RESPONSE OF THE GASTROINTESTINAL TRACT OF THE MOUSE TO 5-FLUOROURACIL

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Of the pyrimidine antagonists used as anti-mitotic agents in the treatment of advanced neoplasms, 5-fluorouracil (FU) has shown the greatest promise. Uracil is taken up preferentially by rapidly growing tissues, such as the intestinal mucosa, bone marrow and many tumors. The synthesis of deoxyribonucleic acid (DNA), and to a lesser extent ribonucleic acid (RNA), is inhibited when the hydrogen in the fifth position in the uracil molecule is substituted with fluorine; severe cellular derangements occur with the administration of such an agent.

A limiting factor in therapy, therefore, has been the severity of the toxic effects which often prevent attainment of dosage levels which are tumor-inhibitory. Although bone marrow depression with resulting leukopenia and granulocytopenia remains the most severe and potentially lethal complication, early alimentary tract side effects such as stomatitis and diarrhea have often proved disabling and dose-limiting. We have studied the effects of FU on the mucosa of the gastrointestinal tract, both in the human subject and in experimental animals, in an effort to understand the nature of this toxicity.

METHODS

Forty-eight albino male mice were placed in cages with free access to food and water.

1. Twelve mice received 5 cc. of saline intraperitoneally each day for 5 consecutive days.
2. Twelve mice received 50 mg. per kg. of FU intraperitoneally each day for 5 consecutive days.
3. Twelve mice received 75 mg. per kg. of FU in the same manner.
4. Twelve mice received 100 mg. per kg. of FU in the same manner.

After the drug had been given for 5 days, each day 1 animal was sacrificed in each group. The animals were examined and specimens were obtained of the esophagus, stomach and the small and large bowel. The tissues were fixed in 10 per cent formalin, embedded in paraffin, and sections stained with hematoxylin and eosin.

Many mice that received the FU in doses of 75 to 100 mg. per kg. died spontaneously between the fifth and the ninth days, and these too were examined. They

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all had developed diarrhea, shaggy hair, an inflamed peritoneum, and many had small bowel perforations.

We chose to administer the drug in a fashion similar to the regimen employed in our patients, giving one injection daily for 5 consecutive days, hoping to obtain in this manner information which would be clinically useful. The conventional dosage of 15 mg. per kg. of FU used clinically produced no changes in mice, so that the dose was increased to 50 mg. per kg., at which point some alterations were detected; higher doses were then also given.

RESULTS

Controls

Histologic examination of the esophagus, stomach and the small and large intestine showed normal mucosal patterns in all animals.

Mice Receiving 50 mg. per kg. FU

In 10 animals the gastrointestinal tract appeared normal. Two animals sacrificed on the seventh and ninth days demonstrated slight focal atrophy of the mucosal glands of the small intestine and a slight increase of the infiltrate in the lamina propria consisting of macrophages, plasma cells, eosinophils and occasional lymphocytes. All the other organs appeared normal, even in these 2 animals.

Mice Receiving 75 mg. per kg. FU

In all animals sacrificed on the fifth day the stomach and esophagus were normal. In the small intestine there was some edema of the submucosa and moderate numbers of macrophages and lymphocytes. Moderate villous atrophy was noted, with focal hyperplasia and hypertrophy of the superficial columnar cells. Nuclei and nucleoli were prominent. Loss of polarity was very slight. The colonic mucosa showed minimal glandular disorganization, epithelial hyperplasia, nuclear atypism, and focal loss of goblet cells.

On the sixth day both the large and small bowel mucosa showed a generally increasing glandular disorganization and epithelial atypism. The esophagus and stomach remained normal.

On the seventh day the aforementioned abnormalities were quite severe throughout the intestinal tract. In addition to marked focal glandular atrophy, loss of nuclear and cellular polarity and atypical hyperplasia approached the appearance of carcinoma *in situ*. By the eighth and ninth days the ileal and colonic mucosa had reverted to a more usual pattern. The villi of the ileum continued to show more clubbing and contained dense lymphocytic infiltrate in the lamina propria. Atrophy and disorganization of the colonic glands were considerably decreased. Dense small round cell aggregates were also present in the underlying tissue.

Alterations in the Paneth cells were often quite remarkable but variable. After 5 days of drug administration, the Paneth cells showed marked dissolution and decreased staining properties, some cells often being extruded into the lumens of the acini. By the seventh day most Paneth cells had disappeared, but the succeeding 2 days showed a return of the Paneth cells to normal numbers, still with poor staining properties.

Mice Receiving 100 mg. per kg. FU for 5 Consecutive Days

All mice receiving this dosage expired between the fifth and the seventh days. Gross pathologic changes in the gastrointestinal tract included perforations and ulcerations of both small and large intestine in all the animals.

The esophageal mucosa was normal except for minimal hypertrophy of epithelial nuclei in some animals.

The gastric mucosa was unremarkable in several animals, but showed slight glandular atrophy in some and disorganization in others. No increase in inflammatory reaction was noted, but the atrophy did not appear to be selective towards a certain group of cells.

The ileum revealed mucosal atrophy with hypertrophy of the lining columnar cells at the apexes of contracted villi (Figs. 1A and 1B). There was severe disorganization of the crypts of Lieberkühn (Figs. 2A and 2B), with marked variation in nuclear size and shape and moderate to marked loss of polarity and stratification of the nuclei. The nucleoli were often multiple, huge, bizarre and hyperchromatic. The changes simulated the appearance of carcinoma *in situ*. Paneth cells were greatly reduced or absent. The lamina propria contained many lymphocytes, macrophages and a few neutrophils. Some of the animals had considerably less atrophy, and in some the epithelial abnormalities were not marked.

In the colon, too, marked disorganization of the glandular pattern and atypical hyperplasia of individual cells could be noted. The goblet cells were severely atrophic, with loss of nuclear polarity and stratification, and with variation in nuclear size and shape. Some of the nuclei were bizarre in appearance. The nucleoli were very prominent and hyperchromatic; numerous mitotic figures could be detected (Figs. 3 and 4).

Discussion

We have studied the response in different levels of the digestive tract to varying dosages of FU in order to assess the relative contribution of each segment to the general picture of gastrointestinal toxicity. We

tried in this experiment to mimic clinical conditions closely by administering the drug in 5 divided doses.

The effect on the gastrointestinal tract of such agents as nitrogen mustard, azaserine, and folic acid antagonists has been extensively investigated in experimental animals. The histologic effect of FU has been examined to a lesser degree. Wong and Benson,[1] giving high doses of FU to mice, found hemorrhages in the stomach and intestine. Heidelberger and colleagues[2] reported cessation of mitotic activity in the mucosa of the ileum and jejunum and regressive changes in the villi.

More recently Ballerini, Bosi, Castoldi and Ricci[3] sacrificed mice at various intervals after intraperitoneal injection of FU in doses of 250 mg. per kg. All animals expired after developing severe diarrhea. Lesions appeared at the bases of the crypts 48 hours after the drug was given, and at the apexes of the villi at 96 hours. This confirmed present concepts of the rate of epithelial cellular regeneration in the intestine, and suggested that the enteropathy produced by FU was one of mitotic arrest. Paneth cells and enterochromaffin cells were present 48 hours after the injection, but had disappeared by 96 hours.

Our studies of the entire gastrointestinal tract in the mouse demonstrated that, as in human beings, there was considerable individual variation in the tolerance of the intestine to a specific dose of FU. Thus, 2 of 10 mice receiving 50 mg. per kg. of FU had changes in the small bowel. The other organs were normal in all the animals in this group. The impression that in mice the small bowel was the portion of the gastrointestinal tract most sensitive to FU did not parallel studies in our human subjects, in 3 of whom small bowel biopsy specimens taken at the height of FU toxicity showed no changes at the same time that colonic specimens exhibited a grossly abnormal appearance.[4] It should be mentioned that the biopsy specimens in our patients were from the duodenum, whereas in the mice we examined ileum and jejunum.

On the other hand the resistance of stratified squamous epithelium in the esophagus to FU confirmed clinical experience that the esophagus was spared all toxic effect of FU.

Dosages of 75 to 100 mg. per kg. consistently had a marked effect on the normal intestinal tract in the mouse, resulting in mucosal alterations which in their atypism and hyperchromatism and glandular disorganization simulated carcinoma *in situ*. At these dosage levels the changes appeared shortly after the 5-day course was completed and were totally reversible, disappearing by the ninth day. Paneth cells were unusually sensitive to FU, disappearing at the height of the effect of the drug, but returning promptly as the mucosa returned to normal.

Summary

Varying dosages of FU were administered to albino mice. Histologic changes appeared first at a dose of 50 mg. per kg. and were first noted in the small bowel, in contrast to human subjects where alterations were first noted in the colon. The drug was administered in 5 divided doses to parallel the method of administration in patients. Resistance of squamous epithelium to the drug was demonstrated, as well as resistance of the gastric mucosa in the mouse, where changes were minimal even at doses of 100 mg. per kg. Severe proliferative and atrophic lesions resembling carcinoma *in situ* were seen in small bowel and colon mucosa, and there was disappearance of Paneth cells.

References

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[*Illustrations follow*]

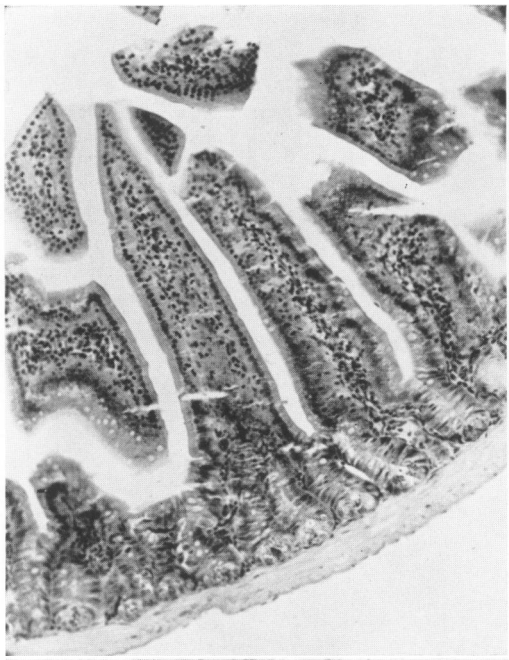
Legends for Figures

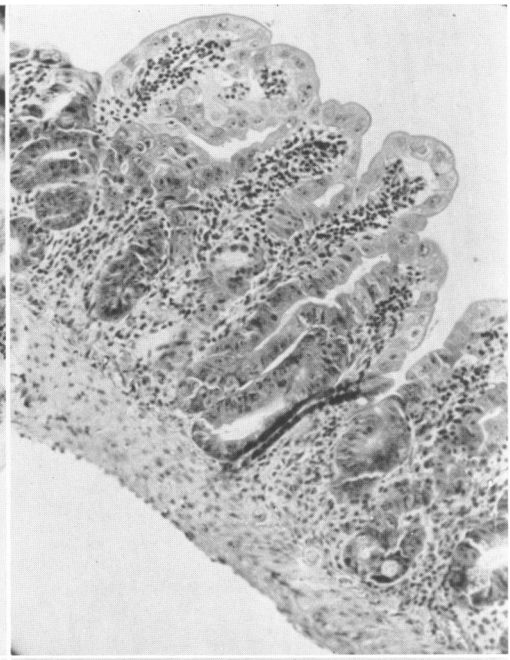
Photomicrographs were prepared from sections stained with hematoxylin and eosin.

Fig. 1. A. Small intestine, normal mouse. B. Small intestine, mouse treated with 5-FU, 100 mg. per kg. Atrophy of the villi and alterations in the nuclei are manifest. × 100.

Fig. 2. A. Small intestine, normal mouse. B. Small intestine, mouse treated with 5-FU, 100 mg. per kg., showing the disorganization of the crypts. × 35.

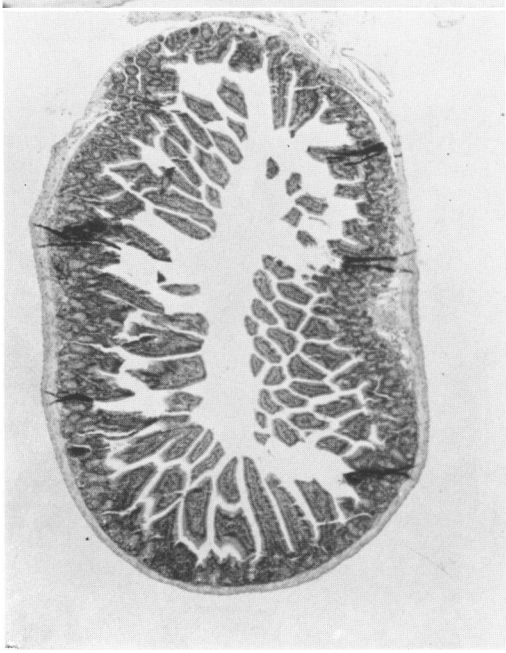
1A

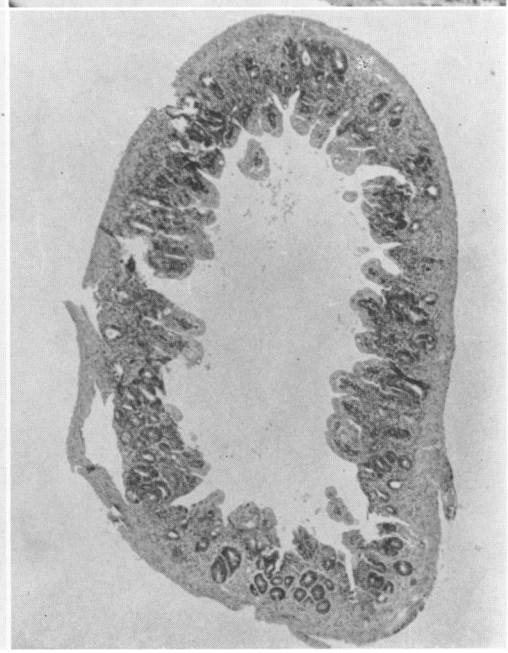




1B

2A





2B

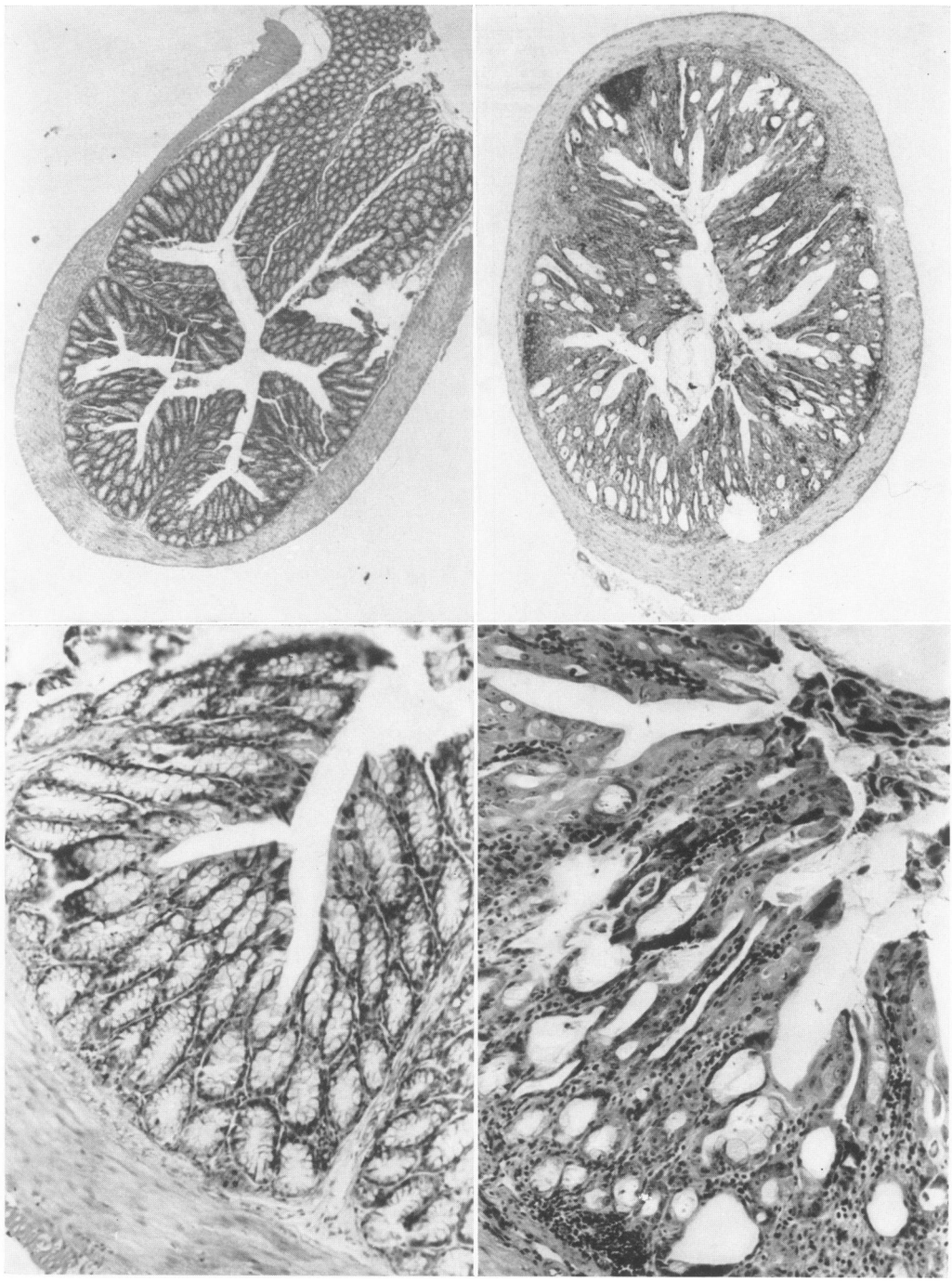


FIG. 3. A. Colon, normal mouse. B. Colon, mouse treated with 5-FU, 100 mg. per kg. There is disorganization of the glandular pattern. × 35.

FIG. 4. A. Colon, normal mouse. B. Colon, mouse treated with 5-FU, 100 mg. per kg. Nuclear alterations and cellular disorganization are manifest. × 100.