Rate of Elimination of Dimethylsulfoxide from Tissues of Euthermic and Hypothermic Rats[1] (34330)

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In addition to analgesic and therapeutic clinical applications, dimethylsulfoxide (DMSO) has been used as a protective agent in low temperature and ionizing radiation studies. The wide variety of uses of DMSO has been reviewed (1, 2). Recently, and of special interest to our study, DMSO was noted to affect the response of animals to hypothermia (3–6). Radiation protection, cryoprotection, and hypothermia effects depend on the concentration of DMSO present in the tissues, although the mechanisms of action are not known.

It has been reported that DMSO accumulates primarily in the soft tissues of the body immediately after injection and accumulates more slowly in the hard tissues (7). Recent work, using autoradiographic analysis, suggested the possibility that DMSO concentrated primarily in the intercellular spaces and did not penetrate the cell membrane in quantity (8). In order to gain further information on the mechanisms which control the action and distribution of DMSO in situations involving the whole animal, a comparative study of the rate of loss of DMSO from selected tissues of the euthermic and hypothermic rat was undertaken.

Methods and Materials. A stock injection solution was prepared by diluting ^{35}S-dimethylsulfoxide (Nuclear Chicago) with drug grade unlabeled DMSO (Crown Zellerbach Corp.) so that the final specific activity of the solution was either 25 or 50 μCi/ml.

Concentration of DMSO in the assayed tissues was determined by liquid scintillation methods, using a Packard Tricarb liquid scintillation spectrometer. The counting fluid was prepared from a commercial concentrate of PPO and POPOP in toluene (Nuclear Chicago).

Sixty male Long-Evans rats (weighing between 240 and 300 g) were placed randomly into two groups. Thirty animals were tested under euthermic conditions, and 30 were tested under hypothermic conditions. Calculations in each condition were based on the averages generated by six groups, each containing 5 rats. Animals in each group were chosen according to weight to insure uniformity of age, the standard deviation of weights within the groups averaging $\pm$ 3.5 g.

Euthermia. Groups of five animals were administered intraperitoneal injections of 1.5 g of DMSO (25% in normal saline) /kg of body weight. After injection, animals were maintained at an ambient temperature of 25° without access to food or water. One animal was killed at each of the first 5 hr after injection. Samples were removed from the brain anterior to the cerebellum, the ventricles of the heart, the liver, the kidney, and the spleen. Each sample was placed in 10 ml of cold toluene and homogenized.

Following homogenization, two 1-ml samples of the homogenate were removed and placed in separate low phosphorous content glass counting vials. Nine ml of counting fluid were added to produce a 10-ml counting volume. Then samples prepared in this way from each animal were maintained at 2° until counting.

Hypothermia. Six groups of five animals were tested. Animals in each group were shaved under light ether anesthesia at least 12 hr prior to initiation of their experimental

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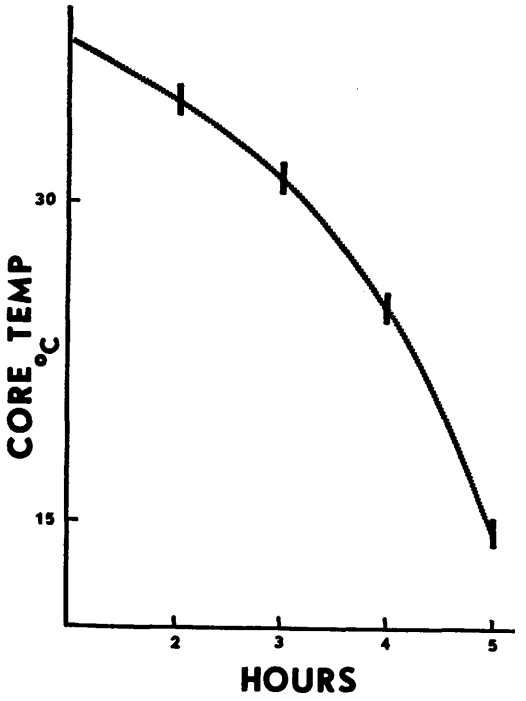

FIG. 1. Standard cooling curve used for experiments under hypothermic conditions; vertical lines indicate the range of body temperatures for the animals sacrificed at the indicated hour.

run. All fur was removed with the exception of that anterior to the ears.

Groups of five animals received 1.5 g of DMSO/kg of body weight intraperitoneally as a 25% solution in saline. Since preliminary analysis of the euthermic data had indicated that a peak concentration of the drug was reached in the tissues on, or before, 1 hr subsequent to injection, hypothermia was initiated 1 hr after injection. Shaved animals were exposed to a 2–4° environmental temperature. The deep colonic temperature of each animal was recorded continuously from a copper–constantan thermocouple. The control and hypothermia experiments were repeated six times. A standard cooling curve which related time after being placed in the cold to animal core temperature was developed (Fig. 1). The core temperature of each test animal followed this curve within an accuracy of $\pm$ 0.8° during the cooling experiments. The rate of cooling of each animal was controlled by varying the velocity of the air passing over its body surface.

As was done previously, animals were sacrificed at 1, 2, 3, 4, and 5 hr after injection. From the standard cooling curve, animal core temperature at these points was respectively 37, 35, 31, 25, and 15°. Tissue samples were removed, weighed, homogenized, and processed in a manner identical to those removed from the euthermic animals.

Sample analysis. Vials to be counted were allowed to stand for at least 3 hr in the refrigerated sample carrier of the counter in order to insure temperature equilibration and to allow for settling of any particulate material. Activity determinations were made on the basis of the time required to generate 10,000 counts. Each sample was counted twice.

Since the counting samples were polyphasic, with interfaces existing between homogenate particles and the counting fluid, quenching of the samples had to be considered. In order to minimize this effect, uniform amounts of tissue were used to prepare each sample of the organs analyzed and was considered as constant within each organ.

Results. An analysis of activity of samples during the euthermic experiments indicated that the peak concentration of DMSO was reached in the tissues studied on or before the first hour following injection. Since injection solutions of varying specific activity were used, it was necessary to convert the raw data to relative values for comparisons between organs. For such comparisons, the values for the first hours were considered as 100% and the values obtained at subsequent times were expressed as a fraction of the 1-hr concentration in an organ. These values, representing the average of six runs of five animals each under euthermic conditions, are given in Table I. The concentration in each tissue decreased from the first to the fifth hour (Fig. 2).

Loss during the second and third hour from all tissues under euthermic conditions is relatively rapid, resulting in a decrease in concentration to approximately 65% of the 1-hr tissue levels of DMSO. Loss over the subsequent hours is slower in all tissues, the concentration decreasing only to an average

TABLE I. Percentage of 1 hr Concentration of DMSO per Milligram of Tissue Wet Weight with Statistical Deviations for Experimental Runs under Euthermic Conditions.

(hr):	2		3		4		5	
	(% per mg)	SE (%)	(% per mg)	SE (%)	(% per mg)	SE (%)	(% per mg)	SE (%)
Brain	79	±4.9	67	±4.4	57	±3.6	52	±3.5
Heart	80	±6.1	64	±4.4	56	±7.4	53	±4.3
Liver	76	±3.4	61	±2.5	54	±4.2	52	±2.5
Kidney	77	±7.3	68	±6.8	61	±6.4	60	±6.8
Spleen	69	±5.0	59	±7.7	50	±2.7	48	±1.8

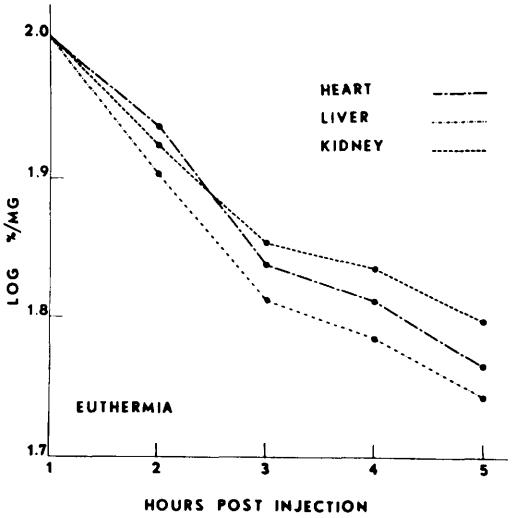

FIG. 2. Loss of DMSO from the heart, liver, and kidney under euthermic conditions during the first five hours following a 1.5 g/kg ip injection; values are expressed as the log of the percentage of the 1-hr concentration per milligram of tissue wet weight present at a given time.

of 53% of peak values over the next 2 hr.

Values at each hour calculated for the experiments conducted under hypothermic conditions are given in Table II. The loss occurring between the first and second hour after injection, which corresponds to the first hour of cold exposure, shows no significant difference from that shown in the euthermic animals. Over the following 3 hr, however, the rate of loss from all tissues is significantly lower than that shown in the euthermic animals (Fig. 3, 4).

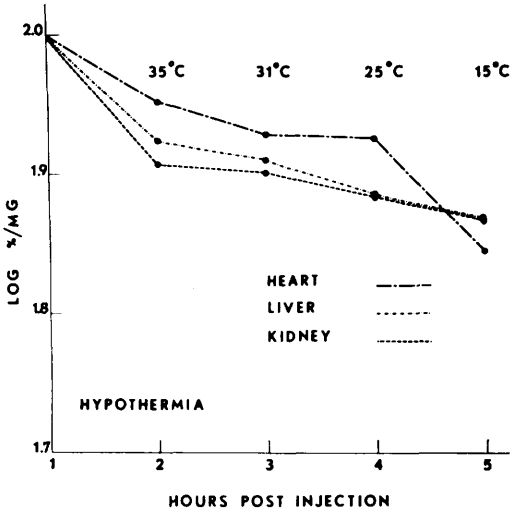

FIG. 3. Loss of DMSO from the heart, liver, and kidney under hypothermic conditions during the first 5 hr following a 1.5 g/kg ip injection.

TABLE II. Percentage of 1 hr Concentration of DMSO per Milligram of Tissue Wet Weight with Statistical Deviations for Experimental Runs under Hypothermic Conditions.

(hr):	2		3		4		5	
	(% per mg)	SE (%)	(% per mg)	SE (%)	(% per mg)	SE (%)	(% per mg)	SE (%)
Brain	91	±5.8	84	±3.0	79	±4.8	72	±2.8
Heart	90	±8.0	85	±2.0	85	±4.0	70	±3.0
Liver	85	±4.7	82	±3.8	77	±3.8	74	±5.2
Kidney	80	±5.7	77	±2.8	77	±3.4	74	±5.5
Spleen	136	±8.7	112	±8.7	97	±8.7	82	±5.0

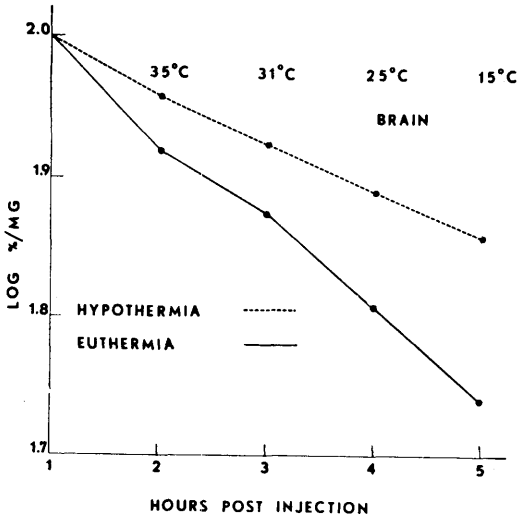

FIG. 4. Loss of DMSO from the brain under euthermic and hypothermic conditions during the first five hours following a 1.5 g/kg ip injection.

The spleen shows an interesting deviation from the results common to the other organs (Table II). It is the only organ under hypothermic conditions which shows an active increase in concentration of DMSO over the peak value obtained during the euthermic experiments, as represented by the 1-hr value. The concentration in the spleen does not drop below the level of peak concentration under euthermic conditions until the third hour of hypothermia, corresponding to the fourth hour after injection. Although the cause of the increase is not known at the present time, increased blood content and decreased rate of blood flow in this organ during hypothermia might be implicated.

Discussion. Observations on the rate of loss of DMSO from the tissues under euthermic conditions show a consistently faster drop in concentration over the first 2-hr period than over the second 2-hr period following attainment of peak tissue levels. The difference is greater than would be expected as a factor of time in a concentration-dependent loss mechanism. It may be that the relatively fast loss observed during the initial 2 hr represents loss of essentially unassociated DMSO, and that this rate decays into a slower loss as the free pool is depleted.

Two basic modes of loss of DMSO from the tissues can be considered. The first is through diffusion of the molecules out of the tissues and into the blood, with subsequent excretion. The second is the breakdown of the DMSO by metabolic pathways and the elimination of the by-products. Any factor tending to stabilize the DMSO by binding, or otherwise making the molecules unavailable for further movement or reaction, will decrease the rates of both of these mechanisms. Thus, if some of the DMSO molecules which pass into the cells become associated with cellular protein, as in the postulated (9) incorporation of the DMSO into hydration sheaths of macromolecules, this fraction of the total will not be characterized by the same freedom to diffuse as the unassociated DMSO. The lower limit of free diffusion out of the tissues then, will be set by the amount of DMSO in some way bound within the tissues. In addition, since the limit of concentration buildup in the tissues by simple diffusion is determined by the free DMSO concentration of the tissue as compared to the blood, such binding may induce concentrations of DMSO in the tissues higher than that in the blood without active transport.

It is interesting to note that during the first hour after initiation of hypothermia, the rate of loss from the liver, kidneys, and heart does not differ from that seen in the euthermic animal. A normal response to cold exposure would be expected to include an increased heat production, reflected in an increase in metabolism. In light of the high urine output during this period, one might reasonably expect an increase in elimination rate. Since there was no increase in elimination rate, it is possible that the animals may not have increased their metabolic rate in response to cold. This is supported by an apparent reduction in shivering in the animals treated with DMSO and exposed to the cold. Certainly this lack of shivering would result in the maintenance of a nonelevated metabolic rate, and consequently a euthermic rate of loss of DMSO over this period. Earlier experiments showed that DMSO prevents an increase of heat production during the cold exposure of both rats and hamsters (4).

After 1 hr of cooling, the decrease in body

temperature was more severe. Under these conditions, a number of physical factors become important. The rate of diffusion decreases with temperature, as does the rate of chemical reaction and consequently the metabolic rate. Blood flow to all organs decreases with decreasing cardiac output. These factors find expression in a decrease in the rate of loss from all the tissues. The rate of loss from the liver and the kidney drops to almost 40% of the euthermic loss rate. The rate of loss from the heart likewise decreases. The drop in elimination rate observed in all the organs probably can be attributed to the effects of decreasing temperature on reaction and diffusion rates directly connected with the elimination of the dimethylsulfoxide.

There are, however, some interesting variations observed in the general pattern of depression seen in the different organs. The first obvious divergence is seen in the brain during the initial hour of hypothermia. The rate of loss is significantly lower in the hypothermic animals over this time period, although the rest of the tissues show no change. The reason for this anomaly is now known. The second, which has already been discussed, is the elevation of DMSO levels in the spleen after initiation of hypothermia.

Summary. It was shown that following the intraperitoneal injection of 1.5 g of dimethylsulfoxide/kg of body weight in rats, the drug reaches peak concentrations in all organs studied within 1 hr under euthermic conditions. Similar results were seen under hypothermic conditions in all organs except the spleen, which continued to increase in DMSO concentration until the second hour after rejection. The rate of loss from the tissues is relatively rapid during the initial 2 hr after peak concentration is reached, and becomes slower during the later periods. The loss curves under hypothermic conditions all demonstrate significantly lower rates of loss than their respective euthermic counterparts. It has been suggested that the observed early fast loss may represent depletion of a free or unassociated DMSO in the tissues.

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