PROGRESS REPORT

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Principal Investigator: John J. Ghidoni
Baylor University, College of Medicine
Houston, Texas
During the past six months, several aspects of our research sponsored by NGR 44-003-018, have made appreciable gains. These will be handled in narrative form rather than in the form of preprints as we have done in the past.

During the past year, the Laboratory of Experimental Pathology in the Department of Pathology has been developed with National Institute of Health funding under the direction of the principal investigator. New facilities, equipment and personnel have been acquired; the technical staff now numbers 10 and the professional staff 8. A breadth of interdisciplinary coverage is now given to the NASA sponsored research under this grant.

A. Gastro-intestinal Tract: We have continued our electron microscopic study of granular cells present in jejunal epithelium as early as 6 hours following irradiation with 138 Mev protons (6,000 rads, whole body). "Karyolytic granules" have been reported in X-irradiated jejunal epithelium\(^1\). The granules in our material have a similar ultrastructure (Fig 1), and are Feulgen and PAS positive in light microscopic preparations; they frequently occupy a basilar position, displacing a deformed nucleus towards the lumen.

These granules contain remnants of nuclei and cytoplasm. These constituents are presumed to be in various stages of degeneration because of the varying vestiges of recognizable
ultrastructure remaining in these sequestered cellular components.

We have seen large numbers of cytolyosomes in epithelial cells in short-term animals (Fig 2). This cellular process of walling off segments of cytoplasm containing mitochondria and endoplasmic reticulum with consequent autodigestion appears to be a continuing process. In later specimens, entire cells appear to be involved; a single membrane surrounds both nuclear and cytoplasmic debris forming a giant cytolyosome. Such sacs of cellular debris are frequently seen to be clearly outside and separate from surrounding epithelial cells (Fig 3); however, comparable granules have been noted wholly within cells, there being only a single membrane between the contents of the granule and the cytoplasmic matrix (Fig 4).

Tentative findings suggest that epithelial cells can undergo various degrees of degeneration following irradiation; the severest degenerative form seems to be a large membrane bound sac of nuclear and cytoplasmic debris -- a giant cytolyosome. Other forms of giant lysosomes containing both nuclear and cytoplasmic debris are present within epithelial cells; it has been impossible thus far to identify the cell types from which these structures are derived. We found that the granular inclusions are Feulgen and PAS positive, and contain nuclear material when viewed with the electron microscope. Hampton, et al.,(1966)², have suggested that similar structures can be
derived from lymphocytes in X-irradiated jejunum. Our study does not preclude this possibility, however, no lymphocytes (degenerating or intact) have been located in proton irradiated jejunum. Most of these sacs of degenerating cellular debris are probably derived from other epithelial cells. Although direct morphologic evidence of phagocytosis of degenerating cells by epithelial cells has not been noted, this probably is the route of entry for the material in the sacs which contained both nuclear and cytoplasmic debris. The entire process requires further investigation and analysis.

B. Ultrastructure of Proton-irradiated Rhesus Liver (2.3 Bev; 3000 rads).

We have studied hepatic tissue from animals that were killed at 6, 12 and 18 hours after receiving 3,000 rads of whole-body irradiation from the 2.3 billion-electron-volt Cosmotron at the Brookhaven National Laboratory.

6 hour interval:

Surveys of the hepatic parenchyma have shown it to be essentially within normal morphologic limits (Fig 5). Glycogen compliment appears usual. Some subtle deviations may be present in slight dilatations of smooth reticulum and occasional swelling of microvilli; however, any definitive statement would be premature at this time.
12 hour interval:

The average hepatocyte had a rather normal appearance (Fig 6). The cytoplasmic glycogen content was sparse; occasional enlarged mitochondria were present. A few cells contained multiple cytolysosomes; some of these cells were contracted and had nuclei with scalloped nuclear envelopes (Fig 7).

Frequently, the microvillar pattern of the plasma membrane along the canalliculus was lost, there being several large blebs rather than conventional microvilli normally present (Fig 8). Blebs were also present in the space of Disse (Fig 9); the appearance was very similar to blebbing of microvilli along the sinusoidal border of hepatocytes in our previous 32 Mev proton study.

Membrane lined channels, that appeared to lay within cells, contained a dense unidentified material as if inspissated within these channels (Fig 10). Accumulations of dense material were also noted around some cells. Figure 11 illustrates this material in a space of Disse.

24 hour interval:

The changes noted in 12 hour specimens were more pronounced and abundant at 24 hours. In addition, several fibrin masses were noted in sinusoids and within spaces of Disse. The latter also contained erythrocytes. These fibrin masses were frequently associated with cells showing signs of cytoplasmic
degeneration (Fig 12). Alterations in nuclear pattern were not common; one nucleus with an unusual chromatin pattern that may represent early chromosome aggregations in prophase or clumping of chromatin is shown in Fig 13; the nuclear envelope is intact.

Electron dense material was frequently present in cytoplasmic spaces (Fig 14). This material is seen in channel-like spaces in the apical cytoplasm adjacent to bile canalliculi. Note the overall increased density of this hepatocyte compared to the three other hepatocytes in the micrograph.

The complete cytoplasmic disarray found in some cells is shown in Fig 15. Note the multiplicity of lipid droplets and widely scattered accumulations of inspissated dense material. Well-formed mitochondria were rare in such cells.

C. Analysis of liver cell mitochondria.

As previously reported, mitochondria from liver cells (6,000 rads; 32 Mev protons) suffer severe focal alterations in their membrane structure. We are working on the methods of demonstrating the elementary particles (ATP related) located along membranes of mitochondrial cristae in the conventional confirmation. Dr. Mayfield, a biochemist, has joined our group in the Laboratory of Experimental Pathology and is participating in this aspect of our study. We are separating out the mitochondrial liver cell fractions by ultracentrifugation.

The mitochondria, as separated in 0.25 molar sucrose,
are in the condensed conformation and must be first converted to conventional form. They are then lightly sonicated and stained negatively with phosphotungstic acid for electron microscopic study. We anticipate modifications in the structure and distribution of elementary particles along altered segments of cristae membranes.

We plan to assay portions of the electron transport enzymic system in these altered mitochondrial fractions if time and funding permit.

REFERENCES:
