Comparative Ultrafiltration of Calcium and Strontium in Serum.*† (24211)

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Comparative metabolism of calcium and strontium in man and animals has become of importance with increase in fallout of Sr^{90}. Metabolic data are available from studies under various conditions for both human subjects(1-3) and animals(4,5) and on bone uptake(6-8). However, little is known about the various stages of passage of the 2 elements through membranes in the body. Once radiostrontium is absorbed and reaches the blood stream, filtration of strontium and of calcium through capillaries into interstitial fluid through the glomeruli, and through various membranes ensues. This filtration is of particular importance in assessing bone uptake and urinary excretion of these ions. The present study deals with *in vitro* filterability of the 2 ions in serum. It was expected that data obtained would permit an estimate of the relative binding of the 2 ions by plasma proteins.

Materials and methods. Blood was drawn by venipuncture from normals as well as from subjects with various pathologic conditions. Several large samples were also obtained uncitrated from the blood bank. In some cases, when special precautions were taken to keep the pH from rising, the blood was collected under oil and maintained under oil during centrifuging and pipetting. In addition, large samples of fresh beef blood were obtained from slaughterhouses. Sr^{85} and Ca^{45} were used as chlorides, the former without carrier, the latter with negligible amounts of stable Ca as carrier. The mixed isotopes were added in proportions of approximately 1 volume of isotope solution to 100 volumes of serum. Ultrafiltration was carried out in Visking cellophane casings, ¼″ diameter when inflated. Approximately 13″ lengths of casing were soaked in saline to separate the sides, and the saline then squeezed out. The outside of casing was wiped dry with gauze and a knot tied in one end. Approximately 1 ml of serum containing isotope solution was used to rinse out the rest of the saline. Five ml of fresh serum were then introduced and the other end tied off. The casing was now bent into U form and the 2 ends passed through the openings of a 2-hole rubber stopper and tied together. The rubber stopper was fitted into 40 ml centrifuge tube and the latter spun 15 minutes to an hour at approximately 600 g. From 0.5 to more than 1.0 ml of ultrafiltrate was obtained, depending on time of centrifuging. On analysis for N, several ultrafiltrates gave values equivalent to 0.2 g protein/100 ml or less, an amount corresponding to non-protein nitrogen. Samples were ultracentrifuged in duplicate and 0.1 ml samples pipetted. The same samples were used for Sr^{85} counting and Ca^{45} determination, high counts of both isotopes being used. Presence of Sr^{85} slightly increased the Ca^{45} count; the ratio of Sr^{85} to Ca^{45} was therefore kept low enough so that minor corrections were needed for both serum and ultrafiltrate, and net interference was negligible. In addition, several sera were ultrafiltered with Ca^{45} alone to ensure that Sr^{85} had no effect. Stable calcium was determined in serum by the method of Kramer and Tisdall as modified by Clark and Collip(9), phosphorus by the method of Fiske and SubbaRow(10). Protein was determined by the Kjeldahl method, a small correction being used for non-protein nitrogen. Sr^{85} was counted in a well scintillation counter and Ca^{45} was determined by precipitation with carrier as the calcium oxalate and counting in a thin window gas flow counter as previously described(11). Once stable Ca had been determined on serum, Ca^{45} was used as a tracer, so that extent of ultrafiltration was followed by determining the Ca^{45} in both serum and its ultrafiltrate. When stable Sr was added, Sr^{85}

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TABLE I. Ultrafiltration of Sr^{85} and Ca^{45} in Serum *In Vitro*.

Diagnosis	% ultrafiltration 37.5°C Sr^{85}	37.5°C Ca^{45}	23°C Sr^{85}	23°C Ca^{45}	Remarks
Carcinoma of uterus	51	36	67	60	10.7 mg % Ca, 6.1 g % protein
" " breast	60	45			10.6 6.8
Cirrhosis, study I	63	46	74	63	9.1 5.7
" , study II (2 mo later)	61	51			
Carcinoma of breast	59	39			9.9 6.3
Multiple myeloma	58	38			9.3 17.0
Hypoparathyroidism	62	38			8.5 7.6
Normal (blood bank)	67	39	72	44	11.5 6.6
Idem	57	40	61	43	10.6 6.8
Saline control			100	100	pH 6.2
Idem	102	98			pH 7.6, 10 mg % Ca, 3 mg % P

was a tracer for it. pH was determined with a Beckman model G pH meter, and a minor correction made for effect of temperature by the method of Rosenthal(12). The pH was lowered when desired with a drop or 2 of HCl. Some experiments were carried out at room temperature, approximately 23°C, and others at 37.5°C in an incubator room.

Results. Table I shows results on a number of serum samples compared with those for saline solutions at different pH used as controls. Every serum tested showed greater ultrafiltration for Sr^{85} than for Ca^{45} at both room and body temperature. Filtration was also higher at 23°C than at 37.5°, but the differences were variable, and in normals inconsiderable for Ca^{45}. An experiment was performed with 2 sera to test whether these temperature variations were due to loss of CO_2 with a concomitant rise in pH at the higher temperature. A serum was first maintained at 37.5° and one portion centrifuged at that temperature; the other portion was then cooled to 23° and also centrifuged. There appeared to be a slight but definite temperature effect, Sr^{85} filtration being 67 and 61% at 23° and 37.5° respectively. Ca^{45} filtrations 50 and 43% respectively.

The effect of small variations of pH was tested by adding slight amounts of HCl to normal human and beef sera (Table II), and found to be small, in accordance with conclusions of McLean and Hastings(13) and other authors(14). The change in pH during ordinary handling of serum could therefore be expected to have a minor effect, slightly greater on Sr^{85} filtration than on Ca^{45}.

Table III shows effect of addition of stable Sr. Amounts used did not appreciably alter either Sr^{85} or Ca^{45} filtration at 23°C, but seemed to increase Sr^{85} filtration at 37.5° for serum A listed in Table III. Five mg% of stable Sr had no effect on serum B at pH of

TABLE II. Effect of pH on *In Vitro* Ultrafiltration of Sr^{85} and Ca^{45} in Serum at 37.5°C.

pH	% Sr^{85} filtered	% Ca^{45} filtered
(a) Normal human serum, Ca 10.5 mg %, total protein 7.2 g/100 ml		
7.47	55	42
7.22	61	45
6.70	68	50
(b) Beef serum, Ca 10.5 mg %, total protein 7.2 g/100 ml		
7.80	49	35
7.52	51	36
7.15	63	40
7.15	57	35
6.71	73	51
6.71	73	53

pH altered by addition of 1 or 2 drops of 2N HCl to 35 ml serum.

TABLE III. Effect of Added Stable Sr on Ultrafiltration or Sr^{85} and Ca^{45} in Serum *In Vitro*.

Serum	Sr added, mg %	% ultrafiltered 37.5°C Sr^{85}	37.5°C Ca^{45}	23°C Sr^{85}	23°C Ca^{45}	Remarks
A	0	58	47	71	51	
	2	64	50	72	49	
	4	69	48	78	43	
	10	65	42	70	46	
	20	71	43	74	50	
B	0	56	43			pH 7.78
	5	64	33			7.91
	0	69	46			6.92
	5	68	44			6.90

TABLE IV. Ultrafiltration of Ca^{45} and Stable P in Presence and Absence of Sr^{85}.

Serum	pH	% ultrafiltration Sr^{85}	Ca^{45}	P
1	7.40	61	38	104
	"		43	102
2	7.42	62	42	102
	"		41	106
3	7.45	58	39	100
	"		39	106

Blood drawn under oil from normal subjects. Temp. of ultrafiltration, 37.5°C.

6.8 or 6.9 but appeared to decrease filtration of Ca^{45} at pH 7.91. This might have been due to formation of a slight amount of colloidal calcium phosphate at this high pH.

The possibility that colloidal calcium phosphate formation was responsible for low Ca filtrations was tested in experiments summarized in Table IV. Blood was collected and centrifuged under oil and inorganic phosphorus determined both on sera and ultrafiltrates. For all 3 sera, approximately 60% of Sr^{85}, 40% of Ca^{45} and 100% of phosphorus were filtered. This indicated that no colloidal calcium phosphate had been formed, and a similar result was found by phosphorus determinations of serum and ultrafiltrate of the hyperparathyroid patient listed in Table I.

Presence of Sr^{85} had no effect on relative Ca^{45} counts, as shown by experiments on sera of Table IV carried out both with and without the Sr radioisotope. Length of centrifugation had no effect on ultrafilterability, the same percentage of both ions being filtered after 15 minutes or 1 hour, although the volume filtered in 15 minutes was 0.5 ml, that in 1 hour 1 ml.

Discussion. Our investigation was aimed to determine comparative ultrafilterability of strontium and calcium in serum, at different pHs, in the presence and absence of stable Sr and at room and body temperature. Under all conditions Sr filtered to a greater extent than Ca. The exact ratio of Ca to Sr filtration varied, but at 37.5°, values for Sr^{85} in several sera of normals averaged approximately 60%, those for Ca^{45} 40%. Similar values were found for sera of male patient with hypoparathyroidism and of a female patient with cancer of the breast. We may conclude that Ca is more completely bound to the proteins of serum than is Sr, more Sr than Ca remaining in a free form.

Values found for calcium are slightly lower than would be indicated by data of McLean and Hastings(13), who found approximately 50% ionized calcium. However, if our data for 23°C are used, and if per cent ultrafiltration is calculated on the basis of amount filtered in a given amount of water instead of serum, the results are in generally good agreement. On the basis of amounts filtered per given amount of water, our average values at 37.5° are approximately 65% for Sr and 43% for Ca, and at 23° approximately 70% and 50% respectively. Percentage of ionized calcium is not quite the same as percentage of filterable calcium, but McLean and Hastings showed that in general the factors that would make for different values would cancel out.

The data of Prasad and Flink(15) are also in general agreement with ours. They ascribe the high values obtained by some authors to the use of CO_2 to stabilize the pH, their own data indicating that use of CO_2 produces artifacts and gives abnormally high values. As our own experiments were carried out chiefly for comparison of Sr and Ca filtrations, we did not attempt to settle this question. We did examine the various factors making for experimental error, and by use of duplicate filtrations, as well as control experiments with saline, eliminated the possibility of radioactive contamination as a factor.

Lack of pH control has also been indicated as a reason for variability of results(16), but the effect of even fairly large changes in pH (Table II), although appreciable, would not account for all the differences. Even at a pH of 6.70, the Ca of blood in Table II was only 50% filterable.

Analytical methods used for Ca have also been examined. The present method for determination of Ca^{45} has a reproducibility of approximately $\pm$ 3%. It lacks the precision claimed for some of the stable calcium methods in the literature, although the number of methods proposed indicates that these claims are rarely substantiated subsequently. It has the great advantage, however, that if a

reasonably high concentration of Ca^{45} is used, the precision is just as good for ultrafiltrate as for serum, and there is unlikely to be a systematic error in analysis applicable to one and not to the other. As shown by McLean and Hastings(13), the ionic and protein-bound Ca in serum are in dynamic equilibrium, so that the Ca^{45} in serum and ultrafiltrate is a measure of relative values of stable Ca. The point was also checked experimentally by allowing several sera to stand for different lengths of time after addition of the radioisotope. No differences were found. Apparently, therefore, use of Ca^{45} as an analytical tool introduced no systematic error.

Whatever the factors contributing to different values for filtration of Ca and Sr, both ions were affected similarly, and the relatively greater filterability of Sr seems definitely established.

Summary. Sera of patients and normal individuals have been ultrafiltered through cellophane to determine relative filterability of Sr and Ca, using Sr^{85} and Ca^{45} as tracers. In every case, Sr was found to filter more completely, values for 37.5° averaging close to 60% for Sr, and 40% for Ca, calculated on the basis of total serum filtered. None of several variables tested affected relative filterability of the 2 ions. Neither addition of stable Sr nor slight changes in pH, such as might result from manipulation during the experiment, produced significant changes in filterability. There appeared to be a slight negative temperature effect, values for both isotopes being lower at 37.5°C than at 23°C, although the effect on Ca^{45} was usually negligible. Results indicate that Ca is more completely bound to protein than is Sr, more Sr remaining in a free form.

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In vivo Fixation of Antibodies in the Adrenal.* (24212)

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Bale and Spar(1) have shown by radiolabelled antibody technics that antibodies prepared in rabbits against rat kidney, when injected into rats, are fixed in the adrenals. This antibody may be an important factor in disease processes which have been attributed to action of anti-kidney antibodies. Its presence in high concentration in a particular site

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