 6).

Limitations of Studies to Date.—The great numbers of fecal, urinary, and blood serum collections necessary to do studies of this kind decidedly limit the number of animals that can be followed experimentally. The major arguments for these experimental designs (within the limitations of the amount of data that can be practicably obtained) are that each dog can be used as its own control and that the relatively short half-life of Ca^{47} permits repetitive radioisotope labeling without interference from the radioisotope administered in the previous study.

The dogs used in this study were in a maximum growth or maturation period and, undoubtedly, hormonal changes occurred in this interval which may have affected their calcium metabolism. It is known that the retention of radioisotopes decreases with increase in age of the dog (21). However, it is felt that such a change would be unidirectional and that when effects of steroid are reversed on recovery, the validity of these effects may be given additional weight. It has been shown that the nature of these effects is similar in the adult dog (1). It is also argued that less variability exists among "litter-mates" during their youth.

Detailed studies should be made with varying steroid dosages to evaluate dose-response relations. Cold calcium assays should be determined simultaneously with Ca^{47} concentrations to correlate changes in specific activity with the influences of adrenal steroid administration and to permit the calculation of exchange rates, r, between compartments rather than the fractional exchange rates, $r/Ca_i = k_i$, which merely permit estimates of relative compartment sizes or the relative calcium content of exchanging compartments. (See *Appendix*.)

The interesting observation of a significant and sudden increase in the apparent resorption rate of Ca^{47} from bone on recovery from steroid should be further investigated. The reproducibility of these Ca^{47} methods without steroid, *i.e.*, the maturation effect on radiocalcium metabolism should be evaluated. Such studies and their results will be reported in a future publication.

Advantages of the Application of the Analog Computer.—The analog computer is an extraordinary tool for the elucidation of the mechanisms and rates of transformations and compartmental exchanges of drugs in the various organs of the body. It permits the establishment of models for gastrointestinal absorption into the blood, diffusion into tissues, incorporation into organs, metabolic pathways, and elimination into the urine and feces. Its use permits the investigation and quantification of distribution and excretion processes for which the application of methods of analytical mathematics is most approximate and difficult.

The philosophy of analog computer programming and application to be consistent with experimental assay, blood level, urinary, and fecal data is based on the use of minimum postulates consistent with physiological reality. The validity of "steady state" approximations and "apparent metabolic half-lives" with respect to the more exact models programmed in the analog computer can be evaluated as is shown in the *Appendix*.

Comparisons of empirical curve fitting and model making by the analog computer were made with respect to Ca^{47} dynamics.

Of course, the digital computer could also be used since numerical methods could approximate the solutions of differential equations established for the appropriate mathematical models. However, the analog computer has the advantages of simpler and more flexible programming for various models. It can generate a series of curves which can be observed by the operator as consistent or inconsistent with the forms and magnitudes of plotted data.

APPENDIX

The total Ca_T^{47} or 100% of the administered dose can be considered as distributed among the various compartments as blood, Ca_B^{47}, soft tissue, Ca_{ST}^{47}, metabolic pool, Ca_{MP}^{47}, gastrointestinal tract, Ca_{GI}^{47}, and bone, Ca_{PB}^{47}, in concurrence with the model of Fig. 1, as

$$100\% = Ca_T^{47} = Ca_B^{47} + Ca_{GI}^{47} + Ca_{ST}^{47} + Ca_{MP}^{47} + Ca_{PB}^{47} \quad \text{(Eq. 13)}$$

where Ca_{GI}^{47} also includes the cumulative amount in the feces and where the negligible amounts in the urine can be ignored. (See Figs. 4 and 6.)

The rates of transfer of Ca^{47} among these compartments before resorption from bone becomes significant can be formulated as

$$d\,Ca_B^{47}/dt = -[k_{B\to GI} + k_{B\to ST} + k_{B\to MP}]\,Ca_B^{47} + k_{ST\to B}Ca_{ST}^{47} + k_{MP\to B}Ca_{MP}^{47} \quad \text{(Eq. 14)}$$

$$d\,Ca_{GI}^{47}/dt = k_{B\to GI}Ca_B^{47} \quad \text{(Eq. 15)}$$

$$d\,Ca_{ST}^{47}/dt = k_{B\to ST}Ca_B^{47} - k_{ST\to B}Ca_{ST}^{47} \quad \text{(Eq. 16)}$$

$$d\,Ca_{MP}^{47}/dt = k_{B\to MP}Ca_B^{47} - (k_{MP\to B} + k_{MP\to PB})Ca_{MP}^{47} \quad \text{(Eq. 17)}$$

$$d\,Ca_{PB}^{47}/dt = k_{MP\to PB}Ca_{MP}^{47} \quad \text{(Eq. 18)}$$

Alternately, these apparent rate constants can be interpreted as fractional turnovers or the fraction of compartmental calcium exchanged in a unit of time as they represent the quotient of an amount of calcium transferred from a compartment in a unit of time, r, and the total amount of calcium, Ca_i, in that compartment, r/Ca_i (14, 15). For example, Eq. 16 can be formulated as the rate of radioisotope transfer proportional to the specific activities of the blood and soft tissue compartments

$$d\,Ca_{ST}^{47}/dt = r[S_B - S_{ST}] = (r/Ca_B)Ca_B^{47} - (r/Ca_{ST})Ca_{ST}^{47} \quad \text{(Eq. 19)}$$

The $S_B = Ca_B^{47}/Ca_B$, $S_T = Ca_{ST}^{47}/Ca_{ST}$ represent the specific radioactivities in the blood and soft tissue compartments, respectively, and Ca_B and Ca_{ST} are the respective total calcium contents of these compartments, so that $k_{B\to ST} = r/Ca_B$ and $k_{ST\to B} = r/Ca_{ST}$. It also follows that $k_{B\to ST}/k_{ST\to B} = Ca_{ST}/Ca_B$ is the ratio of the calcium in the soft tissue to the amount in the blood. It may also be interpreted as the ratio of the apparent effective volumes of soft tissue and blood with respect to calcium if the concentration per unit volume is considered to be the same in the equilibrated compartments.

Inspection of Figs. 4 and 6 indicates that at 1–3 days after the intravenous administration of Ca^{47}, pseudo or quasi steady state conditions can be assumed for the model given in Fig. 1. This assumption implies that the rates of Ca^{47} transfer between blood and soft tissue and blood and metabolic pool are no longer rate determining in the decrease of Ca_B^{47}, and the amounts of radioisotope in these various compartments are proportionately related, as

$$Ca_{MP}^{47} = (k_{B\to MP}/k_{MP\to B})Ca_B^{47} = (Ca_{MP}/Ca_B)Ca_B^{47} \quad \text{(Eq. 20)}$$

$$Ca_{ST}^{47} = (k_{B\to ST}/k_{ST\to B})Ca_B^{47} = (Ca_{ST}/Ca_B)Ca_B^{47} \quad \text{(Eq. 21)}$$

These equations also state that the specific radioactivities, S, in all three compartments are the same in the pseudo or quasi steady state as from Eqs. 20 and 21

$$S = Ca_B^{47}/Ca_B = Ca_{MP}^{47}/Ca_{MP} = Ca_{ST}^{47}/Ca_{ST} \quad \text{(Eq. 22)}$$

The rates of increase of radioisotope in the gastrointestinal tract and bone (Eqs. 15 and 18) may be formulated as equal to the rates of decrease in metabolic pool, soft tissue, and blood (Eqs. 14, 16, and 17) before any return of Ca^{47} from bone to metabolic pool significantly contributes

$$d\,Ca_{GI}^{47}/dt + d\,Ca_{PB}^{47}/dt = -d[Ca_{MP}^{47} + Ca_{ST}^{47} + Ca_B^{47}]/dt = k_{B\to GI}Ca_B^{47} + k_{MP\to PB}Ca_{PB}^{47} \quad \text{(Eq. 23)}$$

Substitution of Eqs. 20 and 21 into Eq. 23 and rearranging gives

$$-d\,Ca_B^{47}/dt = \left[\frac{k_{B\to GI} + k_{MP\to PB}\,k_{B\to MP}/k_{MP\to B}}{1 + k_{B\to MP}/k_{MP\to B} + k_{B\to ST}/k_{ST\to B}}\right] Ca_B^{47} = k_4 Ca_B^{47} \quad \text{(Eq. 24)}$$

The estimated apparent first-order rate constant for the pseudo or quasi steady state elimination of radioisotope from the blood could also be expressed as

$$k_4 = \left[\frac{r_{B,\,GI} + r_{MP,\,PB}}{Ca_B + Ca_{MP} + Ca_{ST}}\right] \quad \text{(Eq. 25)}$$

by substituting the appropriate r_i/Ca_i for the k_i of Eq. 24 and simplifying the result. The r_i values are the calcium exchange rates between the two compartments given in the subscripts of the k_i; the Ca_i values are the total calcium in the compartments identified by the subscript.

It can also be shown (16, 22) from Eqs. 13, 20–22 that on pseudo steady state conditions

$$d\,Ca_{GI}{}^{47}/dt + d\,Ca_{PB}{}^{47}/dt = k_4[Ca_T{}^{47} - Ca_{GI}{}^{47} - Ca_{PB}{}^{47}] \quad \text{(Eq. 26)}$$

This estimated apparent first-order rate constant, k_4, for the pseudo steady state elimination of radioisotope from the blood could also be expressed in terms of apparent compartmental volumes (16, 22, 23).

The term, k_4 (as used in Eqs. 24–26) should be analogous to the k_4 in Table I, the apparent decay constant for the fourth exponential of the polyexponential fit of the $Ca_B{}^{47}$ *versus* time data. Substitution of the k_i values given in Table IV for the model of Fig. 1 will give calculated k_4 values from Eq. 23 of 3×10^{-6} sec.$^{-1}$, whereas the k_4 values of 5×10^{-6} sec.$^{-1}$ are listed in Table I.

The model used in the programming of the computer was necessarily a simplification of the physiological reality. The estimates of $k_{B\to GI}$ representative of apparent rates of radioisotope transfer from the blood to the gastrointestinal tract were actually determined by analog computer fitting to the cumulative fecal Ca^{47} at 5–10 days since no actual data were available for the amount in the intestine. The true value of $k_{B\to GI}$ is undoubtedly greater than the first approximation from fecal data since intestinal capacity for Ca^{47}, possible fecal and mucosal binding of radioisotope, and lag in defecation would contribute to an underestimation of the rate of removal of $Ca_B{}^{47}$ from the blood to the intestine.

An increased value of $k_{B\to GI}$ in Eq. 23 would estimate a k_4 value more consistent with the listed values of Table I. Further, the model and program of Fig. 1 ignore the probable equilibration of Ca^{47} in the intestinal tract with that of the blood as was indicated in the more complex model given in Fig. 2.

REFERENCES

(1) Garrett, E. R., Johnston, R. L., and Collins, E. J., THIS JOURNAL, **51**, 1050(1962).
(2) Collins, E. J., Garrett, E. R., and Johnston, R. L., *Metabolism*, **11**, 716(1962).
(3) Robertson, J. S., *Physiol. Rev.*, **37**, 133(1957).
(4) Aubert, J., Milhaud, G., Manoussos, G., and Dormard, Y., *Compt. Rend.*, **246**, 2817(1958).
(5) Heaney, R. P., and Whedon, G. D., *J. Clin. Endocrinol. Metab.*, **18**, 1246(1958).
(6) Eisenberg, E., in "Biological Activities of Steroids in Relation to Cancer," Academic Press, Inc., New York, N. Y., 1960, p. 190.
(7) Aubert, J., and Milhaud, G., *Biochim. Biophys. Acta*, **39**, 122(1960).
(8) Armstrong, W. D., Johnson, J. A., Singer, L., Lienke, R. I., and Premer, M. L., *Am. J. Physiol.*, **171**, 641(1952).
(9) Thomas, R. O., Litovitz, T. A., Rubin, M. Z., and Geschickter, C. F., *ibid.*, **169**, 568(1952).
(10) Thomas, R. O., Litovitz, T. A., and Geschickter, C. F., *ibid.*, **176**, 381(1954).
(11) Neuman, W. F., and Neuman, M. W., "The Chemical Dynamics of Bone Material," The University of Chicago Press, Chicago, 1958, and references therein.
(12) Solomon, H. K., "Compartmental Methods of Kinetic Analysis," in Comar, C. L., and Bronner, F., "Mineral Metabolism," Part A, Academic Press, New York, N. Y., 1961, and references therein.
(13) Bauer, G. C. H., Carlsson, A., and Lindquist, B., "Metabolism and Homeostatic Function of Bone," in Comar, C. L., and Bronner, F., "Mineral Metabolism," Vol. I, Part B, Academic Press, New York, N. Y., 1961, and references therein.
(14) Comar, C. L., "Radioisotopes in Biology and Agriculture," McGraw-Hill Book Co., Inc., New York, N. Y., 1955, Chapter 1 and references therein.
(15) Sheppard, C. W., "Basic Principles of the Tracer Method," John Wiley and Sons, Inc., New York, N. Y., 1962.
(16) Garrett, E. R., Thomas, R. C., Wallach, D. P., and Alway, C. D., *J. Pharmacol. Exptl. Therap.*, **130**, 106(1960).
(17) Albright, F., *Harvey Lectures*, **38**, 123(1942–1943).
(18) Reifenstein, E. C., *Metabolism*, **7**, 78(1958).
(19) Freeman, S., and Breen, M., *Clin. Orthopedics*, **17**, 1868 (1960).
(20) Harrison, H. E., and Harrison, H. C., *Am. J. Physiol.*, **179**, 265(1960).
(21) Glad, B. W., Mays, C. W., and Fisher, W., *Radiation Res.*, **12**, 672(1960).
(22) Teorell, T., *Arch. Intern. Pharmacodyn.*, **57**, 205,2268 (1937).
(23) Dominquez, R., in Glasser, O., "Medical Physics," Vol. II, Year Book Publishers, Inc., Chicago, 1950, p. 476.