Ultrastructural Changes in Hemorrhagic Shock *
Electron Microscopic Study of Liver, Kidney and Striated Muscle Cells in Rats

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DURING the past century and well into this one, great strides have been made in the knowledge of many diseases and biologic responses to injury as a result of detailed examination of cells, tissues and organs with the light microscope. With the measurement of function of cells and organs subjected to disease or trauma, physiologic aberrations superimposed upon structural changes provided a comprehensive concept of disease. During the past three to four decades more sophisticated technics have been developed for measuring functions that in many instances cannot be related to any morphologic change observable with the light microscope. Although the electron microscope was introduced more than 30 years ago, adequate resolving power of the microscope and technics for the satisfactory fixation, embedding and staining of tissues have been developed only within the past 15 years. Measurable functional biologic changes now can be related to structural alterations in the subcellular components of the cell. It is very likely that all functional changes representing cell dynamics can be related ultimately to specific subcellular structural change.

The general concept of hemorrhagic shock that exists today is an abstraction dealing with multiple interrelated physiologic and biochemical changes. It is very likely that a comprehensive perspective of hemorrhagic shock will be attained only when the measurable intracellular oxidative and enzymatic processes can be related to the physical changes in the subcellular elements observed with the electron microscope. Attention has been directed primarily to the ultrastructural changes produced by hypoxia, but a few investigators have described alterations in various organs at some phase of shock produced by different methods.

Hift and Strawitz [13, 14] compared isolated hepatic mitochondria of dogs dying in a state of irreversible shock with controls and observed enlarged mitochondria characterized by a swollen matrix.

Dalgaard [6] examined the kidneys of three patients dying in a state of shock, one after hemorrhage and two after barbiturate poisoning. His observations demonstrated that the ultrastructure of the glomeruli was normal.

Martin, Hackel and Kurtz [20] studied the ultrastructural changes of myocardial cells in five dogs subjected to hemorrhagic shock. Marked structural abnormalities were present in many myocytes in the region of the intercalated discs. These lesions were characterized by distortion of the

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sarcomeres at the ends of the myocytes on each side of the intercalated disc with fragmentation of the Z bands and displacement of the filaments; translocation of mitochondria from the center to the periphery of the lesion with packing of mitochondria between adjacent normal myofibrils; and tight "scalloping" of the sarcolemma and contraction of the damaged myofibrils. No intrinsic structural alterations were observed in the intercalated disc, myofilaments or sarcolemma, and there was only slight alteration of mitochondrial structure. They also observed by appropriate stains and the light microscope a loss of succinic dehydrogenase and cytochrome oxidase adjacent to the intercalated discs with an increase at the site of mitochondrial dislocation.

Vitali-Mazza, Missale and Ferioli [31] studied the ultrastructural changes of the kidneys of rabbits subjected to ischemic shock. They found that changes in the kidneys were confined to the proximal convoluted tubules. In these cells there were swelling of the mitochondria, alteration in the density of the matrix and distortion of the cristae. There was some disruption of the brush border and frequently cytoplasmic material was seen in the lumen of the tubule. Rarely there was disruption of the basement membrane with extracellular escape of cytoplasmic components.

The purpose of the present study was to examine various organs simultaneously and to record progressive alterations in fine structure and cytoplasmic organelles which occurred during prolonged hemorrhagic shock. These structural changes were then related to the changes in oxidative metabolism which have been found during hemorrhagic shock.

Methods

Male albino Wistar rats weighing 175 to 225 Gm. were used. All animals were fed a high carbohydrate diet until just prior to the time of the experiment. Anesthesia was produced by the intraperitoneal instillation of pentobarbital (4 mg./100 Gm. of rat) and maintained when required by additional instillations. The external iliac arteries were cannulated; one cannula led to a mercury manometer and the other to a 10-ml. syringe used as a reservoir. The cannulae, tubes, syringe and animals were heparinized. Rectal temperature was monitored in all animals; external heat was applied periodically from a light source to maintain the temperature between 35.5 and 37.5° C.

Eight rats were subjected to hemorrhagic shock of variable intensity and duration. Blood was withdrawn into a syringe during a 10-minute period until the mean arterial blood pressure (MABP) was reduced to 50 mm. Hg. This pressure was maintained for 60 minutes by withdrawal of additional blood as necessary. The animals were then bled to a MABP of 30 mm. Hg which was maintained for an additional 60 minutes. In order to determine the progressive structural change in cells with prolonged shock it was necessary to obtain specimens at intervals during the 120 minutes of hypotension. Accordingly, animals were sacrificed after 30 and 120 minutes of shock.

Controls were included in the experimental design to preclude, as well as possible, the interpretation of artifactual changes as significant structural changes produced by hypovolemia. *Anesthesia.* On each day of the study a rat was anesthetized and treated in all respects similar to the experimental animal except that it was not bled. The tissues of this rat served as a control for all studies performed on that day. *Hypoxia.* Two nonanesthetized rats were subjected to an hypoxic environment by placement in an atmosphere of 93% nitrous oxide and 7% oxygen. After 60 minutes these rats, and an anesthetized control rat that had been maintained in room atmosphere, were sacrificed.

The tissues of all rats were treated in a similar manner. When the rats were sacri-

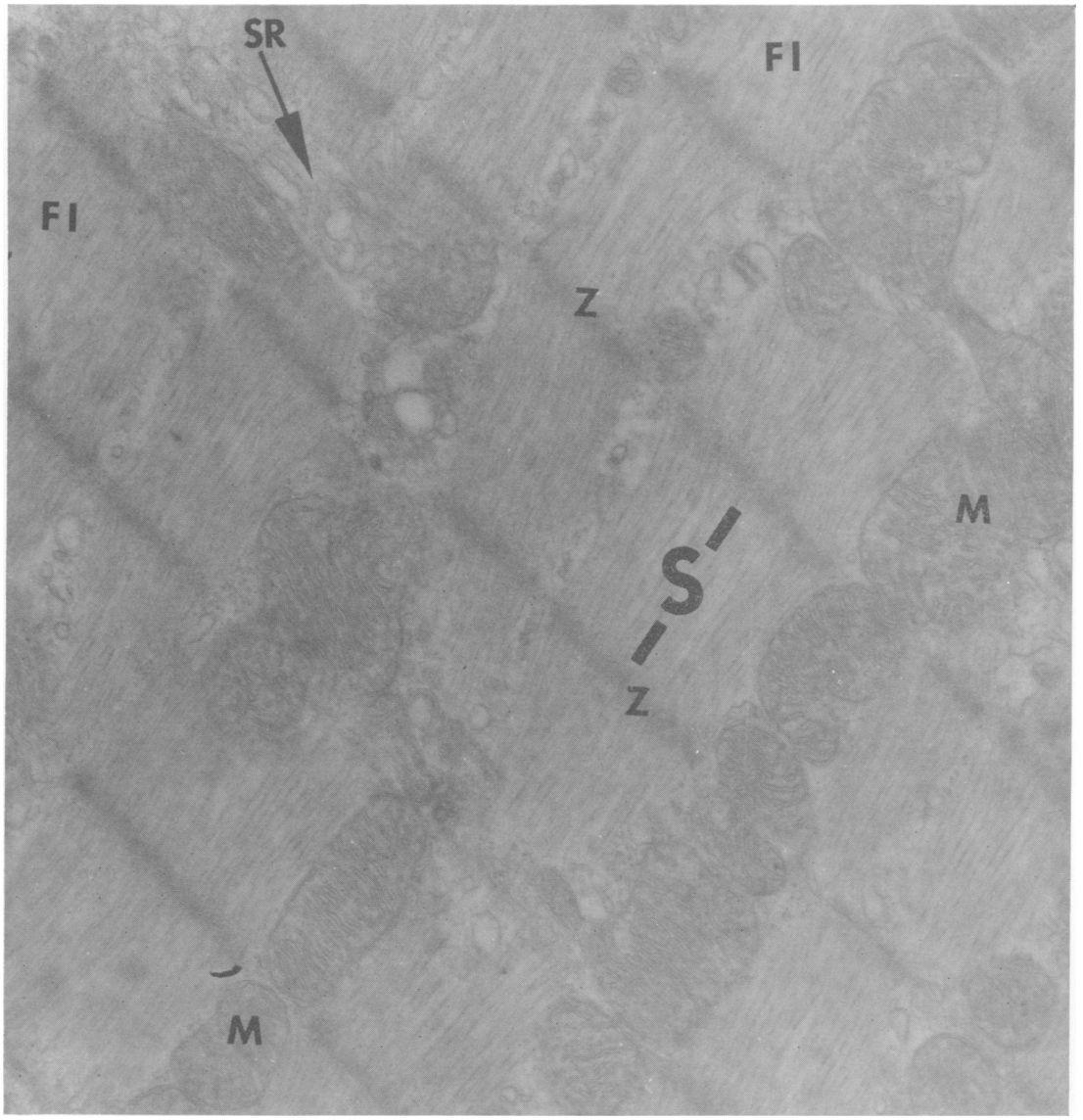

FIG. 1. Normal skeletal muscle, diaphragm. × 30,000. Myofibrils (FI), bands (Z), sarcomere (area between two z bands) (S), mitochondrion (M), sarcoplasmic reticulum (SR).

ficed small pieces of liver, kidney and diaphragm were removed and fixed in either 1% osmium tetroxide or 6.25% glutaraldehyde. Specimens fixed in 6.25 glutaraldehyde were transferred to a 50% buffered sucrose solution prior to refixation in 1% osmium tetroxide. After dehydration in progressive concentrations of ethyl alcohol and clearing in propylene oxide, the tissues were embedded in Maraglas. Sections were cut in an LKB ultramicrotome and stained with either uranyl acetate, 5% in 50% alcohol and 50% H_2O, or in uranyl acetate and 1% potassium permanganate. The sections were examined with an RCA 3G electron microscope.

Results

Striated Muscle, Diaphragm

Of all tissues studied the most striking findings were seen in this tissue. The struc-

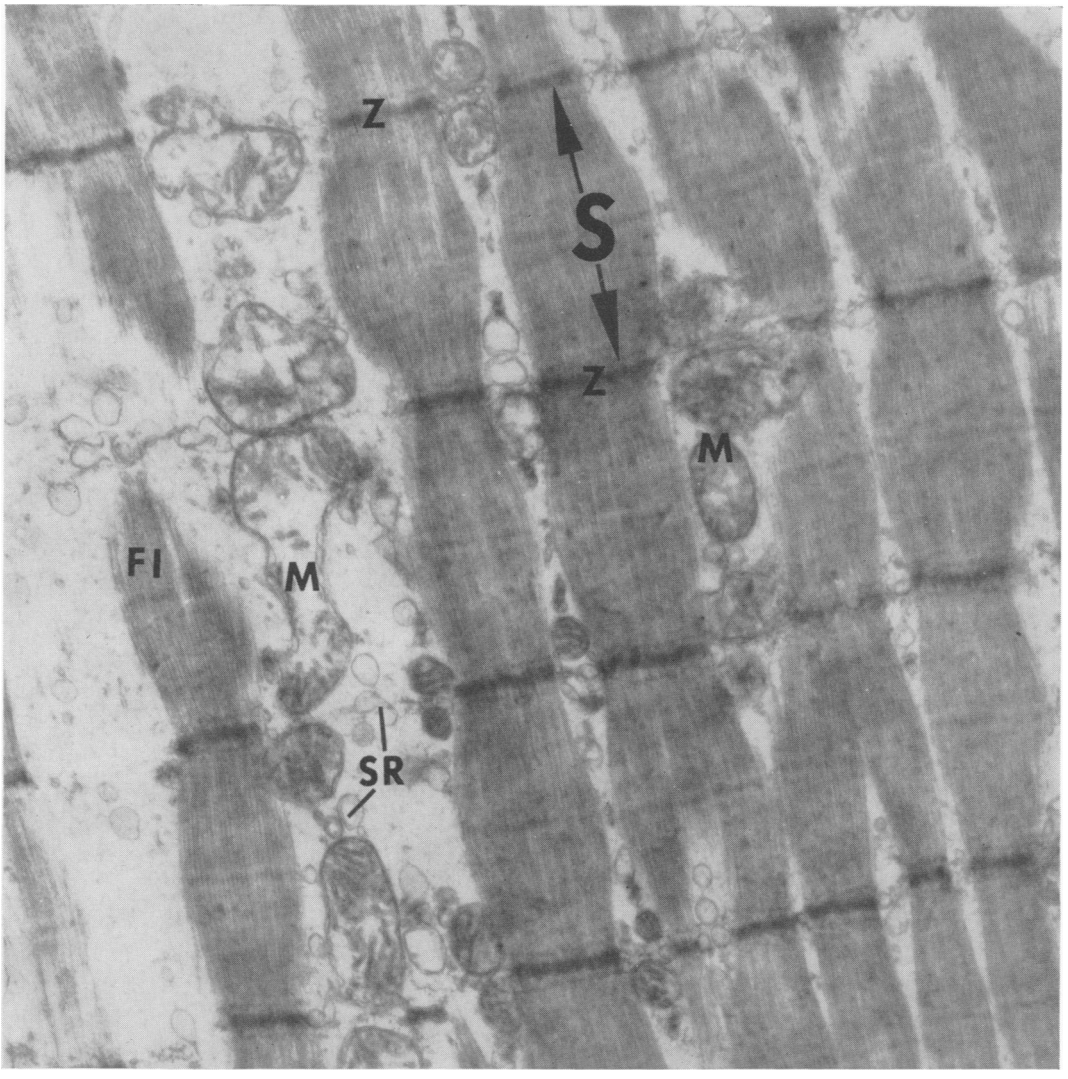

FIG. 2. Skeletal muscle, diaphragm, 120 minutes shock. Note marked intracellular edema, changes in mitochondria and "bow tie" appearance in the repeating sarcomeres. × 22,000. Key as in Fig. 1.

tural details of normal skeletal muscle are seen in Figure 1. The contractile elements, myofibrils, are evenly punctuated by Z bands, giving rise to a repetitive pattern of sarcomeres. The mitochondria and sarcoplasmic reticulum are located between the myofibrillar elements.

After 120 minutes of shock intracellular edema developed with consequent separation of the bundles of myofibrils and more prominent sarcoplasmic reticulum (Fig. 2). This separation of the individual myofilaments within their bundles led to bulging in the center of the sarcomere and a relative constriction in the area of the Z band. At lower magnification this imparted a characteristic "bow tie" appearance to the repeating sarcomeres. The mitochondria became swollen and disorganized.

A comparison of the control muscle with that from animals subjected to 30 or 120 minutes of hypovolemia (Fig. 3A–C) indicates that a temporal progression developed in the spectrum of changes. The

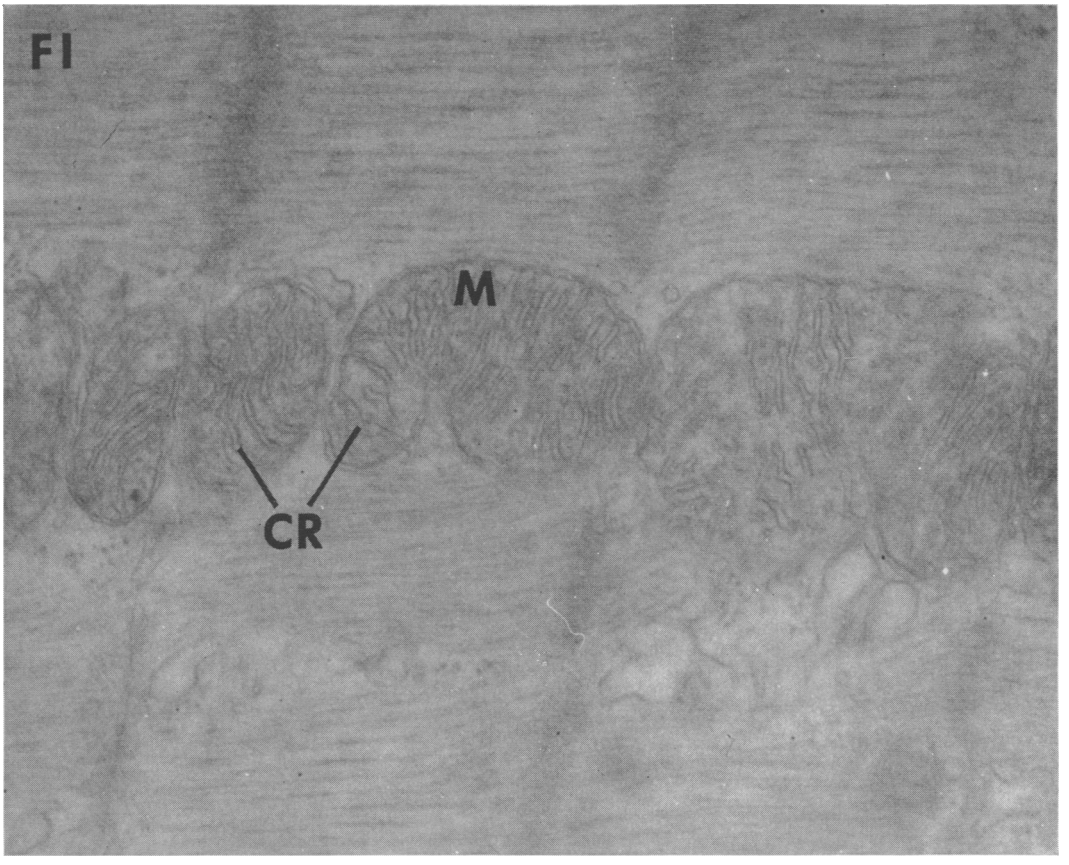

FIG. 3. A. Mitochondria in normal diaphragm. ×45,000. Note mitochondrion (M), cristae within the mitochondria (CR) and myofibrils (FI).

cristae became disorganized as manifest by a loss of their usual parallel or radial arrangement and by condensation of material within the matrix (Fig. 3B). In more prolonged shock (Fig. 3C) a variety of changes became apparent; marked swelling of the mitochondria, occasional distortion, actual rupture of the mitochondrial membrane and profound disorganization of cristae. In some mitochondria the cristae were entirely absent or obscured by a stippled electron-dense matrix.

Suggestive evidence of nuclear change was manifest by alterations in the disposition of chromatin within the nucleus after 120 minutes of hypovolemia (Fig. 4). There was clumping or aggregation of material in the area of the nuclear membrane with consequent poor visualization of the inner lamina of the nuclear membrane.

Liver

Details of a normal hepatic cell and its organelles are illustrated in Fig. 5. After 120 minutes of shock intracellular edema became manifest by widening or distortion of the endoplasmic reticulum (Fig. 6, 7). A relative depletion of glycogen granules was apparent, although the sampling problem inherent in the selection of sections for electron microscopic study does not permit accurate localization of the glycogen-depleted cells within the hepatic lobule. As in muscle the mitochondria were found to be swollen and disorganized (Fig. 6).

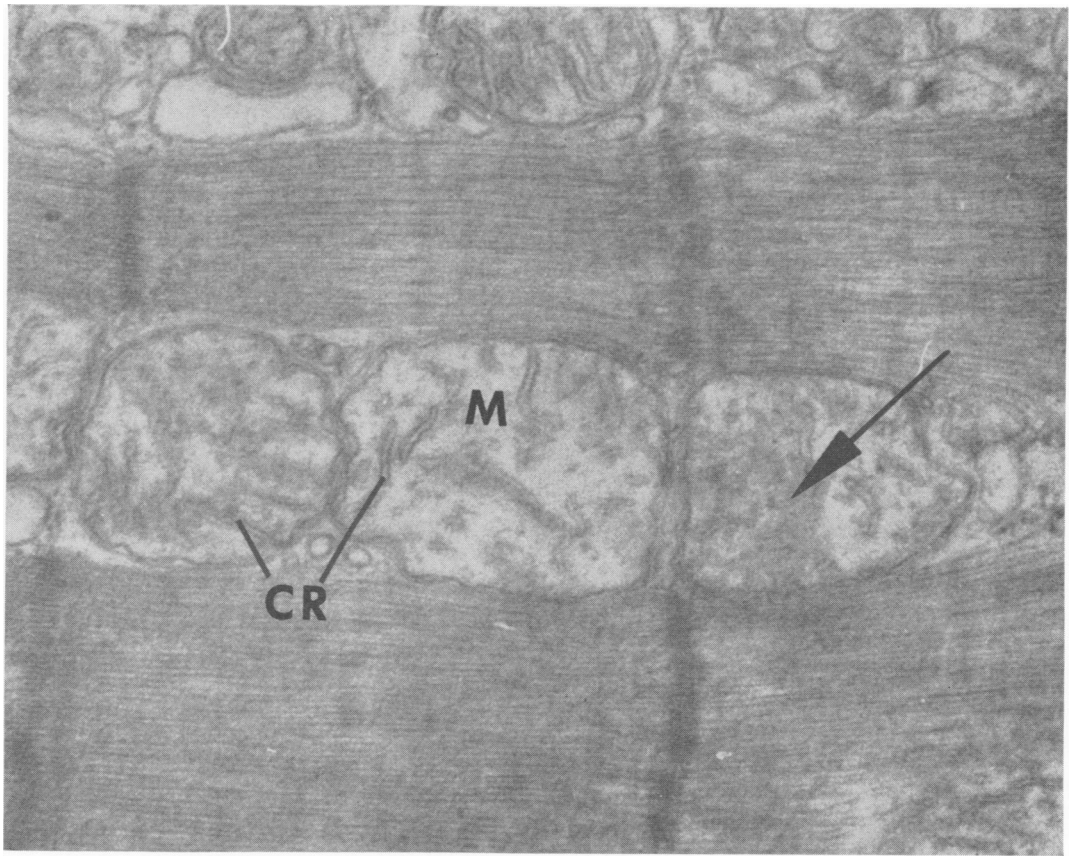

FIG. 3. B. Mitochondria in diaphragm, 30 minutes shock. × 42,000. Note changes in matrix and early disorganization of cristae. Arrow indicates condensation in matrix.

In some cells (Fig. 7) there was an apparent increase in the number and size of peribiliary bodies interpreted to be lysosomes. Their appearance is suggestive of swelling and possibly escape of the contained material of these organelles. It was not possible on the basis of this study to demonstrate consistently sequential changes of ultrastructure in the hepatic cell during prolonged shock.

Kidney

Details of the characteristic intraluminal brush border of a normal proximal convoluted tubular cell are presented in Fig. 8. Changes in the kidney produced by hypovolemia were limited to the proximal convoluted tubules. There was focal intracellular edema, and in some cells the brush border was interrupted with escape of the supranuclear portion of the cytoplasm into the lumen of the tubule (Fig. 9). The mitochondria were altered; the usually elongated mitochondria in the basilar portion of the cell were more spherical, and there was marked disorganization of the cristae and stippling in the matrix. The basement membrane was intact; however, the finger-like projections of the inner surface of this membrane appeared distorted by the mitochondrial swelling. The most significant difference between the animals subjected to 30 and 120 minutes of hypovolemia was discontinuity of the brush border of the proximal convoluted tubular cell after 120 minutes of shock. The changes were similar

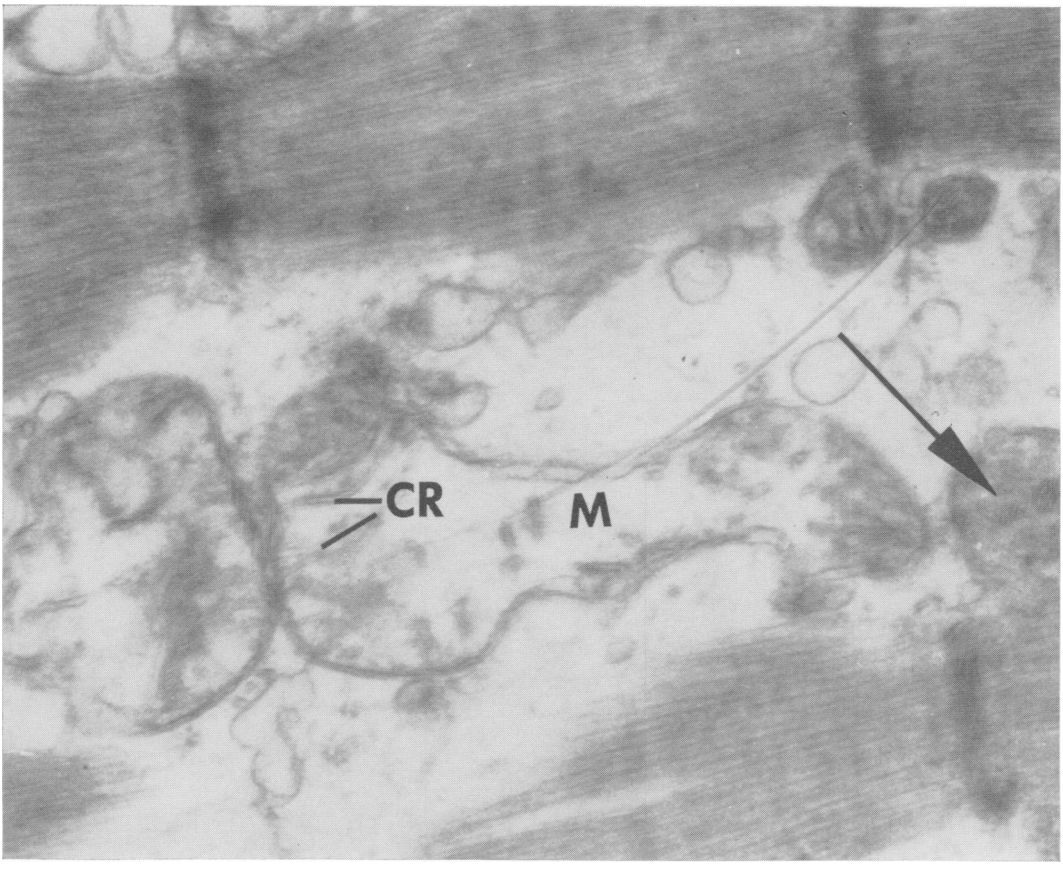

FIG. 3. C. Mitochondria in diaphragm, 120 minutes shock. × 44,000. Note profound disorganization of these structures. Arrow indicates condensation in matrix.

to those described by Vitali-Mazza *et al.*[31] in their shock model in the rabbit.

No significant alteration was observed in the glomeruli or distal convoluted tubules after 120 minutes of hypovolemia. The well preserved fine structure of these areas are illustrated in Fig. 10 and 11.

Hypoxia

The tissues obtained from the hypoxic animals did not demonstrate striking changes in ultrastructure. The appearance of the muscle and kidney sections were quite similar to those obtained from the anesthetized control animal that had been maintained at room atmosphere. However the hepatic cells of the hypoxic animals did demonstrate some edema, and vesicles (Fig. 12) of varying electron density appeared within the cytoplasm. The mitochondria appeared to be preserved after a 60-minute exposure to an atmosphere of 7% oxygen. The hepatic intracellular changes observed after 30 or 120 minutes of hypovolemia were not observed in the hypoxic animals in these studies.

Discussion

Hemorrhagic shock is a physiologic disturbance characterized by a discrepancy between effective circulating blood volume and the total capacity of the vascular bed and manifest by the total change in the physical and chemical composition of extracellular fluid resulting from diminished capillary perfusion. It has become abun-

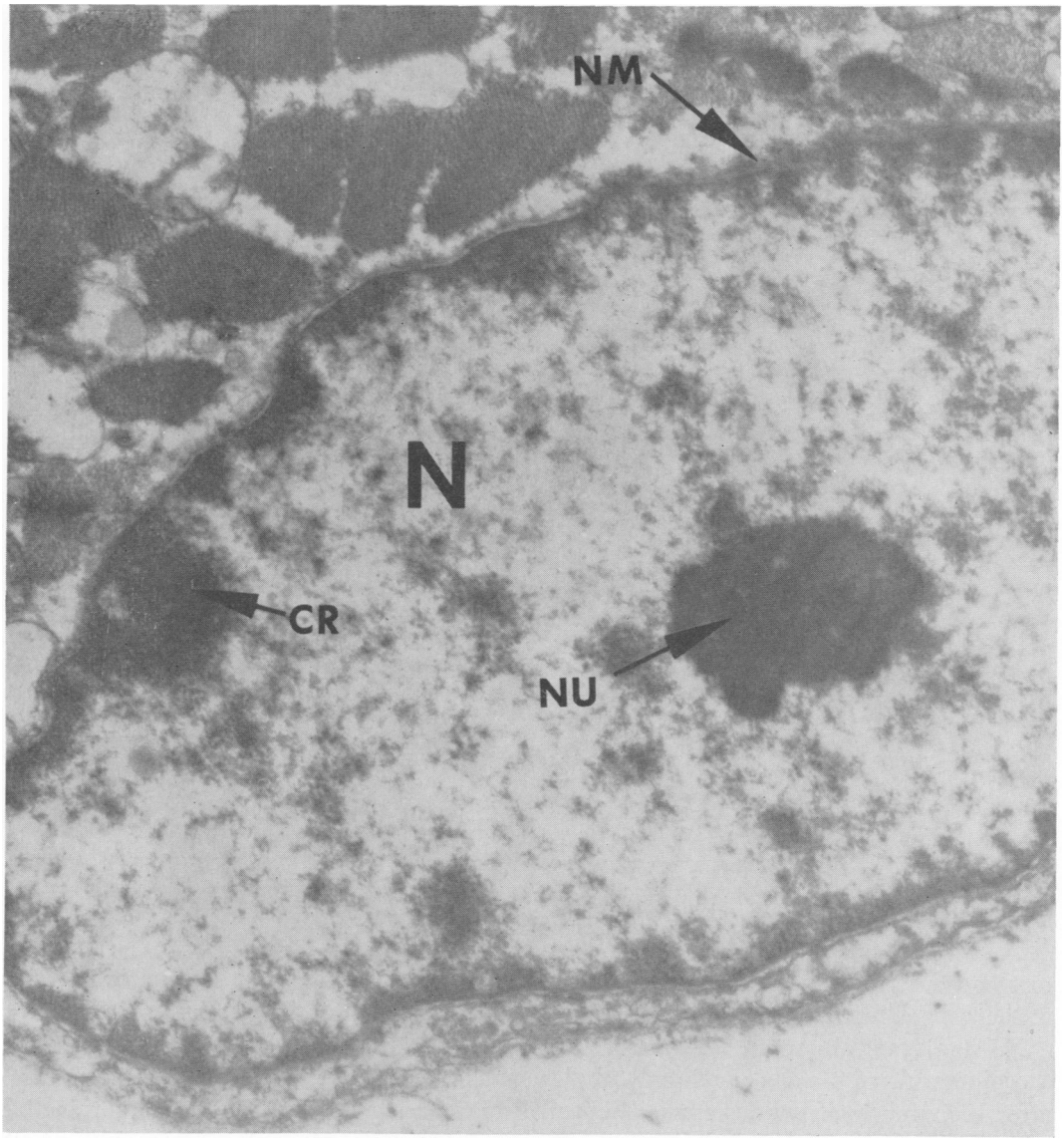

FIG. 4. Skeletal muscle, diaphragm, 120 minutes shock. Nucleus demonstrates margination of nuclear chromatin. × 22,000. Nucleus (N), chromatin (CR), nucleolus (NU), nuclear membrane (NM).

dantly clear that death from hypovolemia must result ultimately from disorganization at a cellular and enzymatic level owing to a failure of energy production. For this reason many studies have been directed toward defining and clarifying the biochemical response to hypovolemia and its attendant cellular hypoxia.[10]

A brief summary of these studies indicates that a reduction of blood volume by hemorrhage will cause an elevation in blood glucose, pyruvic and lactic acids, serum inorganic phosphorus and alpha amino nitrogen and a reduction in the blood pH, pCO_2 and ketone levels.[10] If the animal is not transfused, death results eventually with marked metabolic alterations.

The character of the metabolic response to hypovolemia has been interpreted as a biochemical adaptation to provide energy

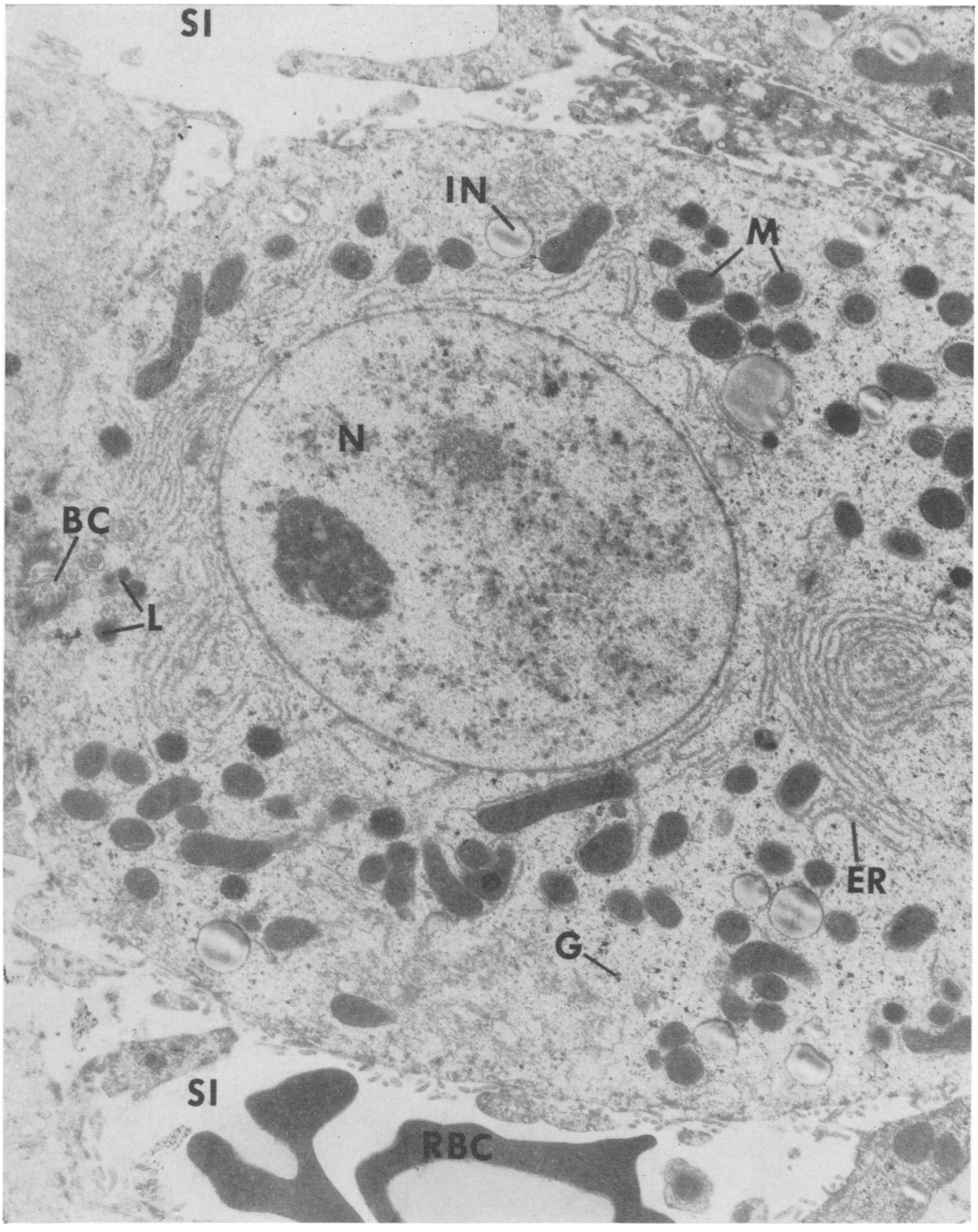

FIG. 5. Normal liver, rat. Relationship of the hepatic cell to the sinusoids and characteristic organelles are seen. The bile canaliculus is located between adjoining cells. × 8,000. Nucleus (N), mitochondria (M), bile canaliculus (BC), sinusoid (SI), lysosomes (L), red blood cell (RBC), endoplasmic reticulum (ER), glycogen (G), inclusion (IN).

in the presence of cellular hypoxia and the neurohumoral response to hypovolemia. It has been observed that hepatic glycogen becomes depleted while peripheral utilization of glucose is accelerated.[7, 26] Thus, since the level of blood glucose at any time

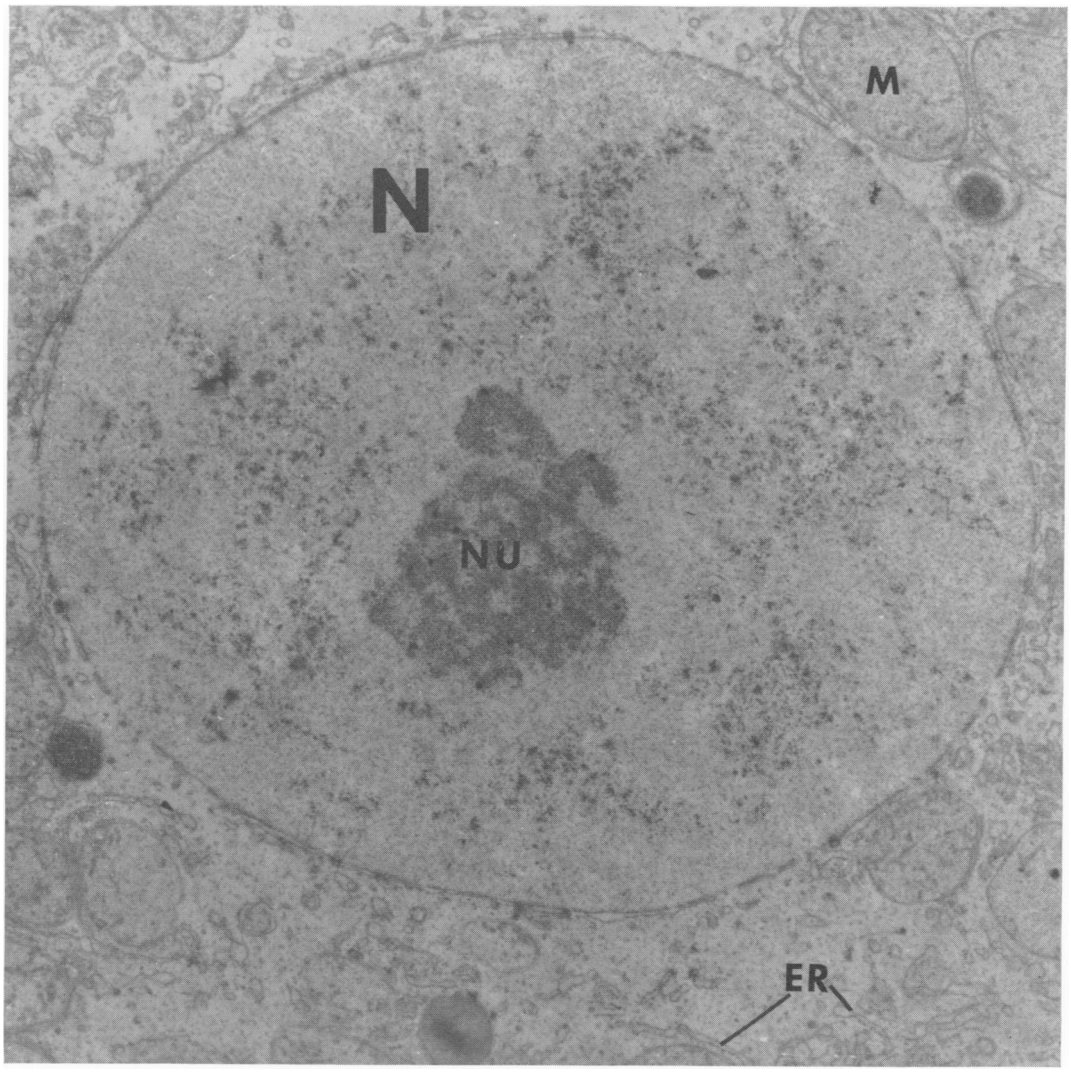

FIG. 6. Detail of hepatic cell, 120 minutes shock, Note absence of glycogen, marked intracellular edema and swollen disorganized mitochondria. × 22,000. Nucleus (N), nucleolus (NU), mitochondrion (M), endoplasmic reticulum (ER).

reflects the balance between hepatic production and peripheral utilization, late in shock there may be marked hypoglycemia as a consequence of the mobilization of a maximal amount of substrate for energy production. The progressive rise of blood lactic and pyruvic acids reflects the increased combustion of glucose to provide energy, whereas the rise of the lactate-pyruvate ratio suggests that energy production is more dependent upon the relatively inefficient pathways of anaerobic metabolism. Since similar changes occur in bled hepatectomized rats [26] it is likely that the accumulation of lactate and pyruvate reflect increased production from the peripheral tissues rather than failure of hepatic removal.

Adequacy of the compensatory biochemical response for energy production during hypovolemia should be reflected by the preservation of tissue stores of high energy

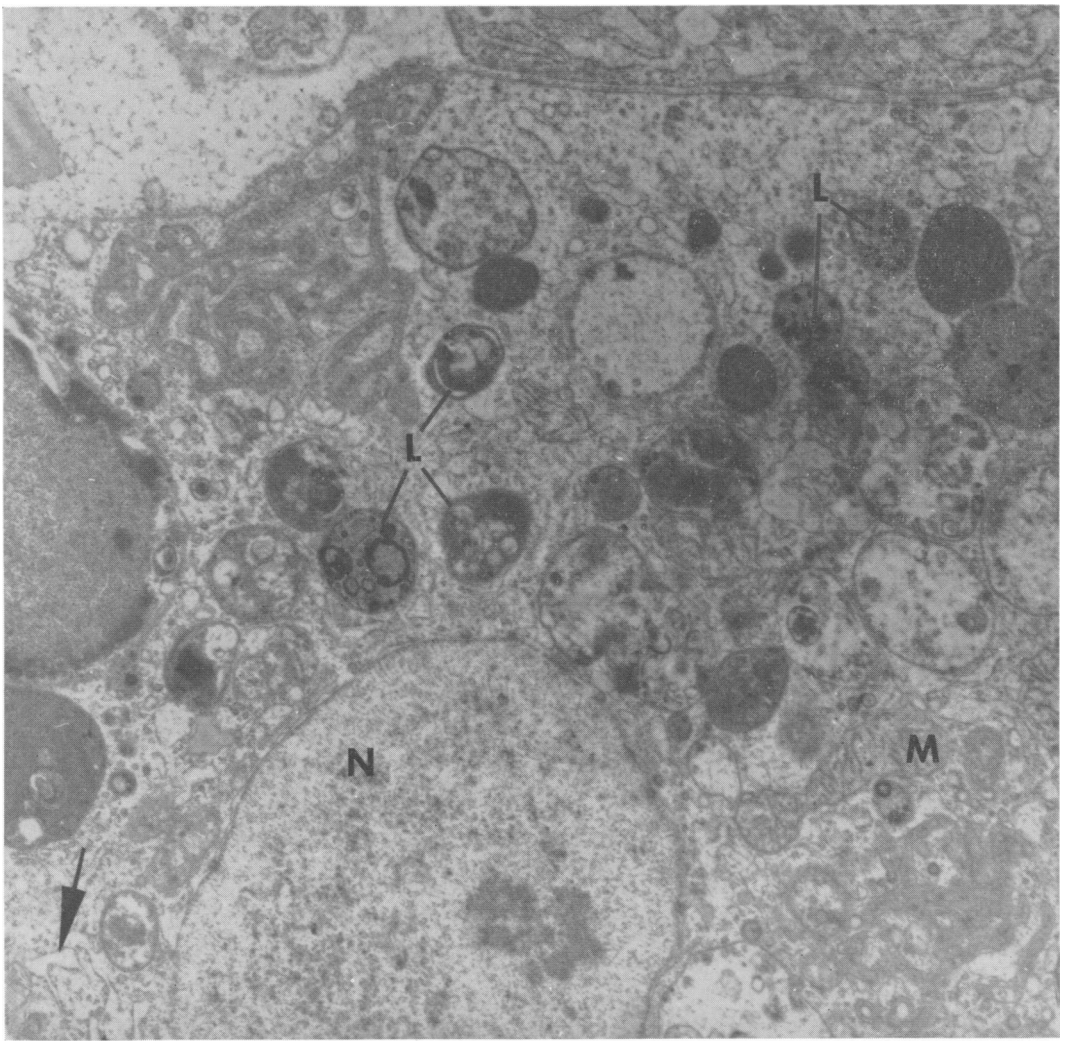

FIG. 7. Liver, 120 minutes shock. Note intracellular edema, distortion of endoplasmic reticulum (arrow) and lysosomes which are increased in number and size. × 14,000. Nucleus (N), lysosomes (L), mitochondria (M).

phosphate compounds. In a detailed study of many tissues during different types of shock, LePage clearly demonstrated a progressive reduction of high energy phosphate compounds during shock.[18] The greatest reduction of ATP was found in the liver, less in the kidney, brain and heart and least in skeletal muscle. The concentration of inorganic phosphorus has been found to rise steadily during prolonged hypovolemia.[8, 10] These observations regarding the unavailability of energy stores and increased production of inorganic phosphorus have been amply confirmed under many circumstances.[17, 22, 24, 25, 30]

While adequate compensatory biochemical changes are essential for providing energy, no critical metabolic reaction has demonstrated the level of tolerance for prolonged hypovolemia. Evidence indicates that despite marked distortion, normal metabolic function and enzyme activity usually can be restored until late in shock.[8, 22, 27]

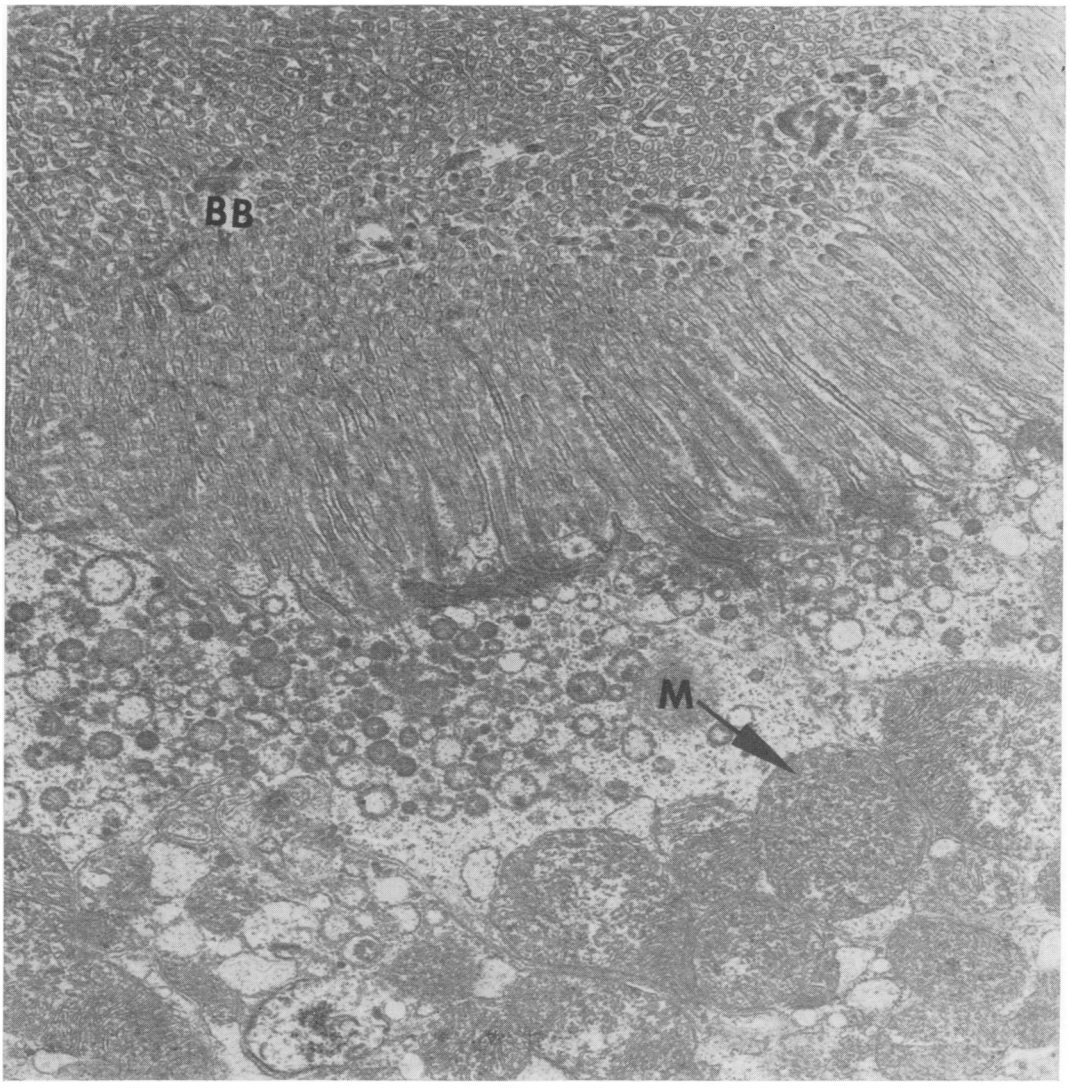

FIG. 8. Normal rat kidney. Detail of apical portion of proximal convoluted tubule cell. × 14,000. Brush border (BB), mitochondria (M).

Since the enzymes involved in oxidative metabolism are localized in the mitochondria, it is reasonable that more recent studies have been concerned with these cellular organelles. The chief function of mitochondria appears to be the formation of high energy phosphate bonds which can be transferred for use elsewhere in the cell. On the other hand the energy derived from anaerobic glycolysis is apparently produced in the cytoplasm rather than in the mitochondria.

While interpretation may differ, morphologic changes do occur in mitochondria during shock.[11, 13, 14, 28, 29] It is interesting that the most significant lesions observed in the kidney after 2 hours of shock remained confined to the proximal tubules (Fig. 9), similar to the changes described by Vitali-Mazza after ischemic shock.[31]

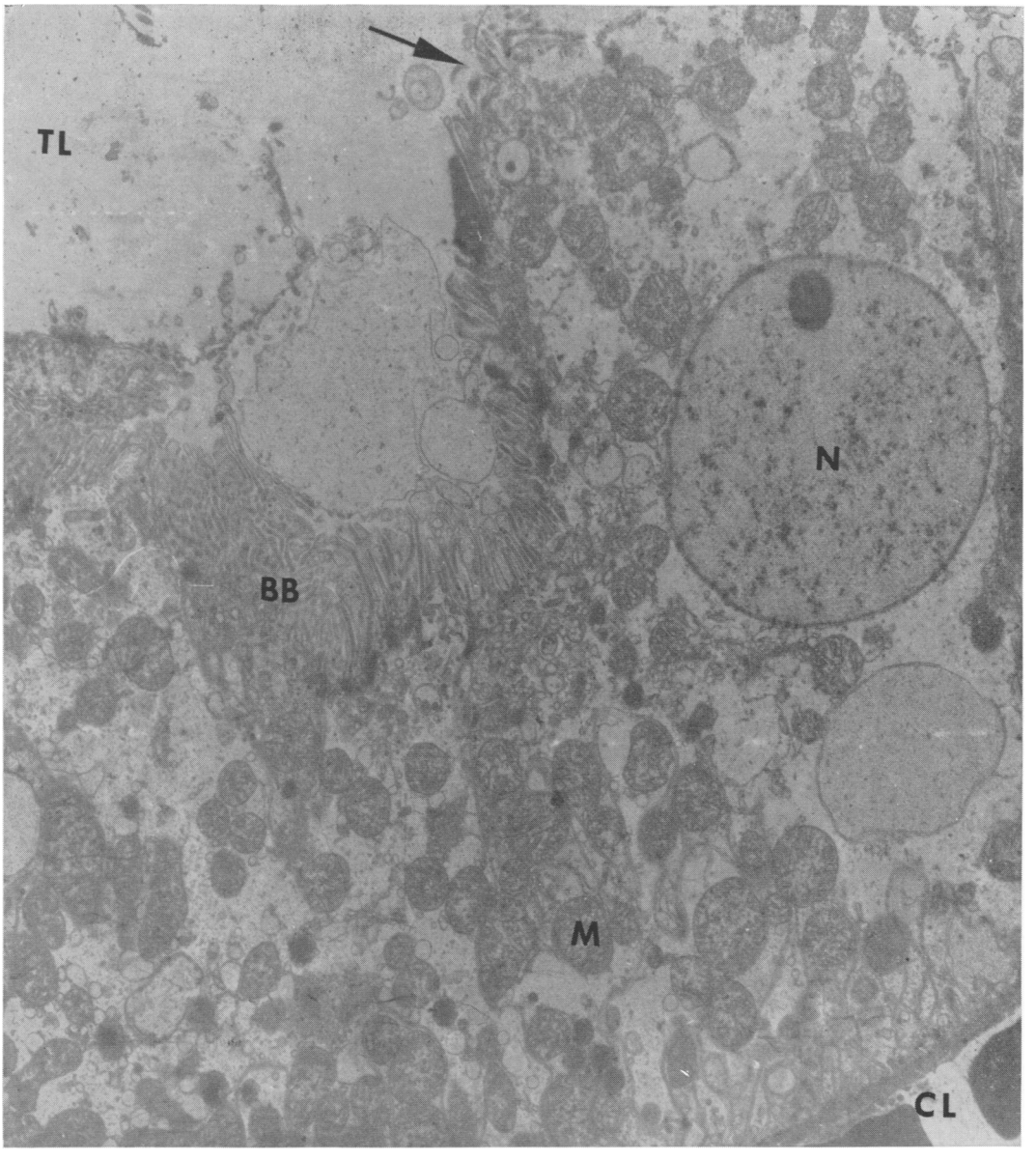

FIG. 9. Kidney, proximal tubular cell, 120 minutes shock. Note swelling and disorganization of mitochondria, and discontinuity of brush border at arrow, with escape of cytoplasm into tubular lumen. × 8,000. Brush border (BB), tubular lumen (TL), mitochondrion (M), nucleus (N), capillary lumen (CL).

Also no demonstrable change was found in the glomeruli, confirming the observation of Dalgaard [6] (Fig. 10).

The most striking structural changes developed in striated muscle; the degree of ultrastructural disorganization became more pronounced in those animals subjected to a longer period of shock. These progressive changes in the mitochondria correlate well with the general metabolic alterations previously observed in rats subjected to hemorrhage of similar duration and severity.[7] The clumping or aggregation of material in the area of the nuclear mem-

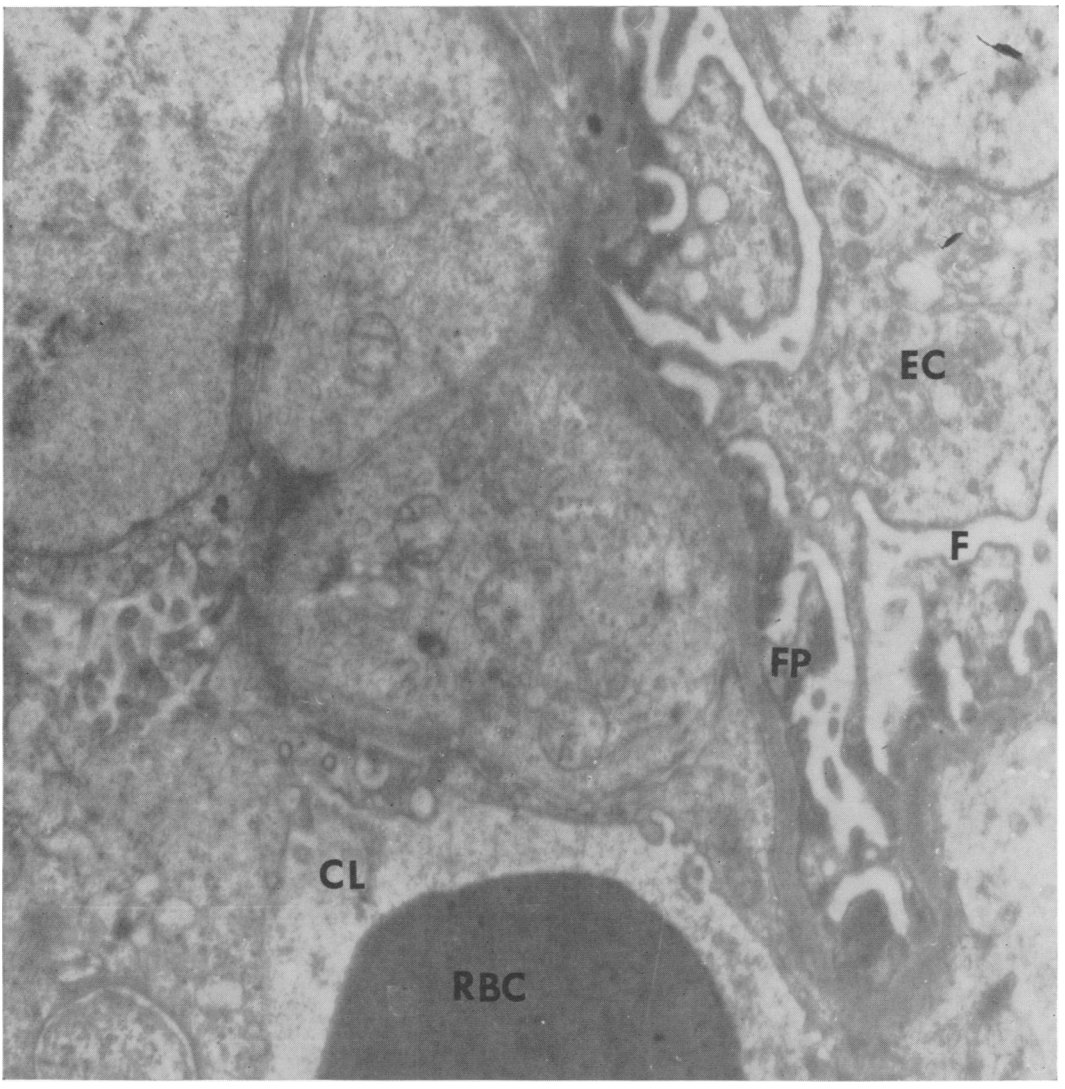

FIG. 10. Glomerulus, 120 minutes shock. The fine structure appears well preserved. × 22,000. Epithelial cell (EC), capillary lumen (CL), red blood cell (RBC), foot process (FP), filtration (Bowman's) space (F).

brane resembles the changes described by Burdette and Ashford in their study of severely hypoxic cardiac muscle.[5]

At present it is difficult to correlate more precisely the metabolic alterations observed during shock with any given modification of ultrastructure. The principal efforts to correlate cellular structure with function largely have been confined to a description of the change in various metabolic products or to a study of the capacity of mitochondria to metabolize various substrates of the Krebs cycle.

Fonnesu [11] measured oxidative phosphorylation in mitochondria obtained from livers, demonstrating "cloudy swelling" resulting from the intraperitoneal injection of lethal doses of S. *typhimurium* toxin into rats or from the subcutaneous injection of diphtheria toxin. Phosphorylation coupled with the oxidation of either succinate or alpha ketoglutarate was partially inhibited

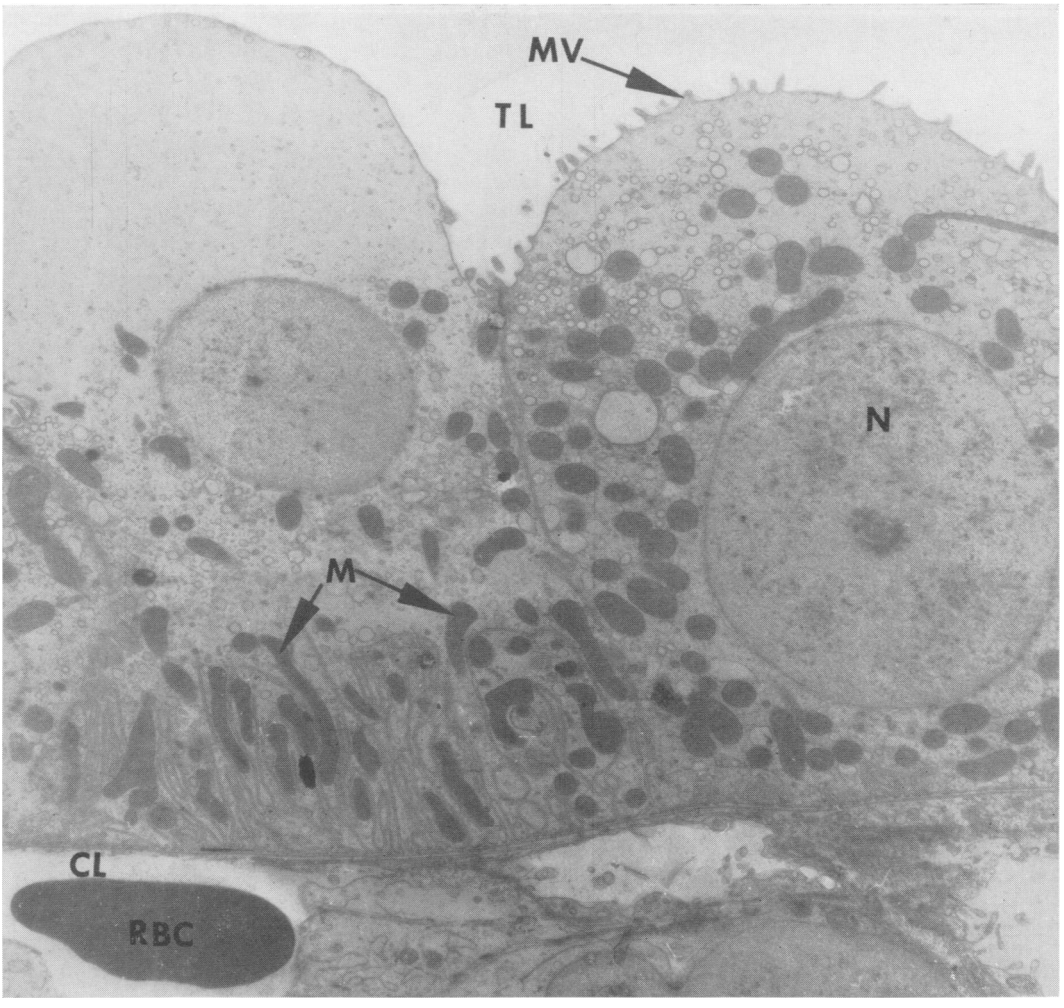

FIG. 11. Kidney. Distal tubule, 30 minutes shock. The fine structure appears well preserved. × 8,000. Tubular lumen, (TL), microvilli (MV), nucleus (N), mitochondria (M), capillary lumen (CL), red blood cell (RBC).

while oxygen consumption was unchanged. Although unrelated to hemorrhagic shock, these chemical findings represent structural changes in mitochondria similar to those observed in hemorrhagic shock.

Myocardial mitochondria isolated from dogs in irreversible shock have been found to contain abnormal amounts of phosphate.[13] Decreased synthesis of ATP with unaltered ATPase activity was found in myocardial mitochondria isolated from rats during drum, tourniquet or hemorrhagic shock.[24] Liver mitochondria in dogs dying from hemorrhagic shock were found to have increased amounts of proteinaceous, nitrogenous and ultraviolet absorbing material.[14] Many studies of mitochondrial activity in the heart, liver and brain of animals during various types of shock have demonstrated a reduction of oxidative phosphorylation.[12, 19, 21, 24, 28, 29] When mitochondria are isolated, however, oxidative phosphorylation proceeds normally.[1, 22]

The observation that mitochondria isolated from animals in profound shock can revert to a normal function *in vitro* if they

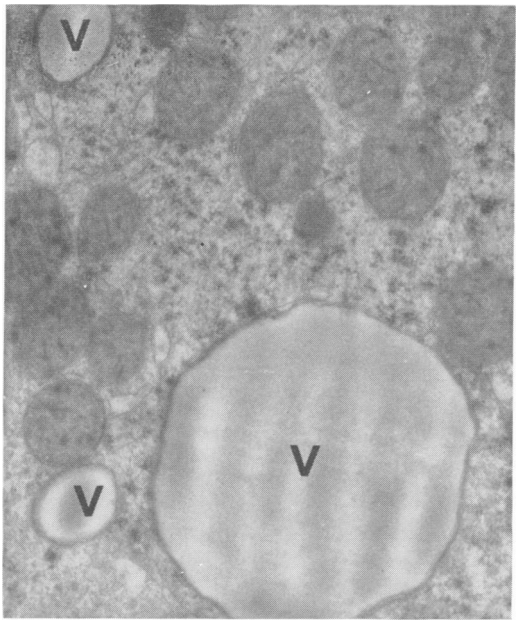

FIG. 12. Detail of hepatic cell after hypoxia showing vesicles (V). × 14,000.

are provided with a proper substrate is consistent with the observations of improved metabolic function following restoration of volume,[8, 27] improved tissue flow by adrenergic blockade [9] or reduction of acidosis.[23] It remains to be determined whether a demonstrable improvement in ultrastructure can be correlated with the metabolic improvement that follows restoration of blood volume. The capacity of mitochondria to resume normal biochemical function after prolonged shock is rather remarkable in light of the extensive distortion and destruction of the cristae and the changes in the matrix where a multitude of interrelated enzymatic processes takes place. Although the mitochondrial membrane is unusually durable, the early swelling of the mitochondria in shock is probably a manifestation of a change in the permeability of the membrane and altered tonicity of the matrix. Such structural changes are compatible with the known influence of different ionic concentrations upon the process of phosphorylation.[15] Durable as the mitochondrial membrane is, a significant number of disrupted membranes were seen in the animals that were hypotensive for 2 hours.

Because of the capacity for mitochondria to recover normal biochemical activity after prolonged shock, it has been suggested that the metabolic alterations of shock reflect not only tissue hypoxia but also the effects of the neuroendocrine response to hypovolemia.[22] This thesis is supported by the significantly less severe ultrastructural alterations observed in tissues of rats subjected to 60 minutes of an hypoxic environment in comparison to the striking changes observed in tissues from rats subjected to only 30 minutes of hemorrhagic shock.

It is noteworthy that Bassi *et al.*[2] found similar relative preservation of hepatic ultrastructure after exposing rats to an atmosphere of 3% oxygen for 120 minutes.

If the differences in ultrastructure in the hypoxic and shocked rats can be confirmed by more detailed studies, an interesting speculation could be advanced. Although hypoxia is present to some degree in both situations, in the hypoxic rats the stimulation of efferent sympathetic activity is via chemoreceptors. In the hypotensive situation there is stimulation via the baroreceptors which probably induces not only efferent sympathetic activity but also a broad endocrine response. Observed differences in structural changes are very likely related to the striking difference in the neuroendocrine response to hypovolemia.

Some information is available concerning the function of lysosomes in shock. Lysosomes are microbodies contained in the cytoplasm of many cells throughout the body. By appropriate staining technics a wide variety of hydrolytic enzymes have been identified in lysosomes. Among these are acid phosphatases, proteolytic enzymes and beta-glucuronidase. Janoff and associates [16] demonstrated in rats and rabbits subjected to traumatic or endotoxic shock that there was a consistent elevation

of plasma acid phosphatase and serum beta-glucuronidase. Traumatic and endotoxic shock produced increases in free cathepsin and beta-glucuronidase in liver homogenates, and both procedures were shown to increase the fragility of the lysosomal particles containing these enzymes.

Bitensky and Galvan [3] found in rats that a laparotomy or manipulation of the liver increased the permeability of hepatic lysosomal membranes to glycerophosphate without necrosis of the cell. Bitensky *et al.*[4] created hemorrhagic shock in rabbits and examined splenic cells for acid phosphatase activity. They found some increased permeability of lysosomal membranes to the substrate beta-glycerophosphate in reversible shock, but diffusion and absence of the enzyme was seen in irreversible shock. The latter suggested disruption of the lysosomal membrane.

It has been postulated that as progressive damage to cells occurs from ischemia, chemicals or toxins, the permeability of the lysosomal membrane and ultimate disruption occur which permit necrosis of the cell to take place from the action of the potent enzymes contained within the lysosomes.[16] In the present study, lysosomal integrity generally was maintained after 2 hours of hypotension. In some instances (Fig. 7) there was a significant change in the hepatic lysosomes characterized by swelling, increased granularity and thickening of the membrane. This was an unusual change and was not characteristic of hepatic lysosomes in general.

While death from hypovolemia ultimately must result from disorganization at a cellular and enzymatic level, the biochemical capacity to produce energy can be restored until late in shock if the environment is suitable. The thesis that the metabolic alterations which occur during hemorrhagic shock reflect an environment in which tissues are exposed to the combined effects of hypoxia, the altered neuroendorine activity and the altered physical and chemical composition of extracellular fluid resulting from poor capillary perfusion is supported by the subcellular changes observed in the present study.

It would be desirable to design experiments in hemorrhagic shock to measure the chemical alterations in the blood stream, oxygen and phosphate exchange in organs, enzymatic and chemical changes in mitochondrial and lysosomal preparations, and enzymatic and ultrastructural changes in the cell at intervals after the onset of controlled hypotension. The difficulties, however, in obtaining reproducible results with many of the technics required discourage such an enterprise at present. Until it is accomplished more information obtained under controlled conditions may lead to a better understanding of shock.

Summary and Conclusions

Progressive changes in the ultrastructure of the cells of the liver, kidneys and striated muscle occur in hemorrhagic shock in rats. These are principally cytoplasmic edema and disorganization of mitochondria, and may be related to the known alterations of aerobic oxidation observed in shock.

It is suggested that the ultrastructural and biochemical changes observed in hypovolemic shock are not solely the result of cellular hypoxia but rather a result of the combined effects of cellular hypoxia, the neuroendocrine response to hypovolemia and the altered physical and chemical composition of extracellular fluid resulting from diminished capillary perfusion.

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Discussion

Dr. Walter J. Burdette (Salt Lake City): This is a perceptive study in depth of an old problem. Electron microscopy is invaluable for directing attention to sites of events most profitable for biochemical inquiry, although the difficulty of assessing results quantitatively is well known. In similar studies in our laboratory Drs. McMurtrey, Floyd, Ashford and I have noted differences in response of various organelles in different tissues