


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. McMurtry, Floyd, Ashford and I have noted differences in response of various organelles in different tissues.
and differences in these findings between animals subjected to shock and those subjected to hypoxia. Shock has been produced by application and release of tourniquets on the hind legs of fasted rats, and the tissues studied most extensively have been liver and muscle. This method of producing shock in the rat has been more reliable than hemorrhage in our hands. We have found that glutaraldehyde fixation is capricious, leading to some misinterpretation, and it has not been used in preparation of tissues from which conclusions are drawn. Otherwise the two studies have been similar as far as the techniques of electron microscopy are concerned.

As the only major observation possibly different than these reported, we have found in the liver that similar changes noted in shocked animals and those subjected to hypoxia are disruption of rough and prominence of smooth endoplasmic reticulum with implied alteration of enzymatic processes and protein synthesis, and in addition changes in the mitochondria (also reported by Dr. Holden) implying alterations in aerobic metabolism, of electronic transport, and phosphorylation. However, the vacuolization encountered with hypoxia has been absent or not striking in animals in shock. In cardiac muscle, similar findings in the states of shock and hypoxia have been damaged mitochondria and late disruption of myofibrillar architectural interrelationships. However, edema has been less pronounced in shock, whereas vacuoles and lysosomes have been more commonly encountered in the myocardium in shock than with hypoxia. Both in heart and liver, nuclear marginalization has been less common and damage in the nuclear membrane has been greater in shock than with anoxia. In general we have found less damage in shock than in hypoxia and the damage tended to be more focal in shock. Possibly the latter, along with the ability of mitochondria to survive and duplicate, even extracellularly, may account for the biochemical recovery possible following even the advanced stages of shock.

Fine structural studies, then, tend to suggest that the effect of shock on tissues cannot be regarded as entirely synonymous with that from hypoxia and anoxia; and the electron microscope offers a means for identifying the loci of greatest impact. In general, conclusions from our studies are consonant with those reported.

Dr. C. Barber Mueller (Syracuse, N. Y.): I, too, had the privilege of reviewing this work, and appreciate well the many difficulties these investigators have experienced in a study such as this.

The common and generally held concept which is currently being utilized, to unify shock concepts, is that of poor tissue flow, and the consequences to the body economy of such poor tissue flow. I think this paper should be one which finally puts to rest the notion that simple hypoxia with adequate flow is the same thing as the complex hypoxia is thought to be due to inadequate capillary perfusion, which is a characteristic finding following hemorrhage.

A few comments as to the significance of the ultrastructure changes—first the cell and then the mitochondria.

In our hands we found that the electron microscope itself was not particularly well suited for the study of the entire cell, particularly in attempts to survey a tissue and choose cells at random. It is difficult to survey enough parts of enough cells to gain a truly objective look at what a tissue really is, and to have that look free from many artifacts, partly of fixation and partly of the personal bias of the investigator who sits at the electron microscope and selects which pictures will be taken.

He discussed the rupture, for example, of the tubule cell associated with these periods of hypotension. We, too, found bits of tubule cells in the lumen of the tubules of the kidney, and spent many years trying to find out where they came from. They were always there, in all of our tissues, except, and only except, when we perfused the renal artery with a fixative and fixed the kidney in situ. So we finally assumed that these were bits of broken cells which were broken by the very act of taking the tissue specimen. This is the kind of artifact which creeps into examination at this level.

Now the mitochondrial changes are certainly fascinating. Dr. Burdette has been very interested in these, and back in 1952 Dr. Rodin reported on a study of the changes in the mitochondria, 15 minutes following the death of the animal. He was using mouse kidneys this time.

He found that the mitochondria at this time became swollen and rounded, they lost their cristae and the characteristic features of the organelle itself began to disappear. There is some suggestion that this is a reversible lesion at this stage. This has not yet been well worked out.

We in our studies have noted similar changes, and we ascribe this, in both the mitochondrion and the cell itself, to a change in the position of water with reference to the cell protein.

And so I think that I have to propose to you today that there are probably major intracellular translocations of water which occur with these situations, and that we are seeing here a reorganization of the intracellular structure. There is no evidence that the total cell mass can gain enough water to do this as an edema contribution from the exterior. And so, as Dr. Moore has talked to us about water translocation in the body economy in general, I think we might here propose that we see the results of translocations of water between the cell cytoplasm and the cell organelles.

Dr. William D. Holden (closing): There are a few technical questions that I would like to answer. All but a few of these tissues were fixed with osmic acid. A few were fixed in glutaraldehyde.
I did not have time to elaborate upon the changes we had observed with hypoxia. However, they do correspond very closely to what Bassi and her colleagues observed after two hours of hypoxia with 3% oxygen and 97% nitrous oxide. The formation of vescicles, especially within hepatic cells, is characteristic of hypoxia. We did not find, nor did she find, any significant mitochondrial changes. Dr. Mueller brought up one of the points that electron microscopists have to face, that is, their personal bias when they are scanning sections with the microscope in order to make a decision as to what pictures they will take. All that I can tell you is that in the course of this study, which has taken 18 months, hundreds of electron micrographs have been obtained and that the changes demonstrated to you today, in so far as we can objectively evaluate them, represent the consistent changes observed in these tissues. In the controls nothing was found that could be ascribed to poor fixation. We always used the same fixatives and embedding material for control and experimental specimens.

Dr. Mueller’s concept of intracellular water shift may be of considerable importance. When several of the micrographs that will appear in print are compared it is quite apparent that there is an intracellular water shift.

The influence of diminished blood flow through capillary beds, the changes in the various hormones that are elicited as a result of hypotension and hypovolemia, as well as the change in oxygen tension itself within the extracellular space surrounding cells make hemorrhagic shock an extremely complex phenomenon.

What we have attempted to do, and we believe as time goes on it will be even more profitable, is to make the effort to relate the presently known functional changes to the structural changes that can be observed at the subcellular level.