latter finding and showed in addition that, as implied by Ratnoff and Margolius(3), surface activation is activation of Hageman factor. Finally, by use of purified Hageman factor devoid of other clotting factors, we mimicked the clot acceleratory activity of asbestos treatment. Comparable preparations from Hageman trait patients were inactive.

Removal of an inhibitor must be considered as an alternative explanation of our data. Shafrir and de Vries(2) believed that an inhibitor was not adsorbed onto glass. We did not feel that asbestos removed an inhibitor, since: 1) treated Hageman deficient plasma did not shorten clotting-times of plasmas containing Hageman factor (Table III); 2) asbestos treatment was mimicked by positive factor addition (Tables III, V); 3) there may have been *increased* inhibition which resulted from asbestos treatment (Table III).

Thus, surface contact may be said to result in Hageman factor activation. In its absence, no activation is noted. The failure of asbestos to activate plasma lacking Hageman factor suggests a *presumptive* test for Hageman trait diagnosis.

Summary. 1. Asbestos treatment of plasma produced activation (clotting-time acceleration) in normal and AHF, PTC, proconvertin, prothrombin, proaccelerin and Stuart deficient plasmas. Hageman deficient plasma was not activated by comparable treatment. 2. Purified Hageman factor preparations from normal sera simulated activation, whereas preparations from sera of Hageman trait patients were inactive in the same test system. 3. The phenomenon of surface activation resulted from Hageman factor activation and was not due to inhibitor adsorption. 4. Asbestos treatment of plasma may be used as a *presumptive* test for Hageman trait.

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Metabolism of Sr^{90} in Adult Beagle Dogs.* (24295)

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This study of Sr^{90} metabolism is part of a large program to compare the chronic toxicities of Ra^{226}, Pu^{239}, Ra^{228}(MsTh), Th^{228} (RdTh), and Sr^{90} in young adult beagle dogs. The experimental design, especially relationship of dose levels to maximum permissible and natural levels in man, has been described (1). The basic plan is to compare the effects of these radioactive isotopes in a larger, longer-lived animal (the dog) than is usually used in the laboratory; then to use this information in conjunction with the knowledge of radium poisoning in humans to reappraise maximum permissible levels for other isotopes. To correlate the biological effects with radiation dose, a detailed knowledge of the metabolism of each radioisotope is needed.

Methods. Sr^{90}, with its daughter Y^{90} in equilibrium, in a citrate buffer solution of pH = 3.5, was given in single intravenous injection. Injected doses ranged from 0.56 to 100 μc/kg; duplicate doses served as injection standards. The beagle dogs, healthy young adults 16 to 17 months old, during the excretion study were housed in stainless steel metabolism cages, with perforated floors which permit urine to drain into plastic bottles so that

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there is good separation of urine and feces. Urines were wet ashed with HNO_3; feces were incinerated in a muffle furnace, then digested with HNO_3. At a time to insure Sr^{90} – Y^{90} equilibrium, 1 ml aliquots of ashed excreta samples were measured using a well-type plastic scintillator to detect the beta particles. There are 2 important factors to consider in using this beta detector, which is similar to that described by Hine and coworkers(2). The first is geometry, for detector response decreases as source is moved from bottom to top of the well: *i.e.*, count rate of 3 ml of a given solution is less than 3 times the count rate of 1 ml of the same solution. Fortunately, the polyethylene tubes used are quite uniform (0.16 cm wall, 0.82 cm inside diameter), and the geometry problem is solved by using a constant volume. The second factor is self-absorption. The Sr^{90} standards are dilute solutions of essentially unit density (density of plasma is also approximately one), but densities of ashed excreta samples are greater and the effect is significant. Polyethylene tubes and liner of scintillator well are sufficiently thick to stop all Sr^{90} betas, so we are actually measuring Y^{90}. Detection efficiency for 1 ml sample of unit density is about 0.14 count/Y^{90} disintegration. Increasing density to 1.5 g/ml decreases efficiency to 0.10 count/Y^{90} disintegration, so that a self-absorption correction must be made. If absorption of the betas is represented by an exponential law, the effect of density on detection efficiency, E, is given approximately by $E = c_1/d\ (1 - e^{-c_2 d})$ (eq. 1), where d is density and c_1 and c_2 are constants. Expanding the exponential gives the following approximate but adequate expression, $E = c_3 - c_4 d$ (eq. 2). To determine c_3 and c_4 experimentally, quadruplicate samples were prepared which contained constant amounts of Sr^{90} and Y^{90} in constant volumes of solutions of varying density, and count rates were determined. The resulting equation, arbitrarily setting $E = 1$ when $d = 1$, is $E = 1.59 - 0.59\ d$ (eq. 3). Blood for the plasma concentration study was heparinized. Soon after injection the Sr^{90} in 1 ml of plasma could be determined directly. As plasma concentration decreased, it became necessary to concentrate the Sr^{90} from a larger volume of plasma by co-precipitation with CaC_2O_4.

TABLE I. Twenty-Two Days Cumulative Sr^{90} Excretion.

Inj. dose (μc/kg)	Inj. age (mo)	Fecal (% dose)	Urinary (% dose)	Total (% dose)	$\Sigma F/\Sigma U$
.593	16	30.3	27.3	57.6	1.11
1.74	"	30.2	29.9	60.1	1.01
3.49	"	32.2	26.6	58.8	1.21
11.2	17	41.1	26.3	67.4	1.56
33.2	"	34.3	20.7	55.0	1.65
105.	"	39.6	24.7	64.3	1.60
Avg	16.5	34.6	25.9	60.5	1.34

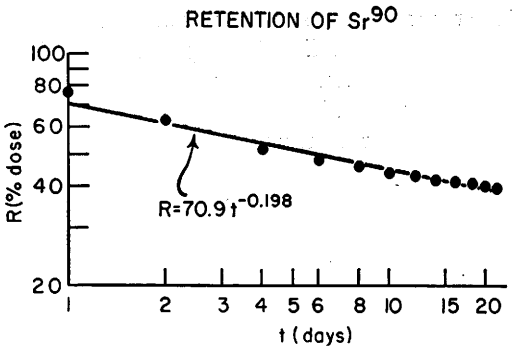

FIG. 1. Retention of Sr^{90} by young adult beagles following a single intrav. inj.

Results. Excretion. Results for each dog are summarized in Table I. Urinary and fecal excretion are nearly equal; average total fecal excretion is 1.34 times average total urinary excretion, and average of individual fecal to urinary ratios is 1.48. There is no apparent dose level effect.

Since this is a continuous excretion study of short duration, the most meaningful presentation of excretion measurements is to calculate retention from cumulative excretion. This has been done for $t = 1$ through $t = 22$ days, and the resulting retention data (Fig. 1)† can be described by a power function. The following equation for retention, R, (% dose) was obtained by applying the method of least squares to logarithms of the data,‡ (t in days), $R = 70.9\ t^{-0.198}$ (eq. 4).

† Indication of a slightly greater slope from day 1 to 4 undoubtedly results from the inherent lag in fecal excretion, which has a significant effect only during initial high excretion period.

‡ Subsequent equations were calculated by the same method.

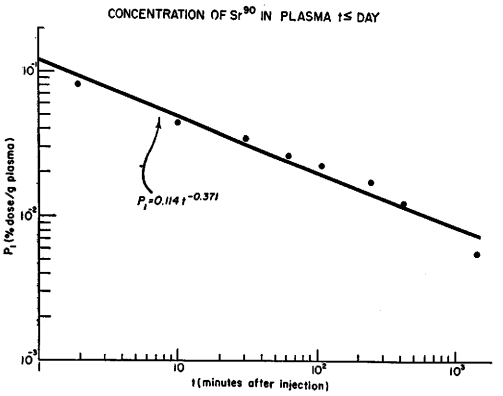

FIG. 2. Plasma concentration of Sr^{90} during first day following inj.

Plasma concentration. Following intravenous injection of soluble Sr^{90}, plasma concentration, as shown in Fig. 2 and 3, decreases rapidly during the first day and continues to decrease through 2 years after injection. To facilitate mathematical treatment, the first day's data and those from one day on were treated separately. Each set can be described by a power function as follows:

$P_1 = 0.114\ t_1^{-0.371}$, $t \leq 1$ day, (eq. 5)
$P_2 = 5.03 \times 10^{-3}\ t_2^{-1.25}$, $t \geq 1$ day, (eq. 6)

where P_1 and P_2 are % dose/g plasma, t_1 is minutes and t_2 is days after injection. From these equations and the average total plasma volume of 450 ml,(3) the per cent dose circulating at any time t after injection can be calculated.

Discussion. Norris and coworkers(4) have used a power function to describe retention of alkaline earth radium in man, and many other studies, (*e.g.*,(1)), have demonstrated the general usefulness of this treatment for retention of alkaline earths in various species. The derivative with respect to time of retention function should be equal to excretion rate and proportional to plasma concentration. Comparing exponents of equations(4) and (6) we observe the expected relationship.§ Since plasma measurements extend over a longer period, the agreement between plasma and retention functions shows that the retention function can be extrapolated without introducing serious error. As further verification, C. W. Mays(5) of this laboratory has determined Sr^{90} retention directly by measuring bremsstrahlung. His data extend to 3 years after injection and his result is very similar to equation(4).

An extensive study of radium metabolism in young adult beagle dogs has also been made at this laboratory(1). Since both groups of dogs were essentially the same age at injection, live in the same environment, and have about the same diet, a reliable metabolic comparison of Sr^{90} and Ra^{226} can be made. Retention, plasma, and excretion results are summarized in Table II. There is a significant difference in retention. Retention of Sr^{90} is slightly less than that of Ra^{226}, but, most interestingly, both decrease at the same rate. During the first day plasma strontium decreases less rapidly than does radium, at one day strontium is about twice that of radium, and thereafter they decrease at the same rate maintaining a ratio of two. Although the fecal to urinary excretion ratio is greater for

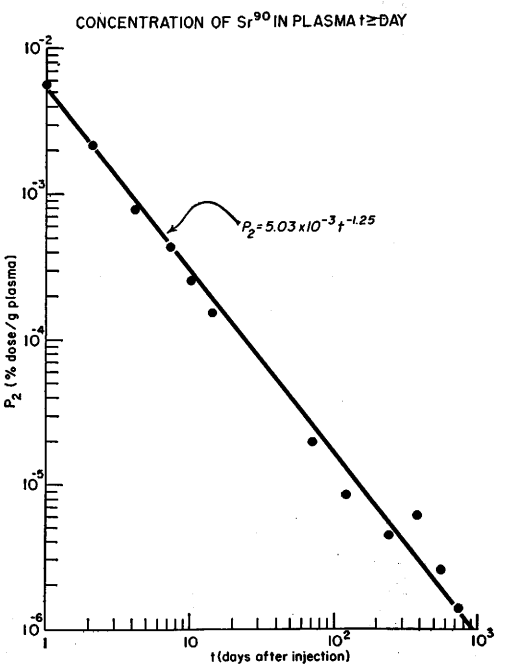

FIG. 3. Plasma concentration of Sr^{90} from 1 day through 2 yr after inj.

§ Actually, plasma concentration decreases a little more rapidly than expected from the derivative of equation(4), which suggests that some of the late plasma values are low.

TABLE II. Comparison of Sr^{90} and Ra^{226}.

Measurement	Sr^{90}	Ra^{226}	Units
Retention	$71\ t^{-.20}$	$79\ t^{-.20}$	% dose, days
First day plasma concentration	$.114\ t^{-.37}$	$.092\ t^{-.45}$	% dose/g, min.
Later plasma concentration	$5.0 \times 10^{-3} t^{-1.25}$	$2.6 \times 10^{-3} t^{-1.24}$	% dose/g, days
Fecal to urinary excretion ratio	1.4	2.5	

radium, the beagle kidney actually excretes radium more effectively than strontium. Renal clearances calculated from data of Table II are 1180 g plasma day for Sr^{90} and 1690 for Ra^{226}. Similarly, we calculate that daily Sr^{90} excretion is 6.5 times the amount in plasma, while 13.5 times the plasma Ra^{226} is excreted. Thus, excretory mechanisms function more effectively for radium, yet radium retention is higher. This results of course from the higher Sr^{90} plasma concentrations, especially during the period soon after injection, when excretion rate is highest. Although Sr^{90} is not excreted quite as effectively, more is available to be excreted with the net effect that more Sr^{90} than Ra^{226} is excreted.

Higher Sr^{90} plasma concentrations can result from several factors. Less efficient excretion is one, but from the above discussion it cannot be the principal one or strontium retention would be higher. A second possible factor is that a larger fraction of plasma Sr^{90} exists as complexes with proteins and the various anions. (Since strontium ion is smaller than radium ion, it should form stronger complexes.) Our preliminary results, using a double tracer technic indicate that a significantly smaller fraction of plasma Sr^{90} is diffusible. The third possibility is greater re-exchange of Sr^{90} from bone to body fluids. These 3 factors, excretion efficiency, protein complexing, and extent of re-exchange, reflect the chemical difference between radium and strontium and most probably account for the observed metabolic differences.

Although in the above discussion we have emphasized the differences, the 2 isotopes actually are very similar metabolically. Both are chemically like calcium and deposit almost exclusively in the skeleton; the retention equations show the same dependence on time as do the late plasma equations. Since we have observed chemically related differences, the common time dependence leads us to speculate that the principal factor affecting time dependence is the overall skeletal metabolism (exchange and remodelling). Small chemical differences affect details of the metabolism, while normal skeletal processes involving calcium are controlling factors in the main aspects of metabolism of these 2 isotopes. If this is so, then similar studies with calcium and barium should give power functions with the same exponents but different coefficients.

Since we have shown previously(6) that Y^{90} produced *in vivo* from decay of Sr^{90} in the skeleton does not translocate to any significant extent, the retention equation(4) can be used to calculate the average dose rate to the skeleton and other tissues within the range of the beta particles.

Summary. 1. Retention and plasma concentration of Sr^{90} in young adult beagle dogs have been determined for 2 years following a single intravenous injection. Retention (% dose) follows the power function (t in days) $R = 70.9\ t^{-0.198}$. 2. Comparison of these results with those from similar studies with Ra^{226} shows the anticipated similarity in metabolism of Sr^{90} and Ra^{226}. However, there are real differences. Sr^{90} retention is less than that of Ra^{226} even though Ra^{226} is excreted more efficiently. This results from higher Sr^{90} plasma concentration. 3. Since Sr^{90} deposits mainly in the skeleton and its daughter Y^{90} does not translocate, this retention equation can be used to calculate average skeletal dose rates for these dogs.

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The Inactivation of Newcastle Disease Virus Hemolysin by Antiserum and High-Energy Electrons.* (24296)

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Previous studies on the hemolysin of Newcastle Disease Virus (NDV) have shown that this property is influenced by the physical treatment of the virus(1), is inhibited by calcium ions(2), and is dependent on pH and the temperature of incubation(3). Radiation studies on NDV(4) have shown that there are about 15 hemolysin units per virus and that the form of these units is probably a flat plate 100-140 Å in radius and 20 Å thick. The experiments described here are concerned with the mechanism of action of the NDV hemolysin. The results of Dulbecco *et al.* (5) and Rubin and Franklin(6) indicate that study of virus neutralization by antibody may yield valuable information on structure and components of the virus. Inhibition of the hemolysin by antiserum and inactivation by high-energy electrons were studied.

Methods. The 87909 strain of NDV was used throughout these studies. High titer virus stocks were prepared by inoculating fertile chicken eggs on the tenth day of incubation with about 10^7 NDV particles. After 30 hours of incubation the infected eggs were chilled to 4°C and the allantoic fluid was harvested. Infected allantoic fluid was then centrifuged for 30 minutes at 30,000 g in a Servall SS-1 centrifuge at 4°C. The virus deposit was resuspended in isotonic saline containing 2% sodium citrate and stored at 4°C until used. *Virus assays.* In the hemagglutinin assay, serial 2-fold dilution of virus in citrate saline were added to .2 ml of a 4% suspension of washed chicken red blood cells (rbc). The highest dilution of virus showing agglutination was scored as the endpoint. All virus samples to be used for hemolysin assays were first dried in citrate saline and resuspended in the same medium. This drying is similar in effect to freezing and thawing and increased the hemolysin titer of the virus preparations about 50-fold. Hemolysin titrations were performed in two ways. The *total hemolysis assay* was adapted from Granoff and Henle(1). 1.0 ml of a virus suspension was placed in a test tube with 1.0 ml of washed rbc in citrate saline (pH 7.2). This mixture was incubated at 37°C for one hour, then 1.0 ml of cold phosphate buffered saline (pbs) was added to stop the reaction. Rbc were removed by centrifugation and optical density of the supernatant fluid was measured at 540 mμ. One tube containing rbc but not virus was used to balance the spectrophotometer and thus eliminate the effect of spontaneous hemolysis. In another tube distilled water was added to give 100% hemolysis. The percent hemolysis for each sample was then calculated from the optical densities. The *hemolysis rate assay* is a modification of the total hemolysis assay. 1.0 ml aliquots of a virus suspension were mixed with 1.0 ml of a 1% suspension of washed rbc and incubated at 37°C. The hemolysis reaction was allowed to proceed in different tubes for 1, 2, and 3 minutes, then stopped by addition of 2 ml of cold pbs. Rbc were removed by centrifugation and optical density of the supernatant fluid was measured at 540 mμ. Rate of he-

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