sity of paracoagulation is closely related to degree of fibrinogen proteolysis by plasmin. In this way, the small amount of acid-soluble material liberated is comparably important to the liberation of fibrinopeptides from fibrinogen by thrombin (3-4% of total N)(11), which gives rise to important changes in the original molecule. It is a matter of further study whether plasmin-liberated peptides play an active role as inhibitor or dissolution agents on the clotting of fibrinogen and on fibrin clot respectively.

Summary. Fibrinogen proteolysis by plasmin and the clotting of lysed fibrinogen were studied. Close relationship was found between increase in NPN and behavior of lysed fibrinogen.

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Comparative Metabolism of Ca^{45} and Ra^{226}.* (22917)

B. J. STOVER, D. R. ATHERTON, AND J. S. ARNOLD
(Introduced by T. F. Dougherty)
Radiobiology Laboratory and Department of Anatomy, University of Utah, Salt Lake City.

In experimental biology, the double tracer technic is a simple but powerful method. It is especially useful for comparative studies of metabolically similar elements, since biological variability is greatly reduced. In the studies described below, the radioactive isotopes Ca^{45} and Ra^{226} were used since the skeleton was of primary interest. The metabolism of radium, while qualitatively the same as that of calcium, quantitatively is unique, and the difference is of value in the study of skeletal metabolism. In addition, knowledge of the metabolism of radium relative to that of calcium is important in the problem of radium toxicity.

Methods. A 5-month-old beagle dog from a colony of several hundred(1) served as the subject. The pup was ½ to ¾ expected maximum size, and its skeleton was still growing rather rapidly. 71.8 μc Ra^{226} and 1.60 mc Ca^{45} in 8.2 ml isotonic saline, pH ca. 2 (HCl), were administered. These amounts, which would not cause detectable radiation damage in a short term study, permitted the measurement of very small samples. The injection standard was measured with the same syringe subsequently used to inject the dog. A conventional proportional counter† was used to measure both Ca^{45} and Ra^{226}. Aliquots of ashed samples were plated on stainless steel discs, which were flamed to evolve radon and its daughters, then allowed to stand 1 to 2 hours to permit decay of any remaining radon daughters. Immediately afterward, alphas (from Ra^{226}) were measured, then both alphas and betas (from Ca^{45}) were counted. The error in determining Ca^{45} by difference was minimized by injecting 22.5 μc Ca^{45} per μc Ra^{226}. Measurement of both isotopes in the same sample eliminates the effect of preparation errors in

* Supported by the U.S. Atomic Energy Commission.

† Nuclear Measurements Co., Model PC-1.

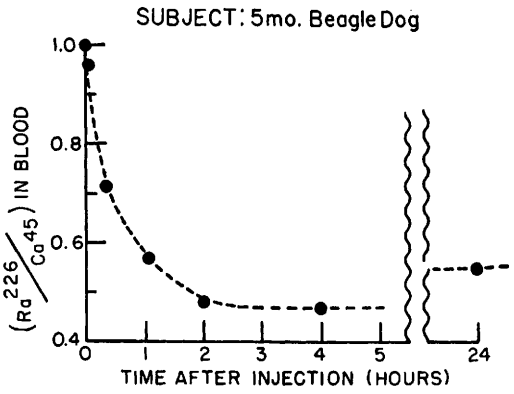

FIG. 1. Relative amounts of Ca^{45} and Ra^{226} in blood at various times following intrav. inj.

determining relative amounts. Appropriate corrections for mass absorption were made, and aliquots of the injection standard were plated and measured with each group of samples. Blood samples were drawn at various times prior to sacrifice, which was at 24 hours after injection. Pre-sacrifice excreta and contents of bladder and gut were collected. The skeleton was completely dissected, and the teeth and individual bones were analyzed.

Results. A. *Blood*: Both alkaline earths serve as tracers for calcium; Ca^{45} quantitatively, and Ra^{226}, qualitatively. Early blood concentrations indicate the rates at which they are diluted by various metabolic processes involving calcium. The concentrations of both isotopes in blood were determined at times ranging from 1.4 min. to 24 hours after injection. Ca^{45} is significantly greater than Ra^{226} at all times except at 1.4 min., when they are about equal. Thus, one or more of the calcium metabolic processes is significantly differentiating between the two alkaline earths. The ratio of Ra^{226} to Ca^{45} in blood which is plotted against time in Fig. 1, decreases rapidly, attaining a minimum value of about 0.48 by 2 hours, which is maintained for several hours. The subsequent increase observed at 24 hours may be real, since additional studies(2) indicate two processes which could cause a slight increase. First, resorption occurring at the epiphyseal lines would release approximately equal amounts of the isotopes to the circulation. Second, Ca^{45} is preferentially incorporated in mature cortical bone, and, if this is a continuous process, it must be accompanied by a preferential release of Ra^{226} to the blood.

Assuming a blood volume of 430 ml, (*i.e.* 7% of body weight of 6.14 kg), the data can be expressed as % of injected dose of blood as a function of time. These data, which appear in Fig. 2, can be described by the following equations, each of which adequately describes all but the earliest point: % Ca^{45} in the blood $= 85t^{-0.70}$, % Ra^{226} in the blood $= 63t^{-0.76}$, (t in min.). The method of least squares was used. (The first 20 minutes can be approximated with the functions % $Ca^{45} = 33t^{-0.42}$ and % $Ra^{226} = 33t^{-0.54}$.)

Using the above equations, VPRC = 0.42, and serum calcium = 0.11 mg/ml, the average specific activity, ($\overline{SA}$) for both isotopes and several intervals has been calculated, and the results appear in Table I. Specific activities are expressed in units % dose/g calcium to facilitate comparison with bone specific activities. Also included are the products $\overline{SA} \times \Delta t$ normalized to the entire period, and ratios of Ra^{226} $\overline{SA}$ to Ca^{45} $\overline{SA}$.

B. *Excreta.* Results of excreta analyses are summarized in Table II, and show that both excretory routes are factors in the more rapid removal of Ra^{226} from blood. Although Ra^{226} excretion is approximately a factor of 10 greater than Ca^{45} excretion, it is still only

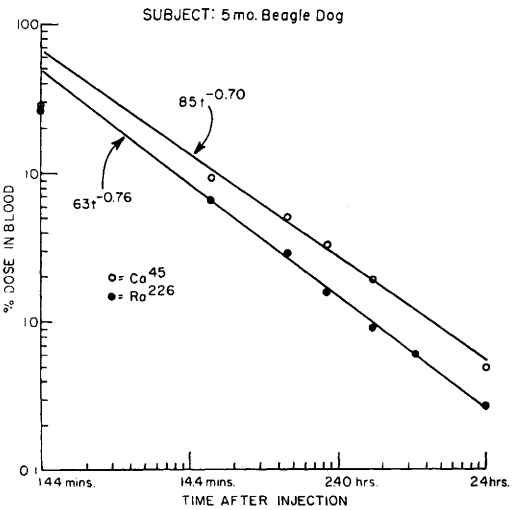

FIG. 2. % of inj. dose of Ca^{45} and of Ra^{226} in blood following intrav. inj.

TABLE I. Average Blood Specific Activities of Ca^{45} and Ra^{226}.

	Ca^{45}		Ra^{226}		
Δt, min.	$\overline{SA}$, % dose/g Ca	Relative $\overline{SA} \times \Delta t$	$\overline{SA}$, % dose/g Ca	Relative $\overline{SA} \times \Delta t$	$\frac{Ra^{226}\ \overline{SA}}{Ca^{45}\ \overline{SA}}$
0– 20	585	.15	516	.23	.88
20–1440	46	.85	25	.77	.53
0–1440	54	1.00	32	1.00	.58

TABLE II. Results of Excreta Analyses.

	% of inj. dose		
	Ca^{45}	Ra^{226}	Ra^{226}/Ca^{45}
Pre-sacrifice excreta	.26	2.85	11
Bladder & contents	.018	.25	14
Small intestine & contents	.14	.19	1.4
Large " " "	1.56	1.90	1.2
Blood at 24 hr	.49	.27	.55

a small fraction of the Ra^{226} injected. Since this was a young animal with a growing skeleton, retention of both alkaline earths is high.

The ratios of Ra^{226} to Ca^{45} in urine and blood at 24 hours, show a preferential kidney excretion of radium by a factor of 26 over calcium. Putnam‡ found that radium in rat blood is 87% ultrafilterable, and the dog calcium value obtained by the same method is about 50%(3). Assuming in this case that the diffusible fraction of Ra^{226} is twice that of Ca^{45}, the observed value of 26 shows that resorption of calcium in the kidney of a young growing beagle is at least 10 times that of radium. The ratio of Ra^{226} to Ca^{45} observed in both large and small intestines at autopsy is about twice that of blood, and probably results from the greater diffusibility of blood radium.

C. *Skeleton and teeth.* At autopsy the entire skeleton was dissected, several long bones were selected for other studies(2), and all others were analyzed individually. Since the radioactive isotopes deposit symmetrically in the skeleton, the special studies were done on one of a pair, and the other was analyzed. The total skeletal retention (including teeth) at 24 hours was found to be about 91% for Ra^{226} and about 94% for Ca^{45}, from which the Ra^{226}/Ca^{45} ratio for the whole skeleton is calculated to be 0.96. The mean of the Ra^{226}/Ca^{45} ratios for the individual bones was 0.96, $\sigma = 0.075$. No significant difference between the Ra^{226}/Ca^{45} ratios of long bones and those of flat bones was observed.

In contrast with the high ratio of the skeleton, the Ra^{226}/Ca^{45} ratio observed in the teeth was 0.49, which is approximately equal to the minimum value observed in blood and is significantly lower than the average blood value for the entire period. Comparing the Ra^{226}/Ca^{45} ratios observed in whole bones, teeth, and blood, it is apparent that the rates and/or mechanisms of isotope incorporation differ in the skeleton and the teeth. The high ratio observed in the skeleton indicates some combination of the following: 1) rapid, irreversible deposition of both isotopes, 2) a higher Ra^{226}/Ca^{45} ratio in the extravascular fluids than in the blood (resulting from the larger fraction of diffusible Ra^{226}), and 3) preferential binding of Ra^{226} on the crystal surfaces. The low ratio observed in teeth, which at this age can be assumed to be depositing dentine, definitely indicates a preferential incorporation of Ca^{45}. The ratio for teeth is too low to be accounted for by rate of fluid movement only, especially since the ratio in the extravascular fluid is probably higher than that in blood.

A process occurring in calcified structures which has been shown to discriminate against radium is recrystallization(2), and possibly in teeth recrystallization is relatively more important in the incorporation of blood calcium than is new crystal formation or ion exchange. If the formation of new crystals is slow compared with those of bone, sufficient time may elapse between the deposition of successive layers for appreciable equilibration to occur between surface and interior (recrystallization), and calcium would be preferentially incorporated.

Summary. The comparative metabolism of

‡ Personal communication.

TABLE III. Specific Activities at 24 Hr.

	% Ca^{45}/g Ca	% Ra^{226}/g Ca	Ra^{226}/Ca^{45}
Blood plasma	18	9.8	.55
Skeleton	2.0	1.9	.96
Teeth	.84	.41	.49

calcium and radium has been studied in a young beagle dog. Ra^{226} left the blood more rapidly than Ca^{45}; the preferential excretion of radium by both kidney and gut is a factor in explaining the blood data, and also is the principal gross metabolic difference. The retention of both alkaline earths was greater than 90%, consistent with the fact that the skeleton was rapidly growing. Analysis of individual bones revealed no significant difference in the relative deposition of the two isotopes, but the teeth showed a preferential incorporation of calcium.

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Effect of Glycerine on Toxicity of Isoniazid in Mice. (22918)

BENJAMIN PRESCOTT, GLADYS KAUFFMANN, WALTER D. JAMES
(Introduced by B. J. Olson)
Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, N.I.H., P.H.S., Bethesda, Md.

Isoniazid (INH) continues in widespread use as a therapeutic agent against human tuberculosis; but dosage is limited by its toxicity. This toxicity has been reduced in laboratory animals by simultaneous administration of appropriate amounts of sodium glucuronate and glycine with the INH(1). Some other chemicals, certain amino and alpha-keto acids, have also been found to be effective in increasing the tolerated dose of INH (2). In addition several organic solvents have now been investigated. One of the most promising is glycerine which has long been recognized as an effective bactericidal agent. As early as 1869 Mueller(3) prepared cowpox vaccine in approximately 75% glycerine solution, and found its activity to be unimpaired whereas extraneous microorganisms were destroyed. A thorough investigation of the antiseptic and germicidal properties of glycerine was reported by Rosenau(4), who concluded that, while 10% by volume favored growth, 50% glycerine inhibited all bacteria studied—some 10 pathogens and a few saprophytes. Locatelli and Bowden(5) and Wood (6) found that sulfa drugs were more effective for topical and surgical application respectively when dispersed in glycerine. Fisher(7) reported that the tuberculostatic activity of streptomycin was enhanced in Youman's medium containing 2% glycerine. Montestruc and Garcin(8) reported successful use of glycerine-suspended BCG to detect allergy in BCG-vaccinated individuals. From these studies it may be deduced that glycerine in suitable concentrations is useful as an adjuvant. Furthermore its low toxicity for human beings was observed by Johnson, Carlson, and Johnson(9) in a group of individuals given 110 g daily for 50 days with no adverse manifestations. However, to our knowledge evidence of its capacity to reduce the toxic action of chemotherapeutic agents in animals has not been presented.

The present study is an investigation of the effect of glycerine on the toxic action of INH in mice. The experiments include: (a) acute toxicity of glycerine solutions in DBA mice, (b) effect of glycerine on acute toxicity of INH in mice, (c) effect of repeated administration of various dosage combinations of INH and glycerine, (d) blood levels in mice following combined administrations, and (e) effect of glycerine on bacteriostatic activity