Cellular Swelling:

I. Hypothermia Graded Hypoxia, and the Osmotic Effects of Low Molecular Weight Dextran on Isolated Tissues

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A RAPID increase in cellular volume occurs in metabolizing *in vitro* tissues in an isotonic medium when subjected to either hypothermia or hypoxia.[4] Because such cellular swelling can be prevented only by continuously supplying the rather stringent metabolic demands of living tissue, it is presumed that the maintenance of cellular volume is a continuously active, energy-consuming process.[5] The cellular swelling of hypothermia appears therefore to be a rather nonspecific phenomenon. Hypoxia or addition of enzymatic toxins such as dinitrophenol produce cell swelling because these also depress those cell functions that maintain both cell volume and the individual electrolyte gradients across the cell membrane. Restoration of normal temperature or oxygen tension promptly restores normal cellular size in such *in vitro* experiments, provided the metabolic depression has not been prolonged sufficiently to be irreversible. It is of further interest that increasing the concentration of the nutrient medium has long been known to reverse promptly cellular swelling.[10]

The salutary effect of low molecular weight dextran (LMWD) in shock or hypothermic perfusion is generally attributed to its blood flow-promoting properties which are well documented.[3] Comparatively little quantitative information is available on the colloidal osmotic effects of LMWD on hypoxic or hypothermic tissues. Actually, the circulatory effects of LMWD are not readily separable from the colloidal osmotic effects in the intact animal or in the isolated perfused organ where a vasculature is involved. The physical properties of LMWD that promote capillary blood flow may increase the oxygen tension of hypoxic cells and thus secondarily exert an osmoregulatory effect. The following experiments were devised to quantitate cellular swelling under controlled hypothermic and hypoxic *in vitro* conditions and to determine the effects of LMWD on this cellular swelling in an experimental preparation that avoids the complexities of a circulatory system.

Methods

In the first series of experiments, the effects of graded hypoxia at 37° C. and 7° C. on total tissue water (TTW) were measured in surviving *in vitro* tissue slices of renal cortex of mongrel dogs. In the second and third series, LMWD was added to the incubation media and the colloidal osmotic effect of the dextran on the tissues observed as a change in TTW of the slices.

The dogs were anesthetized with intravenous Nembutal after 12 hours without

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TABLE 1. *Comparison of Hypoxic and Hypothermic Effects in % Change of Total Tissue Water*

	Hypoxic Effect				Hypothermic Effect	
	37° C.		7° C.		7° C.	
% O_2	% Change TTW from 37° C. 100% O_2 Control	P* Value	% Change TTW from 7° C. 100% O_2 Control	P Value	% Change TTW from 37° C. 100% O_2 Control	P Value
100	—	—	—	—	+3.5 (43)	<0.005
20	+3.1 (14)	<0.005	+0.7 (15)	N.S.	+1.1 (33)	N.S
10	+3.9 (18)	<0.001	+0.3 (18)	N.S.	+0.6 (18)	N.S.
5	+4.4 (11)	<0.001	+1.2 (10)	<0.025	+0.4 (10)	N.S.

* Significance of change from control.
Number of pairs of observations in parentheses.

food or drink. The kidney was removed and the cortex sectioned in the Stadie-Riggs hand microtome within a few minutes of the clamping of the renal pedicle. Slices were cut 0.3 mm. thick and weighed about 25 to 50 mg. Experimental and incubated control slices were then incubated for 30 minutes in 3 ml. of medium with constant and vigorous shaking at 180 strokes/min. while the gas-oxygen mixture was constantly bubbled into the medium via a 22-gauge needle at 50 ml./min. gas flow per tube. After incubation, the slices were carefully blotted on a #541 Whatman filter paper, placed in a small stoppered weighing bottle and weighed on a Mettler H-16 semimicro balance. The tissues were then dried overnight at 105° C. in a vacuum drying oven, cooled in a desiccator, reweighed and the weight loss expressed as TTW in percentage of wet weight. Non-incubated control slices were made of each kidney and similarly blotted, weighed, desiccated and reweighed to ascertain the reproducibility of the determination of TTW. In addition, non-incubated control TTW values served as a baseline from which to judge the adequacy of shaking and oxygenation of the incubated controls. All incubated control slices were shaken for 30 minutes using 100 per cent oxygen and either 37° C. or 7° C. medium except as indicated.

The incubation medium was Krebs Ringer phosphate solution, modified by reducing the calcium chloride to half that given by Umbreit [13] and adding 100 mg. of glucose per 100 ml. of medium. The pH was 7.4 and the final concentration was adjusted to 300 millios./Kg. by determining the freezing point depression with a Fiske Mark III osmometer. The LMWD used was Rheomacrodex * in dry powder form, having an average molecular weight of 40,000. The powder was added to the medium in one of two ways. In the second series of experiments, the LMWD powder was added to the Krebs Ringer phosphate glucose medium of 300 millios./Kg. concentration to provide a final concentration of 330 millios./Kg. In the third series, the osmotic concentration was adjusted to 300 millios./Kg. after the addition of LMWD to the medium.

The three experimental series were derived from the data on 12, 13 and 20 ani-

* Generously supplied by Pharmacia Laboratories, Inc., Rochester, Minn.

mals, respectively. Observations of TTW were made on at least three slices for each control or experimental condition in each experiment of the series. Standard deviations of the means of the observations in each experiment were determined and the t-test applied to judge the statistical significance of differences within the series.

Results

Changes in TTW due to graded hypoxia or 7° C. hypothermia or both determined in the first experimental series are given in Table 1, and the combined mean values of TTW are graphically presented in Figure 1. Hypothermia at 100 per cent O_2 increased TTW from 77.1 to 80.9 per cent. At reduced oxygen concentration levels of 20 per cent or below, there was no statistically significant further increase in TTW due to hypothermia of 7° C.

Graded hypoxia of 20, 10 and 5 per cent oxygen at 37° C. incubation produced progressive increases in TTW with increasing hypoxia. At 7° C., oxygen concentration of 20 and 10 per cent did not cause significant further increase in TTW, but significant additional swelling did occur at 5 per cent oxygen concentration. A quantitative comparison of tissue swelling due to 7° C. hypothermia and that due to graded hypoxia can be made from this series of experiments. Reduction of oxygen concentration to 10 to 20 per cent produces approximately the same degree of TTW increase as hypothermia of 7° C. Whether or not the cellular swelling effects of hypothermia and those of hypoxia are additive is a point of semantics. In the sense that further swelling may be produced by hypoxia even at hypothermic temperatures, the effects are cumulative. Hypothermia and hypoxia are not, however, quantitatively additive because far less hypoxic swelling occurs at the lower temperature.

Addition of LMWD to an isotonic medium results in a decrease in cellular size

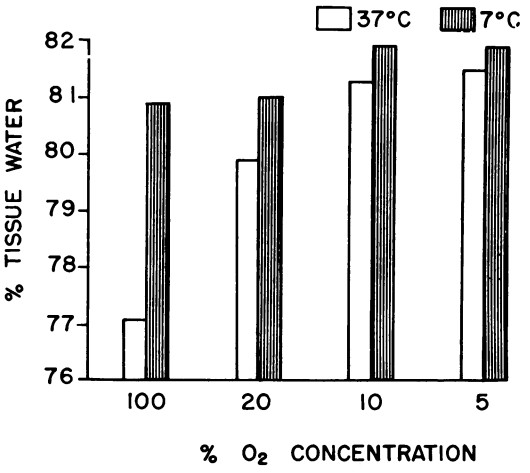

FIG. 1. Effects of graded hypoxia at 37° C. and 7° C. on total tissue water.

that is essentially proportional to the amount of colloid added at either normothermic or hypothermic temperatures. The results obtained from the second series are presented in Table 2 and Figure 2. Concentrations of LMWD used in this experiment are greater than the 3 or 4 per cent

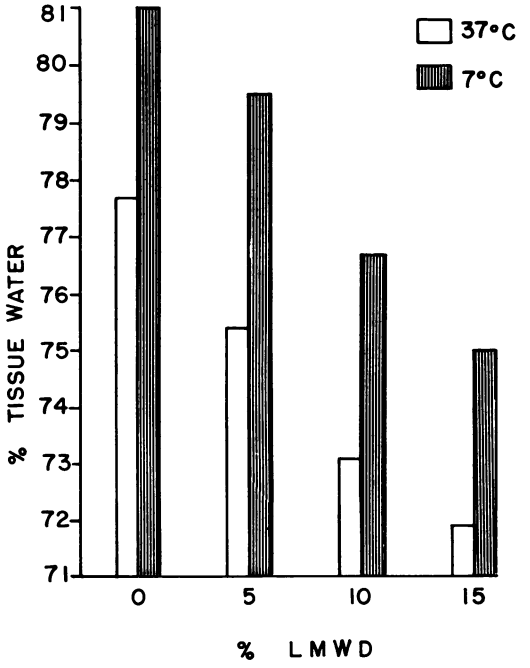

FIG. 2. Osmotic effect of LMWD added to an isotonic medium at 37° C. and 7° C.

TABLE 2. *Effect on Total Tissue Water of LMWD Added to 300 millios./Kg. Medium*

TTW*				TTW		
37° C. Control	LMWD Added	P** Value	Conc. LMWD	7° C. Control	LMWD Added	P Value
77.7 ± 0.7 (12)	75.4 ± 0.5 (12)	<0.01	5%	81.1 ± 0.8 (12)	79.5 ± 0.6 (12)	<0.025
77.7 ± 0.7 (9)	73.1 ± 0.3 (12)	<0.001	10%	80.8 ± 1.1 (8)	76.7 ± 0.6 (11)	<0.001
77.8 ± 0.7 (15)	71.9 ± 0.6 (15)	<0.001	15%	81.2 ± 0.7 (13)	75.0 ± 1.0 (15)	<0.001

* Mean value in % ± standard deviation.
** Significance of difference between control and experimental TTW values.
Number of observations in parentheses.

concentrations one may expect in the blood of a patient after the administration of a maximal dose of LMWD. From these data one may estimate that the concentration of LMWD required to counteract completely the hypothermic cellular swelling is between 5 and 10 per cent. As expected, the osmotic effect of the colloid addition is quite independent of the hypothermic cellular swelling so that about the same degree of dextran dehydration effect is noted at 7° C. as at 37° C. In the third series of experiments when the final concentration of the media was brought to 300 millios./Kg. concentration after the addition of the LMWD, the experimental and control media differed in their colloidal osmotic pressures but not in their total osmotic pressures. TTW values obtained (Table 3) were about 1 per cent higher than in the preceding method of adding LMWD to the isotonic medium.

Discussion

Cellular swelling *in vitro* is readily demonstrable, but direct comparisons to the intact animal or patient in hypothermic or in an hypoxic state should be made with considerable caution. Despite the consistent finding of a decrease in circulating plasma volume and extracellular fluid in hypothermia [6] and shock,[11] good evidence that this fluid enters the cellular space in the intact organism in comparable conditions is curiously lacking. There are two possible explanations for this apparent disparity between the *in vitro* and *in vivo* findings. First, an increase in intracellular fluid in these *in vivo* conditions may not occur, either because there is inadequate available fluid or because of some protective readjustments within the extracellular spaces related to the known heterogenicity of the vascular circulation.[11, 12] Clearly, the *in vitro* preparation differs most markedly from the intact organism in the absence of a vascular circulation and the almost infinite available volume of the artificial "extracellular" fluid. Secondly, intracellular swelling may occur in *in vivo* hypothermia or hypoxic states but is not readily detectable. Shires [11] recently indicated by radioactive sulphate dilution that a reduction of extracellular fluid persists after full restoration of blood volume in hemorrhagic shock. To establish by direct measurements on sequential biopsies taken from *in vivo* experiments that an intracellular fluid increase has occurred, one encounters certain technical obstacles. The greatest problem is that volume changes of the tissue cannot be measured but only compositional determinations made. If one measures TTW in a control biopsy sample, imposes an experimental condition such as hypothermia, and takes a second biopsy, the percentage of

TABLE 3. *Effect on Total Tissue Water of LMWD Addition with Final Medium Adjustment to 300 Millios./Kg. Medium*

TTW*				TTW		
37° C. Control	LMWD Added	P** Value	Conc. LMWD	7° C. Control	LMWD Added	P Value
78.3 ± 0.3 (5)	76.5 ± 0.7 (8)	<0.025	5%	81.9 ± 0.6 (7)	79.7 ± 0.3 (7)	<0.010
77.9 ± 0.4 (6)	75.2 ± 0.5 (13)	<0.001	10%	81.8 ± 0.7 (10)	78.2 ± 0.5 (13)	<0.001
78.2 ± 1.1 (3)	73.8 ± 0.6 (8)	<0.005	15%	81.9 ± 0.7 (6)	76.4 ± 1.0 (8)	<0.005

* Mean value in % ± standard deviation.
** Significance of difference between control and experimental TTW values.
Number of observations in parentheses.

water *per gram of tissue* may show no discernible change even though a considerable shift of fluid to the intracellular space may have occurred. A 2 per cent increase in total tissue water, for example, although undetectable by the *in vivo* biopsy method, represents a loss of over a liter of extracellular fluid in a 70-Kg. man. This approaches the volume of "lost fluid" observed by indirect measurements of the extracellular fluid in human shock.[11] In contrast, the *in vitro* isolated tissue preparations such as used in this study permits precise and direct detection of considerably smaller volume shifts because the identical tissue fragment is measured before and after the experimental condition is imposed. In addition, the method permits compositional measurements of the same tissue.

Most experimental observations of *in vitro* cell swelling have been made by investigators interested in determining the osmolality of intracellular fluid with respect to the extracellular milieu. Assuming that the weight gain represented the ingress of water into the metabolically depressed cell, cellular swelling in an isotonic medium was taken as evidence for the relative hyperosmolality of intracellular fluid.[9] Although J. P. Peters [8] reasoned that intracellular fluid must have the same tonicity as extracellular fluid, his evidence for this was admittedly inconclusive. When the argument for hyperosmotic intracellular fluid was presented by Robinson, Leaf [4] demonstrated that swollen cells gain not water *per se* but gain essentially isosmotic fluid. Moreover intracellular osmolality measurement has confirmed the isotonicity of intracellular fluids, provided the tissue is either heated [1] or frozen [7] to arrest autolysis before the freezing point or melting point is measured.

It is not the purpose of the foregoing experiments to equate the colloidal osmotic properties of LMWD with the established effects on the capillary circulation. Under the *in vitro* conditions of these experiments, however, it is of interest that the osmotic properties appear of such significance in reversing cellular swelling. Direct cellular contact with LMWD is, of course, grossly "unphysiologic." Subsequent data [2] indicate cellular respiration may be inhibited by LMWD contact and a more appropriately "physiologic" alteration of the cell environment would be a comparable increase in concentration of the nutrient medium.

Summary

Cellular volume is maintained by an active energy-requiring process of the cell. Hypothermia, hypoxia or other metabolic deprivations produce an increase in cellular volume that is readily observed in surviving

tissue slices *in vitro*. Reduction of oxygen concentration to 5 per cent was required to produce significant cell swelling at 7° C. At 20 per cent or less oxygen concentration, there was no significant hypothermic swelling effect. LMWD exerts a marked colloidal osmotic effect on *in vitro* tissues with reversal of hypothermic cellular swelling.

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