Neoplasms of the Glandular Stomach in Mice Irradiated with X-Rays or Fast Neutrons*

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The incidence of gastric adenocarcinoma in animals is extremely low (13,14), and attempts to produce such tumors with various carcinogenic agents (11), including ionizing radiations (8), have been only moderately successful, at best.

The present report deals with a number of malignant and premalignant lesions of the glandular stomach of the mouse occurring after whole-body irradiation with x-rays or fast neutrons. The findings suggest that these two types of radiation may act by different biological mechanisms to produce gastric neoplasms.

MATERIALS AND METHODS

The mice used in these experiments were LAf₁ (C57L×AJF₁) hybrids, both males and females.

One group of 162 mice received, at age 4-5 months, a single whole-body dose of fast neutrons (8 Mev) in the Berkeley 60-inch cyclotron. The dose was in the sublethal range (270-520 rep), and the irradiation procedure and characteristics of the neutron beam have been described elsewhere (9). The mice were then followed until natural death, as previously reported (9), up to 21 months after irradiation.

A second group of 250 mice, a composite from several subgroups of mice, received at the age of 8-4 months a single whole-body "supralethal" dose of 250 kVp x-rays (790-810 r). The mice then received a single intraperitoneal injection of mouse spleen homogenate to protect them against acute radiation death (3). Of the treated group, 192 (76 per cent) mice survived and were followed until natural death—up to 21 months postirradiation.

A group of 412 normal LAf₁ mice served as a nonirradiated control population and were followed simultaneously with the irradiated animals. These mice survived up to 34 months of age.

In all groups, the stomach was carefully examined at autopsy, and only those stomachs with gross mucosal lesions were examined microscopically. These stomachs were fixed in 15 per cent formalin, processed routinely, and stained with hematoxylin and eosin. The Congo Red stain for amyloid was also used when indicated.

RESULTS

Group 1 (neutron-irradiated).—In total, 67 mice, or 41 per cent, of this group exhibited gastric lesions at death (Table 1). All lesions but one appeared between 11 and 21 months after irradiation, and no sex difference in incidence was noted.

Fifty-eight of these lesions were classified microscopically as "hyperplasia." Grossly, these consisted of single or multiple mucosal nodules, 2-4 mm. in diameter, usually near the pylorus. Although ordinarily they were sessile, an occasional nodule assumed a polyploid configuration, with prolapse through the pylorus in a few instances. Generally, these lesions did not appear to have contributed to the death of the animal, but in a few cases pyloric obstruction had apparently occurred.

Microscopically, the mucosal nodules consisted of heaped-up, disoriented hyperplastic glands, lined by mucous cells or tall, columnar, indifferent cells with uniformly pink cytoplasm. Chief cells and zymogen cells were not observed in the lesions. In 45 of the 58 cases, the hyperplastic glands had penetrated the muscularis mucosae and invaded the submucosa (Fig. 1). These invasive glands were generally well differentiated and extended into the muscularis propria in only two instances, so that this lesion was considered premalignant, rather than truly malignant.

In three cases, mucosal nodules contained areas of poorly differentiated cells without definite...
glandular structure (Fig. 2). In another nodule, a small region of squamous metaplasia was observed in the hyperplastic glands.

There was a moderate inflammatory reaction associated with almost all these hyperplastic lesions, with both acute and chronic inflammatory cells present. Superficial acute ulceration was noted in a few instances. The stromal elements of the gastric wall, including the blood vessels, generally appeared normal, although some of the invasive hyperplastic glands were supported by a loose, fibrous stroma.

Four mice, or 2 per cent, of this group were found to have true adenocarcinomas of the glandular stomach. Grossly, these lesions were slightly larger than the hyperplastic nodules and had obviously invaded the stomach wall extensively. Under the microscope, these tumors consisted of atypical glandular acini lined by irregular columnar cells with hyperchromatic nuclei showing occasional mitotic figures. Cords and nests of vessels. In twelve of the eighteen hyperplastic lesions in this group, the small arteries and arterioles of the gastric mucosa and submucosa showed extremely severe degenerative changes. This was characterized by a markedly eosinophilic thickening of the vessel walls, to the point of actual occlusion in some instances. The endothelial cells showed degenerative nuclear changes, and the Congo Red stain revealed the presence of amyloid deposits in the vessel walls. This vascular change was usually most striking in the areas of hyperplasia, involving either the mucosal vessels within a hyperplastic nodule, or the submucosal vessels just beneath it (Figs. 3-4). Four of the other six mice in this group showed less severe sclerotic vascular changes.

These degenerative arteriolar lesions in the x-radiated mice were not limited to the stomach. Similar changes were observed in other organs, particularly the kidney, where severe arteriolosclerosis, either with or without amyloidosis, ap-
lesions in the control animals, fourteen occurred in mice more than 25 months of age (e.g., older than any of the neutron-irradiated or x-radiated animals).

**DISCUSSION**

The findings reported here indicate a slight but definite carcinogenic effect of fast neutrons on the glandular mucosa of the mouse stomach. Although the incidence of frank adenocarcinoma is only 2 per cent, there are a large number of atypical hyperplastic lesions (36 per cent), with submucosal invasion present in most of them. Whether such hyperplastic gastric lesions can eventuate in true cancer has long been debated. In man, of course, the chronic gastritis associated with carcinoma is of the atrophic, rather than the hypertrophic type. In animals, hypertrophic gastritis, whether spontaneous or experimentally produced, has not been considered a premalignant lesion (6). However, examination of our material, as well as the studies of others (5, 11), certainly suggests that hyperplastic mucosal lesions such as we have described, with progressive invasion of the submucosa, may well represent early steps in the development of true carcinomas.

The first stages in the pathogenesis of these invasive hyperplastic nodules are not known. Lushbaugh (7) has suggested that “intestinalization” of gastric glands with fusion of surface cells may cause trapping of secretions and microcyst formation in the deeper layers of the mucosa. Subsequently, local increases in pressure would tend to force glandular elements through the muscularis mucosae into the submucosa. The well differentiated character of the invasive glands in such lesions indicates that this explanation is more accurate than the assumption that these are actively invasive, frankly malignant cells at the time they first penetrate the muscularis mucosae. In his study of mice exposed to an atomic bomb explosion, Lushbaugh encountered no definite adenocarcinoma. However, the lesions which he classifies as “benign adenomatous gastritis” appear essentially identical with those which we have considered premalignant “hyperplasia with invasion.”

Adenocarcinomas of the stomach in our neutron-irradiated mice are considerably less frequent than adenocarcinomas of the cecum and small bowel (9). In all, eighteen, or 11 per cent, of this group developed mucoid carcinomas distal to the stomach, eleven in the cecum and seven in the small intestine. None occurred in mice receiving an 8 Mev neutron dose of less than 400 rep, and all but two appeared between 7 and 14 months after irradiation. The greater number and earlier appearance of these tumors are probably related to the fact, observed in this laboratory and elsewhere (1), that fast-neutron irradiation results in greater acute damage to the cecum and small bowel than to the stomach. It is of interest that the gastric lesions did not occur until 11–21 months after irradiation, and no definite dose effect was observed in the stomach over the range studied (290–580 rep).

In comparing the number of gastric lesions in the neutron-irradiated mice to the number in the x-radiated animals, the higher incidence in the former group is probably again related to differences in the amount of acute damage produced. As observed previously (9), fast neutrons appear to cause greater damage to all parts of the gastrointestinal tract of the mouse than do comparable doses of x-rays. Although the basis for this differential action is still not clear, it may result from different mechanisms of ionization of these two types of radiation. It appears from purely physical studies (10) that, at least for a small animal like the mouse, the major reaction in tissue-like material, of fission-spectra neutrons is proton recoil. Thus, the gastrointestinal tract of the fast neutron-irradiated mouse is actually receiving densely ionizing proton irradiation, resulting in many more ion pairs per unit volume than from a comparable rep dose of x-rays. For larger animals, a similar depth dose of these same fast neutrons yields a much higher proportion of the radiation as gamma rays (n, y reaction), with considerably less dense local ionization. This difference helps to explain the observed fact (2) that intestinal injury is not exceptionally great in the neutron-irradiated dog (or rat); on this basis, fast neutrons, at least with these energies, would not necessarily produce gastrointestinal tumors in large animals, including man. On the other hand, proton irradiation, if it were to reach the organ as such, might well be definitely carcinogenic for the gastrointestinal tract of any animal.

As compared with the control animals, the x-radiated group shows a significant increase in gastric glandular lesions. Although only one definite adenocarcinoma was observed, the incidence of hyperplastic lesions was nearly twice that of the control group; and they appeared on the average, in mice 8 months younger than the comparable controls.

The importance of the observed vascular changes in the pathogenesis of these mucosal lesions is difficult to evaluate. As noted by Stewart and Lorenz (12), the possible significance of local vascular lesions in the production of cancer by x-rays or other carcinogens has been discussed for
some time. Although there is no statistical correlation between senile vascular changes and tumors occurring spontaneously at different sites in man and animals, the possibility that local vascular embarrassment could act as a promoting agent upon cells already conditioned by ionizing radiation cannot be excluded. Certainly, the coincident occurrence of severe occlusive arteriolar changes and markedly atypical mucosal hyperplasia in our series is striking. Grant and Ivy noted similar changes in submucosal blood vessels beneath areas of mucosal hyperplasia following the introduction of methylcholanthrene-impregnated thread into the wall of the rat stomach.

In a previous report by Furth et al. (4), it has been observed that, for the production of renal arteriolosclerosis and glomerulosclerosis in LAf1 mice, a minimum dose of 500 r is required. This apparent "threshold" phenomenon probably accounts for the absence of gastric arteriolosclerosis in the control mice and neutron-irradiated (290-580 rep) mice of our series.

SUMMARY

Groups of LAf1 mice received whole-body irradiation with fast neutrons (290-580 rep) of x-rays (800 r). Of 162 neutron-irradiated mice, 36 per cent developed hyperplastic lesions of the glandular stomach, and 2 per cent had adenocarcinomas. Only 9 per cent of 192 x-irradiated mice developed such hyperplastic lesions, and only one adenocarcinoma was observed. Severe occlusive arteriolosclerosis was associated with the mucosal lesions in two-thirds of the x-irradiated mice, but was present in only one of the neutron group. Of 412 nonirradiated control mice, 5 per cent developed gastric hyperplasias. No adenocarcinomas and no vascular lesions were noted in this group.

Whereas fast neutrons appear to exert a direct carcinogenic effect on the glandular mucosa of the mouse stomach, x-rays may, at least in part, act indirectly through local vascular damage to produce gastric neoplasms.

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REFERENCES

Figs. 5-8 are of the same tumor.

**Fig. 5.**—Adenocarcinoma of glandular stomach of LAf1 mouse 19 months after whole-body irradiation with fast neutrons. Neoplastic glands deeply invading stomach wall. \( \times 90 \).

**Fig. 6.**—Neoplastic acini within muscularis propria and approaching serosa (bottom left). \( \times 130 \).

**Fig. 7.**—Another section of same stomach showing heaped-up, atypical mucosal glands (right), and tumor extending along serosal surface (left). \( \times 32 \).

**Fig. 8.**—High-power view from Figure 7 showing tumor within serosal lymphoid nodule. \( \times 160 \).
Fig. 9.—Well differentiated gastric adenocarcinoma in neutron-irradiated mouse 20 months after irradiation. Tumor acini are invading all coats of the stomach wall including serosal tissues adjacent to pancreas (lower left). \( \times 82 \).

Fig. 10.—High-power view from Figure 9. Well differentiated neoplastic acini in loose serosal tissues. \( \times 160 \).

Fig. 11.—Adenocarcinoma of glandular stomach 15 months after neutron-irradiation. Note well differentiated acini in submucosa, as well as dilated, atypical glands extending through the serosa. \( \times 90 \).

Fig. 12.—Squamous-cell carcinoma arising in forestomach of neutron-irradiated mouse. Cords of tumor cells are invading the adjacent liver in which the parenchymal cells (bottom left) are compressed and distorted. Note the clump of tumor cells (arrow) within a hepatic blood vessel. \( \times 130 \).
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