Cellular Swelling II: Effects of Hypotonicity, Low Molecular Weight Dextran Addition and pH Changes on Oxygen Consumption of Isolated Tissues

DANIEL M. ENERSON, M.D., HOWARD M. BERMAN, B.S.

From the Department of Surgery, University of Pittsburgh and the Veterans Administration Hospital, Pittsburgh, Pennsylvania

EXPANSION of intracellular fluid volume is observable in a variety of clinical and experimental conditions. As a generalized phenomenon, it occurs in water intoxication,[5] salt depletion or adrenal insufficiency.[1] Darrow and Yannet[3] produced acute generalized cellular swelling in a dog in a classical experiment by intraperitoneal injection of glucose thus reducing extracellular electrolytes without comparable water loss.

Localized cellular swelling may be produced experimentally by application of a tourniquet to an extremity. On release of the constriction, water, sodium and chloride shifts into the cellular space and potassium is released by the cells.[10, 11] These shifts of fluids and electrolytes typify nonspecific response to a variety of cellular injuries due to metabolic deprivation of cells as occurs in hypoxia. Essentially identical changes are observable in tissue fragments surviving in a nutrient medium and which are subjected to hypoxia or other metabolic depressions.[15, 16] From such *in vitro* studies, it is clear that the cause of these fluid and electrolyte shifts is depression by hypoxia of those active metabolic processes of the cell that maintain electrolyte gradients across the plasma membrane of the cell and also maintain cell volume.

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Shock produces tissue hypoxia by underperfusion, but it is much more difficult to determine if similar fluid shifts also occur in shock. Although Fischer[9] long ago inquired "Where then is the blood hiding?" in clinical shock, nine decades of subsequent clinical observation, laboratory investigation, and the recent application of tracer dilution technics for measuring plasma and extracellular fluid spaces have not yet satisfactorily answered Fischer's question. Using S^{35} tagged sulfate, Shires[22] reported that extracellular fluid was reduced 18 per cent in splenectomized dogs following hemorrhagic shock and reinjection of the shed blood. In 18 patients following hemorrhage, Shires also observed extracellular fluid loss of 4,414 ml. more than could be attributed directly to blood loss. The fate of this "lost" extracellular fluid is at present not known. If some or all of this "lost" fluid enters the cellular compartment, it would produce only a small percentage change of tissue hydration from the normal because of the large mass of cellular fluid, and it may, therefore, be difficult to detect directly by tissue analysis.

Regardless of the cause, it is important to know if swelling *per se* is injurious to the cell. If swelling affects the metabolism of the cell, this could represent a self-potentiating pathophysiologic mechanism in which this metabolic impairment may in turn produce additional swelling. Robinson[21] dem-

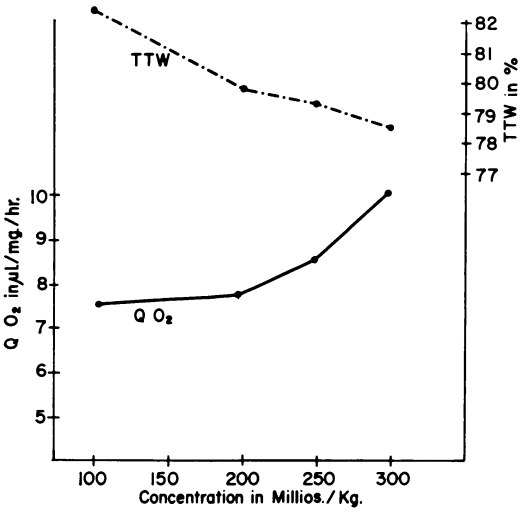

FIG. 1. Hypotonic medium produces moderate increase in total tissue water (TTW) and distinct interference with oxygen consumption (Q O_2).

onstrated that respiration of surviving renal cortex slices was depressed significantly when the osmolarity of the nutrient medium was reduced to the unphysiologic level of 60 millios./L. Metabolic impairment from hypotonic swelling of more moderate range has not been either documented or quantitated.

Low molecular weight dextran (LMWD) is of established benefit in circulatory disorders that produce tissue hypoxia. This effect is attributed to the ability of LMWD to reduce blood "sludging" and thus improve flow in the capillary circulation. The degree to which osmotic properties of LMWD contribute to its beneficial effects in hypoxic conditions remains unknown. It is interesting that hypoxia is characterized by cellular swelling and that LMWD injections *in vivo* exert a pronounced osmotic effect with expansion of plasma volume.[12, 13] Fahraeus[8] suggested that "sludging" of blood in the capillary circulation may be a secondary phenomenon of physiologic benefit rather than of pathologic importance. To separate osmotic effect from circulatory effect of LMWD, it is necessary to observe

TABLE 1. *Effects of Decreased Osmolality of Medium on Q O_2 and TTW of Isolated Rat Diaphragm*

Osmolality	Q O_2			TTW		
	Mean*	S.D.	No.**	Mean*	S.D.	No.**
100	7.59	0.50	43	82.4	0.7	44
200	7.61	0.68	20	79.8	0.4	20
250	8.52	0.97	23	79.1	0.4	24
300	9.99	0.94	81	78.5	0.6	85

* All values differ significantly from 300 millios./Kg. control values ($p < 0.001$).
** Number of observations.

changes in tissue metabolism and hydration in an *in vitro* preparation.

Recently, metabolic acidosis as a sequela of hypoxia, shock or related conditions has been considered.[17] Although acidosis is known to alter cardiac output, it is also of interest to know the direct effect of acidosis on tissue metabolism more generally. DeRoetth[4] found that pH alterations from 5.7 to 7.5 of nutrient medium did not change the oxygen consumption of isolated *in vitro* cornea, but the present authors are unaware of other quantitative information of direct effects of pH on oxygen consumption of isolated tissues.

The following experiments were devised to observe effects of cellular swelling and acidosis on oxygen consumption of isolated tissues. Conditions of the experiments were selected prinicipally because of their relationship to hypoxic states. In the first experimental series, cellular swelling was produced by dilution of nutrient medium; in the second series, 10 per cent LMWD was added in an attempt to counteract the effect of dilution; and in the third series, pH of the medium was varied but osmolality of the medium was maintained. In each series, oxygen consumption and total tissue water were measured.

Methods

The hemidiaphragms of 60–100 Gm. Charles River C.D. Strain female rats were

TABLE 2. *Effects of LMWD on Q O_2 and TTW of Isolated Rat Diaphragm Swollen by Hypotonic Medium*

	Q O_2			TTW			
Osmolality	Mean	S.D.	No.**	LMWD	Mean	S.D.	No.**
100	7.42	0.59	36	0	82.2	1.1	39
100	6.44*	0.69	38	10%	79.7*	1.3	39
300	9.05	1.05	15	0	77.8	0.7	16
300	6.26*	0.88	15	10%	73.0*	0.6	15

* All values differ significantly from corresponding control value without LMWD addition ($p < 0.001$).
** Number of observations.

used for the surviving tissue in these experiments. Following rat sacrifice by cervical cord dislocation, the diaphragm was removed as quickly and atraumatically as possible, divided, and blotted on #541 Whatman filter paper to remove blood and fluids. The tissue was immediately placed into a tared weighing bottle, weighed on a Mettler H-16 semimicrobalance and inserted into a Warburg respirometer flask within 5 minutes after sacrifice. All time relationships, particularly in blotting and weighing, were held as constant as possible to achieve a consistent wet weight. A circular Aminco Warburg respirometer was used at a water bath temperature of 37° C. The respirometer flask was of the center well type, containing 0.2 ml. of 10 per cent potassium hydroxide with a small fan of filter paper in the center well, and 2.0 ml. of modified Krebs Ringer phosphate glucose solution with the hemidiaphragm in the outer chamber. Flasks were agitated at 112 strokes per minute. Manometer readings were taken at 5-minute intervals for 30 minutes and Q O_2 was expressed as oxygen consumption in microliters per milligram of dry weight of tissue per hour.[25] Hemidiaphragms were then removed from the Warburg flask, weighed and desiccated overnight at 105° C. in a vacuum drying oven. After cooling in a vacuum desiccator to a constant weight, the tissues were reweighed and the weight loss of desiccation expressed as percentage total tissue water (TTW) of final tissue wet weight.

In the first series of experiments, the concentration of the Krebs Ringer phosphate glucose medium was lowered by dilution from 300 millios./Kg. to 250, 200, and 100 millios./Kg. as determined by freezing point measurements using the Fiske Mark III osmometer. In the second series, Rheomacrodex,* LMWD as a dry powder, was added to the hypotonic and isotonic media. In both series, pH was maintained at 7.4.

For the third series of experiments, pH of the medium was lowered from 7.4 to 7.0, 6.6, and 6.2 by addition of hydrochloric acid. The pH was raised to 8.2 by addition of sodium hydroxide. Base or acid additions were made dropwise using a Radiometer Model pHM-4 pH meter. Concentration of the medium in this series was maintained at 300 millios./Kg.

All oxygen consumptions and TTW's were determined in duplicate diaphragm samples for each experimental condition. In all, 167 hemidiaphragms were used in Series I, 104 in Series II, and 122 in Series III. Standard deviations were determined for individual observations in each series and the *t*-test was applied to experimental differences.

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

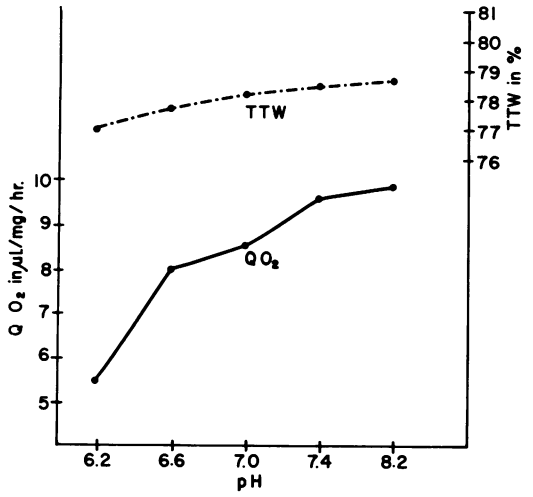

FIG. 2. Acid medium of pH 6.2 markedly impairs oxygen consumption and produces a significant decrease of total tissue water.

Results

In Series I, when surviving hemidiaphragms were placed in a *hypotonic medium* of 100 millios./Kg., the oxygen consumption was significantly reduced from the control level of 9.99 to 7.59 µl./mg./hr. At 200 and 250 millios./Kg. concentration, the Q O_2 was 7.61 and 8.52, respectively, each statistically significantly lower than the 300 millios./Kg. control values (Fig. 1, Table 1). The amount of tissue swelling produced by even the most severe hypotonic medium changes was similar to that produced *in vitro* by hypoxia or hypothermia.[7] A hypotonicity of 100 millios./Kg. increased the TTW from a control value of 78.5 per cent to 82.4 per cent of final tissue wet weight and intermediate dilutions of the medium produced tissue swelling approximately proportional to the degree of dilution.

In Series II, 10 per cent LMWD was added to both *hypotonic and isotonic media* to determine the oncotic osmotic effects of the colloid (Table 2). The LMWD addition further depressed the Q O_2 in the 100 millios./Kg. hypotonic medium from 7.42 to 6.44 µl./mg./hr. The LMWD reduced the TTW from 82.2 per cent in the hypotonic swollen tissues to 79.7 per cent and similarly decreased the TTW of the tissues in the isotonic medium from 77.8 per cent to 73.0 per cent. The LMWD addition thus failed to reverse the impaired respiration of the hypotonically swollen tissues although cellular swelling *per se* was reversed. Of interest is the reduction by LMWD addition of Q O_2 of tissues in an isotonic medium from 9.05 control value to 6.26 µl./mg./hr. This effect on tissues in an isotonic medium may explain to some degree the failure of LMWD to reverse the depressed Q O_2 of the hypotonically swollen tissues.

In Series III, *acidification* of the Krebs Ringer phosphate glucose medium to a pH of 6.2 reduced the oxygen consumption of the surviving rat diaphragms from a control Q O_2 of 9.62 to 5.47 µl./mg./hr. (Fig. 2, Table 3). Depression of the pH to 6.6 and 7.0 effected lesser but statistically significant Q O_2 reductions to 8.08 and 8.61, respectively. When pH was lowered to 6.6 or 6.2, there was a significant reduction of TTW from 78.5 per cent control level to 77.9 per cent and 77.1 per cent, respectively, although osmolality of the medium was strictly maintained at 300 millios./Kg. under all pH conditions. Raising pH of the medium to 8.2 by addition of sodium hydroxide produced no significant change in either oxygen consumption or water content.

Discussion

Self-potentiation is a distinguishing feature of hypoxic circulatory states. In clinical or experimental shock, this presumably contributes to the established tendency toward irreversibility of shock regardless of cause. Moore[18] enumerated ten such identifiable cyclical events in clinical shock representing the principal self-aggravating effects of low blood flow on the heart, kidneys, liver, blood vessels and peripheral

TABLE 3. *Effects of pH Changes on Q O_2 and TTW of Isolated Rat Diaphragm*

pH	Q O_2				TTW			
	Mean	S.D.	No.*	*p* Value**	Mean	S.D.	No.*	*p* Value**
6.2	5.47	0.69	20	<0.001	77.1	0.4	20	<0.001
6.6	8.08	1.07	12	<0.001	77.9	0.6	12	<0.005
7.0	8.61	0.77	11	<0.005	78.3	0.4	11	N.S.
7.4	9.62	1.06	60	—	78.5	0.6	62	—
8.2	9.73	0.93	19	N.S.	78.6	0.5	20	N.S.

* Number of observations.
** Significance of difference from pH 7.4 control value.

tissues. A vascular circulation was deliberately omitted in the design of these experiments in the hope of elucidating some self-aggravating mechanisms at the cellular or tissue level that may play a sustaining role in the events of hypoxia due to circulatory disorders.

While in the past much attention was focused on the homeostatic features of circulatory disturbances, it is now clear that there is inherent in many normal physiologic mechanisms a basic instability that may, given the proper set of circumstances, provide a *cycle of self-aggravating events* to the total detriment or even destruction of the organism.[14] In the language of cybernetics, homeostatic physiologic mechanisms may be regarded as examples of negative *feedback systems,* while virtually all pathologic processes are positive feedback systems. In both normal physiologic and in pathophysiologic states, these opposing systems coexist in various balances.

Results of Series I and Series III in these studies indicate tissue metabolism is significantly impaired *in vitro* by hypotonic cellular swelling or acidification of the medium. These findings suggest two positive feedback mechanisms at the tissue level that may contribute to the events of progressive deterioration that follow in circulatory hypoxic states. If hypoxia produces cellular swelling, and tissues swollen to the same degree have decreased oxygen consumption, the cycle may be described as follows:

Hypoxia → Decreased oxygen consumption
↓ ↑
Cellular swelling

Despite the large number of "vicious cycles" attempting to explain the phenomena of shock, cellular swelling has not previously been described in a self-potentiating role in hypoxic states to the best of the present authors' knowledge. One may further conjecture that at the tissue or organ level, cellular swelling may restrict capillary flow and provide another level of self-potentiating pathophysiologic changes:

Tissue hypoxia ⟶ Cellular swelling
↑ ↓
Decreased capillary flow ← Increased capillary resistance

No evidence is, of course, obtainable directly from these experiments that the second relationship exists, but circulatory impairment secondary to tissue swelling occurs in such clinical conditions as the anterior tibial compartment syndrome and the above is thus a concept familiar to surgeons. Circulatory compromise secondary to increased pressure within the fascial compartment and improvement of circulation with surgical relief of the confining pressure by fasciotomy may be observable clinical correlaries of the above feedback relationships.

Depression of cell respiration by decreasing the pH of the milieu may constitute still another positive feedback system contribut-

ing to the deterioration in hypoxia that can be described:

Hypoxia → Decreased oxygen consumption
↓ ↑
Anaerobic metabolism ⟶ Metabolic acidosis

Progressive metabolic acidosis characterizes acute hypoxic or shock states, presumably because of excessive lactic acid production during anaerobic carbohydrate metabolism.[17] Because of a parallel reduction of cardiac output, peripheral tissues become hypoxic and produce increased amounts of lactic acid, thus potentiating metabolic acidosis. Although decreased oxygen consumption accompanies shock,[2, 14] this is probably a manifestation of oxygen debt and from this one cannot judge the direct effect of shock or acidosis on tissue oxygen consumption.

In addition to myocardial depression, it is of interest to quantitate the deleterious effects of acidosis acting directly on other organs and tissues in hypoxic states. The present studies indicate that *lowering the pH* of the nutrient medium sharply reduces oxygen consumption of an isolated tissue preparation. The effect seems to be independent of depression of tissue metabolism secondary to cellular swelling because TTW was slightly reduced rather than increased by the acid medium. Observed changes in TTW with pH shifts agree with previous observations both *in vivo* and *in vitro* that alkalosis increases and acidosis decreases water content of tissues.[6]

Objections may be raised to applying quantitatively data obtained from isolated *in vitro* tissues to an *in vivo* environment. Yet biologic mechanisms are often so complex as to defy directional as well as quantitative analysis even when reduced to the simplest *in vivo* experimental model. The fact that shock and hypoxia in intact animals or patients tend to be self-potentiating and irreversible adds interest if not credence to the cellular feedback systems described. The extreme complexity of the "standard hemorrhagic shock" laboratory preparation cannot be overstated. It seems worthwhile to elucidate some of the pathophysiologic phenomena of tissue hypoxia in the simplest model possible.

According to Wynn[23, 24] the body as a whole functions as a predictable, if somewhat imperfect, osmometer in a manner that may be compared quantitatively to the behavior of erythrocytes *in vitro*[20] or isolated tissue fragments *in vitro*.[16] In quantitatively applying data from these experiments to *in vivo* conditions, it must be appreciated that TTW values in this study were expressed as a percentage of the *final wet weight of tissue*. These values may be expressed as percentage of "original" tissue wet weight and in this form are more useful in comparison to *in vivo* situations—particularly in a quantitative comparison of the effects of LMWD addition in these *in vitro* experiments and in the intact animal or patient. A 10 per cent *addition of LMWD*, for example, reduces TTW of tissues in an isotonic medium by 4.8 per cent from 77.8 per cent control value to 73 per cent of final wet weight of tissue. These same values, converted to percentages of "original" or control wet weight, are 77.8 per cent and 60.0 per cent, respectively, a decrease of 17.8 per cent of original wet weight.

For experimental clarity, the LMWD in these experiments produced a 10 per cent concentration, an eight-fold exaggeration of the 1.25 per cent concentration obtained by Gelin[12] in the plasma of adult human subjects following the administration of 500 ml. of 15 per cent LMWD. Because osmotic effect is proportional to concentration of the colloid, the *in vitro* and *in vivo* osmotic effects of LMWD can be compared. On the basis of the observed 17.8 per cent decrease of original wet weight by 10 per cent LMWD in the *in vitro* experiments, one may estimate the *in vivo* tissue weight

change produced by 1.25 per cent concentration of LMWD in the plasma should be:

$$17.8\% \times \frac{1.25\%}{10\%} = 2.25\%.$$

The *in vivo* "tissue mass" from which LMWD extracts water presumably consists of the body cell mass (40% × body weight) and the extracellular fluid (23% × body weight). The change in "tissue mass" for a 70-Kg. human can thus be estimated: [19]

$$\begin{aligned}\Delta \text{ tissue mass} &= ((40\% + 23\%) \times 70 \text{ Kg.}) \times 2.25\% \\ &= 44.1 \text{ Kg.} \times 2.25\% \\ &= 0.992 \text{ Kg.}\end{aligned}$$

From these *in vitro* data, one would predict that the osmotic effect of 500 ml. of 15 per cent LMWD in a 70-Kg. human would be the shift of 0.992 Kg. or 992 ml. of fluid into the vascular compartment. The actual plasma expansion measured by Gelin in humans given 500 ml. of 15 per cent LMWD was 900 ml. Although Gelin administered the colloid in 500 ml. volume, control saline injections produced almost no detectable plasma expansion. Thus the diluent volume probably is not a significant variable in the comparison.

The extent of *plasma expansion* produced by LMWD *in vivo* thus appears to parallel the corresponding decrease in *tissue hydration in vitro*. Recently, much attention has been given to those properties of LMWD that reverse blood "sludging" and thus appear to some observers to improve capillary blood flow. If LMWD does improve blood flow and, hence, the oxygen tension of hypoxic tissue, it should effect a shift of fluid from the intracellular space by reversing hypoxic cellular swelling in addition to its colloidal osmotic effect. Conversely, one may speculate from the present *in vitro* experiments that LMWD *in vivo* may improve the metabolic status of hypoxic tissues strictly because of osmotic ability to reverse cellular swelling that is *per se* metabolically deleterious to the cell. In these *in vitro* experiments, *LMWD* provided such protection from cellular swelling produced by hypotonic media, but metabolic benefit to the isolated tissues was not observed. Presumably, in the absence of a vascular compartment, the LMWD was free to contact the tissue fragment and impair cellular respiration. Increasing osmolality of the medium by some other means may thus be a more suitable *in vitro* counterpart of the *in vivo* administration of intravenous LMWD in assessing metabolic effects.

Summary

Lowering the concentration of the medium containing isolated rat diaphragms from 300 to 250 millios./Kg. reduced oxygen consumption from 9.99 to 8.52 μl./mg./hr. although tissue water was only slightly increased from 78.5 per cent to 79.1 per cent. Further lowering of concentration produced even more marked changes.

Addition of LMWD reversed the cellular swelling caused by hypotonic medium but further lowered rather than reversed depressed oxygen consumption of the tissues.

Acidification of the medium reduced both tissue oxygen consumption and tissue water.

The possible significance of the findings in clinical or laboratory *in vivo* conditions is discussed.

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