Binding of ^{85}Sr to Homogenate and Subcellular Fractions of Rabbit Tissues[1] (36888)

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Little attention has been given to soft tissue metabolism of strontium nuclides, although extensive studies concerning the deposition of ^{90}strontium and other alkaline earth elements in mineralized tissues of mammals have been carried out (1, 2). Kidman, Tutt and Vaughan (3) found less than 1% of the total dose, following parenteral injection of strontium isotopes, in soft tissues of rabbits. More recently, Brues *et al.* (4) have shown that within a few hours after a single injection of ^{90}strontium to male mice, soft tissues may contain higher concentrations of the nuclide than present in the circulating blood. Seminal vesicles (that secrete calcium citrate) and salivary glands (that maintain a high calcium content) contained the highest concentrations of strontium relative to blood. Åberg and Gillner (5) found that iv administration of $^{89}SrCl_2$ to rams results in spermatozoal uptake of the radionuclide. They also found (6) that 10^{-1} atoms ^{89}Sr/spermatozoan is sufficient to kill the cells on Day 50 after administration of the isotope. Åberg and Gillner also suggested that the nuclide was most probably bound to spermatozoal DNA although RNA and proteins were not excluded. Wacker and Vallee (7) found strontium tightly bound to isolated RNA preparations from various sources and, to a lesser extent, to DNA preparations.

These data strongly suggest (8) that radiation effects may directly result from fixation of strontium radionuclides to nucleic acid molecules in nucleoprotein-rich tissues in addition to the long-range radiation effects from bone storage of alkaline earth nuclides. The present study, using equilibrium dialysis, provides additional information concerning the *in vitro* binding of ^{85}Sr to homogenates and subcellular organelles of selected rabbit tissues.

Materials and Methods. Frozen adult rabbit tissues were obtained commercially[2] and maintained at −20° until used. Liver and kidney tissues from individual animals of both sexes were initially studied with no apparent sex differences. In most experiments, liver from individual animals of both sexes, kidneys from 1 to 4 animals of mixed sex, testes from 4 to 20 animals, and ovaries from 10 to 40 animals were pooled for study. Tissues were thawed for 2 hr at room temperature and 1 vol of tissue (wet wt) was homogenized in 6 vol of 0.154 *M* NaCl when only whole homogenates were studied or in 0.25 *M* sucrose when subcellular fractionations were desired. All tissues were maintained in ice and subsequent procedures were performed in a cold room at 4°.

Liver and kidney tissues were homogenized with a Teflon motor driven pestle but fibrous testes and ovaries were homogenized in a Waring blender. All tissues were strained through two layers of cheesecloth to remove fibrous material. Nuclei and cell debris were sedimented at 900*g* for 10 min, mitochondria at 10,000*g* for 15 min, and microsomes at 100,000*g* for 1 hr. The subcellular fractions were reconstituted to original volume with 0.104 *M* NaCl containing 0.05 *M* Tris (pH 7.3 or pH 8.5) or 0.104 *M* NaCl containing 0.05 *M* acetate (pH 5.0 or 6.0). The tissue preparations were dialyzed exhaustively overnight with frequent changes of the appropriate buffer before undertaking equilibrium dialysis. For competitive binding studies, analytical reagent grade chloride salts of mag-

[1] This investigation was supported in part by U.S. Public Health Service Grant EP 00102 from the Environmental Protection Agency.

[2] Pel-Freez Biologicals, Inc., Rogers, AR.

nesium, calcium, strontium, and barium were added to appropriate buffers (pH 7.3).

Equilibrium dialysis was performed in duplicate, with 6 ml of tissue preparation in cellulose dialyzing tubing (1 in. flat width) that had previously been boiled and thoroughly washed in distilled water. The bags were tied at both ends and placed in screw-capped 24 × 200 mm tubes containing 24 ml of appropriate buffer with added ^{85}Sr. Appropriate controls, in duplicate, with and without tissue or ^{85}Sr, were included in each experiment. Equilibrium dialysis was achieved by gently shaking the tubes horizontally at about 40 strokes/min on a mechanical shaker in the cold room at 4° for 42–65 hr.

After dialysis, a 1–5 ml aliquot of the bag contents and external solutions were counted for ^{85}Sr activity in a well-type scintillation detector with automatic sample changer. In all runs, a sample of the original ^{85}Sr was counted in order to correct for decay and a minimum of 40,000 counts were obtained to yield relative counting errors of less than 1%. After counting, 5 ml aliquots of the bag contents were dried at 85° for 24 hr in preweighed aluminum cups to obtain dry weight of the material. Control buffers were dried and weighed to correct for buffer salt content.

The RNA and DNA samples were dialyzed exhaustively versus the appropriate buffer before equilibrium dialysis. The final solutions contained about 5 mg/ml. RNA samples consisted of a crude commercial yeast preparation[3] and a highly purified calf liver S-RNA[3]. DNA samples were from two different lots of Levene's alcohol extracted calf thymus[4] and a highly polymerized calf thymus preparation.[3]

Estimations of RNA and DNA were made by the method of Wannemacher, Banks and Wunner (9) and proteins by micro Kjeldahl using 6.25 to convert nitrogen to protein. Alkaline earth elements were measured by atomic absorption spectrometry. Solutions of ^{85}Sr contained between 1 and 1.35 mg Sr/ml (sp act between 2.95 and 8.15 mCi/mg Sr)[5] as purchased.

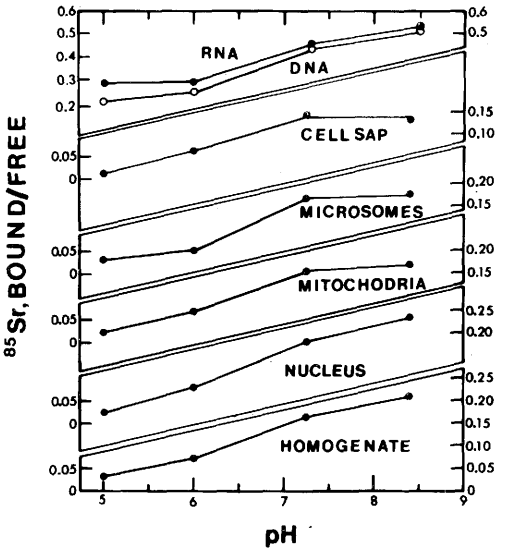

FIG. 1. Effect of pH on the binding of ^{85}strontium to rabbit testes tissue components and to isolated RNA and DNA preparations. The data are expressed as (μg bound ^{85}Sr/g dry wt)/(μg free ^{85}Sr/liter).

Results. The binding of ^{85}Sr to whole tissue homogenates, subcellular fractions, and nucleic acids is pH dependent (Fig. 1). Although more definitive studies were not performed, it is apparent that maximum binding of ^{85}Sr atoms for all substances lies above pH 7. These data are in accord with accepted pK values for nucleotides, nucleic acids, and organic phosphate groups (10). With these data at hand, binding studies were subsequently performed at pH 7.3.

The binding of ^{85}Sr to all tissue homogenates and subcellular fractions is essentially linear for all concentrations of ^{85}Sr up to at least 20 μg/liter. A typical experiment for rabbit testes is shown in Fig. 2. Similar experiments performed with preparations of RNA and DNA (Fig 3) again demonstrate linearity with concentration.

The quantitative binding of ^{85}Sr to tissues and tissue components is shown in Table I. Testes and testicular fractions generally have the highest binding capacity with ovaries next in line and with liver and kidney next

[3] Sigma Chemical Co., St. Louis, MO.

[4] Nutritional Biochemicals Corp., Cleveland, OH.

[5] New England Nuclear Corp., Boston, MA.

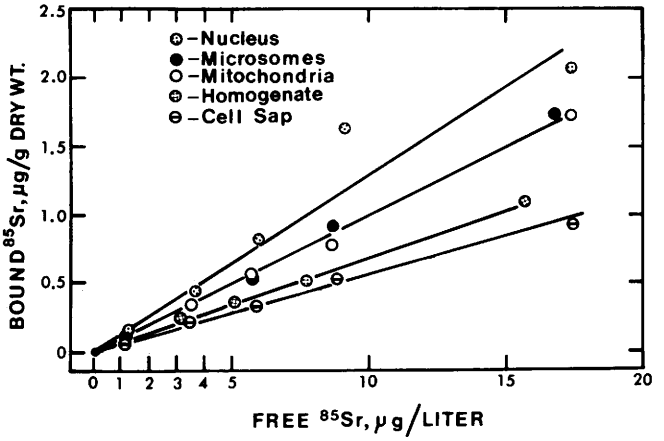

FIG. 2. Linear relationship of ^{85}strontium bound to rabbit testes tissue components vs free ^{85}strontium at equilibrium and pH 7.3. Note single line for similar values of microsomes and mitochondria.

following. For each particular tissue (except liver), the nucleus shows the greatest binding and the cell sap the least binding. Although microsomal fractions from liver bind more strontium than mitochondrial fractions, mitochondria and microsomes from other tissues bind approximately equal amounts within experimental error. It may be noted that the standard error for ovarian nuclei is much greater than that for other tissue nuclei. Although the ovaries were obtained from nonpregnant rabbits, the variation may be due to various stages of follicular development as grossly observed. Similar binding data for RNA and DNA samples are shown in Table II. Although RNA samples isolated from yeast and liver bind similar amounts of ^{85}Sr, DNA binding is more variable and generally less than that for RNA. Thus, two different preparations of Levene's hot alcohol extracted DNA varied between 175 and 456 ng ^{85}Sr/g.

Analyses of RNA, DNA, and protein in the tissue homogenates before and after exhaustive dialysis and for subcellular fractions after exhaustive dialysis are shown in Table III. It is apparent that the RNA, DNA, and protein content of homogenates is significantly lower following exhaustive dialysis compared to undialyzed homogenates, thus suggesting the loss of dialyzable nitrogen-containing tissue constituents. Although the dialyzable material may represent some enzymatic hydrolysis during tissue preparation

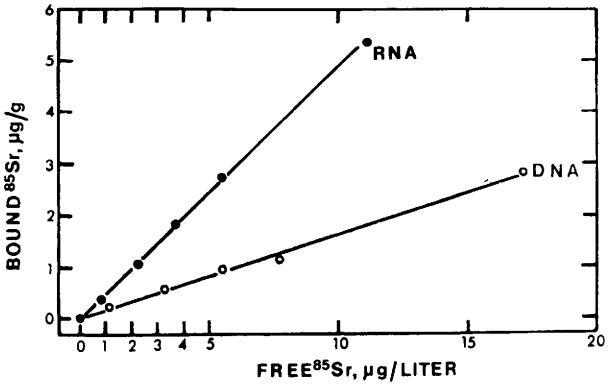

FIG. 3. Linear relationship of ^{85}strontium bound to RNA and DNA vs free ^{85}strontium at equilibrium and pH 7.3.

TABLE I. Binding of ^{85}Sr to Homogenates and Subcellular Fractions of Rabbit Tissues.[a]

Tissue	Testes	Ovaries	Liver	Kidney
Homogenate	107 ± 6 (13)	108 ± 14 (11)	82 ± 6 (13)	74 ± 4 (13)
Nuclei	155 ± 12 (5)	150 ± 34 (4)	82 ± 6 (4)	110 ± 11 (4)
Mitochondria	115 ± 12 (5)	83 ± 20 (4)	68 ± 3 (4)	65 ± 3 (4)
Microsomes	115 ± 14 (5)	83 ± 12 (4)	105 ± 17 (4)	89 ± 7 (4)
Cell sap	90 ± 19 (4)	56 ± 11 (4)	74 ± 10 (4)	38 ± 5 (4)

[a] The data are expressed as (ng bound ^{85}Sr/g dry wt)/(μg free ^{85}Sr/liter) at equilibrium and pH 7.3. The values are averages ± SE for the number of trials given in parentheses.

and dialysis, all procedures were performed at 4° or in ice in order to minimize these factors. The relative constancy of replicate determinations also suggests that the dialyzable tissue components have little, if any, effect on the binding of ^{85}Sr to nondialyzable tissue components. It may also be observed that the total nucleic acid ratios (PNA/protein) are essentially similar for the tissue homogenates before and after dialysis.

Typical data demonstrating the inhibition of ^{85}Sr binding for testes homogenates by alkaline earth elements are shown in Fig. 4. The inhibition of ^{85}Sr binding by Mg, Ca, Sr, and Ba is adequately expressed by the logarithmic equation of the form $Y = bX^{-m}$. As shown in Table IV, the slope (m) and the intercept (b) of the equation are the same (within experimental error) for all tissues with each particular element. It is pertinent

TABLE II. Binding of ^{85}Sr to Nucleic Acids.[a]

RNA		DNA	
Yeast	473 ± 13 (4)	Levene's hot alcohol No. 1	456 ± 14 (2)
Liver	464 (1)	Levene's hot alcohol No. 2	175 ± 5 (2)
		Calf thymus	216 (1)

[a] Binding expressed as (ng bound ^{85}Sr/g)/(μg free ^{85}Sr/liter) at equilibrium and pH 7.3. Average values ± SD for number of determinations in parentheses.

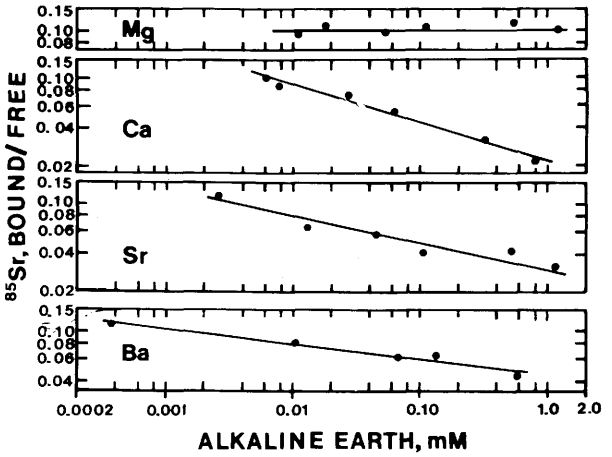

FIG. 4. The effect of alkaline earth elements on the binding of ^{85}strontium to rabbit testes homogenates at pH 7.3. The data are expressed as in Fig. 1. Note the logarithmic scale of the ordinate.

TABLE III. Chemical Composition of Rabbit Tissues and Subcellular Fractions.

Fraction	Testes	Ovaries	Liver	Kidney
		Before dialysis (8 samples)[a]		
Homogenate				
RNA	6.29 ± 0.27	2.23 ± 0.11	4.32 ± 0.17	3.38 ± 0.29
DNA	3.94 ± 0.30	2.36 ± 0.08	1.41 ± 0.08	3.32 ± 0.21
Protein	72.39 ± 1.48	47.84 ± 0.88	67.57 ± 1.94	80.28 ± 1.42
PNA/prot.	0.141	0.096	0.085	0.083
		After dialysis (2–3 samples)[a]		
Homogenate				
RNA	3.09 ± 0.09	1.97 ± 0.10	3.52 ± 0.31	1.68 ± 0.01
DNA	3.52 ± 0.08	1.37 ± 0.09	1.32 ± 0.12	2.22 ± 0.27
Protein	50.62 ± 2.7	28.20 ± 4.6	60.40 ± 5.0	56.82 ± 0.4
PNA/prot.	0.131	0.118	0.080	0.069
Nucleus				
RNA	2.62 ± 0.54	2.09 ± 0.13	3.79 ± 0.47	1.76 ± 0.19
DNA	4.21 ± 0.45	3.21 ± 0.06	1.98 ± 0.19	3.02 ± 0.39
Protein	41.79 ± 2.1	27.11 ± 3.4	51.17 ± 4.8	53.24 ± 5.8
PNA/prot.	0.164	0.195	0.113	0.090
Mitochondria				
RNA	2.44 ± 0.20	1.49 ± 0.03	2.50 ± 0.28	0.92 ± 0.07
DNA	3.82 ± 0.08	1.78 ± 0.18	0.71 ± 0.12	0.83 ± 0.26
Protein	39.21 ± 1.8	18.42 ± 0.5	37.46 ± 3.9	34.04 ± 5.1
PNA/prot.	0.159	0.178	0.086	0.051
Microsomes				
RNA	3.96 ± 0.15	2.34 ± 0.20	3.90 ± 0.15	1.02 ± 0.03
DNA	1.39 ± 0.26	0.48 ± 0.02	0.40 ± 0.02	0.23 ± 0.03
Protein	37.23 ± 1.0	27.86 ± 1.9	46.46 ± 1.0	19.21 ± 2.2
PNA/prot.	0.143	0.101	0.093	0.065
Cell sap				
RNA	0.82 ± 0.17	0.49 ± 0.07	1.10 ± 0.01	0.30 ± 0.03
DNA	0.26 ± 0.07	0.17 ± 0.03	0.34 ± 0.00	0.16 ± 0.02
Protein	40.35 ± 1.1	17.44 ± 3.5	42.10 ± 1.6	19.86 ± 2.2
PNA/prot.	0.026	0.038	0.034	0.023

[a] Data expressed as percentage of dry weight. PNA represents sum total of RNA and DNA. Average values ± SE for number of samples given in parentheses.

to note that magnesium ion does not interfere with the binding of ^{85}Sr to any of the tissues studied up to concentrations of about 1 m*M* while calcium ion inhibits the ^{85}Sr binding to the greatest extent and even more than strontium itself. It appears therefore, that ^{85}Sr is binding to the same site(s) as calcium and that this binding site(s) has a greater affinity for calcium ion. Ions that are smaller or larger than calcium have a lesser affinity for the binding site(s).

Discussion. These results demonstrate that strontium nuclides are bound, under static nonmetabolizing conditions, to testicular and ovarian tissues more than to liver and kidney tissues. In subcellular fractions of testes and ovaries, the nuclide is preferentially bound to nuclei followed by intermediate binding to mitochondria and microsomes with least binding to cytosol components. For liver and kidney cell fractions, the binding of strontium nuclides is less than that found in gonadal tissue fractions and appears to be more evenly distributed throughout the various cell fractions. Crude preparations of ribonucleic acid from yeast or highly purified preparations from calf thymus bind the greatest amount of strontium nuclides compared to all

TABLE IV. Effect of Alkaline Earth Elements on ^{85}Sr Uptake by Rabbit Tissue Homogenates.[a]

Ion	Liver	Kidney	Testes	Ovary	Av
			Slope (m)[b]		
Mg^{2+}	—0.04 ± 0.00	—0.04 ± 0.08	—0.02 ± 0.00	—0.02	—0.031 ± 0.05
Ca^{2+}	—0.36 ± 0.03	—0.38 ± 0.03	—0.34 ± 0.02	—0.42 ± 0.01	—0.368 ± 0.04
Sr^{2+}	—0.18 ± 0.03	—0.24 ± 0.01	—0.20 ± 0.01	—0.16	—0.200 ± 0.04
Ba^{2+}	—0.10 ± 0.05	—0.13 ± 0.01	—0.14 ± 0.03	—0.08	—0.117 ± 0.04
			Intercept (b)[b]		
Mg^{2+}	0.060 ± 0.007	0.054 ± 0.002	0.114 ± 0.006	0.111	0.081 ± 0.031
Ca^{2+}	0.018 ± 0.003	0.014 ± 0.003	0.020 ± 0.001	0.016 ± 0.001	0.017 ± 0.003
Sr^{2+}	0.028 ± 0.003	0.014 ± 0.001	0.030 ± 0.001	0.020	0.023 ± 0.008
Ba^{2+}	0.045 ± 0.014	0.036 ± 0.001	0.048 ± 0.006	0.108	0.052 ± 0.027

[a] Tissue values ± SD for duplicate experiments with 5 or 6 points as shown in Fig. 4 except for some single experiments with ovary.

[b] Slope (m) and intercept (b) from equation $Y = bX^{-m}$ and graph of Fig. 4.

tissue components tested and the binding appears to be independent of the biological source. The binding of ^{85}Sr to DNA preparations is variable and may depend on the state of polymerization of the substance. The presence of binding sites on other molecular species can not however be ruled out, although nucleic acids appear to be the predominant binding species in the cell. In this regard, Wacker and Vallee (7) have calculated that bound strontium in isolated RNA can not be entirely due to available phosphate groups from nucleic acids, thus suggesting that other binding groups also play some role in this phenomenon.

Attempts to correlate the ^{85}Sr binding with tissue components indicates that the binding appears to be associated with the total nucleic acid concentrations of tissue although cytosol, that contains very little nucleoprotein, exhibits appreciable ^{85}Sr binding. In general, tissues rich in nucleoprotein such as testes and ovary tend to bind more strontium than liver and kidney that have lower PNA/protein ratios.

With due recognition of the inherent difficulty in translating data obtained from *in vitro*, static experiments to living animals, the data suggest that strontium nuclides are preferentially bound to nuclear material in reproductive and somatic organs in equilibrium with body fluids. The presence of strontium nuclides in close proximity to genetic material increases the possibility of genetic damage even though the concentration of nuclide is relatively small compared to the quantity of the nuclide pool in bone. It is conceivable therefore that the large bone pool, with a turnover rate of about 7%/yr for ^{90}strontium (11), would tend to maintain an equilibrium concentration of the nuclide with cell nuclei for a long period of time, thereby increasing the likelihood of genetic damage and increased mutation rate.

We thank Ms. Sylvia Raymond and Katherine L. Chen for their excellent technical assistance.

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Received July 13, 1972. P.S.E.B.M., 1972, Vol. 141.