Fate of Radiosulfate in Multiple Myeloma.* (24829)

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Evidence establishing presence of an abnormal protein-bound polysaccharide as one of the metabolic abnormalities associated with multiple myeloma has been reported from this and confirmed by other laboratories(1,2,3,4). Studies with radiosulfate in myeloma were initiated as part of a program designed to investigate mucopolysaccharide metabolism in this disease. This report describes the turnover, dilution, and excretion of inorganic radiosulfate in patients with myeloma and, for comparison, in patients with other malignant disorders.

Methods. Carrier-free S^{35} as sodium sulfate in doses ranging 0.867 to 2.07 millicuries was administered intravenously to 9 patients with multiple myeloma and 10 subjects with other neoplastic diseases (Table I). Assays of radioactivity were performed with a gas flow counter, since S^{35} is a pure, weak beta emitter (0.168 mev maximum energy; 87 days half-life). Suitable corrections for self-absorption and backscattering were made for specimen geometry adopted as well as for physical decay. The equipment was calibrated against S^{35} standard solutions supplied by the National Bureau of Standards. Venipunctures were performed at intervals of 5 minutes, one hr, 4 hr, 8 hr, 24 hr, and 48 hr following administration of radiosulfate. Blood specimens were placed in test tubes containing 2 drops of versene to prevent coagulation. Total urinary output was collected during first and second day following injection of S^{35}. *Calculations.* Concentration of S^{35} (% dose/liter) in whole blood and in plasma was determined (Fig. 1), as was the fraction of S^{35} dose excreted (% dose excreted) in urine during the first 2 days following S^{35} administration (Table I). The apparent space of dilution was calculated from blood and plasma data. This computation involved finding net concentration at zero-time by graphical extrapolation, to obtain the so-called zero-time radiosulfate space(5). The body surface of each participant was determined from body weight and height, using the conventional DuBois relationship.

Results. 1. *S^{35} blood levels.* S^{35} concentration in blood as a function of time falls rapidly (Fig. 1). This is true for both whole blood and plasma, and for multiple myeloma patients as well as other subjects. The initial whole blood maxima, obtained 5 minutes following injection of radiosulfate, range from 8.04% dose/liter to 15.7% dose/liter for multiple myeloma patients and from 6.12% dose/liter to 13.1% dose/liter for control subjects. The corresponding plasma concentrations at 5 minutes range from 7.38% dose/liter to 14.4% dose/liter for multiple myeloma and from 6.35% dose/liter to 13.4% dose/liter for the others. By 8 hours, however, these S^{35} levels dropped to almost a third of their respective initial values.

For 16 of 19 patients, whole blood and plasma S^{35} concentration data, when graphed semi-logarithmically, could be resolved into 3 distinct exponential components. Fig. 2 illustrates such a plasma concentration S^{35} curve, together with its 3 exponential decay rates, obtained from a multiple myeloma patient. From analysis of such plots, obtained from both whole blood and plasma concentrations, it appears that an initial rapid turnover, corresponding to half-disappearance time (T_1) of about an hour, governs the fall of S^{35} for the first few hours. For the ensuing hours of first day, the decrease is described by half-time (T_2) of approximately 3 hr. A third half-time (T_3), about 17 hr, characterizes the second day following the dose. Mean values of these various half-disappearance times for myeloma and other patients as derived from blood and plasma data are listed in Table II. For relating these half-disappearance times to corresponding turnover constants (k), the

* This investigation was supported in part by grant from Nat. Inst. of Health, U.S.P.H.S.

We wish to acknowledge the excellent technical assistance rendered by Messrs. Joseph V. Marino, Jack Sokol, and Atis Folkmanis.

TABLE I. S^{35} Urinary Excretion and Zero-Time Whole Blood Spaces of Dilution in Patients.

Sex	Age	Diagnosis	% urinary excretion /24 hr 1st	2nd	Space (liter)	Space / Body surface [liter / meter²]	Space / Wt [l × 100 / kg]
			Group A				
♀	52	Multiple myeloma	26.8	1.01	7.12	6.98	12.0
♀	57	*Idem*	69.6		12.0	7.15	18.6
♂	39	"	33.6	18.6	12.0	7.15	20.3
♂	54	"	60.3	17.2	10.4	5.20	12.5
♀	73	"	79.0	5.19	6.08	3.80	9.57
♂	73	"	39.4	9.10	8.00	4.15	11.3
♂	61	"	42.7	20.0	11.7	6.83	18.3
♂	74	"	54.4	7.30	7.50	4.84	15.5
♂	65	"	57.0	20.2	8.75	4.97	13.2
		Avg	51.4 ± 16.1*	12.3 ± 7.1	9.29 ± 2.16	5.67 ± 1.27	14.6 ± 3.5
			Group B				
♂	57	Hypernephroma	72.2	16.5	8.40	5.12	14.7
♀	33	Ca breast	39.8	12.1	13.7	7.34	15.9
♂	69	Ca sigmoid	51.8	11.6	12.2	6.17	15.1
♂	78	Osteogenic sarc.	71.2	9.91	9.85	6.20	16.6
♂	76	Ca sigmoid	80.2	9.10	9.20	5.44	15.5
♀	53	Ca breast	15.4	5.84	16.0	8.80	22.0
♀	41	Hodgkin's sarc.	46.7	6.50	15.2	9.50	27.6
♀	62	Ca breast	80.7	6.60	11.0	7.05	17.8
♀	46	Ca breast	62.1	12.1	7.40	5.52	18.7
♀	62	Met. bone dis., primary unknown		18.9	10.7	6.04	14.6
		Avg	57.8 ± 20.3	10.9 ± 4.08	11.4 + 2.87	6.72 ± 1.39	17.9 ± 3.9

* Stand. dev.

approximate relationship $k = 0.693/T$ holds. For the 3 other patients (2 of whom had myeloma), the initial rapid rate (T_1) was absent; T_2 and T_3 were both present, in good agreement with values tabulated.

2. *S^{35} urinary excretion.* The combined first and second 24 hr urinary excretion of S^{35} range from almost 30% of dose to maximum of 90%, with no apparent difference between the 2 groups studied (Table I).

3. *Radiosulfate space of dilution.* The zero-time radiosulfate spaces and the ratio of these for whole blood and plasma spaces to body surface and to weight, were similar for myeloma patients and other subjects (Table I). Since concentration of S^{35} in whole blood does not differ appreciably from that in plasma, the respective spaces are almost identical, and, for brevity, only the whole blood radiosulfate spaces are tabulated.

Discussion. The turnover, spaces of dilution, and urinary excretion of S^{35}-sulfate in patients with multiple myeloma and other malignant disorders were similar. Although utilization of inorganic sulfate by the mammal is not fully understood, it is known that elementary sulfur cannot replace cystine or methionine for incorporation into tissue protein of the rat(6,7). Other studies, however, demonstrated that S^{35}-sulfate is utilized by the cytoplasm of the myeloid series of bone marrow cells(8,18). Also, there is a significant incorporation of S^{35}-sulfate in the sulfomucopolysaccharide of mammalian tissues such as cartilage, aorta, sclera, cornea, and intestinal mucosa(9,10,11,12). Indeed, Boström suggests that it may be possible to consider S^{35} sulfate metabolism as reflecting the metabolism of the whole molecule of sulfomucopolysaccharide(13). He demonstrated that for the rat, the principal uptake of S^{35} sulfate was by mucopolysaccharides containing ester sulfates, with a low, but significant uptake in taurine isolated from liver. There was no uptake in cystine or methionine(14). In the rat, Smith and coworkers have shown that about 40% of administered S^{35} sulfate activity was bound to a serum constituent having

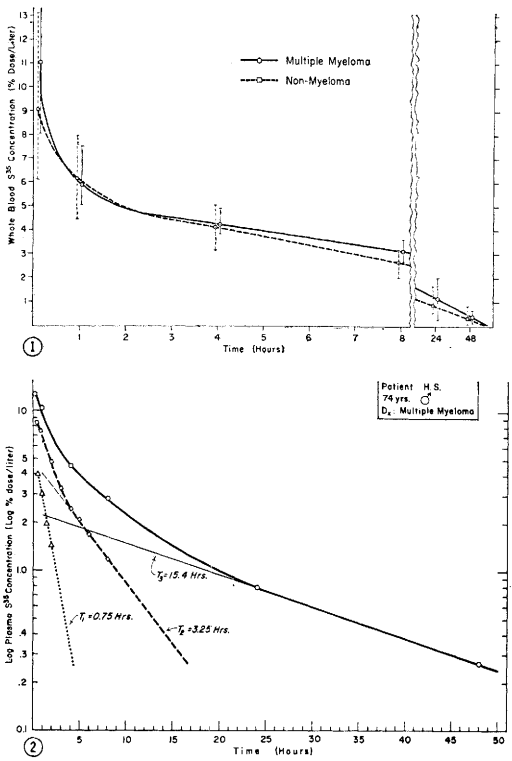

FIG. 1. Variation of whole blood S^{35} concentration with time. Individual points represent mean values in 9 patients with multiple myeloma and 10 patients with other neoplasms. Range (max and min values) is indicated by vertical lines extending from points.

FIG. 2. Resolution of a plasma S^{35} concentration curve into 3 exponential components. T_1, T_2, and T_3 are corresponding half-disappearance times.

the electrophoretic mobility of α_1-globulin; they suggest that the binding occurs in a protein bound sulfomucopolysaccharide(15).

The S^{35} concentration in blood and plasma, turnover times and fraction excreted are in general agreement with the findings of others for animals and normal man. The almost identical whole blood and plasma concentrations suggest that an appreciable amount of S^{35} enters the red cell, a conclusion in accord with data reported for the rat(16,17). Values of the various half-disappearance times (Table II) indicate a rapid turnover of administered radiosulfate. Our values for the intermediate half-time (T_2), and for radiosulfate space for both groups of patients are in close agreement with those reported for normal human subjects(5,19).

TABLE II. Initial, Intermediate, and Late Half-Disappearance Times Obtained from S^{35} Whole Blood and Plasma Concentration Curves.

	Avg half-disappearance times (hr)		
	T_1	T_2	T_3
Whole blood			
Multiple myeloma	.635 ±.205*	2.90 ±.274	15.9 ± 2.2
Other neoplasms	.778 ±.308	3.23 ±.44	17.4 ± 3.9
Plasma			
Multiple myeloma	1.05 ±.29	3.73 ±.36	17.0 ± 3.0
Other neoplasms	.981 ±.451	3.54 ±.64	17.9 ± 4.4

* Stand. dev.

It would appear that administered inorganic sulfate is rapidly turned over and quickly excreted with little, if any, being retained for tissue incorporation. The rapidly excreted S^{35} may be the original sodium sulfate or in the form of sulfomucopolysaccharide, which also is rapidly excreted after administration (personal communication, R. Soberman). In the human, one may conclude that only a small portion of the original dose is retained for utilization and for incorporation into sulfomucopolysaccharide, and, with the technics used, an increased retention could not be demonstrated in multiple myeloma as compared to other neoplasms.

Summary. Radiosulfur (S^{35}) as sodium sulfate was administered intravenously in doses ranging from 0.867 to 2.07 mc, to 9 patients with multiple myeloma and to 10 patients with other neoplasms. The fate of administered sulfate was apparently the same for both groups of patients. S^{35} was rapidly turned over and excreted; 30 to 90% appeared in urine within 48 hours. Blood levels of S^{35} closely paralleled those of plasma and decreased rapidly. It was possible to demonstrate that 3 exponential rates governed the disappearance of S^{35} from whole blood and plasma. The mean zero-time radiosulfate space of dilution was 9.29 ± 2.16 liters for patients with multiple myeloma and 11.4 ± 2.87 liters for those with other neoplasms. By technics employed, a preferential retention of sulfate could not be demonstrated in multiple myeloma as compared to other neoplasms.

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Received February 16, 1959. P.S.E.B.M., 1959, v101.

Effect of Close Arterial Injections of KCl on Gastric Potential Difference.* (25830)

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Numerous studies on a variety of organisms have shown that there is in general an inverse relationship between K^+ ion concentration of ambient fluid and magnitude of transmembrane potential difference (P.D.). In the frog skin(1), resting nerve and other tissues, it has been suggested that the potential difference is due primarily to K^+ ion gradient from inside of cell to the nutrient medium. There are exceptions to this general rule, for example, the findings of Tasaki *et al.*(2) with respect to K^+ ion gradient in fluids of inner ear, where the gradient is opposite to that predicted by the classical scheme. In the gastric mucosa, it has been suggested that the P.D. arises from EMF's associated with active transport of Cl^- ion(3,4). However, the possibility exists that the actual EMF of gastric mucosa is dependent on K^+ ion gradient from the cell to the interstitial fluid (ISF). Our primary purpose was to determine the effect of increasing K^+ ion concentration in ISF on gastric P.D. Because of systemic toxic effects of elevated plasma K^+ ion concentration, the method of close arterial injection was used. A preliminary report has been published(5).

Methods. Amytalized dogs were used with chambered gastric segment technic(6). Approximately 20 cm^2 of the secretory portion of stomach with blood supply intact, is placed in a lucite chamber. Two pairs of reversible electrodes were used, one pair for measuring P.D. and the other pair for sending current (1 ma/20 cm^2). P.D. was measured with Brown recording potentiometer. Direct current resistance was determined as change in P.D./unit of applied current(7). One branch of splenic artery was canulated for close arterial injections. All other branches except those going to stomach were ligated. Injections were made with motor driven syringe. All experiments were performed on resting stomachs with 0.16 M NaCl solution bathing the mucosal surface.

Results. Fig. 1 illustrates a typical experiment showing effect of injection of several iso-

* This work was supported by Grants from Nat. Inst. Health and from the Nat. Science Foundation.