GASTRIC LESIONS IN EXPERIMENTAL ANIMALS FOLLOWING SINGLE EXPOSURES TO IONIZING RADIATIONS *

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Past studies indicate that the stomach is less sensitive to ionizing radiations than the small intestine. In the intestine, functional changes have been noted within minutes,[1] and inhibition of mitosis is evident within hours of exposure to low dosages of whole body irradiation. Lethal † dosages produce partial denudation of the intestinal mucosa, but regeneration is usually complete within a week. At supralethal doses, complete loss of epithelium from the small intestine leads to extensive fluid loss and, in most species, to death in 3 to 4 days.[2,3] Guinea pigs and hamsters differ according to the propensity of the remaining epithelial cells to stretch out and cover the flattened villi.[4] The resulting reduction in loss of fluid lengthens survival to 5 to 6 days. However, the basic process of extensive destruction of the epithelium of the small intestine is similar in all species studied. The lesions represent the direct effects of radiation, since they can be reproduced by single local exposures of the abdomen or the intestine itself.[5]

No similar early lesions have been observed in the stomach following single exposure of the whole body to radiation. Changes in gastric motility have been noted.[6] Anatomic lesions and alteration in gastric secretion have not been described except after local and usually multiple exposures with cumulative doses greatly in excess of those that will produce severe lesions of the small intestine.[7-11]

Recently, plasma transfusion, marrow transplants and the administration of antibiotic agents have prolonged the survival of dogs,[2] rats[12] and hamsters,[4] following exposures that killed unprotected animals in 3 to 6 days. In these animals gastric lesions developed 2 to 3 weeks after irradiation. Following the single exposures, the time sequence of the development and healing of gastric lesions was studied and compared with the lesions of the small intestine in the same animals. The complex conditions of multiple dosages, with different rates of partial recovery of various tissues between exposures were avoided.

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† The term "lethal" is used for doses that kill 1-99 per cent of animals, "supralethal" for uniformly killing doses.

Material and Methods

Dogs were given whole body irradiation by a Co^{60} source with 1,200 to 1,700 r. Of 11 dogs given electrolytes, plasma, and protein hydrolysates by infusion, 4 survived 6 to 12 days.[2] Twenty dogs without such infusion died 3 to 4 days after exposure from acute dehydration and vascular collapse. In another experiment, 6 dogs were given local irradiation to the stomach, the source being a 200 KVP Picker therapy roentgen machine (25 ma, 1 mm. Al, 0.15 mm. Cu, TSD 50 cm., HVL 0.8 mm. Cu, dose rate in air 44 r/min). Radiation was applied with or without eventration of the stomach through a midline incision, followed by replacement of the stomach and surgical closure. In 2 of the dogs biopsy specimens of the gastric mucosa were taken 3 weeks later, and 4 dogs were sacrificed between 2 and 4 weeks after exposure.

Rats, too, were given roentgen irradiation; the machine qualities are described above. Within an hour of exposure to 800 or 900 r., the rats were joined in parabiosis with nonirradiated litter mates.[12] Single irradiated animals died in 3 to 5 days. Parabiosis prolonged survival of 50 per cent of the animals to 30 days or longer.

Hamsters were exposed to 1,160 r. with a clinical roentgen machine operating at 200 KVP (20 ma, HVL 0.76 cm. Cu.). Some of these animals had one leg shielded during irradiation. Others received an intracardiac injection of one homogenized spleen and the marrow from 4 long bones of a 2-week-old normal hamster within a few hours of irradiation.[4] Approximately half of the animals so treated survived longer than 30 days while all animals not receiving the spleen and marrow homogenate died in 4 to 8 days.

Histologic studies were made on animals that died spontaneously or were sacrificed. Both those receiving protective substances and those not so treated were included. Tissues were fixed in buffered formalin sublimate solution[18] and paraffin sections were stained with hematoxylin and eosin.

Results

Dogs

Gastric and intestinal lesions were compared 3, 6 and 10 to 12 days after whole body exposure to 1,200 r. At 3 days, the architecture and appearance of individual cells of the fundic and pyloric mucosa of dogs were normal (Figs. 1 and 2). In contrast, the mucosa of the small intestine was extensively denuded. The remaining lining cells were stretched out and flattened in an apparent attempt to maintain continuity of the epithelium (Fig. 3). Cells with enlarged vesicular nuclei

and prominent central nucleoli were conspicuous. At 6 days only one dog was available for study. Its fundic mucosa was normal. In the pyloric mucosa the base and pits of the glands were unchanged but the mid thirds were lined by hyperchromatic cells with centrally located, occasionally swollen nuclei and prominent nucleoli reminiscent of early radiation changes in the small intestine. These altered cells were closely packed, with no significant diminution in number (Fig. 4). At that time the mucosa of the small intestine had been reconstituted. In most areas, a moderate hyperplasia of the lining epithelium was the only remaining abnormality (Fig. 5). In a few areas, hyperplastic crypts and glands alternated with others lined by the flattened, stretched-out cells seen earlier (Fig. 6). Ten to 12 days after receiving 1,200 r., the fundic glands were partly denuded of epithelium and the remaining cells were hyperchromatic, with swollen nuclei and, often, distinct central nucleoli (Fig. 7). Some parietal cells could be identified by their eosinophilic staining quality. Occasional mitotic figures were present, some of them of abnormal nature. Similar changes were seen in the pyloric mucosa. At this time the mucosa of the small intestine was fully regenerated and indistinguishable from that of control dogs (Fig. 8). No lesions were found in the mucosa of the colon of the dogs in this series.

None of the dogs given 1,200 r. whole body irradiation were kept alive longer than 12 days. Consequently the later fate of the gastric lesion could not be followed in this group of animals. The gastric mucosa was therefore studied after local irradiation with 1,200 to 1,700 r. Dogs so irradiated generally continued in good health and did not suffer from the diarrhea attending denudation of the small intestine following whole body irradiation. Anorexia was noted in one of the dogs.

Three dogs sacrificed 2 weeks after the local exposure to 1,700 r. showed lesions of the fundic glands similar to those described following whole body irradiation with 1,200 r. The lumens of many of the glands were widened because of flattening of the lining cells. Many of these cells were eosinophilic. Polymorphonuclear leukocytes were present in some of the glands. Occasional foci of hyperchromatic cells with scattered mitotic figures were present at the level of the foveolas, apparently representing early attempts at regeneration. Regeneration was more prominent, however, in the pyloric region (Fig. 9), again originating in the pits of the glands. The cylindrical cells with basal nuclei characteristic of the pyloric glands were well preserved in some

glands, whereas in others transition to cuboidal cells with more centrally located nuclei was seen. In 3 of the 4 dogs on which biopsies were performed or which were sacrificed at 21 days, regeneration of the fundic and antral epithelium proceeded from the neck of the glands where the rare mitotic figures in normal mucosa are located. However, no regeneration was evident in one dog in a biopsy specimen procured at 21 days. Extensive local ulceration had developed (Fig. 10). The few remaining groups of epithelial cells were arranged in an acinar pattern. The individual cells were large, with "owl eye" nuclei. When this dog was sacrificed 32 days after irradiation, a wide area at the margin of the ulcer was covered with a single layer of epithelium, except for a few newly formed hyperplastic glands. The very abnormal cells seen in the 21-day biopsy specimen were no longer present.

Rats

Sections were made from the stomachs of 3 rats sacrificed or found dead 3 weeks following exposure to 800 or 900 r. and subsequent experimental parabiosis. There was spotty loss of cells with flattening of the remaining epithelium in many of the gastric glands. A prominent feature was the presence of hyperplastic epithelium with frequent mitotic figures in the neck of some of the glands (Fig. 11). Complete repair was prompt. No gastric lesions were seen in animals surviving 40 days or longer. Sections were also made from the tissues of numerous animals which died during the first 2 weeks after exposure to 800 and 900 r. No convincing evidence of damage to the gastric epithelium was revealed. There were focal dilatation of glandular lumens and apparent loss of the epithelium, but these conditions were occasionally found in controls and were often indistinguishable from artifacts. The small intestine, as in dogs, underwent rapid denudation and re-epithelization with complete restitution by the end of 7 to 10 days.

Hamsters

The time sequence of injury to the small intestine and recovery was similar to that in rats and dogs. Gastric lesions were not seen during the first week following exposure to 1,160 r. At 3 to 4 weeks, however, lesions were found in the glandular stomach of each of 8 animals examined. Hyperplastic epithelium with frequent mitotic figures was always present in at least some of the gland pits. Occasionally, large areas exhibited this clear-cut evidence of regeneration. There were ulcerations in some stomachs, suggesting failure of regeneration (Fig. 12). In one experiment, adequately preserved tissues were secured from 10

animals dying between the 28th and 56th days. In 7, gastric ulcers were present and were probably the cause of death. In another series, very few deaths occurred during the 2nd and 3rd month after similar exposure and treatment, indicating that lethal progression is not a necessary sequela of the gastric ulceration.

Discussion

Inhibition of mitotic activity and death of cells as they enter the next mitosis are presumably the common features of radiation damage in both the stomach and small intestine. A detailed review of the pathogenesis of the intestinal lesions has recently been published by Quastler.[14]

In the small intestine the crypt cells provide the reservoir of regenerating epithelium. The few Paneth cells at the very bottom of the crypts and the goblet and chief cells covering the villi represent the progeny of these formative elements. The life span of the mature cells is estimated at 36 hours, the oldest cells being shed into the lumen from the tip of the villus, while new cells gradually migrate up its sides. Denudation of the small intestine would be expected within 1½ days of cessation of mitotic activity. This is postponed, however, by failure of the usual migration upward.[15] Old cells cling to the shortened villi upon which they stretch out, thus providing a continuous epithelial lining.* Differences in the efficacy of this clinging property may account for differences among species. In rats denudation is complete 3 days following exposure to 1,000 r., but in golden hamsters complete denudation is absent as late as 6 days even after the application of 4,000 r.

Quastler's thesis[14] is presumably applicable to the gastric mucosa. In the fundus, 4 types of cells can be distinguished, the mucus-secreting surface epithelial cells, the neck cells, the parietal cells and the chief or zymogenic cells of the tubular glands. The surface epithelium is replaced by dividing cells in the pits of the glands. The neck cells, contiguous with but differing from the first type of mucus-secreting cells, also divide, but the parietal and chief cells probably do not.[16]

The arrest of mitosis by colchicine has been used to determine the number of cells entering division in a given time. In the rat stomach, Stevens and Leblond[17] found 2.6 per cent of mucous neck cells and 5.9

* Recently, H. Quastler and F. G. Sherman studied radioautographs of mouse small intestine after labeling of deoxyribose nucleic acid by incorporating tritium into thymidine. In these as yet unpublished experiments the migration out of the generative zone was not appreciably modified by irradiation, and the "clinging ability" of the epithelium appeared to be the major feature which increased survival of the cells and delayed denudation and death.

per cent of surface epithelial cells in mitosis 4 hours after colchicine administration. From these data they computed the renewal times of surface epithelial cells as 3 days and of mucous neck cells as 6½ days. The computations presuppose that the cells replace only themselves.

There is considerable support, however, for the thesis of Bizzozero[19] that only the cells in the pit and neck of the glands divide, and that both the surface epithelium and the parietal and chief cells regenerate from these formative cells. The present observations tended to confirm this theory. Areas of regenerative hyperplasia were prominent in the neck of the glands. It is not known, however, what the normal life span of parietal and chief cells is, or whether they are replaced only in response to injury. If the neck cells, which are greatly outnumbered by the chief and parietal cells, replace them continuously, then the life span of chief and parietal cells must be much longer than the 6-day renewal time computed for the neck cells themselves. Since the survival of surface epithelium following irradiation considerably exceeded the 3-day life span suggested by Stevens and Leblond, there must be retention of cells that would normally be shed and replaced. This would also apply to the pyloric mucosa for which Leblond and Walker[16] suggested renewal times of just under 2 days. The apparent prolongation of life span would be comparable to the greater average age of automobiles in wartime due to nonavailability of new ones. Alternatively, the renewal time as derived from observation of mitotic activity may underestimate average life span. The mitotic index as a measure of cell renewal may be likened to the reticulocyte count as an indicator of production of red cells. Estimates of red cell life span from reticulocyte data are notoriously unreliable.

The development of gastric lesions in experimental animals was comparable to the lesions observed in serial gastric biopsy specimens from 3 patients with duodenal ulcers who received a fractionated total exposure to 1,600 r. to the fundus of the stomach.[18] The delay in actual loss of damaged cells, the appearance of swollen cells with abnormal nuclei and nucleoli, and the hyperplastic regeneration proceeding from the pits of the glands were common to both the human and experimental lesions. In contrast to the present experiments, the delay in the onset of the lesions of human subjects was less significant since the irradiation extended over a 10-day period. Of greater interest was the observation of coagulation necrosis in the depth of the tubular glands of the human fundus and the very slow repair. Coagulation necrosis was noted early but persisted nearly 5 weeks in 2 of 3 patients. Differences between species, variation in depth dose, and the utilization

of single or fractionated exposures to irradiation could play roles in these varied responses.

The appearance of more atypical cells 2 to 3 weeks following irradiation may have represented the late development of degenerative changes, or these cells may have undergone one division or more and then assumed bizarre shapes during unsuccessful attempts at further division. The second possibility is suggested by the observation of undifferentiated cells with vesicular nuclei and occasional abnormal mitotic figures lining the entire length of tubular glands (Fig. 6). Degeneration *in situ* incident to entering the next mitosis appears unlikely, since fully differentiated parietal and chief cells do not normally divide. It appears more likely that these cells represented recent progeny of damaged neck cells incapable of continued normal development.

Progression of the gastric lesion to frank ulceration was observed occasionally in hamsters after full recovery of bone marrow function. It occurred after local irradiation of one dog with normally functioning bone marrow. We have pointed out previously that regeneration of small intestine epithelium following whole body irradiation is usually complete by the time dogs develop pancytopenia and die from infection and hemorrhage.[3] Ulceration of the gastrointestinal tract secondary to hemorrhage and agranulocytosis may occur at that time. In dogs, the tonsils and anal mucosa are usually diseased. In guinea pigs the stomach is frequently the site of hemorrhagic lesions which may ulcerate. "Agranulocytic ulcers" of the small intestine have been noted in this laboratory in mice, rats, and hamsters. These lesions were clearly distinct from those representing the delayed but direct radiation damage to the gastric mucosa here described.

Summary

Epithelial changes in the gastric mucosa developed about two weeks following single supralethal exposures to irradiation. These changes were quite similar to those which occur earlier in the small intestine. Regeneration proceeded from the neck of the glands and was comparable to the regeneration of small intestine mucosa from surviving crypt epithelium. Occasionally, the direct damage was severe enough to lead to ulceration, even after local irradiation and in the absence of agranulocytosis and hemorrhage. This was in contrast to the late ulcers appearing in the regenerated mucosa of the small intestine which were associated with hemorrhage or represented direct sequelae of the pancytopenia that follows lethal irradiation.

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[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Dog. Fundus of stomach. Normal appearance 3 days after receiving 1200 r. Hematoxylin and eosin stain. × 38.

FIG. 2. Dog. Pylorus. Normal appearance 3 days after receiving 1200 r. Hematoxylin and eosin stain. × 38.

FIG. 3. Dog. Small intestine, showing loss of epithelium with remaining cells flattened. Hematoxylin and eosin stain. × 38.

FIG. 4. Dog. Pylorus 6 days after exposure to 1200 r. Early lesion. Hematoxylin and eosin stain. × 50.

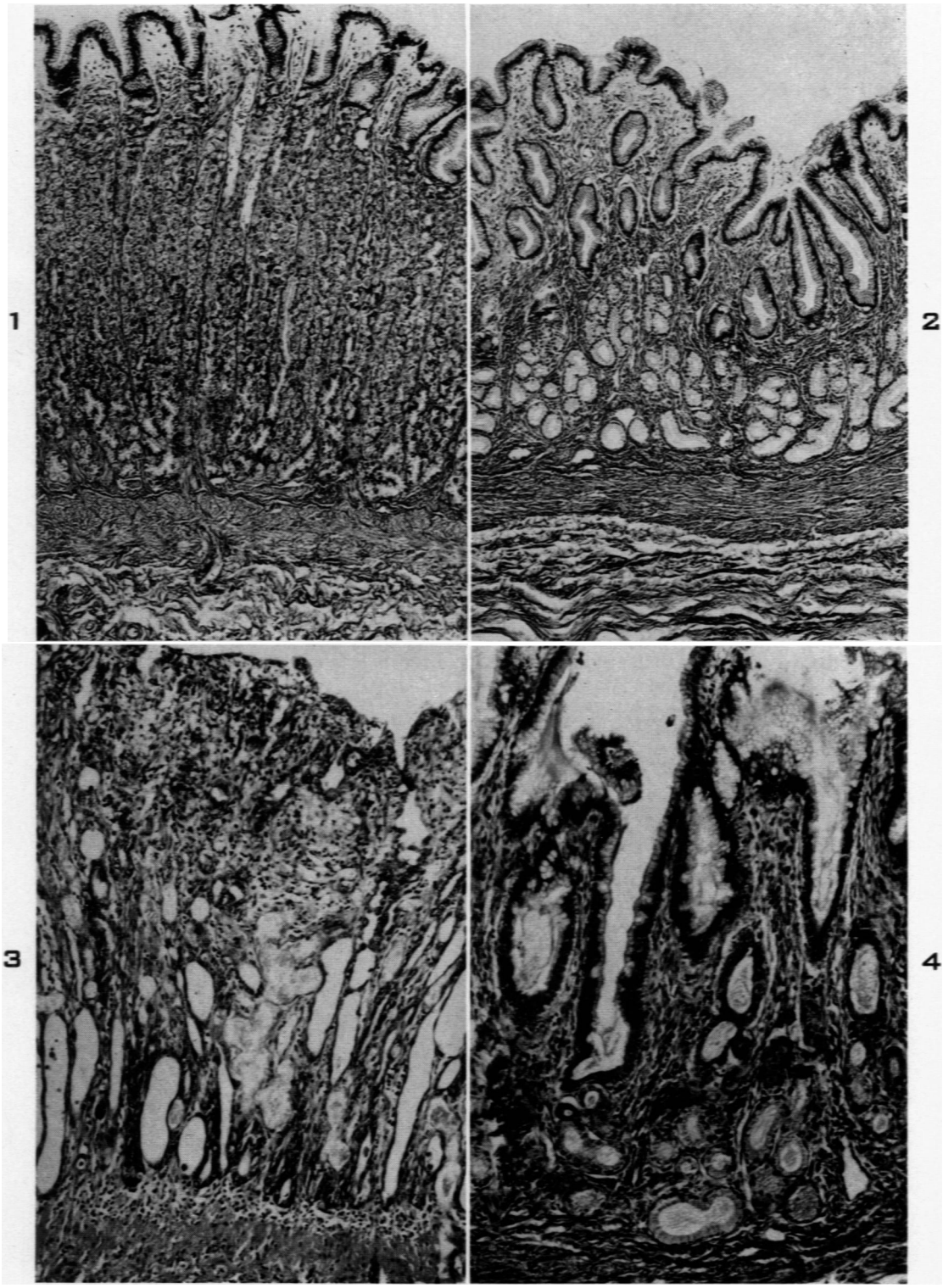
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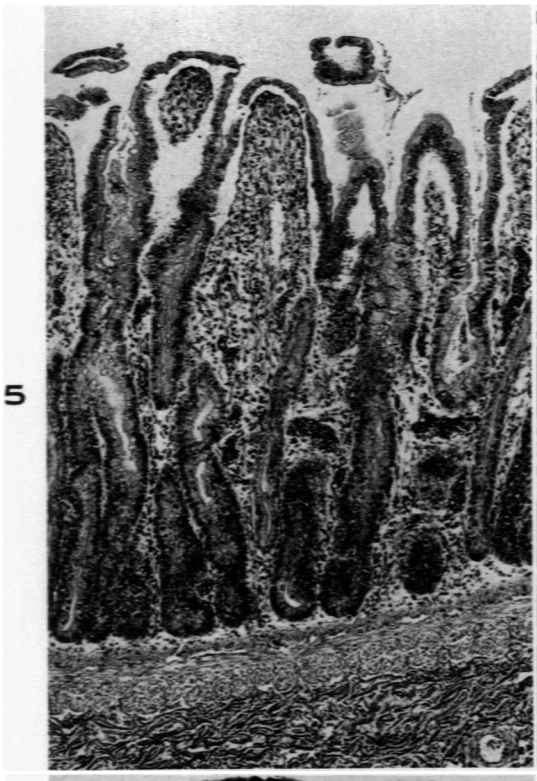
FIG. 5. Dog. Small intestine. Slightly hyperplastic, regenerating epithelium 6 days after exposure to 1200 r. Hematoxylin and eosin stain. × 38.

FIG. 6. Dog. Small intestine, revealing delayed regeneration compared with area shown in Fig. 5. Hematoxylin and eosin stain. × 38.

FIG. 7. Dog. Partial destruction of fundic glands 11 days after irradiation with 1200 r. Hematoxylin and eosin stain. × 80.

FIG. 8. Dog. Fully regenerated small intestine. Hematoxylin and eosin stain. × 38.



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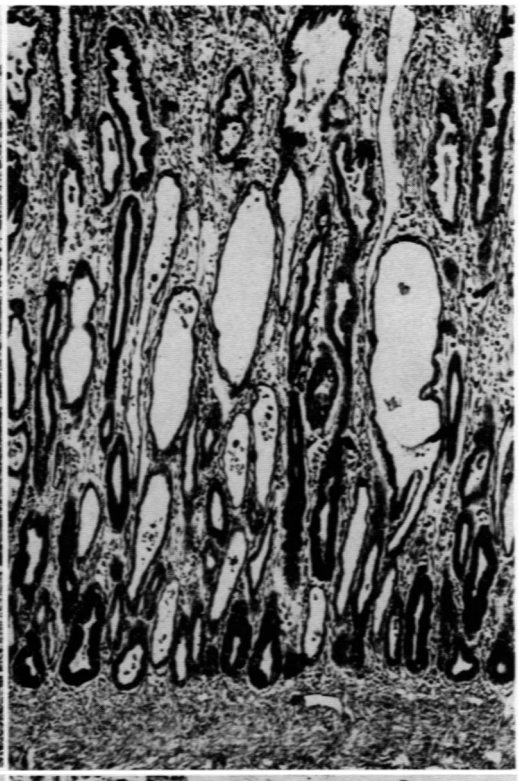

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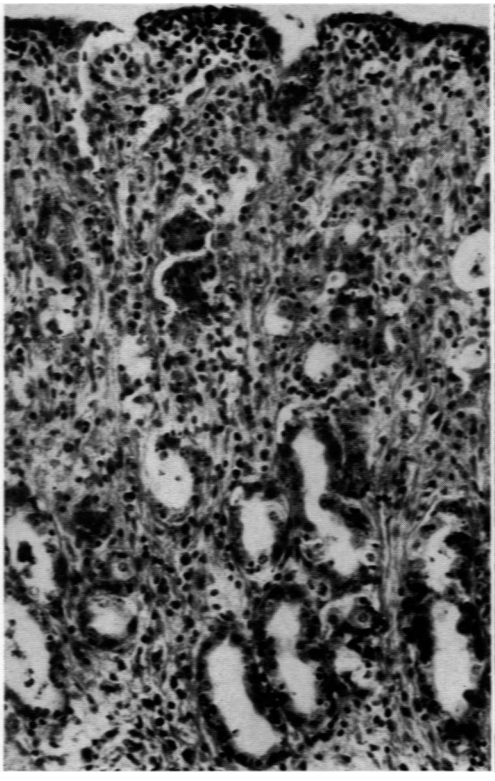

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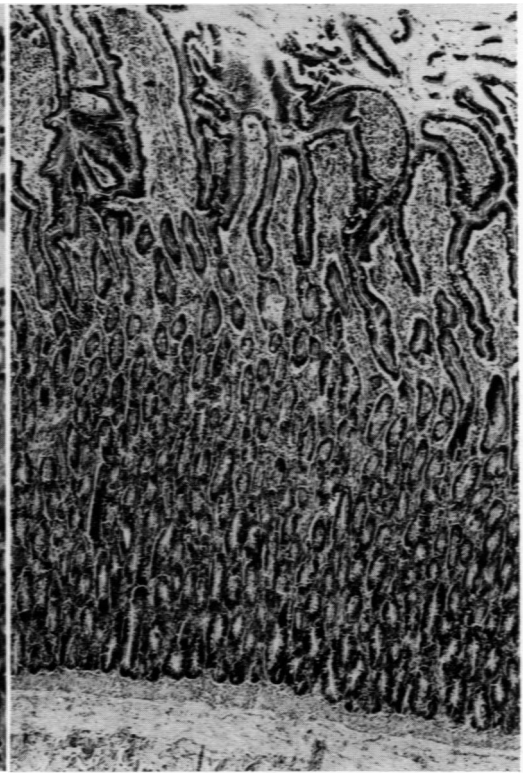

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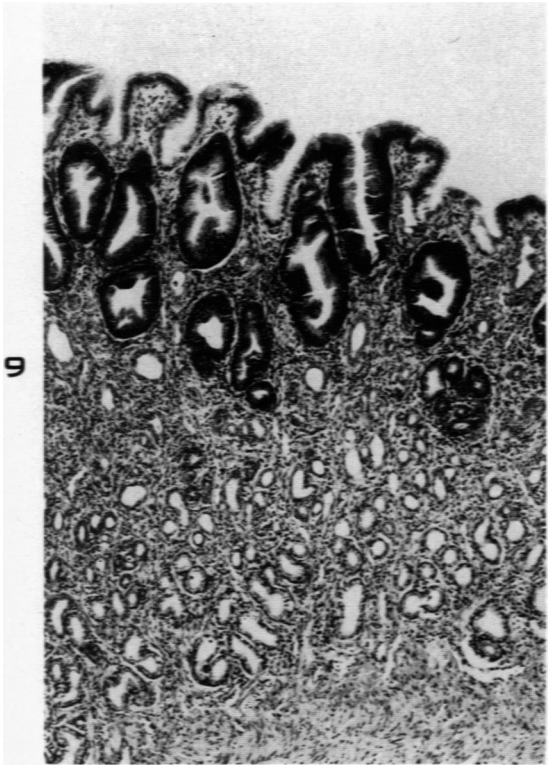
FIG. 9. Dog. Hyperplastic, regenerating foveolas of the pylorus, 14 days after local irradiation with 1700 r. Hematoxylin and eosin stain. × 38.

FIG. 10. Dog. Biopsy of stomach 21 days after local irradiation with 1700 r. Hematoxylin and eosin stain. × 80.

FIG. 11. Rat. Hyperplastic neck glands of stomach, 21 days after exposure to 900 r. Hematoxylin and eosin stain. × 50.

FIG. 12. Hamster. Ulceration and regeneration of gastric mucosa, 21 days after irradiation with 1150 r. Hematoxylin and eosin stain. × 38.



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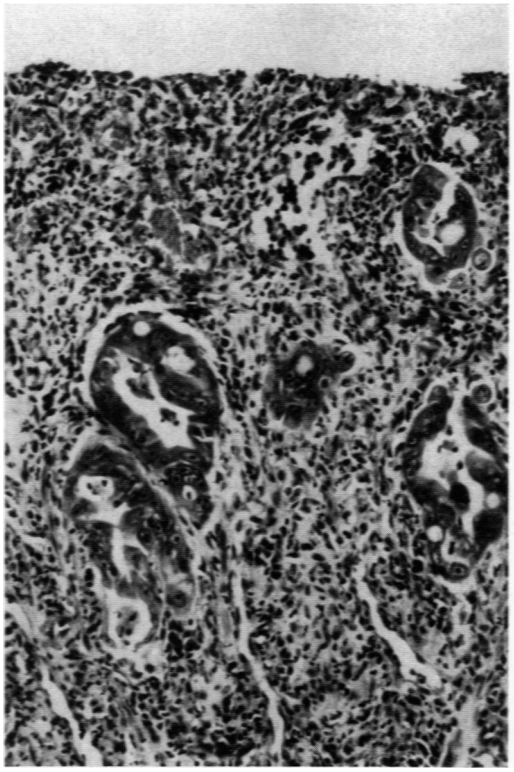

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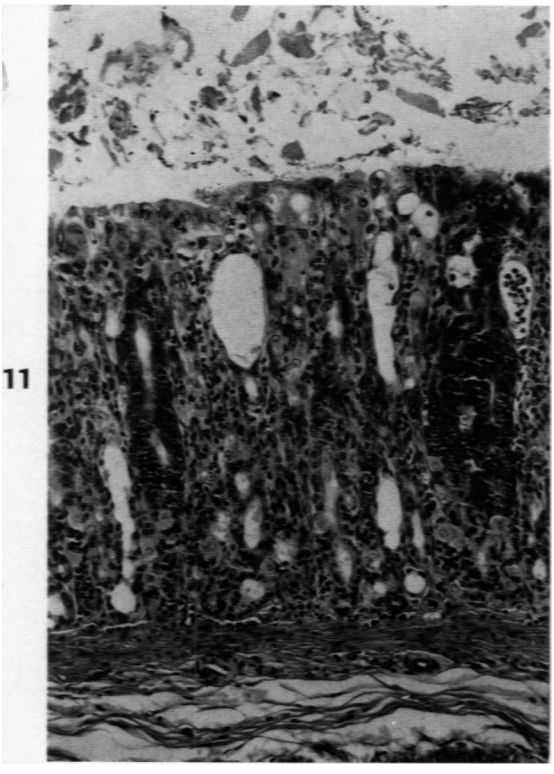

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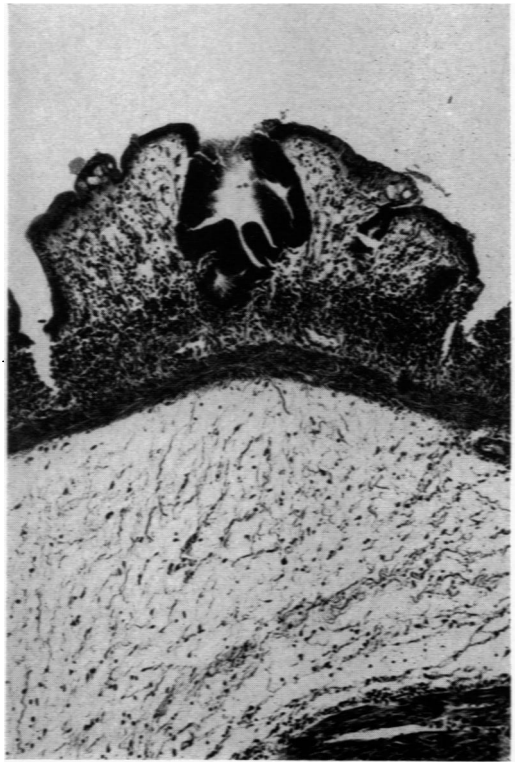

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