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RADIOTOXICITY OF INJECTED Sr^{89} FOR RATS, MICE, AND RABBITS

PART I. INTRODUCTION: METHODS

BY

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ABSTRACT

The toxic effects and metabolism of radiostrontium (Sr^{89}-Sr^{90}) were studied. Determinations were made of the acute lethal doses for rats, mice, and rabbits, and of the comparative toxicity and metabolism following intraperitoneal and intravenous administration. Distribution studies of radiostrontium in various organs were made.

A method is described for handling and preparing radioactive strontium for administration to experimental animals. Procedures are given for preparing tissues and excreta for measurement of Sr^{89} - Sr^{90} with a Geiger-Müller counter, and for correcting these measurements for radioactive decay back to the time of injection.

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1. Introduction

In the various chain-reacting piles constructed for the plutonium project, enormous amounts of radioactive materials were produced by uranium and plutonium fission, and of course, such concentrations of radioactive materials presented a very great health hazard. Many of the radioactive isotopes were of such short life that they could be eliminated as a health hazard by allowing them to decay before the isotopes were separated. Other fission products, like radiobromine, were slightly-lived so that they survived the cooling period in amounts sufficient to be dangerous for a few days or weeks. However, it was necessary to study materials of this class in the vicinity of the pile so that the time between production of the radio-isotope and its use in biological experiments was at a minimum.

A third class of radioactive fission products, those with half-lives of many days, were potentially very dangerous for those who worked at the piles and for those in plants associated with the piles during the processing of uranium or plutonium. These long-lived fission products, if taken into the body, might continue to radiate and injure the tissues for months or years - much as radium is known to do. However, since there were so many fission products in this class, and because speed in obtaining answers to at least few urgent questions was so essential, it was necessary to select a limited number of representative radio-isotopes for biological experimentation.

As soon as the physical properties of Sr^{89} and Sr^{90} were elucidated, it became apparent that they might be among the most important potential health hazards arising from uranium or plutonium fission. These isotopes were present in high yield after fission, the energies of their radiations (or that of a

radioactive daughter) were considerable, and their half-lives were long. Table 1 gives the best values available for these and other characteristics of Sr^{89} and Sr^{90} (1).

TABLE I

	Sr^{89}	Sr^{90}
Fission yield	4.6 %	5 %
Half Life	55 days	Ca. 30 years
Max. energy	1.7 Mev	0.65 Mev

Kr^{89} —2.5 min, β→ Rb^{89} —15 min, β_1 3.8→ Sr^{89} —55 days, β_1 1.7→ Y^{89} stable

Kr^{90} —short, β_1→ Rb^{90} —short, β→ Sr^{90} —r 30 yr, β 0.65→ Y^{90} —60 hr, β 2.5→ Zr^{90} stable

Although both Sr^{89} and Sr^{90} are produced in large yield, in any fresh or moderately young preparation of fission strontium, the radioactivity is due almost entirely to Sr^{89}, since the rate of disintegration of Sr^{89} is more than 100 times that of Sr^{90}. After a few half-lives of Sr^{89}, the radiation from Sr^{90} (or from its radioactive Y^{90} daughter) does begin to represent a significant proportion of the total, and by 1 1/2 to 2 years after fission, virtually all of the radiation from a sample of strontium is due to the Sr^{90} - Y^{90}. Throughout this report, references to radiostrontium or to Sr^{89} indicate a mixture of the two isotopes. The actual composition of radiostrontium samples at the time of injection is discussed in Section 4.3 of this report.

In addition to the physical characteristics of radiostrontium, certain features of the known metabolism of strontium indicated its probable importance as a health hazard. Strontium was known to be very readily absorbed and tenaciously retained in the skeleton of experimental animals. In studies on

the metabolism of radiostrontium, Pecher[2] found that within 48 hours after treatment with Sr^{89} an average of 33 to 34 per cent of an intravenously injected dose, and 10 to 12 per cent of an orally administered dose were present in the skeleton of mice. In fact, the maximum skeletal deposition occurred within 8 hours after intravenous injection. There appeared to be no difference in the metabolism of the chloride, lactate, and glucoate when given by either route. In all cases, the only significant retention was in the skeleton.

The available information showed an expected similarity between the metabolism of strontium and that of radium, hitherto the only substance of which the radiotoxicity had been extensively studied. Radium was readily absorbed following ingestion of radium salts [3,4]. Absorbed radium was retained in measurable amounts for many years and only slowly excreted. The retained radium, like strontium, was largely located in the skeleton[5].

It was known that extremely minute amounts of radium (1 to 100 micrograms) could cause anemia, spontaneous fracture of bones, osteogenic sarcoma, and death in human subjects[6,7,8]. All of this damage was produced by a slowly disintegrating element (the half-life of Ra is about 1600 years) which gives rise to several α, β, and γ radiations. It was not known, even approximately, what would be the effect of a similarly metabolized element that was disintegrating much more rapidly than radium and was giving off only β radiation.

Because of the physical characteristics of radiostrontium and the similarity of its metabolism to that of radium, a large part of the investigation

of fission products in this laboratory was devoted to a study of the toxic effects of radiostrontium and to a further study of its metabolism.

This paper will deal only with the acute or relatively short-time effects of exposure to radiostrontium. Other papers, now in preparation, will discuss the chronic toxic action of Sr^{89} and Sr^{90}.

2. Preparation of Radioactive Material

2.1 Materials. The Sr^{89} used by the Experimental Biology Section came from uranium bombarded in the pile at Oak Ridge, Tennessee. Methods used for separation are discussed in detail in another report (9). Analytical procedures have also been discussed elsewhere(10).

2.2 Purity of Materials. The earlier samples contained Sr of 75 per cent to 93 per cent radiochemical purity. The impurities were mostly Ba^{140}, La^{140}, Y^{90}, (daughter of Sr^{90}) and other rare earths. The hydroxide insoluble impurities were removed with ferric hydroxide resulting in 95 per cent to 97 per cent radiochemical purity. The resin prepared materials were 97 per cent to 99 per cent radiochemically pure strontium containing 3 per cent to 1 per cent Y^{90} in equilibrium with Sr^{90}.

3. Methods and Administration

The stock solutions were highly radioactive (several millicuries per milliliter) and were frequently in acid. Usually, the acid was driven off by evaporation to dryness after which the solids were redissolved in water and

the pH adjusted to 3-5 with sodium carbonate or acetic acid. Occasionally, however, any excess acid was neutralized to the proper pH by the direct addition of carbonate without any previous evaporation treatment. Dilutions with saline or water were made such, that isotonic solutions resulted with an activity of about 0.25 mc/ml for mouse injections, 1 mc/ml for rats, or 5 mc/ml for rabbits. With solutions of such concentrations, doses of 3 to 10 uc/g were given with variations in volume of 0.2 to 1.0 ml in the mice, 0.3 to 5.0 ml in rats, and 1.0 to 10 ml in the rabbits. When lower or higher doses were given, the concentration of the solutions was adjusted so that injections of similar volume could be given.

From each solution used, the first half milliliter and the last half milliliter to pass through the syringe were delivered to volumetric flasks and the radioactivity was measured to estimate the actual dose administered. These measurements showed that strontium was not significantly adsorbed on the frosted glass surfaces or metal needle of the syringe. This is in contrast to the results with barium-lanthanum[11].

Since the bottles emitted 0.01 to 0.1 r/hr which made direct handling hazardous, the radioactive solutions were kept in serum bottles that were held by means of a single burette clamp. In fact, with the more active solutions, sufficient secondary radiations of a penetrating nature (soft gamma or X rays) were produced to require further safety measures. The bottles containing very active solutions were stored in thickwalled, transparent Lucite containers [12] or were kept behind lead bricks except when actually in use. At all times during injections, all the experimenters wore rubber gloves. They also wore film badges and pocket electroscopes to determine the degree of exposure to external radiation.

The radioactive solutions and the saline for the controls were administered via intraperitoneal injection to mice, rats, and rabbits. Only in the rabbit injections were sterile solutions, needles, and syringes employed. Intravenous injections were given to a few animals of each species, but since the results were so similar to the much larger intraperitoneal series, in most cases, the discussion of results will be limited to the intraperitoneal series alone. In a number of rabbits and in a few rats, but never in the mice, it was found that the injection had been made inadvertently wholly or in part directly into the gut. In these animals, a very large percentage of the injected dose was excreted within 24-48 hours and more than 90 per cent of this excretion was in the feces. Furthermore, the retained dose in such animals was always well under half of the average for the species and usually was virtually nil. Such animals were not included in tabulations of mortality, excretion, and retention, but do appear in Table 2.

4. Methods of Analysis of Biological Materials

4.1 Analysis of Tissues: When an animal died or was sacrificed, tissues were taken for Sr^{89-90} analysis. The wet weight of the tissues was recorded and then the tissues were transferred to procelain evaporating dishes. A few milligrams of inert strontium carrier were added in the form of a $SrCl_2$ solution in dilute HCl. After the tissues had been dried for 24 to 48 hours at 150° C, they were ashed in an electric muffle furnace at 700° to 800° C for 24 to 48 hours. The length of drying and ashing time depended on the size of the tissue sample. The longest times were required for rabbit carcasses and the shortest, for soft tissues from mice.

The tissue ash was dissolved in 6 N HCl and was transferred quantitatively to a volumetric flask or mixing cylinder with frequent rinsings of water. After the tissue ash solution was made up to volume, the entire solution was transferred to 2 or 4-ounce, screw-top, wide-mouth bottles for storage. The storage bottles,

with tops removed, were placed under a survey counter. From the radiation intensity observed, the proper aliquot for radioactivity measurements could be estimated. This aliquot was placed in a Coors #1 porcelain capsule and was evaporated to dryness under a heat lamp. The capsule containing the dried sample was allowed to cool to room temperature and then the radioactivity was measured with a Geiger-Muller counter. All counting was done under an aluminum absorber of 50 mg/cm^2 thickness in order to minimize errors due to self-adsorption.

4.2 Analysis of Excreta: Excreta were analyzed for their content of Sr^{89-90} at intervals after the injections. A small amount of dilute HCl and a few milligrams of inactive strontium were added to the collection containers to reduce adsorption of the radiostrontium. Urine was diluted to a known volume with dilute HCl and an aliquot was then taken and dried for direct measurement of radioactivity. Mixed excreta and feces were dried, ashed, and dissolved in exactly the same manner as the tissues. Small amounts of acid-insoluble residue were present in the diluted rat and mouse feces and mixed excreta samples. This residue was found to retain varying (5 to 50 per cent) proportions of the total radioactive strontium. Accordingly, when pipetting an aliquot of such a solution for measurement, every effort was made to suspend the residue and thereby obtain an aliquot of it as well as of the solution. Such suspensions were frequently checked by making duplicate or triplicate analyses.

The amount of insoluble residue from rabbit feces ash was so large that a homogeneous suspension could not be made. Further treatment of rabbit feces ash, according to a method developed by W. P. Norris and H. E. Evans in this laboratory, was employed to reduce the amount of residue [13]. By this method, the feces ash was heated with aqua regia (about 100 ml for each day's collection) until NO_2 was no

longer evolved. A 24 per cent HF solution containing 16.5 per cent H_3BO_3 was then added (about 40 ml for each day's collection of feces) to the hot aqua regia suspension, and the mixture was boiled for 2 to 5 minutes. The resulting nearly clear solution was diluted and an aliquot was pipetted for measurement.

4.3 Correction for Decay: Prior to tabulation, all measurements were corrected for decay back to the time of injection, using a 55 day half-life up to about four months after injection. After this time, there was sufficient long-lived Sr^{90} present to require a correction. This correction was determined from theoretical decay curves of Sr^{89} and Sr^{90}, after a calculation of the original Sr^{90} content at the time of injection had been made.

The original Sr^{90} content was calculated from the activity of an aliquot of the injection solution at two times, P_1 and P_2. The two times should be as widely separated as possible (perhaps 100 to 200 days apart), with the first preferably on the day of injection. Since the rate of decay of Sr^{90} is essentially zero over a period of this length, and since the rate of decay of Sr^{89} is accurately known, the following relations for the activity, A_1 and A_2 at the two times hold:

A_1 = Activity of Sr^{89} Activity of Sr^{90} (1)

A_2 = Activity of Z x Sr^{89} at P_1 Activity of Sr^{90} (2)

(where Z is the fraction of Sr^{89} which has not decayed in the time between P_1 and P_2) as read from a Sr^{89} decay curve

subtracting (2) from (1) gives $A_2 - A_1$ = Activity of Sr^{89} (1 - Z) (3)

Since Z and the activity at P_2 and P_1 are all known, the activity of Sr^{89} at P_1 (the day of injection) and the activity of Sr^{90} by difference can be obtained immediately.

TABLE 2

ESSENTIAL DATA

EXPERIMENT	AVERAGE DOSE (uc/g)	NO. ANIMALS PER GROUP	SEX	STRAIN	EXCRETION MEASUREMENTS	HEMATOLOGICAL OBSERVATIONS	HISTOLOGICAL EXAMINATION	MAIN PURPOSE OF EXPERIMENT
				MICE				
				ABC - wh				
CD	3.2	4	M	WBH	Yes	No	Yes	Preliminary study of acute toxicity and metabolism
	0.86	4	M	WBH	Yes	No	Yes	
	17 mg inactive Sr/mouse	4	M	WBH	No	No	?	
	0	4	M	WBH	No	No	Yes	
CE	13.5	4	M	WBH	Yes	No	No	Continuation of experiment CD
	7.0	4	M	WBH	Yes	No	Yes	
				ABC - BR				
CY	10.0	6	F	BBH	Yes	No	Yes	Det'n comparative toxicity and metabolism of Sr^{89} in mice, rats, and rabbits.
	5.0	6	F	BBH	Yes	No	Yes	
	2.0	12	M&F	BBH	Yes	No	Yes	
	0	12	M&F	BBH	No	No	Yes	
BH	14.5	18	M	BBH	Yes	Yes	Yes	Histological study series
	6.8	6	M	BBH	Yes	Yes	Yes	
	3.6	68	M&F	BBH	Yes	Yes	Yes	
	1.9	6	M	BBH	Yes	No	Yes	
	1.0	45	M&F	BBH	Yes	Yes	Yes	
	0.3	12	M	BBH	No	No	No	
	0	?	M&F	BBH	No	Yes	Yes	
FI	7.0	12	M	B&W BH	No	No	No	Repeat of CY
	5.0	12	M	B&W BH	Yes	No	No	
	3.0	12	M	B&W BH	Yes	No	No	
	2.0	12	M	B&W BH	No	No	No	
	0	12	M	B&W BH	No	No	No	

TABLE 2 (Cont'd)

ESSENTIAL DATA

EXPERIMENT	AVERAGE DOSE	NO. ANIMALS PER GROUP	SEX	STRAIN	EXCRETION MEASUREMENTS	HEMATOLOGICAL OBSERVATIONS	HISTOLOGICAL EXAMINATION	MAIN PURPOSE OF EXPERIMENT
	uc/g							
	12.0	6	M	WBH	No	No	No	
	10.0	6	M	WBH	No	No	No	
	8.0	6	M	WBH	Yes	No	No	
	8.0	6	M	BBH	Yes	No	No	
FS	8.0	6	M	BlBH	Yes	No	No	Final determination
	8.0	6	M	AF_1Bl	Yes	No	No	of lethal dose and
	6.0	66	M	WBH	No	No	No	prelim. study of
	4.0	6	M	WBH	No	No	No	some factors affect-
	0	6	M	WBH	No	No	No	ing this dose
	8.0 (I.V.)	6	M	WBH	Yes	No	No	

TABLE 2 (Cont'd)

ESSENTIAL DATA

EXPERIMENT	AVERAGE DOSE	NO. ANIMALS PER GROUP	SEX	EXCRETION MEASUREMENTS	HEMATOLOGICAL OBSERVATIONS	HISTOLOGICAL EXAMINATION	MAIN PURPOSE OF EXPERIMENT
	uc/g						
				RATS			
	19	2	M & F	Yes	No	No	Preliminary study of toxicity and metabolism
	10.5	3	M & F	Yes	No	No	
	5.3	3	M & F	Yes	No	No	
CR	2.5	3	M & F	Yes	Yes	Yes	
	1.3	3	M & F	Yes	No	Yes	
	0.63	8	M & F	Yes	Yes	Yes	
	0.25	8	M & F	Yes	Yes	Yes	
	10.0	4	F	No	No	Yes	Determination of comparative toxicity and metabolism of Sr^{89} in mice, rats, and rabbits
	5.0	4	F		No	Yes	
CY	2.0	7	M & F		No	Yes	
	0	8	M & F		No	No	
CZ	0.3	10	M	Yes	No	No	Determination of precision of various measurements. Study of conc. of Sr^{89} in blood
	8.	6	M	No	No	No	
	5.	6	M	Yes	No	Yes	
FI	33.	6	M	Yes	No	No	Repeat of Cy
	2.	6	M	No	No	No	
FS	3.0 (I.V.)	9	M	Yes	No	No	Study of metabolism after I.V. and I.P. injections.
	3.0 (I.P.)	6	M	Yes	No	No	

TABLE 2 (Cont'd.)

ESSENTIAL DATA

EXPERIMENT	AVERAGE DOSE	NO. ANIMALS PER GROUP	SEX	EXCRETION MEASUREMENTS	HEMATOLOGICAL OBSERVATIONS	HISTOLOGICAL EXAMINATION	MAIN PURPOSE OF EXPERIMENT
	uc/g						
			RABBITS				
CY	20	2	M & F	No	No	Yes	Det'd of comparative toxicity and metabolism Sr^{89} in mice, rats, and rabbits.
	5	2	M & F	No	No	Yes	
	2	6	M & F	Yes	No	Yes	
	Control	8	M & F	No	Yes	Yes	
	8	6	M	No	No	No	
	5	6	M	Yes	No	Yes	
FI	3	10	M	No	Yes	No	Repeat of Cy
	2	6	M	No	No	Yes	
FX	1	24	M	Yes	No	No	Improvement in recovery of injected Sr.

5. Animals

The mice employed in most of the experiments were brown or white ABC males from the Jackson Memorial Laboratory. A few black ABC males and some black AF_1 males (a strain developed in laboratory by inbreeding of several groups of ABC mice) were also used. The mice were about 30 days old and weighed approximately 20 (17-24) grams each at the time of injection. The rats used in these experiments were albino males of the Sprague-Dawley strain, 10 to 12 weeks old and weighing about 200 (165-240) grams each at the time of injection. The rabbits were males weighing 1800 to 2500 grams each, were 60 to 90 days old, and were obtained from Mr. Harold Swift, Lakeside, Michigan. A few females of each species were used in certain experiments (Table 2).

The arbitrary experimental code letter has been retained in many of the tabulations to avoid confusion, since several experiments, involving many dose levels, were made with each of the species of animals.

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