TABLE I. Lipid Analyses (mg/g) of Liver Samples from Groups of Normal, Injected Control and Dilantin Treated Rats.

	No. animals	Total cholesterol	Phospholipids	Neutral glycerides
Normal	6	1.91 ± .15	27.3 ± 1.31	3.47 ± .73
Control	6	2.01 ± .14	26.4 ± 3.09	7.08 ± .58*
Dilantin	6	1.85 ± .14	24.4 ± .92*	1.23 ± .24†

* Significantly different from normal value.
† Significantly different from both control and normal values ($p < .01$).

These results suggest that the effect of Dilantin upon tissue lipids was primarily confined to the glycerol containing lipids. A similar effect of this drug has also been previously described(2) for rat skin. The mechanism through which Dilantin affects the lipid content of such different tissues as skin, liver and aorta remains obscure. However, it would appear to be of great interest to explore the possible effects of Dilantin upon atherosclerosis.

TABLE II. Lipid Analyses of 2 Pools of Rat Aorta (mg/g) in Normal, Injected Control and Dilantin Treated Animals.

		Total cholesterol	Phospho-lipids	Neutral glycerides
Normal	a)	.95	7.2	13.2
	b)	.78	6.2	9.5
Control	a)	1.41	8.0	16.5
	b)	1.33	8.4	12.2
Dilantin	a)	1.07	6.3	2.69*
	b)	1.21	5.9	2.85*

* Differ by more than 3 standard deviations from the mean of normal plus control values (12.8 ± 2.5).

Summary. Twelve daily intraperitoneal injections of one ml suspensions of 25 mg of sodium bi-phenyl hydantoinate (Dilantin) in isotonic saline to rats resulted in a significant decrease in tissue concentration of neutral glycerides of both the aorta and liver when compared with the tissue from both normal and saline injected control animals.

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Effect of Dimethyl Sulfoxide and Glycerol on Cation Content of Freeze-Thawed Cardiac Muscle.* (27651)

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Dimethyl sulfoxide (DMS) has been reported to be effective in protecting against cellular damage and death in freeze-thawed tissues, including isolated hearts(1-7). Since the preservation of mammalian tissue by low temperatures and freezing is in part limited by the deleterious effects of intracellular dehydration and subsequent concentration of intracellular ions(8), the protective effect of DMS on heart might be related to its ability to prevent or modify this critical change of water and salt concentration. Experiments were therefore performed on isolated cardiac tissue preparations, frozen and thawed with and without DMS. Analysis of total tissue potassium (K) and sodium (Na) was made and results compared to freshly dissected tissue. For comparison, glycerol treated tissue was also studied since this substance is known to be an effective protective agent in the freeze-preservation of a variety of cells and tissues(8).

Methods. Hearts from white New Zealand rabbits were used. The animals were sacrificed by a blow on the head and the heart

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quickly removed and placed in a dissecting dish containing oxygenated Ringer solution of the following composition (in mM/L): NaCl, 154.0; KCl, 5.6; $NaHCO_3$, 5.9; $CaCl_2$, 2.2; Dextrose, 5.5. The right atrium and a strip of the free wall of the left ventricle were then dissected free of all visible fat and vascular tissue. Experiments were divided into 5 groups:

Group I. Freshly dissected right atria and left ventricular tissue were blotted on filter paper, and immediately dried in an oven at 110°C in preparation for analysis for K and Na. *Group II.* Right atria and left ventricular tissue were placed into test tubes containing 2 ml of Ringer solution. These were then immersed and frozen in a container filled with a solid CO_2-ethanol mixture (–79°C). After 5 minutes at –79°C, the tubes were removed and the tissue rapidly thawed at 37°C. Preparations were then removed from the tubes, blotted on filter paper, and placed in a drying oven. *Group III.* Tissue handled in same maner as Group II, except that DMS (final concentration, 10% v/v) was added to the Ringer solution, and tissue was "incubated" for 2 minutes before placing in the –79°C container for freezing. *Group IV.* Same as Group III, except that glycerol (15%) was used. *Group V.* Left ventricular tissue handled in same way as Group IV except that tissue was not frozen after incubation with glycerol.

After tissues had dried to a constant weight, dry weights were recorded, and nitric acid digests prepared for flame photometer analysis of total tissue K and Na using an internal lithium standard. Changes in cation concentrations were expressed as per cent of those values recorded in fresh tissue (Group I).

Results. Fig. 1 illustrates changes in K and Na concentrations occurring in right atrial tissue. Fig. 2 summarizes changes in K and Na content in left ventricular tissue.

In both right atrial and left ventricle tissue, freeze-thawing was associated with a marked loss of tissue K and gain of Na (Group II), compared to untreated freshly dissected preparations (Group I). The presence of 10% DMS in the freezing medium (Group III)

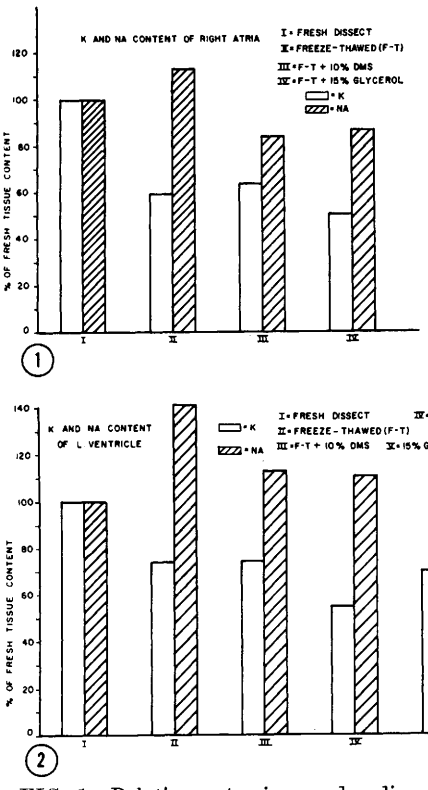

FIG. 1. Relative potassium and sodium content of isolated rabbit right atria freeze-thawed with and without dimethyl sulfoxide (DMS) and glycerol. Cation content is expressed as per cent of those values seen in freshly dissected, untreated tissue (designated as 100%). Values based on 6-20 experiments.

FIG. 2. Relative potassium and sodium content of isolated rabbit left ventricular tissue freeze-thawed with and without DMS and glycerol. Values based on 6-10 experiments.

had no significant protective action in preventing K loss when compared to the change in K seen in freeze-thawing without DMS (Group II). The increase in Na content which occurs with freeze-thawing was only slightly prevented by the presence of 10% DMS ($P>0.05$). Right atrial Na in Group III was found to be lower than that seen in the freshly dissected untreated group. The increase in Na content in the DMS-treated left ventricle preparations, while less than that seen in freeze-thawing without DMS, was still higher than the Na values obtained in freshly dissected left ventricle.

The presence of 15% glycerol in the Ringer solution in which the ventricular tissue was freeze-thawed does not prevent the K loss

normally seen with freeze-thawing without preservative. Sodium gain was found to be slightly ($P>0.05$) less in the glycerol treated ventricles compared to tissue freeze-thawed without glycerol. Sodium content in the glycerol treated ventricles was not significantly different from Na values seen in freshly dissected ventricle. The presence of 15% glycerol without freezing (Group V) produced a loss of K, but no significant change in Na content compared to values seen in Group I.

The presence of 15% glycerol during the freeze-thawing of right atrial tissue produced results qualitatively similar to those seen with ventricular tissue. Thus, K loss in freeze-thawed glycerol treated preparations was greater than that seen with freeze-thawing alone. The sodium content in the presence of glycerol was slightly less than that seen in freezing without this agent, however.

Discussion. These results offer no support for the view that the protective action of DMS in freeze-preservation of isolated hearts (7) might be related to the ability of this agent to prevent or significantly modify the marked loss of tissue K and gain of Na associated with freeze-thawing of muscle. Under the conditions of these experiments, glycerol also had no protective action in preventing K loss associated with freeze-thawing. Moreover, the presence of glycerol actually increased K loss during freeze-thawing. This might help explain the fact that 10-20% glycerol-Locke solutions are toxic in isolated rabbit hearts, and do not protect against freezing injury (9). It should be emphasized, however, that the methods used in these experiments might not represent the optimum conditions for demonstrating the protective action of DMS. On the other hand, it has been established that 15% DMS is an apparently effective concentration for the cold preservation of cells and tissues(1-7) under any given experimental conditions of freezing. The fact that DMS diffuses rapidly into and out of cells *in vitro*(1) also suggests that lack of penetration is not a major cause of its being unable to protect against ion changes.

Measurement of total tissue content of K and Na does not, of course, allow conclusions about changes in intracellular cation concentrations. But based on the analysis that damage to cells caused by freeze-thawing is related in part to a decrease in the normal K/Na ratio in tissues, DMS and glycerol appear to be ineffective in preserving this balance under the conditions of these experiments. Whether or not DMS has a more subtle effect on membrane permeability and cation transport mechanisms during freezing remains to be determined.

Summary. The effects of dimethyl sulfoxide (DMS) and glycerol on K and Na content of freeze-thawed rabbit heart muscle have been determined. Both right atrial and left ventricular tissue were found to lose K and gain Na after freeze-thawing compared to freshly dissected tissue. The presence of 10% DMS or 15% glycerol during freeze-thawing did not prevent this loss of K or gain of Na. Glycerol was also found to produce a loss of K in ventricular tissue even in the absence of freeze-thawing.

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