Fluid Therapy in Experimental Hemorrhagic Shock:
Ultrastructural Effects in Liver and Muscle

RALPH G. DePALMA, M.D., WILLIAM D. HOLDEN, M.D.,
ANN V. ROBINSON

From the Department of Surgery, Case Western Reserve University of Cleveland, Ohio

IN THE PAST, the efficacy of intravenous fluid therapy for hemorrhagic shock was evaluated largely on an empiric basis. It was accepted that therapy was most effective when blood loss was replaced by an equal volume of blood. Within the past 3 decades it has been shown that there are alternative choices of solutions for the therapy of hemorrhage. Optimal combinations of crystalloid solutions or blood may enhance survival.[11, 12, 14, 28, 30, 32] Although the use of balanced salt solutions has improved clinical results,[27, 33, 39] a precise rationale for employing crystalloid solutions is not yet delineated. The overenthusiastic or inappropriate use of crystalloid solutions may increase morbidity and mortality in some instances.[22]

Impetus for the use of balanced salt solutions, generally Ringer's solution was based on experimental findings of Shires and associates [31] published in 1960. Using radioactive tracers, they demonstrated loss of extracellular fluid exceeding the volume of blood lost. Moyer and associates,[11] in extensive bioassay studies, demonstrated the value of additional crystalloids in hemorrhage; they described possible sequestration of fluid in altered interstitial matrix.[3]

Application of postulates derived from these experiments to the treatment of hemorrhagic shock appeared valid since enhanced survival was clearly demonstrated. However, limitations inherent in the use of radioactive tracers and their distribution in the fluid compartments of the body made interpretation of experimental findings difficult. There is still disagreement as to whether or not there is an actual deficit in the extracellular fluid in excess of the volume of shed blood.[26, 29] If there is, where does this excess fluid loss occur?

The present study was done to demonstrate the alterations in cellular structure resulting from controlled hemorrhage with various types of fluid preparations used for replacement. It was possible to correlate ultrastructural changes in hepatocytes and myocytes with survival using a standard murine shock model. Reproducible alterations of cellular ultrastructure occurred with shock and with fluid therapy of various types and amounts. Reversal of the ultrastructural changes appeared to be important correlates of survival.

Materials and Methods

Young adult male Sprague-Dawley rats weighing between 250–350 Gm. were subjected to hemorrhagic shock for 120 minutes; 60 minutes at 50 mm. Hg blood pressure and 60 minutes at 30 mm. Hg blood pressure. The rats were anesthetized with intraperitoneal pentobarbital 4 mg/100 Gm. body weight. The iliac arteries were cannulated and 500 μ of heparin were given via the cannula. The details of this reservoir model for the production of hemorrhagic shock have been described previously.[13, 17]

TABLE 1. *Hemorrhagic Shock: Effects of Various Infusions on Survival*

	# Rats	% Survival	Pvs no Rx	Pvs Blood
Untreated hemorrhage blood loss of 47% ± 14% volume at 120 minutes	30	36%	—	< 0.05
Reinfusion of shed blood	30	90%	< 0.05	—
Normal saline: volume equal to shed blood	30	63%	NS	< 0.05
3× volume of shed blood	30	83%	< 0.05	NS
L. Ringer's: volume equal to shed blood	30	70%	< 0.05	NS
3× volume of shed blood	30	80%	< 0.05	NS
5% dextrose: Volume equal to shed blood	30	47%	NS	< 0.05
3× volume of shed blood	30	60%	NS	< 0.05
5% D. L. Ringer's 3× volume of shed blood	30	60%	NS	< 0.05

In each experiment four animals were used. One anesthetized and heparinized control animal was cannulated but not bled; three animals were bled to produce hemorrhagic hypovolemia for 120 minutes, two of these were infused with whole blood or various crystalloid solutions over a 20-minute period, and one was not infused with any fluid. The relative effect upon survival of whole blood, normal saline, Ringer's solution, 5% dextrose in Ringer's solution, and 5% dextrose in water was assessed in 240 animals. The efficacy of these treatments has been reported.[7] The therapeutic infusions are reviewed here in relation to cellular changes observed in a second group of 32 animals. For this study, the animals were sacrificed immediately after reinfusion of blood or the infusion of various crystalloid solutions. Simultaneous laparotomies were performed through midline incisions in the control, the bled, and the two treated

rats. A 1 × 2 mm. portion of the right hepatic lobe was removed, minced, and fixed for electron microscopy. Then the entire right hepatic lobe was removed rapidly; a section 1–2 mm. thick was quickly frozen in liquid nitrogen cooled isopentane for histochemical studies. A 1 × 2 mm. portion of the right diaphragm was then obtained for electron microscopy. All tissues were processed identically and simultaneously.

Tissues for electron microscopic examination were placed immediately in ice cold veronal buffered 2% osmium tetroxide for one hour,[24] were progressively dehydrated through graded alcohols, and were embedded in Maraglas.[36] Ultrathin sections were cut, stained with uranyl acetate and citrate, and examined on an RCA EMU 3G electron microscope.

Histochemical determination of adenosine triphosphatase (ATPase) activity and glucose 6 phosphatase (G6Pase) activity was carried out on 8 μ sections of the frozen portion of the right hepatic lobe. ATPase activity and G6Pase activity were assessed as described in a previous publication[6] using the methods described by Ashworth,[1] Chiquoine[4] and Wachstein and Meisel.[40] Sections were viewed by light microscopy and activity estimated by intensity and distribution of reaction products. The slides were viewed as unknowns and graded on a 1–4 basis.

Results

The survival of treated rats is shown in Table 1. Reinfusion of shed blood resulted in the highest survival rate. This, however, was not statistically different from an infusion of a volume of normal saline equal to 3 × the volume of the shed blood or Ringer's solution in either volume. Survival was decreased when 5% dextrose in Ringer's solution, a hyperosmolar solution, was infused rapidly. The infusions of 5% dextrose in water did not enhance survival over the control rate.
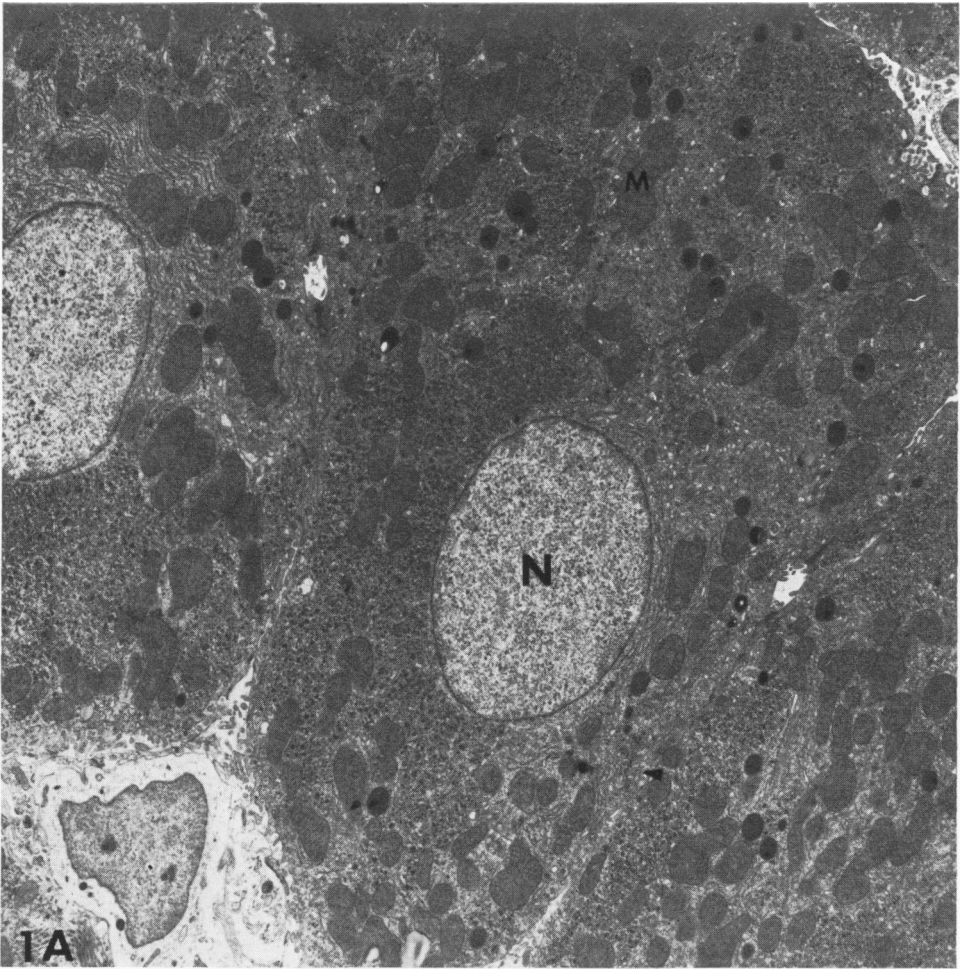
FIG. 1A. Control hepatocyte 4,700 ×. Nucleus, N., Mitochondria, Mt. The nuclear chromatin is fine; mitochondria are filamentous. Note opaque quality of cytoplasm and glycogen granules.

The ultrastructure of the hepatocytes after hemorrhage revealed consistent changes: marked intracellular edema with widening and distortion of the endoplasmic reticulum, swelling of mitochondria with conversion of the normal ellipsoid or filamentous forms to globular configuration, alteration of the rough endoplasmic reticulum with dispersion of ribosomes into the hyaloplasm, and clumping of the nuclear chromatin (Fig. 1).

Approximate quantification of the degree of widening of cytoplasmic areas between parallel lamina of the endoplasmic reticulum was possible. This was done by mea-surement along lines drawn at right angles to parallel reticular arrays in 12 comparable areas of each of three cells selected at random from shock and control groups. This distance was increased by a factor of 1.8 in the shocked rats indicating more fluid within the cytoplasm of the cell (Fig. 2).

After blood transfusion, mitochondria were still predominantly globular. The over-all density of the cell was greater and there appeared to be less intracellular edema than in the cells of untreated animals. The number of lysosomes and microbodies appeared to be increased. Residual separation of the
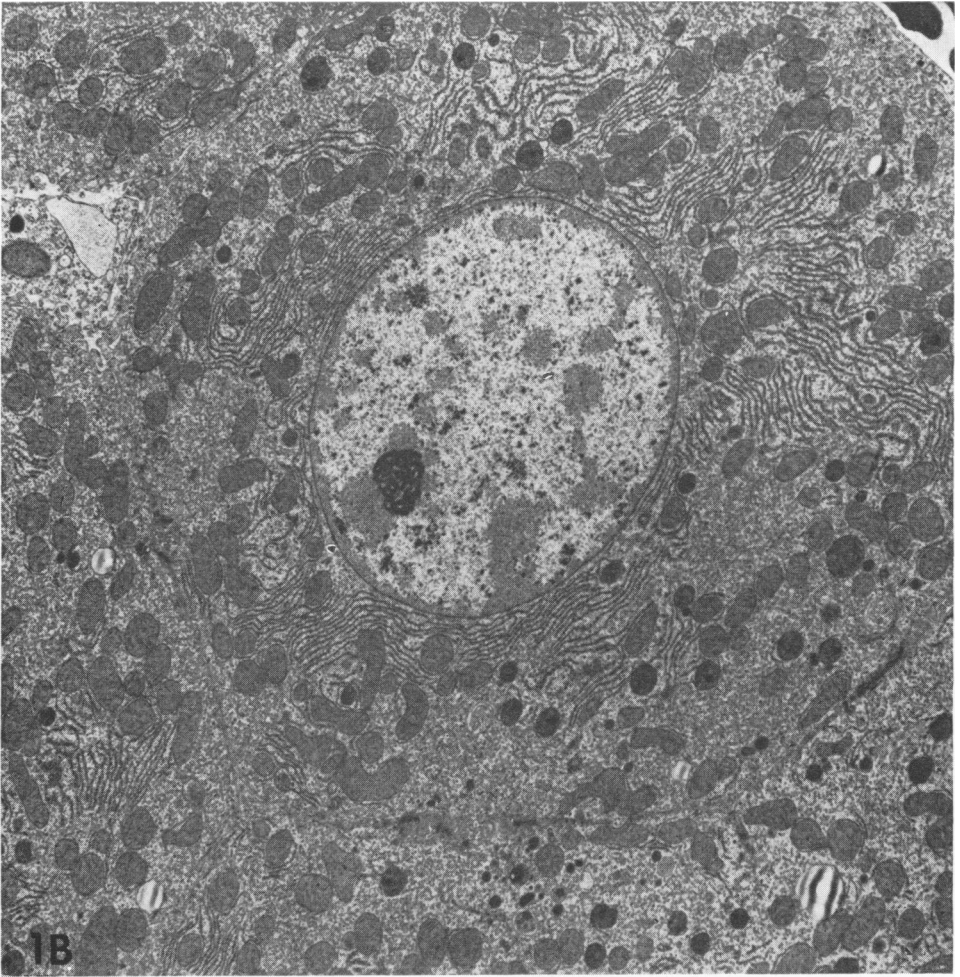
FIG. 1B. Hepatocyte after 120 minutes hypovolemia 4,700 ×. Note globular mitochondria. The lucency of the cytoplasm suggests increased water content of the cell. Glycogen is absent.

endoplasmic reticulum was seen. Free ribosomes were present in the hyaloplasm. Clumping of nuclear chromatin was not seen. The appearance of hepatocytes after reinfusion with Ringer's solution or normal saline equal to 3 × volume of the shed blood was similar to control hepatocytes. In contrast to animals treated with transfusion, increased numbers of microbodies and lysosomes were not observed. Rare cytoplasmic vacuoles were noted in the hepatocytes after reinfusion of blood, Ringer's solution or saline (Fig. 3).

Treatment with 5% dextrose in Ringer's solution failed to provide evidence of ultrastructural repair, but did lead to the development of large cytoplasmic vacuoles. There was marked distortion of intracellular structures. Clumping of nuclear chromatin was also prominent after the infusions (Fig. 4). To a lesser degree similar findings were observed after the infusion of 5% dexterose and water.

Ultrastructural changes in skeletal muscle as a result of hemorrhage appeared to be intracellular edema with spreading of
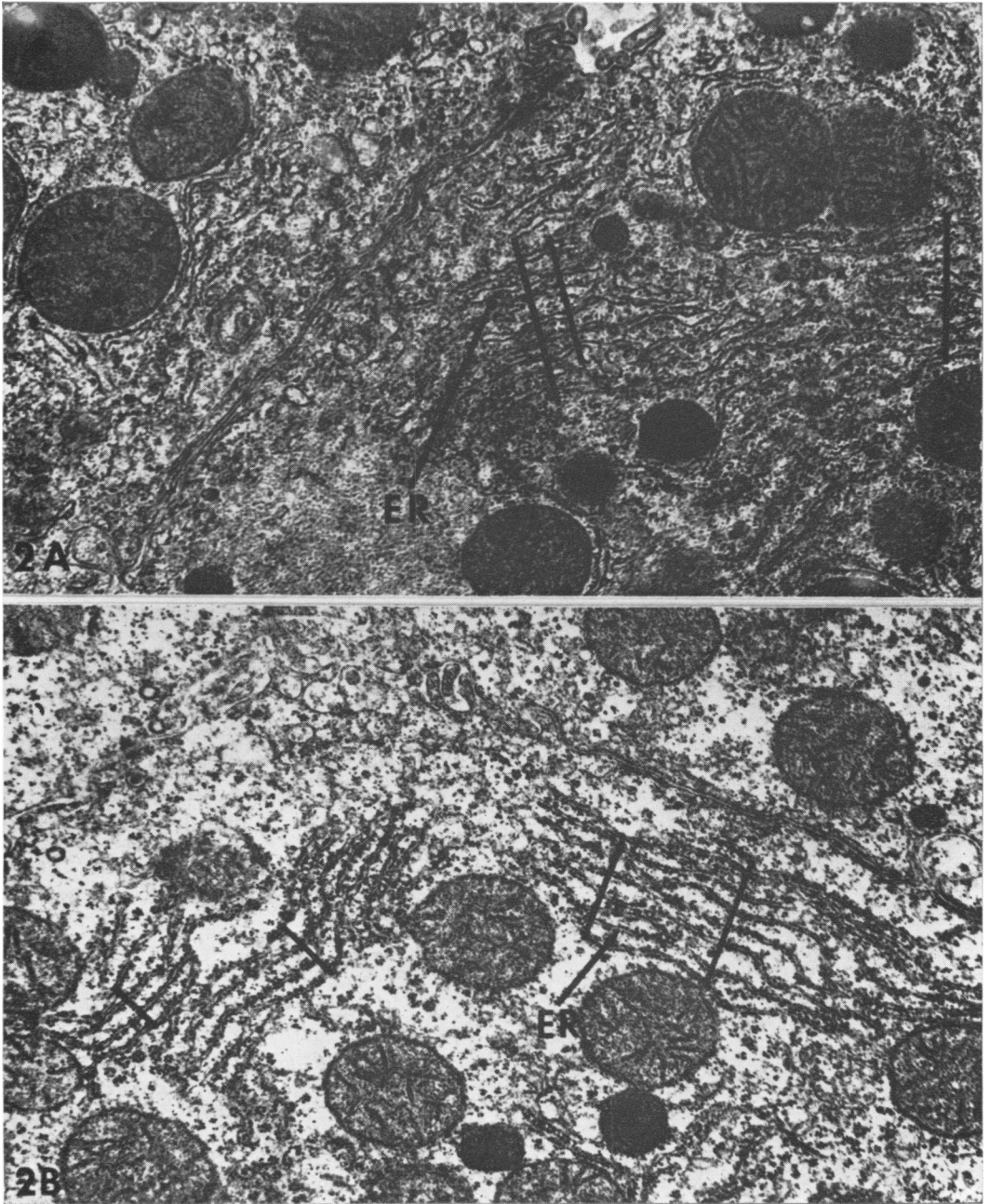
FIG. 2A. Portion of control hepatocyte 19,900 ×. Detail of rough endoplasmic reticulum, E. R. B. Comparable area in hepatocyte after 120 minutes hypovolemia 19,900 ×. Note widening of spaces between lamellae of E. R. and lucency of cytoplasm.

myofibrillae and distortion of mitochondria (Fig. 5). After reinfusion of shed blood, or treatment with Ringer's solution or normal saline, the ultrastructural appearance of the myocytes was indistinguishable from control sections. After infusion of 5% dextrose
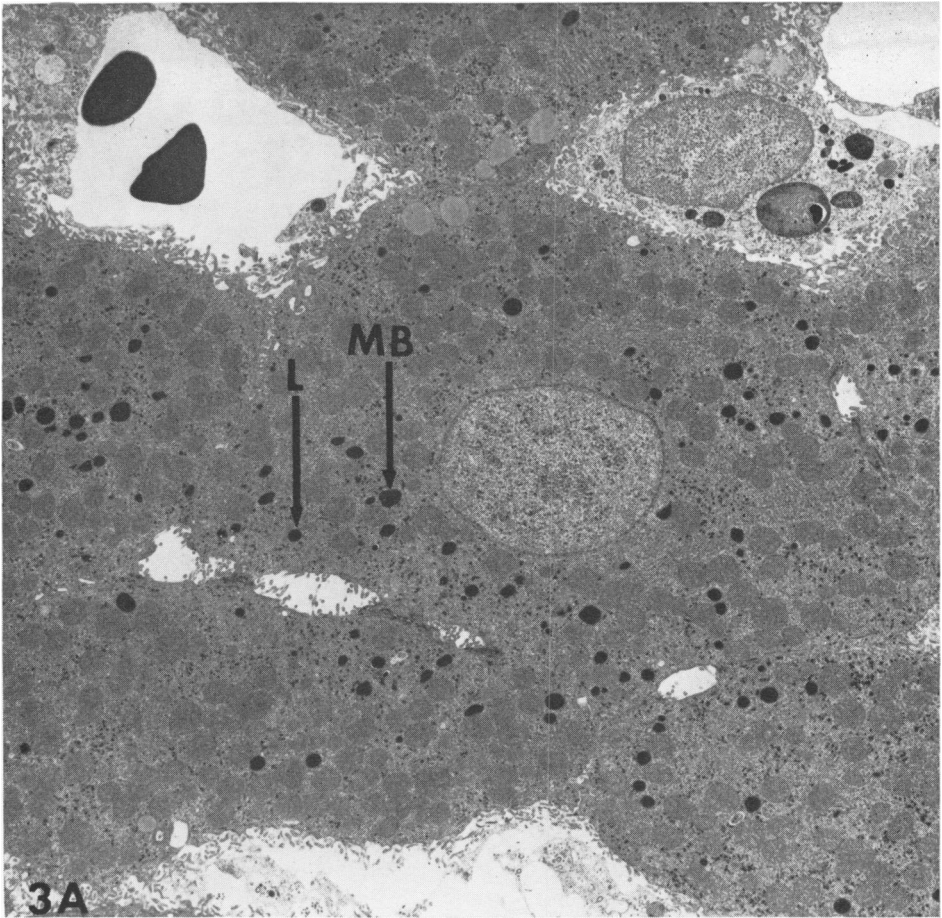
Fig. 3A. Hepatocyte after reinfusion of blood 4,700 ×. Note that mitochondria remain globular. Glycogen is present—note opacity of cytoplasm. Increased numbers of lysosomes (L) and microbodies (Mb) are present.

Ringer's solution vacuoles and intracellular distortion with edema persisted in the myocytes (Fig. 6).

As previously reported, consistent increases in ATPase and G6Pase activities were seen after hemorrhage.[6] The increased reaction products were seen at the time of sacrifice with no detectable difference between treated and untreated animals.

Discussion

Swelling of cells, presumably owing to increased hydration, has been observed in a variety of injurious circumstances. These include: incubation in an hypoxic environment,[17] incubation at low temperature,[25] ex-

perimental carbon monoxide and cyanide poisoning,[16] and ischemia due to vascular occlusion.[19, 38] In previous studies of the effect of hemorrhage on ultrastructure,[8, 9, 18] we reported the appearance of cellular swelling. These studies were done with the same murine shock model[7] employed in this study; 2 hours of hypotension associated with a blood volume loss of $47 \pm 14\%$ consistently produced these changes. The most probable cause of increased cellular hydration is a shift of interstitial fluid into the cell during hypovolemia. The changes were most clearly visible in hepatocytes; a significant increase in distances between the arrays of endoplasmic reticulum was
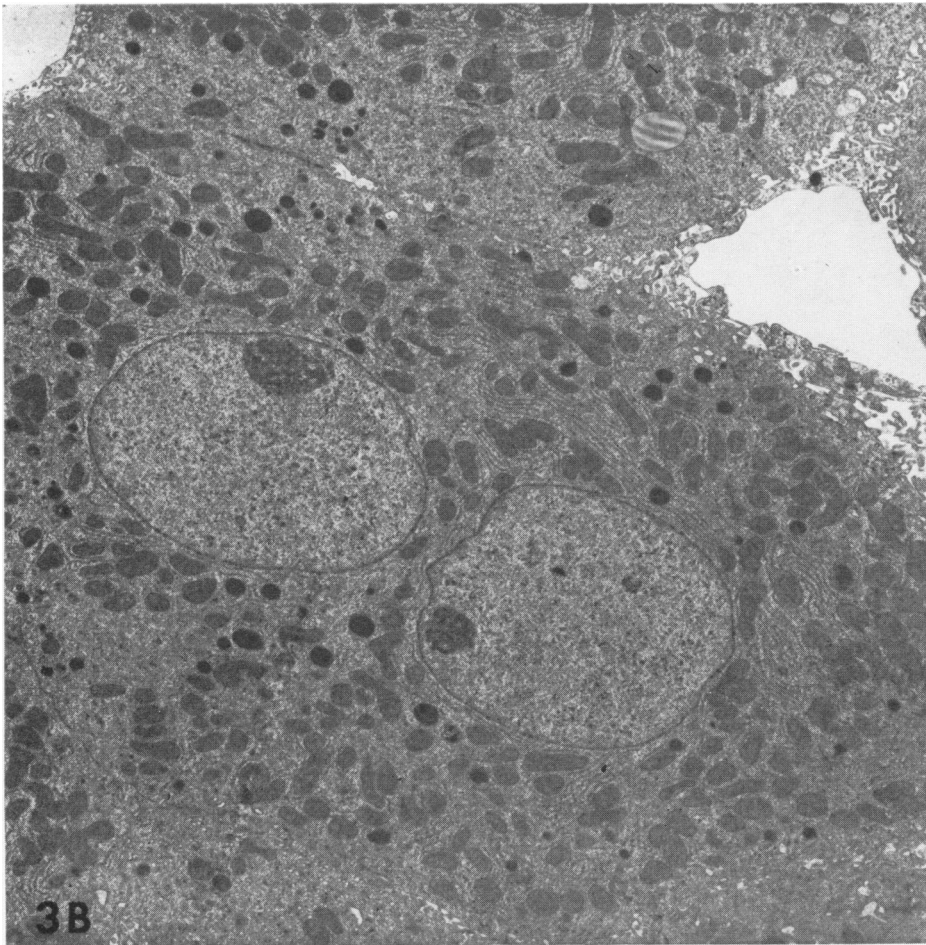
FIG. 3B. Hepatocyte after shock and reinfusion of 3 × volume of shed blood as normal saline 4,700 ×. The ultrastructural appearance is normal.

observed. The visual evidence of swelling in myocytes was also evident, though distances between the myofibrillae were not readily measured owing to disparities in the degree of muscular contraction at the time of sampling.

A shift of interstitial water into cells during shock has been suggested before. The evidence has been indirect and based primarily upon: (1) studies with radioactive isotopes, (2) examination of plasma Na+ content and osmolality, (3) measurement of K+ concentration in cells and interstitial fluid, and (4) measurement of transmembrane potentials of cells. It is useful to review briefly the implications of

these studies. The determination of sulfate [35] space, using 20 minutes as a time for equilibration, demonstrated a discrepant reduction in functional extracellular fluid.[31] It should be emphasized that the application of this concept by Shires [32] generated the current clinical interest in the use of crystalloids. In studies using a longer equilibration time, 12 hours with Br[82], changes in extracellular fluid other than those expected from hemorrhage were not documented.[20] In experiments using Br[82] and osmometry, Batey and associates,[2] and Singh and Flear [34] postulated increased permeability of cell membranes in both hemorrhagic and endotoxic shock, with
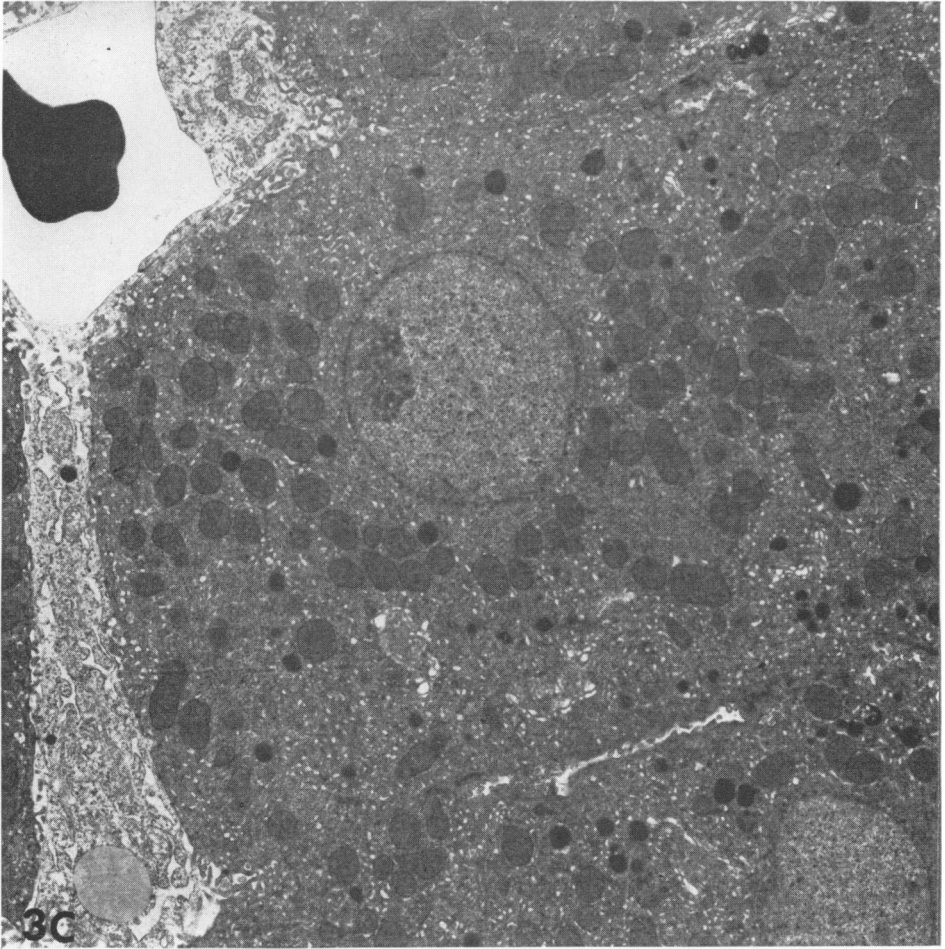
FIG. 3C. Hepatocyte after shock and reinfusion of 3 × volume of shed blood as lactated Ringer's 4,700 ×. Note occasional small vacuoles, V; these may also be seen after transfusion of blood.

shrinkage of the extracellular fluid compartment owing to the entry of water, Na+, and Cl− into the cells. These investigators thought that the Br^{82} technic might not be appropriate for measurement of extracellular fluid because of entry of some Br^{82} into cells. The elegant experiments of Haljamäe,[15] showed K+ loss by cells and a disproportionate increase in interstitial K+, demonstrating transcellular fluid and electrolyte migration during shock. These "hidden cellular electrolyte responses" which cannot be documented by changes in plasma K+ concentration have long escaped clinical notice. Haljamäe emphasized the importance of alterations of hydration of the interstitial ground substance. Cunningham, Shires and Wagner [5] showed decreased transmembrane potential of skeletal muscle in hemorrhagic shock suggesting impairment of cell membrane pump function. In their studies, the leakage of K+ from cells into the interstitial fluid was also observed. These investigators concluded that an intracellular shift of Na+ and Cl− occurred. The ionic changes were interpreted as being consistent with cellular swelling.

The implications of the concept of cellular swelling for the treatment of hemorrhage are important if optimal modes of
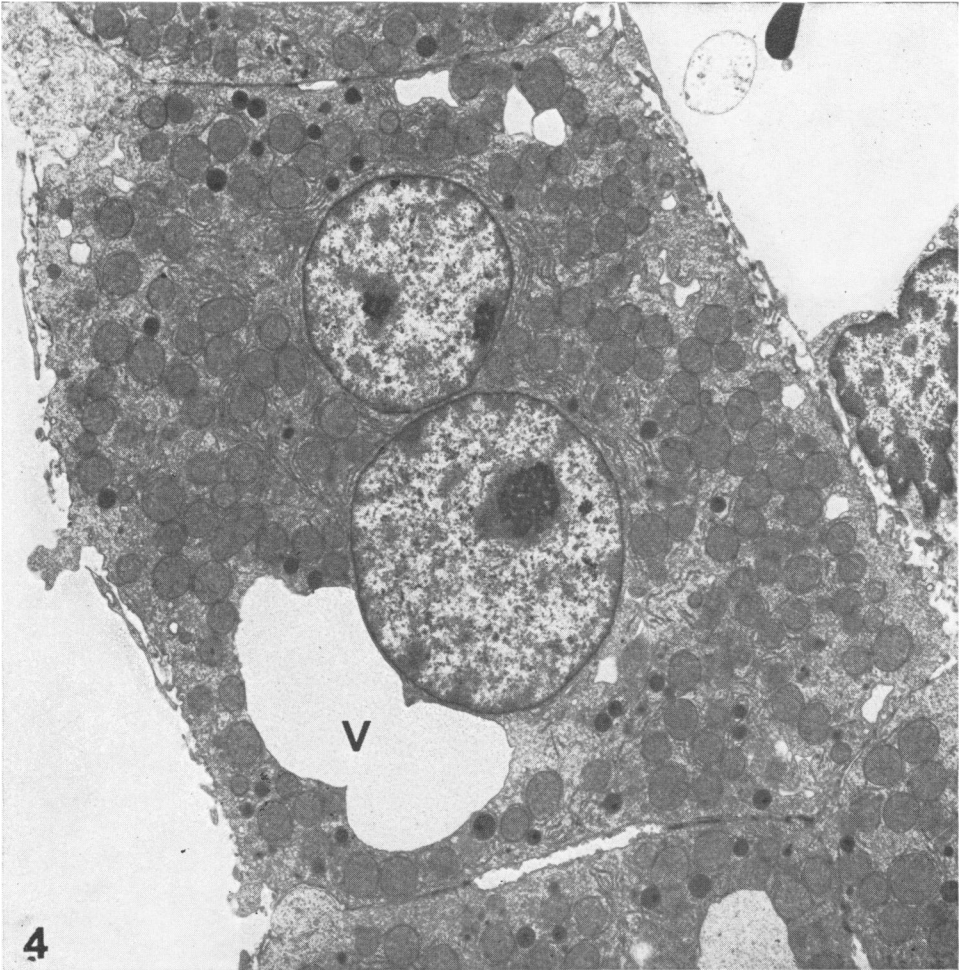
FIG. 4. Hepatocyte after shock and infusion of $3 \times$ volume of shed blood as 5% dextrose lactated Ringer's $4,700 \times$. Note distortion of fine structure by vacuoles. V. These range from 0.3μ to 14μ in size in different liver cells. The nuclear chromatin is quite coarse.

therapy are to be selected. While much has been done to improve the therapy of hypovolemia, it is probable that optimal treatment in varying hypovolemic states has yet to be defined. The understanding of partition of extracellular fluid in shock is still incomplete. Of importance in magnitude and direction is the transcapillary flow of water, salt, and protein into the intravascular compartment which occurs following hemorrhage. This major movement of fluid supports the circulation. The measurement of magnitude and time intervals involved in this shift have been extensively documented.[22, 35, 37, 42] However, the apparently paradoxical shift of interstitial fluid into cells, at a time when plasma volume is reduced, still requires accurate measurement and further characterization. While this shift may be of lesser magnitude, it may alter function of the subcellular organelles. It is here that proper selection of crystalloid solutions may influence success or failure in a marginal situation.

In these studies, Ringer's solution appeared to be slightly superior to normal saline in promoting survival after hemorrhage. Of interest was the reversal of cel-
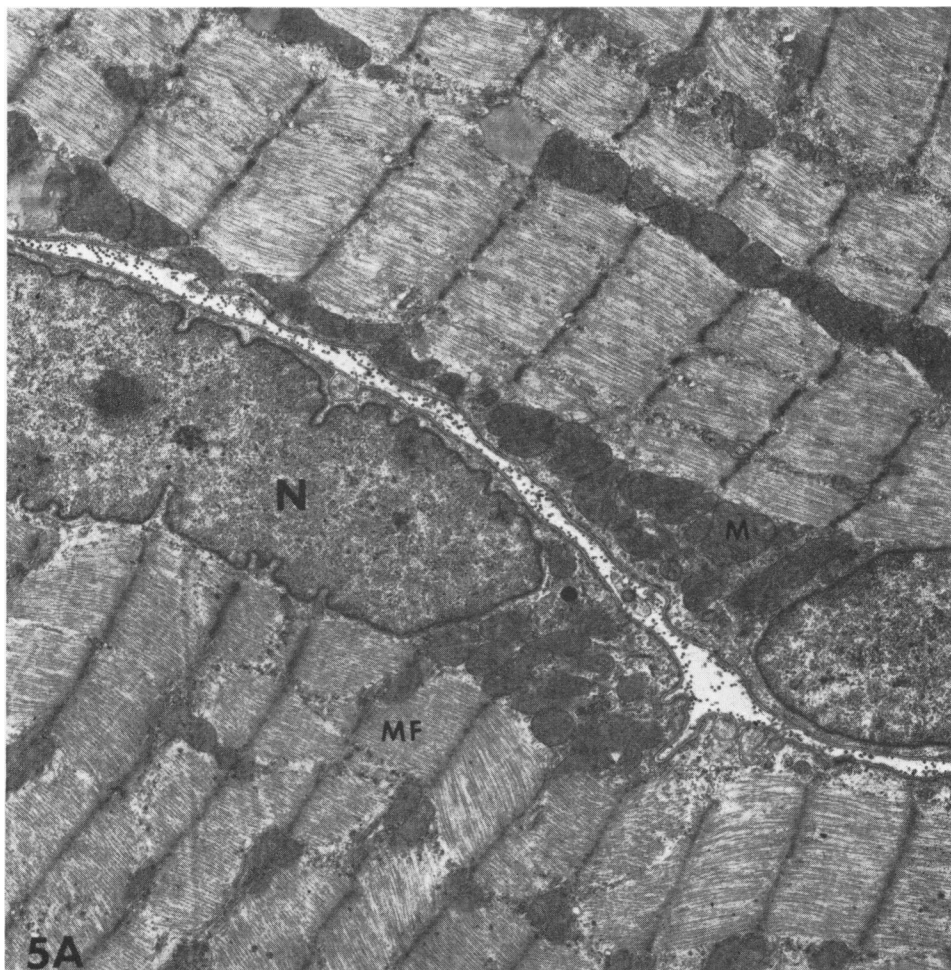
FIG. 5A. Detail of control myocyte, diaphragm 9,700 ×. Note myofibrillae,
Mf, Nucleus, N, Mitochondria, M.

lular ultrastructural changes occurring with
reinfused blood and Ringer's solution or
normal saline equal to three times the vol-
ume of shed blood. The documentation of
deleterious effects of rapid infusions of 5%
dextrose in Ringer's solution is of ther-
apeutic importance. Survival was decreased
and there was marked distortion of ultra-
structure of cells. The changes were espe-
cially dramatic in hepatocytes. Severe os-
motic disequilibrium was suggested in the
appearance of vacuoles and distortion of
cell membranes. This unfavorable effect of
rapid glucose infusion was also observed in
survival experiments by Dillon and asso-

ciates.[10] McNeill, Williams and Moore[20] re-
ported an unusual response to infusion of
20% glucose in the dog which was inter-
preted to be the result of osmotic mobiliza-
tion of cell water. It is therefore suggested
that rapid infusion of hyperosmolar dex-
trose and crystalloid solutions be avoided
in the initial treatment of hemorrhagic
shock. However, it should be recognized
that in late shock or sepsis, hypoglycemia
may occur. Under these circumstances, glu-
cose, an important energy source,[21] is re-
quired.

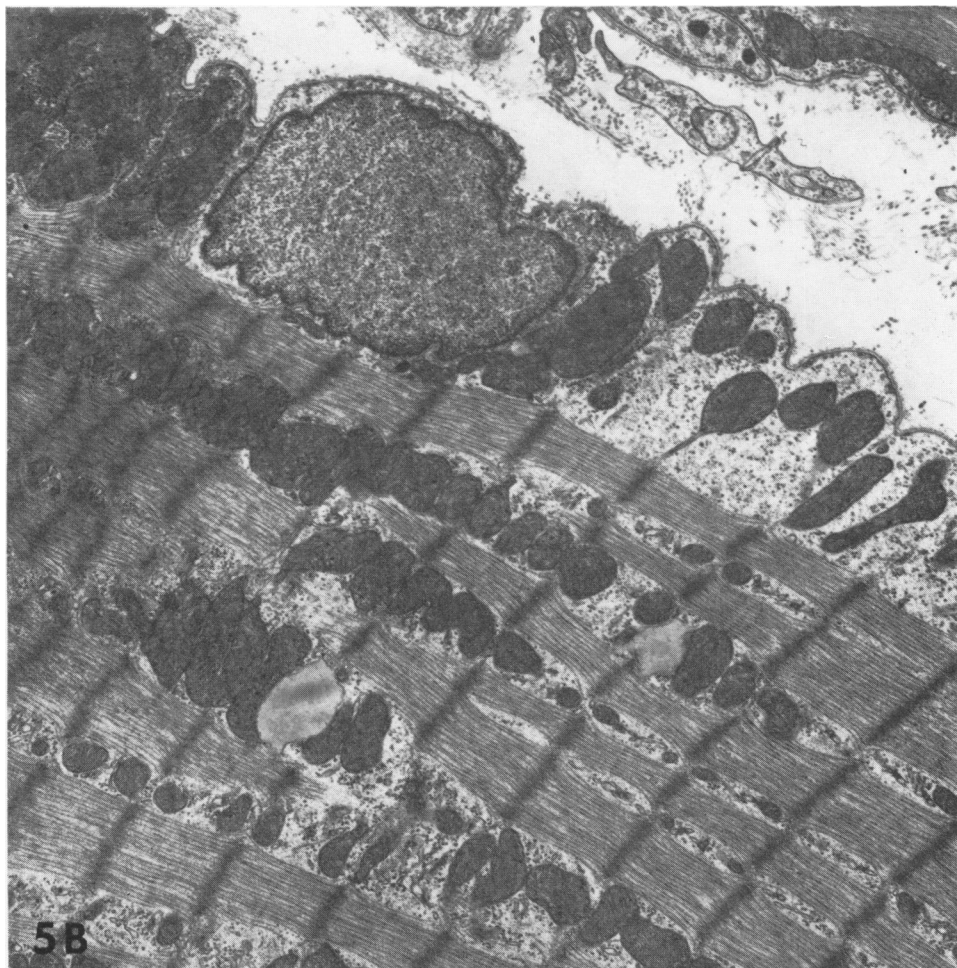While the main purpose of the present
experiments was to correlate changes in

FIG. 5B. Detail of myocyte after 120 minutes hypovolemia 9,700 ×. Note lucency
of cytoplasm suggesting edema and widening of spaces between myofibrillae.

ultrastructure with survival after treatment, histochemical data were obtained in an effort to understand alterations in enzyme activity in cells and membranes. Increased histochemical ATPase and glucose 6 phosphatase activities were not reversed 20 minutes after completion of infusion. Within the limits of histochemical methodology, it is reasonable to conclude that changes in the activity of cell membranes persist for some time after correction of the hypovolemia.

Of great potential importance are recently described stereologic and morphometric methods for quantitation of changes in the fine structural components of hepatocytes.[41] The extension of these methods to measurement of other tissues and to interstitial spaces may yield direct and quantitative evidence to support indirect tracer studies, measurement of cellular electrolyte responses, and determination of transmembrane potential. Application of morphometric ultrastructural technics may permit estimation of time sequences for fluid shifts into cells. Undoubtedly extension of these technics to other tissues will be laborious and time consuming. Once the magnitude, direction and sequence of fluids shifts are
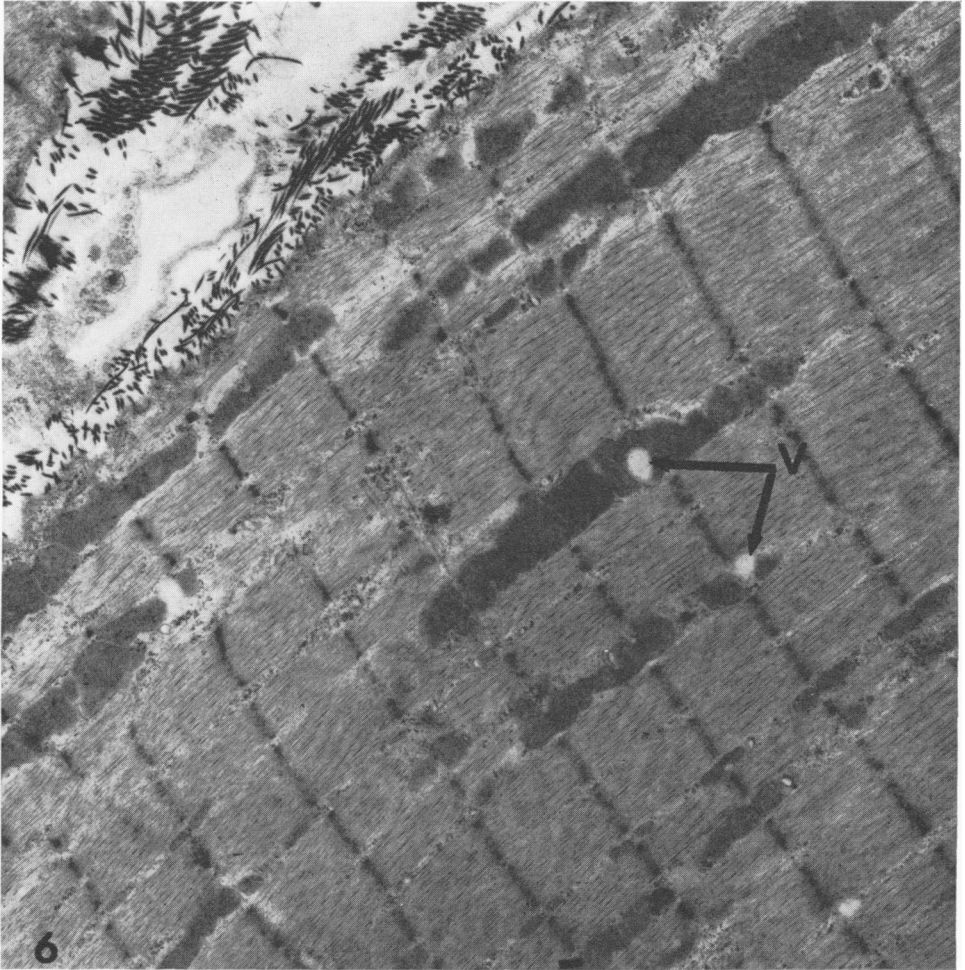
FIG. 6. Detail of myocyte in diaphragm after shock and infusion of 5% dextrose
lactated Ringer's 9,700 ×. Note vacuoles V within cytoplasm.

measured, more precise definitions of optimal therapy for hypovolemia will ensue.

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