inhibitor activity. Thus, inhibition of 15%-20% involves uncertainty factors of an order which would make it difficult to compare accurately the degree of inhibition obtained.

Excretion of total sulfur and phosphate (as P) in "normal" human urine is reported as approximately 1.0 g and 1.1 g, respectively, per day(6). Inasmuch as sulfate ions constitute 92% of total sulfur excreted daily(6), it is conceivable that the number of sulfate ions present in the urinary dialysate are sufficient to produce the described inhibitory effect. Quantity of sulfate ions in concentrated urinary dialysates is approximately 8.0 mg/ml or 8×10^{-2} M. This concentration of sulfate was found to inhibit DNase II effectively (Fig. 3).

The data (Fig. 3) suggest that in charcoal-treated urinary concentrates sulfate ions might be largely responsible for inhibition of DNase II. Phosphate ions probably contribute little to inhibition of DNase II in the case of the preparations used since removal of phosphate by charcoal did not affect degree of inhibition. Thus, although phosphate ions may have an inhibitory effect, their contribution to inhibition under our experimental conditions (Table I) appears to be small.

Summary. 1. Urinary dialysates were found to retain inhibitory activity toward DNase II after ashing. 2. Chemical analysis of the charcoal-treated dialysate before and after ashing indicated presence of detectable quantities of sulfate ions but not of Mg^{++} and Cu^{++}. 3. Various divalent ions, in contrast to monovalent ions, had a marked inhibitory effect on DNase II. 4. The data suggest that a major portion of urinary inhibitory activity with respect to DNase is due to presence of sulfate ions in the concentrated urinary dialysate.

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Received March 30, 1960. P.S.E.B.M., 1960, v104.

Protection of Mouse Bone Marrow by Inorganic Compounds During Freezing and Thawing. (25846)

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It has long been known that glycerol protects mammalian cells against freezing and thawing damage(1). Lovelock showed that human red blood cells are protected against hemolysis not only by glycerol but also by a number of neutral organic solutes of low molecular weight(2). He attributed freezing damage to the increase in electrolyte concentration that occurs when pure ice is formed in and around the cells, and the protective action of organic solutes to reduction of electrolyte concentration in and around the cells because of the colligative properties of solutions. However, low concentrations of inorganic ions will protect bacteria against freezing and thawing damage(3). A similar protection, if it could be demonstrated for mammalian cells, would be contrary to Lovelock's hypothesis. We tested a number of inorganic compounds for ability to protect mouse bone marrow cells against freezing and thawing

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death. Mouse bone marrow cells have been preserved by the Polge-Smith-Parkes technic of slow-freezing in glycerol solutions and, after thawing, used successfully to promote recovery of lethally irradiated mice(4,5). It has been shown that recovery is the result of replacement of the host's damaged hematopoietic system by proliferation of the donor's cells(6). Our previous experiments showed that mouse bone marrow cells could also be successfully slow-frozen in presence of organic molecules other than glycerol. Many other polyalcohols and mono- and disaccharides are effective(7,8,9).

Materials and methods. The technical details of these experiments are identical to those described previously(7). Suspensions of femoral bone marrow from (C57BL X 101)F_1 donor mice were made in saline containing 3.5% polyvinylpyrrolidone (PVP)‡ and the compound to be tested. PVP, previously shown to be nonprotective, was used to prevent cell clumping. Samples of suspension (1 ml) were frozen in a bath whose temperature dropped at a rate of 1°C/min from +20° to −25°C(10). The tubes were then plunged into liquid nitrogen and kept at −196°C for 1 hour. Samples were thawed by agitation in a 37°C water bath, the cells were counted in a hemocytometer, and the suspension was diluted with saline to a concentration of 2 X 10^6 nucleated cells/ml. Lethally irradiated (900 r) isologous host mice were each given 0.5 ml of this suspension *via* the tail vein. Eosin-uptake measurements(11) were also made to determine the percentage of nonstaining (and presumably viable) cells. Seven simple inorganic molecules were tested using 8 different concentrations of each.

Results are given in Table I. The mice not given bone marrow all died, but 75% of control mice that had received 10^6 fresh bone marrow cells survived more than 30 days. Mice given marrow frozen in either physiological saline (0.154 M NaCl) or in Tyrode's solution did not survive. Of 7 neutral salts used, treatment with 6 (NaI, NaBr, $NaNO_3$, Na_2SO_4, $Na_2S_2O_3$ and NaSCN) gave fairly good 30-day survival at some concentrations, and even the seventh, $NaNO_2$, gave a few survivors. The eosin-uptake measurements seemed to be of little value in estimating recovery-promoting power of cells frozen in these solutions.

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Discussion. The data in Table I suggest that there are 2 concentrations of each compound that give high protection. Thus, with NaSCN, survival is obtained between 0.003

TABLE I. Thirty-Day Survival of Lethally Irradiated Mice after Infusion of 10^6 Nucleated Bone Marrow Cells Frozen and Thawed in Solutions of Inorganic Salts.

Solution	Conc. (M) of salt in 3.5% PVP in saline	No. of mice	Eosin-negative cells (%)	30-day survival (%)
None		210		0
Fresh		288	84	75
Frozen in saline		81	3	0
Frozen in Tyrode's		58	5	0
Frozen in NaI	.003	18	11	6
	.006	"	11	6
	.013	"	19	0
	.025	20	35	50
	.05	"	23	35
	.1	"	3	10
	.2	23	2	0
	.4	20	0	0
Frozen in NaBr	.003	16	5	25
	.006	20	16	55
	.013	"	9	40
	.025	"	6	45
	.05	"	4	40
	.1	"	1	25
	.2	"	2	35
	.4	"	1	0
Frozen in $NaNO_3$	.003	"	19	40
	.006	"	14	65
	.013	"	12	95
	.025	"	13	45
	.05	"	17	45
	.1	"	9	15
	.2	22	3	59
	.4	20	0	0
Frozen in $NaNO_2$	.003	"	7	5
	.006	"	11	0
	.013	"	10	0
	.025	"	7	0
	.05	"	12	5
	.1	"	9	0
	.2	"	5	0
	.4	"	2	0
Frozen in Na_2SO_4	.003	"	6	60
	.006	"	2	0
	.013	"	1	50
	.025	"	1	5
	.05	"	7	15
	.1	"	5	0
	.2	"	2	5
	.4	"	0	0

TABLE I (continued).

Solution	Conc. (M) of salt in 3.5% PVP in saline	No. of mice	Eosin-negative cells (%)	30-day survival (%)
Frozen in NaSCN	.003	20	13	30
	.006	"	15	40
	.013	"	10	5
	.025	"	9	0
	.05	"	6	0
	.1	"	1	0
	.2	"	0	5
	.4	"	0	5
Frozen in $Na_2S_2O_3$	.003	"	2	15
	.006	"	4	0
	.013	"	3	30
	.025	"	3	25
	.05	"	5	20
	.1	"	6	35
	.2	"	16	5
	.4	"	2	0

and 0.013 M and between 0.2 and 0.4 M but not at intermediate concentrations. Although the data are not extensive enough to establish a bimodal concentration-protection curve for any one compound, the fact that all the compounds tested showed some effect seems significant. This phenomenon has been noted previously with protective organic compounds (7). Its significance, however, remains obscure.

A small amount of protective compound was injected into each mouse along with the test cells. The dose of compound varied from a minimum of 0.056 mmole/kg up to 12.8 mmole/kg. It seems extremely unlikely that this quantity by itself could have any protective effect on the mice.

It is difficult to explain the protection of mammalian cells against freezing and thawing damage by inorganic salts. It cannot, however, be attributable to a simple lowering of electrolyte concentration during freezing, as was suggested by Lovelock(2) for organic solutes. Neither can it be the result of vitrification of cellular fluid as suggested by Luyet (12). More quantitative physical data, preferably on individual cells, may help to explain the observed phenomena.

Summary. The ability of inorganic compounds to protect mouse bone marrow cells against freezing and thawing damage has been investigated, using a lethally irradiated isologous recipient as a functional assay of viability. NaI, NaBr, $NaNO_3$, $NaNO_2$, Na_2SO_4, NaSCN, and $Na_2S_2O_3$ were tested. All showed protective action against freezing and thawing. The effectiveness of different inorganic salts varied greatly. Each compound was effective only at certain concentrations.

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Received April 4, 1960. P.S.E.B.M., 1960, v104.