MORPHOLOGICAL ANALYSIS OF DENUDATION OF THE VILLI IN THE INTESTINAL FORM OF ACUTE RADIATION SICKNESS

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Portions of the duodenum, jejunum, and ileum of sexually mature rats and mice were studied under light and electron microscopes on the third and fourth days after irradiation in doses of 1000, 1500, 7000, and 15,000 R. Denudation of the stroma of individual villi was observed in paraffin sections of the mucous membrane of the small intestine 3-4 days after irradiation. However, examination of thin and semithin Epon sections under the light microscope and of ultrathin sections in the electron microscope showed that the villi always were covered by enterocytes, the cytoplasm of which was loaded with lipid droplets and showed loss of its structural details. In the intestinal form of acute radiation sickness intravital denudation of the villi thus does not take place and its existence in paraffin sections is the result of unsuitable treatment of the material.

KEY WORDS: villi of the small intestine; irradiation; enterocytes.

It has been asserted in the recent radiobiological literature that 3-4 days after irradiation of animals in doses of 1000 R or more considerable areas of the stroma of the mucous membrane of the small intestine become denuded [1, 2, 8, 9], with a resulting disturbance of the water and salt balance in the body [10] and leading ultimately to death of the animals [5, 8]. During the investigation of paraffin sections of the small intestine from rats and mice, the present writers also observed denudation of the villi, but on analysis of thin and semithin Epon sections in the light microscope and also of ultrathin sections in the electron microscope, a different picture was observed. These findings are discussed below.

EXPERIMENTAL METHOD

Experiments were carried out on 20 sexually mature Wistar and August rats, 25 noninbred mice, and 15 CBA mice. The animals were irradiated on the Gamma-Cell-220 apparatus in doses of 1000, 1500, and 7000 R (dose rate 30 R/sec) and on the Gub-20,000 apparatus in doses of 1000 and 15,000 R (dose rate 83.4 R/min). Pieces of tissue for histological and electron-microscopic investigations were taken from the duodenum, jejunum, and ileum 72 and 96 h after irradiation. In the first case, areas of intestine were fixed in 10% neutral formalin solution and in Bouin's and Carnoy's fluids. The material was embedded in paraffin wax. Sections 5-6 μ in thickness were cut longitudinally and transversely and stained with hematoxylin–eosin and Schiff's reagent and counterstained with methyl green. Pieces of tissue for electron microscopy were fixed by Palade's method and embedded in Epon 812 after the standard treatment. These Epon sections were cut under a binocular loupe by means of a safety razor blade and studied under the light microscope unstained. Semithin (1-2 μ) sections, and later ultrathin sections (500-700 Å) from the same block were cut on the LKB ultratome. Ultrathin sections were studied in the IEM-5y electron microscope.

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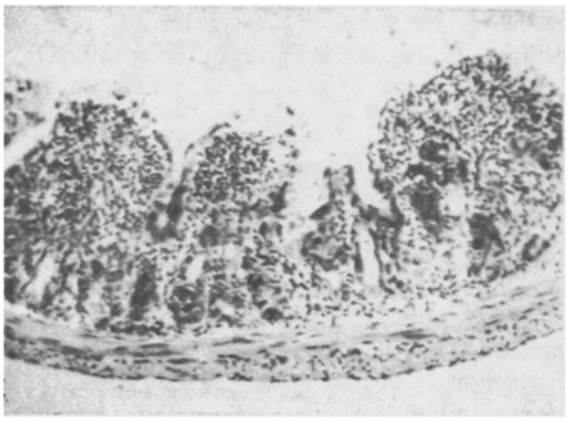

Fig. 1

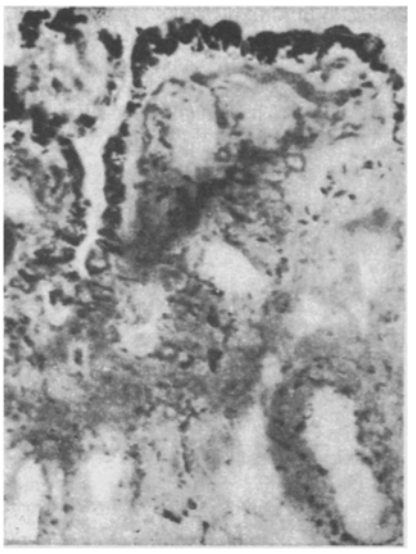

Fig. 2

Fig. 1. Denudation of apices of villi on third day after whole-body irradiation of mice in a dose of 1000 R. Paraffin, hematoxylin–eosin, 120 ×.

Fig. 2. Osmiophilia of epithelium covering villi on third day after whole-body irradiation of mice in a dose of 1000 R. Epon 812, unstained section, 180 ×.

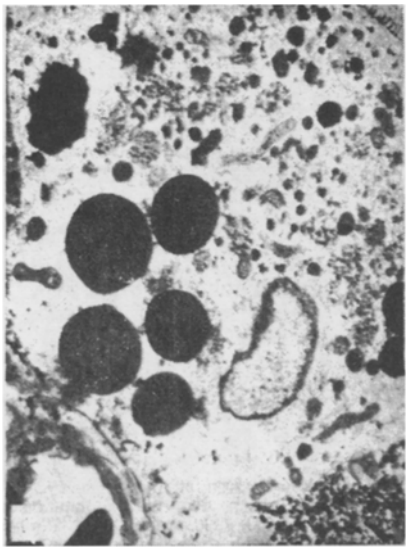

Fig. 3. Enterocyte from apex of villus. Cluster of lipid drops and edema of basal part of cell on third day after whole-body irradiation of mice in a dose of 1000 R. Epon 812, ultrathin section, 2200 ×.

EXPERIMENTAL RESULTS

Examination of histological sections from wide areas of the duodenum, jejunum, and ileum showed that 3-4 days after irradiation the villi were covered by enterocytes of the third type [8]. The apices of individual villi were denuded of their covering epithelium and the stroma were in direct contact with the intestinal contents (Fig. 1). This histological picture of the mucous membrane of the small intestine is the same as that given in the literature and it shows that immediately before death of the animals certain parts of the stroma of the mucous membrane were denuded. Destructive changes in the epithelium covering the villi and the denudation of their stroma were more marked in animals irradiated with smaller doses 1000-1500 R) than with high doses (7000-15,000 R).

However, the study of thin and semithin Epon sections of the intestinal mucosa of these same irradiated animals failed to reveal any denuded villi. In these sections the apices of the villi and their lateral surfaces were covered by strongly osmiophilic cells (Fig. 2). The osmiophilia of the apices of the villi were more marked after irradiation in doses of 1000-1500 R. Electron-microscopic investigation also showed that the stroma of the villi were always covered with enterocytes, although their ultrastructural organization was extremely modified (Fig. 3).

It can be concluded from these results that denudation of the villi does not take place after irradiation in the doses specified. The phenomenon of denudation of the villi observed in histological paraffin sections is always the result of removal of the enterocytes during processing of the material. This happens as follows. As Fig. 2 shows, the apices of the villi are covered with strongly osmiophilic cells; these cells, as Fig. 3 shows, are loaded with lipid granules and their organoids are greatly reduced. The degree of lipid loading varies between individual cells and also in the villi as a whole. Considering this fact, and knowing that lipid drops are readily dissolved in organic compounds (during histological treatment xylol or chloroform is always used) removal of the lipid drops from the enterocytes must certainly be expected. In fact, in paraffin sec-

tions large numbers of enterocytes with vacuoles in their cytoplasm can be seen. These vacuoles correspond to areas of cytoplasm which have lost their lipid granules. If the cell is extremely overloaded with lipid granules (Fig. 3) and contains virtually no organoids, after solution of the lipids the residual part of the cytoplasm will be so structurally disorganized that it is readily destroyed during histological treatment or can no longer be positively stained. As a result the false impression of denuded villi is created. With more massive doses of irradiation (7000-15,000 R), when lipid loading of the enterocytes is slight in degree, no denuded villi are seen in the sections.

Yet another factor that could produce denudation of the villi must also be considered. This is the degenerative change and edema of the stroma in the tunica propria of the mucous membrane which develop in the late stages after irradiation [3, 4, 6, 7]. Edema fluid accumulates chiefly around the capillaries of the basement membrane of the tunica propria and between it and the enterocytes, producing intracellular edema and displacing the organoids into the perinuclear zone. This makes the covering epithelium, already damaged by radiation, additionally vulnerable. These two factors, high lipid loading of the enterocytes and necrobiotic changes in the stroma, which take place in the terminal stage of acute radiation sickness, lead to denudation of the villi during processing of the material, and this phenomenon has been looked upon by many investigators as taking place during life. Considering the observations described in this paper, the writers consider that caution is necessary when interpreting this fact.

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