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Comparative Passage of Sr^{85} and Ca^{45} from Plasma into Body Fluids in Man.* (25599)

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(Introduced by B. A. Sachs)
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In vitro studies on comparative ultrafiltration of strontium and calcium(1) have shown that a lower percentage of strontium than of calcium is bound to plasma proteins. However, the passage of the 2 elements from plasma into cerebrospinal fluid in man occurred at similar rates(2), indicating that the membranes through which the ions pass discriminate in favor of calcium. In an extension of these investigations, transfer of calcium and strontium across membranes of other body cavities was studied and passage of both ions from plasma into peritoneal, pleural and interstitial fluid was investigated.

Materials and methods. Transfer of either Sr^{85} alone or of Sr^{85} and Ca^{45} from plasma into various body fluids was studied in 9 patients. The diagnoses of the patients and types of studies performed are listed in Table I. In patients with ascites in whom a paracentesis was indicated, a polyethylene catheter was inserted into the peritoneal cavity through a No. 14 needle after anesthetizing the skin. Ascitic fluid was obtained for analyses of protein and calcium by aspiration from the polyethylene catheter, the open end of the tubing being clamped afterwards. A tracer dose of either Sr^{85} or of Sr^{85} and Ca^{45} as the chloride was injected intravenously and samples of plasma and peritoneal fluid were then obtained serially. The patients were kept on a slowly oscillating bed for 4-8 hours in an attempt to obtain thorough mixing of peritoneal fluid with the radioisotopes. The peritoneal fluid was withdrawn 24 hours after injection of the tracers, to measure the fluid volume. Transfer of calcium and strontium from peritoneal fluid into the blood stream was studied in one patient by injecting the tracers into the peritoneal cavity and obtaining samples of ascites and plasma as indicated above. Transfer of the 2 elements from plasma into pleural fluid was also studied in 2 patients in a similar manner. Comparative concentration of radiocalcium and radiostrontium in plasma and interstitial fluid was studied in a patient with massive edema of the lower extremities (Patient 5) following intravenous injection of the tracers. Interstitial fluid was obtained by Southey tubes inserted into the subcutaneous tissue. Samples of plasma, peritoneal, pleural and interstitial fluid were analyzed for Sr^{85}, Ca^{45}, calcium, phosphorus and total protein. Sr^{85} was counted in a well-type scintillation counter and Ca^{45} was counted after precipitation with carrier calcium as the oxalate in a gas flow counter(3). Stable calcium was determined by the method of Kramer and Tisdall as modified by Clark and Collip(4), inorganic phosphorus by the method of Fiske and SubbaRow (5), and protein in serum and fluid by the Kjeldahl method, a small correction being made for non-protein nitrogen. Rates of flow

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TABLE I. Nine Patients Studied.

Age, sex	Diagnosis	Studies performed
76, ♀	Carcinoma of ovary	Sr^{85} transfer into ascites fluid
53, ♀	*Idem*	*Idem*
61, ♀	Carcinoma of breast	Sr^{85} + Ca^{45} transfer into ascites fluid
55, ♀	" of ovary	*Idem*
51, ♂	Cirrhosis of liver	"
60, ♂	*Idem*	" *
36, ♀	Hodgkin's disease	Sr^{85} + Ca^{45} transfer into interstit. fluid
54, ♀	Carcinoma of breast	Sr^{85} + Ca^{45} transfer into pleural fluid
51, ♀	Rheumatic heart disease, cirrhosis of liver	*Idem*

Tracer doses of Sr^{85} and/or Ca^{45} were inj. intrav. to all patients.

* Patient 6 received, in addition to intrav. dose, an intra-abdominal inj. of Sr^{85} + Ca^{45}.

of fluid from plasma into the peritoneal cavity were calculated on the assumption that amount of radioisotope passing from the vascular space into the peritoneal cavity was proportional to the difference of its concentration in plasma and ascites at any time, no allowance being made for binding of the isotopes to protein. Rates were calculated for each collection period from this difference of the isotope concentration. If R is rate of passage of fluid into and out of the peritoneal cavity in ml/min, t time in minutes, V total volume of peritoneal fluid in ml, assumed to be constant, $\overline{P}$ and $\overline{F}$ are average radioisotope concentrations in plasma and peritoneal fluid, expressed in percent of dose/ml, respectively, and ΔF is increase of isotope concentration in peritoneal fluid during the period in question, increase of radioisotope in the peritoneal fluid in a given period is $V \Delta F = Rt(\overline{P} - \overline{F})$, and $R = \dfrac{V \Delta F}{t(\overline{P} - \overline{F})}$.

Ultrafiltration studies were carried out on plasma and peritoneal fluid of Patient 6 at 37°C by a modification of the method of McFarland and Hein(6,7), as previously described(1).

Results. Table II presents results obtained on passage of radioactive tracers from plasma into peritoneal fluid of 2 patients with carcinomatous effusions. In Patient 3, rate of flow calculated from Sr^{85} data differed from rate calculated from Ca^{45} data, Sr^{85} entering the peritoneal fluid at approximately half the rate of Ca^{45}, while in Patient 4, both radioisotopes entered at approximately the same rate. Plasma levels for Sr^{85} and Ca^{45} were identical within limits of experimental error in Patient 3, while plasma Ca^{45} values of Patient 4 were initially lower than those of Sr^{85}, reaching approximate equality at 24 hours.

Table III shows data obtained on a patient with cirrhosis of the liver. An intravenous injection of Sr^{85} alone was first given followed by an intravenous injection of Sr^{85} and Ca^{45} 3 months later. Sr^{85} plasma and ascites levels of these 2 studies agreed well. Sr^{85} and Ca^{45} data obtained in the second study (Table III) indicate a slightly greater rate of passage of Sr^{85} than of Ca^{45} from plasma into ascites, comparative rate of passage of Sr^{85}/Ca^{45} being 1.14/1.03 or 1:1. Ultrafiltration studies carried out on plasma and peritoneal fluid of this patient revealed that 61% Sr^{85} and 51% Ca^{45} were ultrafilterable from plasma (ratio 1.2) and 76% Sr^{85} and 67% Ca^{45} from peritoneal fluid at 37°C. The lower half of Table III presents data on passage of the radioisotopes from peritoneal fluid into plasma following an intracavitary injection of both radioisotopes. Both tracers

TABLE II. Passage of Sr^{85} and Ca^{45} from Plasma into Peritoneal Fluid Following Intravenous Injection of Tracers in Patients with Carcinomatous Effusions.

Time (hr)	Plasma, % dose/l Sr^{85}	Plasma, % dose/l Ca^{45}	Plasma Sr^{85}/Ca^{45}	Peritoneal fluid, % dose/l Sr^{85}	Peritoneal fluid, % dose/l Ca^{45}	Ascites Sr^{85}/Ca^{45}
			Patient 3			
1/3	4.54	4.54	1.00	.10		
2/3	3.19	3.30	.97	.12	.20	.60
1½	2.44	2.43	1.01	.22	.40	.55
4	1.65	1.57	1.05	.37	.65	.57
8	1.26	1.19	1.06	.50	.89	.56
24	.72	.64	1.13	.70	1.13	.62
		Avg	1.04		Avg	.58
			Patient 4			
½	7.02	6.11	1.15	.81	.66	1.23
1	5.64	4.84	1.17	1.08	.95	1.14
2	4.59	4.02	1.14	1.55	1.36	1.14
3	4.02	3.49	1.15	1.89	1.63	1.16
4	3.73	3.11	1.20	2.08	1.86	1.12
8	3.11	2.73	1.14	2.60	2.27	1.15
24	2.37	2.17	1.10	2.44	2.23	1.09
		Avg	1.15		Avg	1.15

TABLE III. Passage of Sr^{85} and Ca^{45} between Plasma and Peritoneal Fluid Following Intravenous and Intracavitary Injection of Tracers in Patient with Cirrhosis of Liver.

Patient 6

Time (hr)	Plasma, % dose/l Sr^{85}	Plasma, % dose/l Ca^{45}	Plasma Sr^{85}/Ca^{45}	Peritoneal fluid, % dose/l Sr^{85}	Peritoneal fluid, % dose/l Ca^{45}	Ascites Sr^{85}/Ca^{45}
Intrav. inj. of Sr^{85} and Ca^{45}						
¼	5.98	5.85	1.02	.17	.17	1.00
½	4.99	4.92	1.01	.33	.27	1.22
1	4.32	3.94	1.10	.47	.40	1.18
4	2.68	2.56	1.05	1.03	.86	1.20
8	2.11	2.00	1.06	1.35	1.16	1.16
24	1.48	1.56	.95	1.32	1.17	1.13
		Avg	1.03		Avg	1.14
Intracav. inj. of Sr^{85} and Ca^{45}						
⅓	.13	.11	1.18	8.29	8.13	1.02
½	.27	.26	1.04	7.71	7.69	1.00
1	.45	.43	1.05	7.54	7.37	1.02
4	1.06	1.02	1.04	5.82	5.74	1.01
6	1.33	1.19	1.12	5.18	4.82	1.07
7½	1.42	1.29	1.10	4.40	4.13	1.07
11	1.48	1.37	1.08	3.45	3.18	1.08
12½	1.53	1.33	1.15	3.02	2.86	1.06
15	1.52	1.35	1.13	2.48	2.30	1.08
24	1.46	1.34	1.10	1.62	1.36	1.19
		Avg	1.10		Avg	1.06

passed into the plasma at approximately the same rate.

Table IV lists data on passage of the radioisotopes from plasma into interstitial fluid in a patient with Hodgkin's disease and marked edema of the lower extremities. Concentrations of Sr^{85} and Ca^{45} in plasma were almost identical for 4 days and in interstitial fluid for 5 days of the study. Sr^{85} and Ca^{45} values in the interstitial fluid appeared to reach a maximum at about 4 hours, indicating a time lag in passage of approximately the same order for both radioisotopes. The edema fluid contained 2.8 g % protein and 10.3 mg % Ca, compared with 6.2 g % and 13.6 mg% respectively in the plasma.

TABLE IV. Passage of Sr^{85} and Ca^{45} from Plasma into Interstitial Fluid Following Intravenous Injection of Tracers in Patient with Hodgkin's Disease.

Patient 7

Time (hr)	Plasma, % dose/l Sr^{85}	Plasma, % dose/l Ca^{45}	Plasma Sr^{85}/Ca^{45}	Interstitial fluid, % dose/l Sr^{85}	Interstitial fluid, % dose/l Ca^{45}	Interstitial fluid Sr^{85}/Ca^{45}
1¼	3.23	2.76	1.17	1.37	1.34	1.02
4	2.16	2.09	1.03	1.68	1.59	1.06
21	1.53	1.52	1.01	1.28	1.34	.96
24	1.35	1.39	.97	1.22	1.27	.96
92	.97	.92	1.05			
120				.69	.70	.99
		Avg	1.05		Avg	1.00

Table V lists rates of passage of fluid between plasma and peritoneal cavity calculated from Sr^{85} and Ca^{45} data. Rates of flow calculated from Sr^{85} in 6 patients ranged from 3 to 28 ml/min. There was good agreement of rates of flow in Studies I and II performed in Patient 6, while there was a 2-fold increase in Study III. The ratio of Sr^{85}/Ca^{45} was approximately 1:1 in Patient 4 and in Study III of Patient 6 and possibly also in Study II of the same patient. The ratio was approximately 1:2 in Patient 3. There was no apparent correlation between rate of flow and protein concentration in plasma or ascites. The net increase in volume of peritoneal fluid over the period studied as determined clinically was relatively negligible, so that rates of passage into and out of the peritoneal cavity could be assumed to be approximately the same.

TABLE V. Rate of Passage of Fluid between Plasma and Peritoneal Fluid Calculated from Sr^{85} and Ca^{45} Data. Intravenous injection of tracers.

Patient	Ascites vol, ml* (× 100)	Rate of flow, ml/min., from data Sr^{85}	Rate of flow, ml/min., from data Ca^{45}	Total protein, g % Plasma	Total protein, g % Ascites
1	70	20		6.6	2.2
2	85	28		6.6	5.5
3	50	3	7	6.5	4.5
4	45	10	10	7.3	4.9
5	47	8		7.6	1.0
6 Study I	80	10		6.2	1.7
II	70	10	8	6.1	1.4
III	110	24†	24†	5.9	1.8

* Removed by paracentesis after completion of the study.

† The tracer was inj. into the peritoneal cavity.

The ratios of pleural fluid/plasma Sr^{85} and Ca^{45} of 2 patients listed in Table VI indicate greater passage of Sr^{85} than of Ca^{45} into the pleural cavity although pleural fluid protein of Patient 8 was high (this patient had a carcinomatous effusion) and pleural fluid calcium of both patients was almost as high as that of plasma. Rates of passage of both Sr^{85} and Ca^{45} into the pleural fluid were higher in Patient 9 than in Patient 8.

Discussion. Concentrations of protein and calcium indicated that none of the body fluids studied were genuine ultrafiltrates. Formation of abnormal body fluids is obscure. It has been suggested that the intrinsic capillaries of the liver are extremely permeable and produce hepatic lymph, high in protein, which may be a possible source of ascitic fluid(8). However, this process of ascitic fluid formation may not be applicable to effusions resulting from various etiologies. The process is all the more obscure because flow of fluid is in both directions. The net rate of formation of ascitic fluid was estimated to be less than 0.5 liter/day, *i.e.*, less than 0.4 ml/minute the slowest rate of passage calculated from the Sr^{85} data being 3 ml/minute (Table V). When tritium-labeled water is used, rate of flow is found to be higher, so that 40-80% of the total ascitic fluid volume enters and leaves the peritoneal cavity per hour(9). This rate of flow would correspond to a passage of 58 to 115 liters of water from plasma into the abdominal cavity within 24 hours in a patient with 6 liters of ascites. In studies carried out with radio-labeled albumin, complete albumin turnover in the fluid has been reported to occur in 2 days(10), whether albumin was injected intravenously or intraperitoneally. Comparing the low rates of passage of fluid derived from Sr^{85} and Ca^{45} data with those obtained with tritium, one may conclude that the 2 ions do not diffuse as freely into and out of the peritoneal fluid as water but may enter the fluid in part combined with protein and in part unbound.

Although ascitic fluid is not an ultrafiltrate, relative Sr^{85}/Ca^{45} values in plasma and ascites of Patient 6 following intravenous and intracavitary injection of the tracers were similar to those found in ultrafiltration experiments (Table III). Studies carried out in 2 patients with carcinomatous effusions revealed a ratio for passage of fluid from plasma into the peritoneal cavity of 3:7 and 1:1 when calculated from Sr^{85} and Ca^{45} data, respectively, (Table V), indicating no preferential passage of Sr^{85}. Rate of flow from plasma into peritoneal fluid varied considerably from patient to patient. In Patient 6, rate of flow following intracavitary injection was much greater than after intravenous administration of the isotopes. This difference may be due to a change in status of the patient during the 3-month interval between studies. The increased volume of fluid in this patient, as well as deterioration of his clinical condition, also indicated that such a change occurred. The extremely low rate of passage in Patient 3 may have been due to dehydration. From the variable amounts of protein in different peritoneal fluids, from the different rates of passage of the isotopes and the different Sr^{85}/Ca^{45} ratios, it may be inferred that many factors affect ascitic fluid formation and that the mechanism of formation may differ from case to case.

TABLE VI. Passage of Sr^{85} and Ca^{45} from Plasma into Pleural Fluid.

Patient	Plasma: Ca, mg %	Plasma: Protein, g %	Pleural fluid: Ca, mg %	Pleural fluid: Protein, g %	Fluid/plasma: Sr^{85}	Fluid/plasma: Ca^{45}
8	9.9	6.5	8.1	3.3	.67*	.55*
9	9.6	5.8	9.4	1.0	.81†	.74†

* 24 hr after intrav. inj. of tracers.
† 27 hr *Idem*

The Sr^{85}/Ca^{45} ratio for passage into pleural fluid from plasma was almost like that of an ultrafiltrate (Table VI). This preferential passage of Sr^{85} is noteworthy since red blood cells were present in the carcinomatous pleural effusion, indicating increased permeability of the capillaries, and both radioisotopes might be expected to pass into the pleural fluid at about the same rate. Of interest are the high values for calcium in the pleural fluid, whether the fluid was an exudate or transudate. This is contrary to what might be expected from the literature(11) but has been confirmed in our laboratory on a larger number of patients. It should be noted that a single reading of the serum calcium value may be far from representative of the patient's true calcium level since considerable fluctuations in serum calcium have been found even in healthy individuals(12) and greater fluctuations may be expected to occur in pathologic states.

Summary. The passage of strontium and calcium from plasma into peritoneal, pleural and interstitial fluids has been studied in man following intravenous injection of Sr^{85} and Ca^{45}. Rates of passage of the 2 isotopes into peritoneal cavity have been calculated. In 1 case, Ca^{45} entered the cavity more rap-

idly than Sr^{85}, in another, Sr^{85} entered more rapidly, and in 2 cases, both radioisotopes entered at the same rate. Both ions passed more rapidly into and out of the peritoneal cavity than the fluid accumulated, but passage of ions was slower than flow of water. Rate of passage varied in different patients as did ratio of rates for Sr^{85} and Ca^{45}. The high protein content indicated that peritoneal fluid was not an ultrafiltrate although the Sr^{85}/Ca^{45} ratio corresponded to that of an ultrafiltrate in some cases. The ratio in pleural fluid approximated that of an ultrafiltrate, Sr^{85} entering more rapidly. The ratio of passage of Sr^{85} and Ca^{45} into interstitial fluid studied in one patient with massive edema indicated increased permeability of the capillaries, both isotopes entering the fluid at about the same rate.

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Lipid Bound Glutamic Acid Deficiency in Aging Arteriosclerotic Subjects.*† (25600)

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A previous report(1) described chromatographic identification of bound glutamic acid in the lipid fraction of rat liver. Our object was to determine whether this factor also exists in human plasma lipoproteins and whether the compound shows variation in the aging arteriosclerotic human subject.

Methods. Twenty ml of blood were introduced into a centrifuge tube containing 1.5 ml acid citrate dextrose solution. Fractionation of alpha and beta lipoproteins was carried out according to method of Lever *et al.*(3). Following fractionation, the tubes containing alpha- and beta-lipoproteins received 75 ml of ethyl alcohol:ethyl ether (3:1) extraction mixture. The suspension was stirred 3 hours at room temperature. Then the clear supernatant was decanted into 125 ml Erlenmeyer flasks. To the precipitate were added another 75 ml of lipid extracting media, suspension stirred, then stored overnight at −25°C(4). After removal from deep freeze, stirring was maintained 3 hours. The tubes were then centrifuged and this second extraction was pooled with the previous one. This extraction procedure was repeated using anhydrous ethyl ether. Pooled extracts were brought to anhydrous state over sodium hydroxide pellets *in vacuo* at 40°C. Phospholipids were extracted from the residue with three 15 ml portions of anhydrous petroleum-ether (b.p. 30°-60°C). If residue and solvent are anhydrous, there is no contamination from amino acids

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