A Study of the Ultrastructural Changes in Two Irradiated Tissues of Differing Radiosensitivities

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Abstract

A micromorphological study of two different tissues was made in which the only experimental variable was inherent tissue radiosensitivity. Mouse hepatocytes and duodenal epithelial cells were exposed to 1350 R of Co-60 gamma radiation. Tissues were collected 24 hours post-irradiation and prepared for electron microscopy. It was found that while changes in nuclear shape and dilation of nuclear envelopes and endoplasmic reticulum were common to both cell types, these changes were much more exaggerated in the duodenal cells. The duodenal cells also showed nuclear alterations and a type of secondary lysosome which were lacking in the hepatocytes. Fatty degeneration appeared solely in the hepatocytes.

Introduction

Accurate ultrastructural characterizations of cells, including irradiated cells, began in the mid 1950's (18). Since then, numerous accounts have described changes in a wide variety of irradiated tissues under just as wide a variety of irradiation conditions. As a result of these studies, it is clear that the nucleus is the focal point of a cell's reaction to ionizing radiation. However, to say that its reaction represents the full story is a gross oversimplification.

There has, as yet, been no study performed in which a radiosensitive cell's reaction has been compared to a radioresistant cell's reaction at the ultrastructural level with the only variable being cell type. The question that this study attempts to answer is: given that the only intended difference between two populations of cells is their radiosensitivity, will one cell type show significantly different micromorphological alterations than the other after irradiation?

Materials and Methods

Conditions of Irradiation

Male mice of the Cox (Swiss) strain weighing 30 to 36 grams were irradiated singly with a U.S. Nuclear 7500 Ci (3-15-66) Co-60 source. An exposure rate of 178 R/min was used to achieve a total exposure of 1355 R. The source was calibrated with the Victoreen R-meter. A total of seven mice were irradiated, and seven were used as controls. Animals were sacrificed by etherization 24 hours after irradiation. Tissues were fixed with glutaraldehyde which had been redistilled from a 25% stock solution in accordance with Fahimi and Drochmans (5).

The primary lobe of the liver and the first centimeter of the duodenum from irradiated and control mice were fixed immediately after sacrifice. The surface of the liver was rinsed with the primary fixative (3% glutaraldehyde in a 0.05 M phosphate buffer, pH 6.8) before being excised. The duodenum was intraluminally perfused with

the fixative before being excised. Liver and duodenum were minced and immersed in fixative at room temperature for 60 to 90 min. After primary fixation, the tissues were rinsed three times with a 0.05 M phosphate buffer followed by postfixation for 2 hours in 2% osmium tetroxide in a 0.05 M phosphate buffer, pH 6.8 at room temperature. The tissues were stained *en bloc* in a 2% aqueous uranyl acetate solution overnight at 4°C. This was followed by dehydration in a graded ethanol series and infiltration with a low viscosity plastic (26). Tissues were sectioned with diamond knives on a Sorvall MT-2B ultramicrotome at a thickness of 600 A. The sections were placed on 300 or 400-mesh bare copper grids, stained with lead citrate (23) and examined on a Hitachi HU-11A electron microscope using an accelerating voltage of 50 KV.

Results

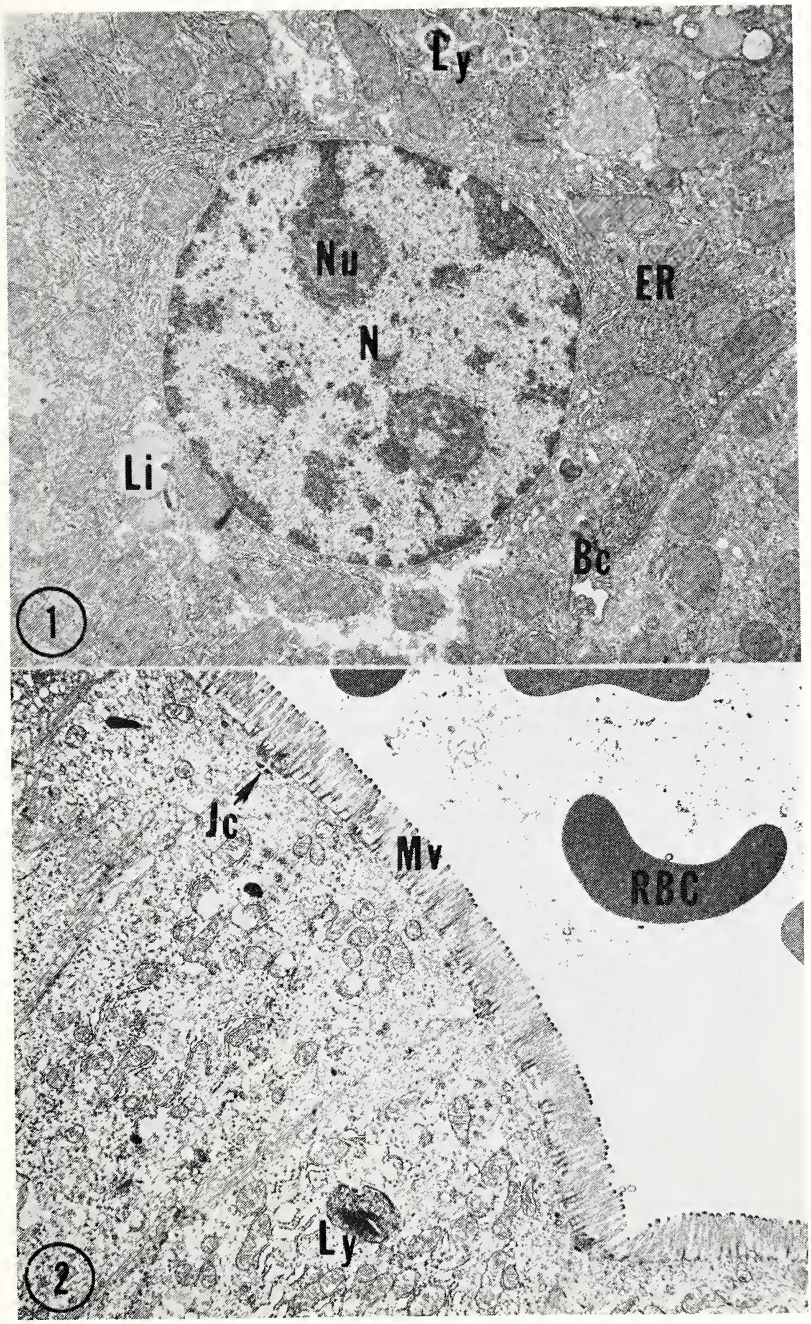
Morphology of Control Tissues

No obvious distortion of the ultrastructure of the mouse hepatocyte was observed in control tissues (Fig. 1). The nuclei were either spherical or near spherical and possessed one or two distinct centrally located nucleoli. The nuclear envelope exhibited no swelling or distension. The heterochromatin appeared as a very thin and consistent border around the inner aspect of the nuclear envelope, avoiding the nuclear pores. Mitochondria were neither swollen nor distorted and cristae appeared linear and unbroken. Rough endoplasmic reticulum (RER) was very abundant and was most often found as rather long, double-membrane units encircling mitochondria or lipid droplets or as a cytoplasmic parallel array of several double-membrane units. The two unit membranes of the RER maintained a strict parallel arrangement. Ribosomes were found either as small, unbound clusters (polyribosomes) or as part of the RER. A small natant population of lipid droplets, primary and secondary lyosomes, and peroxisomes was common.

Control fixations of the duodenal epithelial cells yielded similar morphological results (Fig. 2). Duodenal nuclei often appeared more elipsoidal than hepatocyte nuclei. The RER of the duodenal cells did not maintain the same parallelism between the two unit membranes that was shown in the hepatocytes but, instead, took on a more circular or vesical-like configuration. There was a great deal of variability in the appearance of the mitochondria. They were apparently still unstable after the primary fixation process and, consequently, showed some swelling and dissolution of the matrix. Ribosomes were predominantly attached to the endoplasmic reticulum (ER) or were found free in the cytoplasm as polyribosome clusters. An occasional primary or secondary lysosome was also found. The apical ends of the cells were covered with a dense population of microvilli.

FIGURE 1. *Control hepatocyte. N, nucleus; Nu, nucleolus; ER, endoplasmic reticulum; Be, bile canal; Li, lipid; Ly, Lysosome; Mag.=3417X.*

FIGURE 2. *Control intestinal epithelial cells. Mv, microvilli; Ly, lysosome; RBC, red blood cell; Je, junctional complex; Mag.=5576X.*


Ly
Nu
ER
N
Li
Bc
1
Ic
Mv
RBC
Ly
2

The Irradiated Hepatocyte

One of the more striking features of the irradiated hepatocyte was the appearance of the reticular network (Fig. 3). The cells had ER that was very dilated sometimes to very exaggerated extremes. When this characteristic appeared at all, it was universal throughout the affected cell. The voids caused by this dilation of the ER did not contain any electron dense debris. Along with the dilation of the ER, there occurred a similar dilation of the nuclear envelope which was in some cases quite exaggerated, appearing only between the nuclear pores. Lobing or "blebing" of the nucleus was frequently observed. The nucleus became more or less amorphous in shape, possessing tabs or lobes which extended into the cytoplasm. Changes in shape of the nucleus and ER occurred together in almost every case. The exceptions were a few instances where swelling of the ER occurred without any nuclear alterations. Increased cytolytic activity was evidenced by an increase in size, complexity and number of secondary lysosomes (Fig. 4). These cytolytic bodies often possessed various sizes of lipid droplets, membrane fragments, and osmiophillic plaques and tended to cluster in small groups. Mitochondrial changes were limited to a slight to moderate loss of density of the matrix or a disruption of the internal membrane system (Fig. 5). No alterations were noticed in the distribution of the ribosomes.

The Irradiated Duodenal Epithelial Cell

As with the hepatocyte, the most obvious changes in the duodenal cells involved the nucleus and the endomembrane system. The ER was very vesiculated (Fig. 6), often so extensively that the cytoplasm appeared somewhat frothy. The nuclear envelope took on a swollen or distended character, and the nucleus became amorphous.

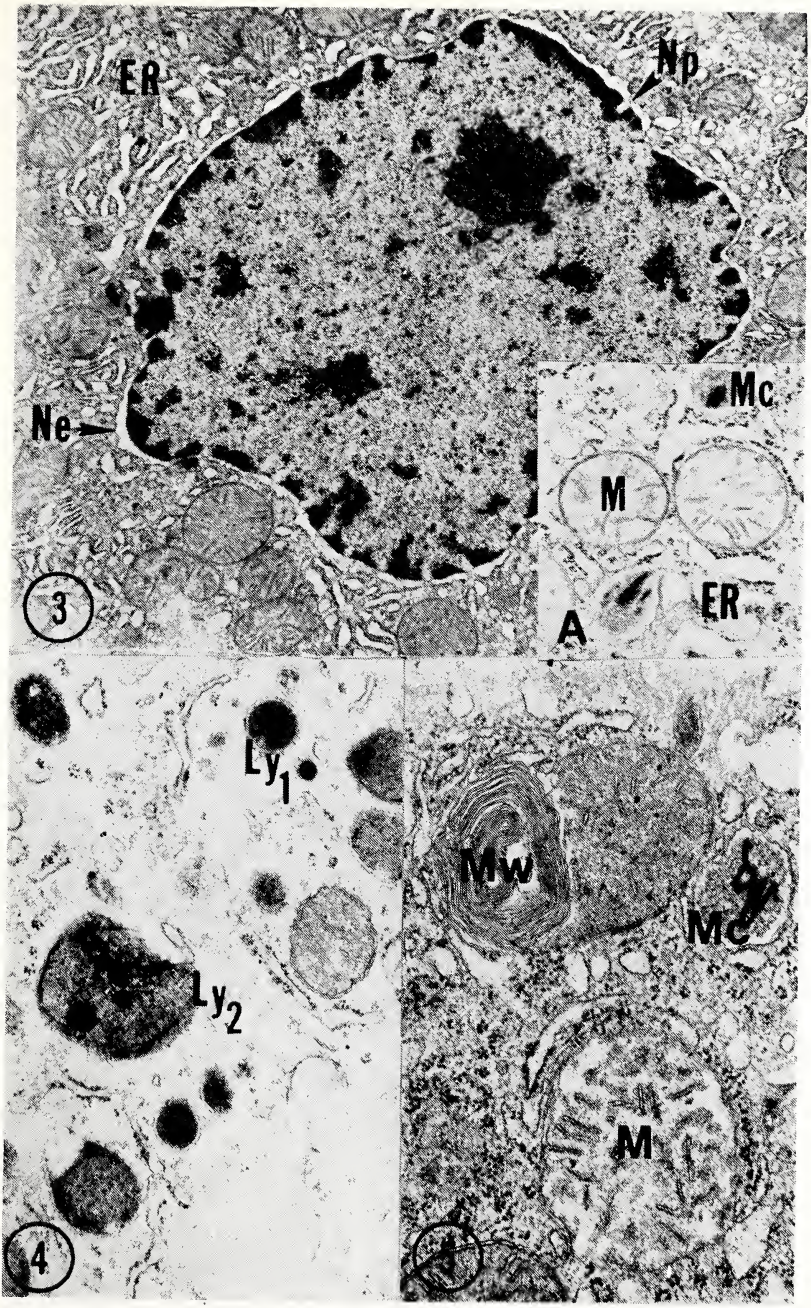
The main difference between the irradiated hepatocyte and the irradiated duodenal epithelial cell was one of degree. While nuclear alterations in the hepatocyte were confined to changes in outline, nuclear changes in duodenal cells were much more elaborate and, on occasion, so extreme as to give the appearance of isolated islands of chromatin, cytoplasmic pockets, or pseudopodia-like extensions of the nucleus (Fig. 7). In some cases, the duodenal nuclear envelopes would swell to the extent of becoming balloon-like. Figure 7 shows one case in which the nuclear envelope had partially dissolved and cytoplasmic and nuclear contents had become mixed. Changes within the nucleus were also more pronounced in the duodenal cells. There were instances of nuclear caps (Fig. 8) and pycnotic nuclei (Fig. 9), neither of which was found in the irradiated hepatocyte.

FIGURE 3. *Irradiated hepatocyte. ER, endoplasmic reticulum; Np, nuclear pore; Ne, nuclear envelope; Mag.=5495X.*

FIGURE 3A. *Irradiated hepatocyte. ER, endoplasmic reticulum; M, mitochondria; Mc, microbody; Mag.=24,074X.*

FIGURE 4. *Lysosome aggregate of irradiated hepatocyte. Ly_1, primary lysosome; Ly_2, secondary lysosome; Mag.=4689X.*

FIGURE 5. *Mitochondria of irradiated hepatocyte. M, mitochondria with a low density matrix; Mw, membrane whorl; Mc, microbody; Mag.=19,428X.*


ER
Np
Ne
3
Mc
M
A
ER
Ly1
Ly2
4
Mw
Mc
M

The lysosomal systems of the two cell types behaved similarly. The hepatocyte lysosomes rarely became more elaborate than those shown in Fig. 4. However, the duodenal lysosomes were more numerous, considerably larger, and more complex. There was a great heterogeneity of contents within the duodenal lysosomes which included whole areas of cytoplasm, extensive collections of membranes, mylenated figures, lipid droplets of various sizes and densities, and occasionally an entire organelle (Fig. 10). Some of the duodenal lysosomes were considerably larger than their hepatic counterparts and in several instances, occupied approximately one-third of the volume of an epithelial cell. Other variants of the lysosomal system present only in the irradiated duodenal epithelial cell were the multivesicular body, residual bodies, and membrane whorls. As with the irradiated hepatocytes, there was no perceptible change in ribosomal distribution; however, the Golgi complex did exhibit isolated incidences of hypertrophy.

Discussion

Cell Population Kinetics

Unless hepatic cells are stimulated by artificial means, such as partial hepatectomy, they will divide rarely, if at all (19). The duodenal epithelial cells, however, divide very rapidly and are known to be very radiosensitive (16). Like many radiosensitive systems, the intestinal epithelium is a cell renewal system (15). The majority of the epithelium consists of crypt cells which move in a coherent sheet up the villi where they are extruded at the tip (21, 27). The generation time of proliferating crypt cells is about 12 hours.

Light microscope studies have shown that mitotic activity is abolished in stem cells shortly after a supralethal dose of x-rays (27). X-irradiation with 1 krad leads to depletion of the intestinal epithelium, "anenterocytosis", through aplasia combined with continued loss of mature cells (16). Movement of the entire epithelial sheet and extrusion of cells from the tips of the villi seems to be blocked for about 0.5 day after irradiation (10). It would seem unlikely then, that ultrastructural changes found in this study were due to any magnification effects resulting from differences in cell cycle times, since mitosis is blocked by the magnitude of exposure that was used.

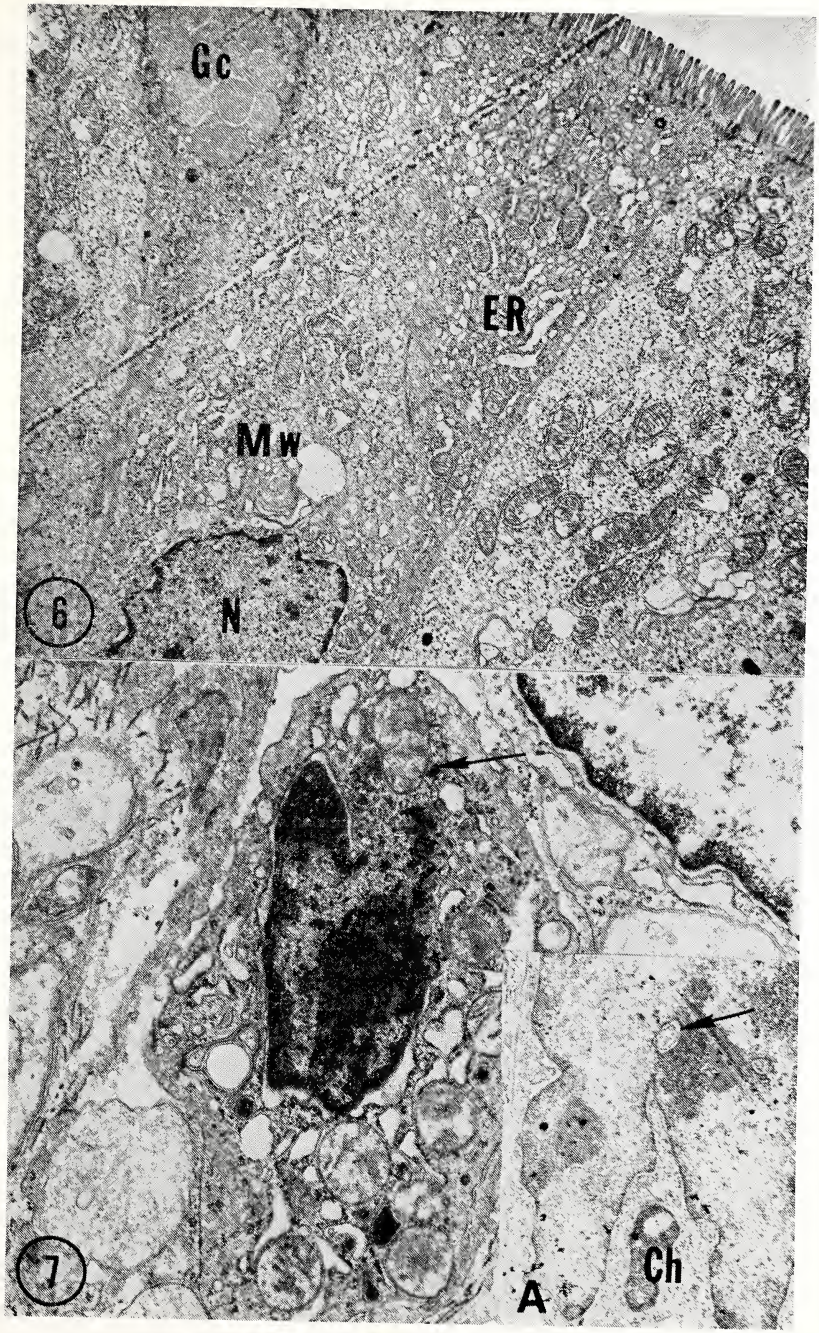
Nuclear Alterations

In both the hepatocyte and the duodenal epithelial cells, nuclear alterations of two main types were found. First, as in several other studies, the nuclear envelope exhibited slight to severe swelling (2,6,7, 11,25). The severity of this alteration was always greater in the duodenal cells. A second alteration which had taken place in the nuclei

FIGURE 6. *Irradiated intestinal epithelium. ER, endoplasmic reticulum (dilated); Gc, goblet cell; N, nucleus; Mw, membrane whorl; Mag.=2728X.*

FIGURE 7. *Transverse section of intestinal epithelial cell. Nuclear contents have partially spilled into cytoplasm (arrow); Mag.=4986X.*

FIGURE 7A. *Extremely lobed nucleus of irradiated intestinal epithelial cell. Cytoplasmic packet (arrow); Ch, isolated island of chromatin; Mag.=1740X.*


Gc
ER
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N
6
7
A
Ch

of both cell types was a change in the outline of the nucleus. This "blebing" or lobing was, again, more exaggerated in the duodenal cells than in the hepatocytes. Both swelling of the nuclear envelope and nuclear blebing are not uncommon to irradiated tissues (2,3,11). Within 1 hour after a 3-krad dose, irregularly shaped nuclei were found to be commonplace in the epithelium of the irradiated intestine along with an increase in the size of the nuclei (20). Nuclear envelopes of irradiated epithelial duodenal cells were "tremendously swollen" as early as 2 hours after an exposure of 1350 R (10). Irregularities in nuclear shape were found as early as 90 min after a 1350 R exposure along with "chromatin-like areas" dispersed within the cytoplasm or bulging from the nuclear surface (13). It is interesting to note that the irregular shape of the nucleus after irradiation is also observed in transformed cells.

Endoplasmic Reticulum

Distention and dilation of the ER was also a very common characteristic of both cell types used. Dilated ER is not uncommon in irradiated tissue (1,6,11,14). In hepatic parenchymal cells ER was observed in varying degrees of dilation at all post irradiation intervals greater than 4 hours after a 3-krad dose of Co-60 gamma rays (8) and was found as early as 2 hours post irradiation in the epithelium of the duodenal crypts in x-irradiated mice (10). Along with the distention and dilation of the ER, an apparent loss of ribosomes was observed in several studies (13,17,20,12). No obvious reduction in the number of ribosomes was found in either of the tissues used here.

Lysosomes

One of the more dramatic changes observed in this study was the change in the lysosomal component of irradiated cells. There was an increase in the number and internal complexity of these organelles in both cell types along with an increase in the number of primary and secondary lysosomes. However, in duodenal cells the size and complexity of secondary lysosomes was far greater than in hepatocytes.

Changes in the lysosome population of hepatic parenchymal cells have been observed as early as 2 min post irradiation (8). These changes were referred to as "membranous annuli" enclosing a variety of cytoplasmic components. Such structures are identical, by definition, to autophagosomes. Rene et al. (22) found a simultaneous increase in both the number of lysosomes and the activity of acid phosphatase in hepatocytes of rats 2 hours after a 2-krad dose. Changes in the lysosomal

FIGURE 8. *Irradiated intestinal epithelial cell. Nucleolar cap, Nc; Mag.=2250X.*

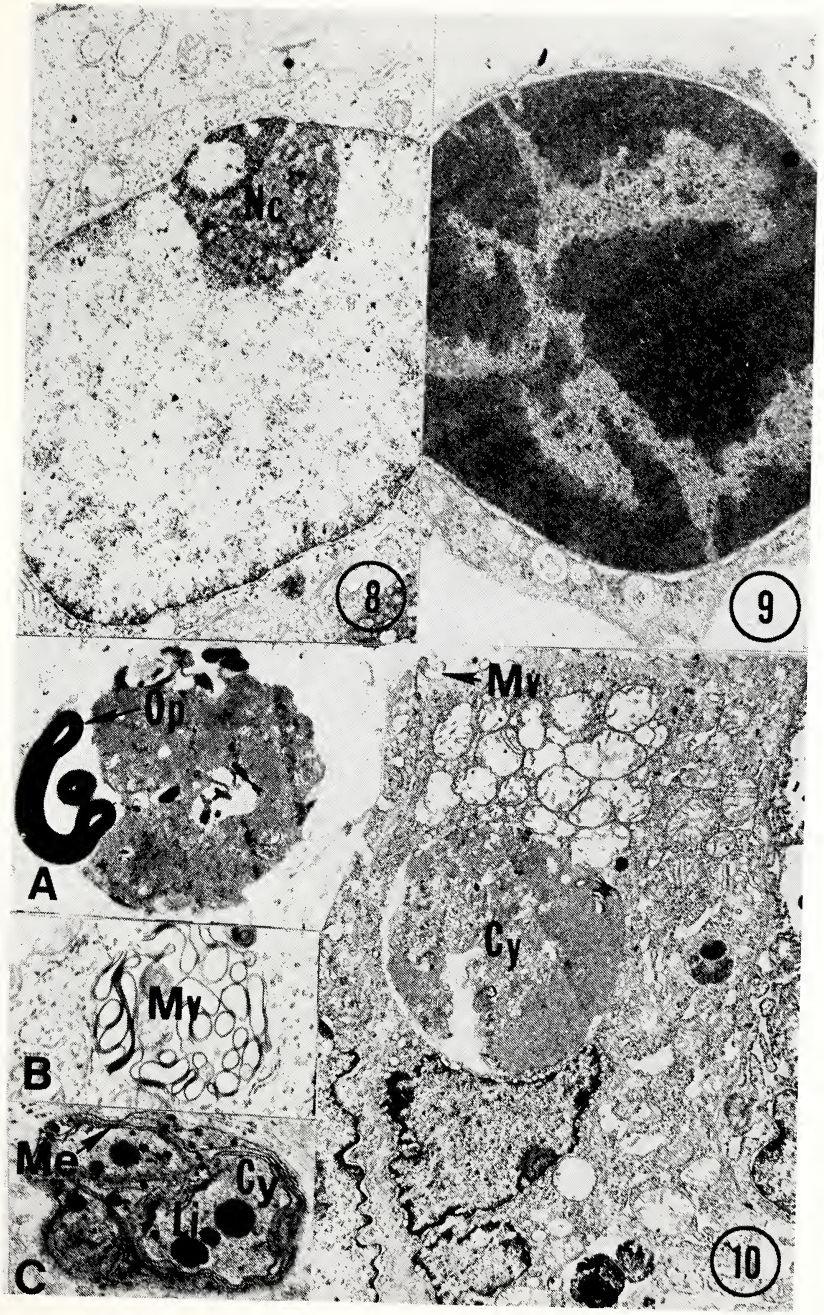
FIGURE 9. *Pycnotic nucleus, irradiated intestinal epithelial cell. Mag.=2931X.*

FIGURE 10. *Large cytolysosome in irradiated intestinal epithelial cell. Mv, microvilli; Cy, cytolysosome; Mag.=3960X.*

FIGURE 10A. *Irradiated intestinal epithelial cell, cytolysosome with osmiophilic plaque, Op; Mag.=20,833X.*

FIGURE 10B. *Irradiated intestinal epithelial cell. Mv, multivessicular body; Mag.=11,505X.*

FIGURE 10C. *Irradiated intestinal epithelial cell, cytolysosome. Me, membranes; Li, lipid droplet; Cy, cytoplasmic inclusion; Mag.=10,500X.*


Nc
8
9
Op
A
My
Cy
My
B
Me
Cy
Li
C
10

content of irradiated duodenal epithelial cells is considerably more elaborate than in irradiated hepatocytes. An increase in the number of multivesicular and dense bodies was observed in these cells as early as 90 min after a 1350-rad dose of x-rays (9,10,12,13). The number of cytolysosomes was also increased beginning at 3 hours post irradiation. These cytolysosomes were found to contain mitochondria, ER ribosomes and nuclear fragments.

Other Cellular Components

In the irradiated hepatocyte, fatty degeneration has been demonstrated (8,19). In this study, it appeared as the deposition of numerous clear vacuoles from which the lipid component was leached out during processing. Hendee and Alders (8) found these lipid vacuoles as early as 4 hours after exposure of rats to 3 or 16 krad.

No obvious changes in the morphology of the Golgi complex was found in this study. Hugon et al. (10) reported a reduction of the Golgi area to a few cysternae. This finding was predominant at 2 hours post irradiation. It is conceivable that what was seen in Hugon's study may have been the result of unusual sectional geometry, or what may have been seen was small clusters of smooth ER.

The significance of the present study lies in the experimental design. If one wishes to consult the literature and compare ultrastructural characteristics of two different irradiated tissue types, one would undoubtedly find the two particular tissues of interest. However, the exposure, exposure rate, the post irradiation time at which the tissue was collected and the species from which the tissue came would all very probably be at variance with one another. In this study the only variables are those inherent in the two different tissues. Even more significant are the differences seen when all the above-mentioned variables are kept constant, for it is these differences that are the net result of how two different cell types cope with exposure to ionizing radiation.

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