Small Intestinal Ulcers and Intestinal Flora in Rats Given Indomethacin

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INDOMETHACIN — (1-p-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid—an anti-inflammatory agent used for arthritis, has been reported to produce peptic ulcers in man [1-4] and animals.[5,6] After one of us experienced epigastric distress while taking indomethacin, a preliminary experiment in rats was undertaken. Surprisingly, the drug did not produce upper gastrointestinal ulcers, but instead, we observed striking longitudinal ulcers on the mesenteric side of the distal jejunum and the ileum.

The purpose of this paper is to describe the lesions produced by single doses of indomethacin in the rat. Further, we have used this model to investigate the role of the intestinal flora in the production of the small-intestinal ulcers.

Materials and Methods

Male Simonsen strain rats weighing 200–250 gm. were housed 4–6 per cage and allowed rat chow and water ad libitum. Indomethacin in single doses of 5, 10, 20, 30, and 40 mg./kg. was given intragastrically in 2 ml. of saline. When antibiotics were given, they were started 2 hr. after indomethacin administration. Intragastrically administered antibiotics were dissolved in 2 ml. of saline. Doses are given under *Results*. Control animals received 2 ml. of saline intragastrically. When antibiotics were placed in the drinking water, concentrations were as follows: neomycin sulfate 1.3 gm./L., polymyxin B sulfate 60 mg./L., and bacitracin 30,000 U./L. Animals separated for mortality studies were kept for 28 days.

Rats used for morphologic and bacteriologic study were killed by cervical dislocation. The abdomen and thorax were opened with sterile instruments, and samples of heart blood, liver, and spleen were cultured in thioglycolate broth. A 12-cm. segment of mid-small intestine, weighing approximately 1 gm., was removed by sterile technique and ground with a mortar and pestle in 9 ml. of saline; serial ten fold dilutions in saline were then prepared. Aliquots of 0.1 ml. of appropriate dilutions were spread on agar plates using a bent glass rod. The lowest plate contained 1/100 of the original sample. The mediums used and incubation conditions are given in Table 1. Organisms were counted and identified by standard techniques. Counts were expressed as $\log_{10}$, and the differences of means analyzed by the one-sided t test. Differences in frequency of isolation were analyzed by Fisher's exact test.

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Table 1. Mediums, Incubation Atmosphere, and Organisms Enumerated From Mid-Small-Intestine Segment and Cecal Content

Medium	Atmosphere	Principal organisms
Sheep blood agar	air, 37°C.	Aerobes
MacConkey agar	air, 37°C.	Gram-negative aerobes
Sabouraud agar	air, 37°C.	Yeasts
SF agar	air, 45°C.	Enterococci
Sheep blood agar	90% H_2, 10% CO_2, 37°C.	Anaerobes
LBS agar	90% H_2, 10% CO_2, 37°C.	*Lactobacillus*
Kanamycin-vancomycin blood agar*	90% H_2, 10% CO_2, 37°C.	*Bacteroides*

* Kanamycin 100 mg./ml., vancomycin 7.5 mg./ml.

For morphologic study the small intestine was rolled on a dowel, opened longitudinally, and fixed in 10% formalin containing 2% sodium acetate. Other organs examined grossly and microscopically included stomach, colon, mesenteric lymph nodes, spleen, liver, kidney, adrenal gland, heart, and lung.

Results

The results of an initial mortality and morphologic experiment are given in Table 2. Groups of 3 animals were killed for morphologic study 3 days after indomethacin administration for each of the doses, and at 12 and 18 hr. and each day of the period of 1–7 days for the 20 mg./kg. dose. One of 3 rats receiving the 5 mg./kg. dose had a small ulcer in the small intestine. All animals receiving 10 mg./kg. had patchy small ulcers in the distal half of the small intestine, and in those given the 20 and 40 mg./kg. doses, the ulcers were severe; they appeared similar for the two doses. The morphologic findings in later experiments using 30 mg./kg. were the same as with 20 and 40 mg./kg. (Table 3). Multiple longitudinally oriented sharp ulceractions were present on the mesenteric side, beginning at approximately the midjejunum and extending to the ileocecal junction. There were fewer ulcers in the distal ileum. The ulcers ranged from a few millimeters to many centimeters in length and averaged 4 mm. wide (Fig. 1). Microscopically, the ulcers were sharply localized to the mesenteric side with absence of the mucosa, partial to

Table 2. Mortality and Severity of Ulcers in the Small Intestine After Various Doses of Indomethacin

Indomethacin (mg./kg.)	Mortality (No. dead/total)	Day of Death		Ulcers*
		Median	Range	
5	0/10	—	—	0 to +
10	0/10	—	—	+
20	10/10	6	2–9	++
40	10/10	2	2–5	++

* 0, absent; +, mild; ++, severe.

complete necrosis of the muscularis, variable penetration of the ulcer with areas of perforation into the mesentery, and surrounding peritonitis. The ulcers were present, but small, at 12 and 18 hr. became larger by 1 and 2 days, and reached a maximum size by 3–4 days (Fig. 2 and 3). The inflammatory reaction was acute during the first few days, but by 5–7 days many chronic inflammatory cells and the beginning of fibroblastic proliferation were evident. After 24 hr. large masses of bacteria were evident on the surface of the ulcers. In the animals that lived longer, adhesions between loops of bowel and walled-off abscesses were present. Some animals had evidence of obstruction with dilatation of the small intestine proximal to adherent loops of bowel. Approximately one-third of the rats receiving the higher doses had small ulcers in the colon, which were not appreciated grossly and appeared to begin as small necrotizing lesions in the region of the muscularis mucosae (Fig. 4). One rat had a small ulceration of gastric mucosa, and 2 had isolated ulcerations of the proximal small intestine. There was no microscopic evidence of vasculitis in the region of the necrotizing ulcerative lesions. In one experiment a 20 mg./kg. dose of indomethacin was given intramuscularly. The mortality and lesions were the same as when the drug was given intragastrically. No lesions other than peritonitis were demonstrated in the other organs examined.

Table 3 presents the results of a series of experiments designed to test the effect of antibiotics on mortality and ulcers produced by indomethacin. When the antibiotics were given two or three times daily intragastrically, there appeared to be some protective effect, and the ulcers definitely were less extensive than in controls. However, even animals with small ulcers sometimes had generalized peritonitis. A few animals, which are excluded from the data presented, were killed immediately by the intubation procedure; these tended to be in the anti-

Table 3. Effect of Antibiotics on Mortality and Ulcers Induced by Indomethacin

Dose of indomethacin, (mg./kg.)	Antibiotic treatment		28-day mortality (No. dead/total)		Severity of ulcers at 2 days ‡	
	I.G.*	In water†	Treated	Placebo	Treated	Placebo
30	b.i.d. × 14 days	none	5/10	9/10	+, 0, 0§	++, ++, ++
30	t.i.d. × 12 days	none	3/8§	9/10	+, 0, 0§	++, ++, ++
30	none	+ 14 days	9/15§	14/15	not examined	
30	b.i.d. × 5 days	+ 14 days	0/11§	10/12	+, +, +§	++, ++, ++
40	b.i.d. × 5 days	+ 14 days	7/12§	12/12	+, +, 0§	++, ++, ++

* Each intragastric dose contained neomycin sulfate 20 mg., polymyxin B sulfate 2 mg., and bacitracin 1000 U.

† Neomycin sulfate 1.3 gm./L., polymyxin B sulfate 60 mg./L., and bacitracin 30,000 U./L.

‡ Animals killed at 2 days to determine the severity of lesions were the same as those used for cultures, but were separated from those used for mortality. 0, absent; +, mild; ++, severe.

§ Less than placebo group, $p < .05$, Fisher's exact test.

Table 4. Rat Mid-Small-Intestinal Flora After a 30 mg./kg. Dose of Indomethacin and Effect of Antibiotics

Organism	Mean* $\log_{10} \pm$ S.D. (No. pos./total) per 12-cm. segment			
	Normal	Indomethacin, 2 days	Indomethacin + antibiotics, 2 days	Indomethacin + antibiotics, 7 days
Lactobacillus	7.9 ± 0.85 (6/6)	8.0 ± 1.1 (8/9)	5.5 (1/9)†	— (0/4)
Yeast	6.1 ± 0.36 (4/6)	— (0/9)	5.9 ± 0.67 (9/9)	7.2 ± 0.26 (4/4)
α-Hemolytic streptococci	4.3 ± 0.67 (3/6)	— (0/9)	2.1 (1/9)	3.9 ± 1.2 (3/4)
Enterococci	6.9 ± 2.6 (5/6)	6.4 ± 1.5 (9/9)	2.1 (1/9)†	— (0/4)
Escherichia coli	2.7 ± 0.45 (5/6)	7.9 ± 1.2 (8/9)‡	2.5 (1/9)†	4.7 ± 2.1 (3/4)
Proteus	2.5 ± 0.48 (4/6)	6.0 ± 2.5 (7/9)	2.3 (1/9)†	2.0 (1/4)
Bacteroides	5.2 (1/6)	7.5 ± 1.1 (8/9)‡§	— (0/9)†	6.3 (1/4)
Clostridium	5.8 ± 3.0 (4/6)	7.6 ± 1.4 (8/9)‡	5.3 ± 2.0 (3/9)†‖	— (0/4)
Staphylococci + *Micrococcus*	4.6 ± 1.9 (4/6)	— (0/9)	— (0/9)	— (0/4)

* Mean and standard deviation of those animals with positive cultures.
† Frequency of isolation less than indomethacin without antibiotics, $p < .05$.
‡ Mean greater than normal, $p < .05$.
§ Frequency of isolation greater than normal, $p < .05$.
‖ Mean less than indomethacin without antibiotics, $p < .05$.

biotic-treated group, since the animals lived longer and were intubated more times. In an attempt to eliminate this problem and get a more uniform administration, antibiotics were next put in the drinking water. However, the disappearance of fluid from the water bottles dropped from a normal level of 40–50 ml. per animal per day to 10–20 ml./day during the first 3 days after indomethacin, regardless of whether antibiotics were present in the drinking water. In surviving animals, intake rose to normal by the sixth day. Using this information in the next experiment, we supplemented the antibiotics consumed in the drinking water with intragastric doses during the first 5 days and achieved 100% protection at the 30 mg./kg. dose of indomethacin. With the same antibiotic schedule, protection was only partial at the 40 mg./kg. dose of indomethacin.

The mid-small-intestinal flora was enumerated in placebo-treated rats 2 days after a 30 mg./kg. dose of indomethacin, and 2 and 7 days after indomethacin administration in antibiotic-treated rats (Table 4). The animals killed 2 days after the 30 mg./kg. dose of indomethacin correspond to those used for morphologic study in Table 3. The results in the normal rats correspond closely with our previous results [7] except for the frequent presence of enterococci and *Clostridium* in large numbers. Two days after indomethacin there was a significant increase in the numbers of *E. coli*, *Bacteroides*, and *Clostridium*, and the frequency of isolation of *Bacteroides* was significantly greater (Table 4). Although the mean and frequency of isolation for *Proteus* did not quite reach statistical significance, 4 of 9 animals had greater than 10^7 *Proteus* per intestinal segment, while the greatest number in control animals was 10^3. The absence of yeast probably is due to crowding of the plates and inhibition by the other organisms, as yeasts were present in normal numbers in histologic sections of small-intestinal content. Two days after antibiotic treatment, the mid-small-intestinal flora was suppressed, except for yeasts (Table 4). The 3 animals with *Clostridium* were all from the first experiment. No other organism was found more than once in 9 animals. At 7 days the yeasts were present in slightly greater numbers ($p < .05$), and moderate numbers of *E. coli* and α-hemolytic streptococci were found.

The antibiotics were also effective in preventing bacteremia (Table 5). The spleen and liver cultures may represent peritoneal organisms, rather than organisms within these organs. We have observed previously the presence of *Clostridium* in the liver and spleen of control and experimental animals, and suspect that these are due to contamination of the cultures by spores. In the experiment in which 100% protection was

Table 5. Blood, Spleen, and Liver Cultures After a 30 mg./kg. Dose of Indomethacin and Effect of Antibiotics

Culture site	No. pos./total, organisms (number of times isolated)			
	Normal	Indomethacin, 2 days	Indomethacin + antibiotics, 2 days	Indomethacin + antibiotics, 7 days
Blood	0/6	8/9*	1/9†	0/4
		E. coli (×6)	*E. coli* (×1)	
		Proteus (×3)		
		Bacteroides (×1)		
Spleen	1/6	9/9*	6/9	3/4
	Clostridium (×1)	*E. coli* (×5)	*E. coli* (×1)	*Clostridium* (×1)
		Proteus (×5)	*Proteus* (×3)	α-Hemolytic streptococcus (×1)
		Clostridium (×3)	*Enterococcus* (×1)	Yeast (×1)
		Klebsiella-Aerobacter (×1)	Yeast (×2)	
Liver	2/6	9/9*	8/9	2/4
	Clostridium (×2)	*E. coli* (×6)	*E. coli* (×1)	*Clostridium* (×2)
		Proteus (×4)	*Clostridium* (×3)	
		Clostridium (×3)	*Enterococcus* (×1)	
		Klebsiella-Aerobacter (×1)	Yeast (×2)	

* Greater than control, $p < .05$.
† Less than indomethacin without antibiotics, $p. < .05$.

observed, the liver and spleen cultures were negative in 1 animal and yielded yeasts in the other 2 animals.

Discussion

This study demonstrates that indomethacin produces a striking and, as far as we know, unique type of longitudinal ulceration on the mesenteric side of the distal small intestine. Ulcers in the stomach, duodenum, and colon were less frequent and focal. Cioli, Silvestrini, and Dordoni [5] found gastric ulcers in 80–100% of fasted rats 18 hr. after administration of indomethacin in doses of 25–200 mg./kg. Although their reported lower doses were in the same range as those used in this study, the fasting may have made their animals more susceptible to gastric ulceration. These authors did not mention other lesions, and we are not aware of any detailed published description of lesions produced by indomethacin in the rat. Menguy and Desbaillets [6] found gastric and duodenal ulcers in 3 of 5 dogs given a 5 mg./kg. dose of indomethacin daily. Peptic ulcers in man have been attributed to indomethacin, especially when the dose was greater than 200 mg./day.[1-4] Shack [8] reported two instances of nonspecific ileal ulceration in patients taking indomethacin. It probably is not reasonable to compare the effects of indomethacin in the rat with the toxic effects in man, since excretion patterns are different,[9] and the doses used in our rats are much greater than required for anti-inflammatory effects.[10]

The pathogenesis of the lesion produced by indomethacin is not clear. The distribution of the lesion along the mesenteric side of the intestine suggests that the anatomy of this region may play a role in localization of the lesion. We were not able to demonstrate a vascular lesion. Hucker *et al.*[9] studied absorption and excretion of indomethacin in rats, as well as in other species. Indomethacin is absorbed rapidly from the intestines and is excreted by both kidney and liver. The fraction of the drug excreted by the liver undergoes an enterohepatic circulation. Although rat small intestine does not concentrate the drug above plasma levels, the small intestine below the entrance of the bile duct is exposed continuously to the drug until it is excreted from the body. In the rat, over half of the dose is excreted by 24 hr., and excretion is divided about equally between urine and feces.

We have been interested in the role of the intestinal flora in ulcerative diseases of the intestines. Previously, we found that whole-body or intestinal X-irradiation in amounts that produced diffuse loss of small-intestinal epithelial cells resulted in a bacterial overgrowth of cecal-type organisms in the small intestine of the rat.[7] The floral overgrowth after irradiation was quite similar to that found after administration of

indomethacin. Prevention of the bacterial overgrowth with antibiotics did not alter the lethal effect or the character of the lesions produced by irradiation. In contrast, prevention of the bacterial overgrowth after indomethacin administration not only reduced the lethal effect but resulted in much less severe ulceration. It is unlikely that the antibiotics interfered directly with the action of indomethacin, since the antibiotics were started 2 hr. after indomethacin administration.

It seems more likely that the flora plays a role in the development of the ulcers. The fact that a similar floral overgrowth in an intestine denuded by irradiation does not result in severe peritonitis like that produced with indomethacin suggests that there is something about the lesion that allows bacteria to get through the intestinal wall. Histologically, indomethacin results in necrosis in all layers of the intestinal wall, although in the early lesion mucosal damage is predominant. Since the antibiotics do not prevent completely the ulceration, we think that these agents reduce the severity of the lesion by allowing healing to start sooner, rather than by preventing some syngergistic effect between the flora and the drug in causing the initial damage.

Summary

Indomethacin given intragastrically or intramuscularly in single doses produced penetrating longitudinal ulcers on the mesenteric side of the distal jejunum and the ileum of rats and, less frequently, slight focal ulceration in the stomach, duodenum, and cecum. Dose of 5 and 10 mg./kg. produced mild, nonfatal ulcers, while doses of 20, 30, and 40 mg./kg. resulted in severe ulcers, and fatal peritonitis and bacteremia. An overgrowth of cecal-type organisms occurred in the small intestine 2 days after administration of a 30 mg./kg. dose of indomethacin. Peroral administration of neomycin, polymycin B, and bacitracin prevented the bacterial overgrowth in the small intestine, greatly reduced the severity of the ulcers, and prevented the bacteremia produced by indomethacin. In one experiment, 100% protection from the lethal effects of indomethacin, 30 mg./kg., was achieved by combined intragastric and oral administration of these antibiotics. The protection from the lethal effect was only partial with a 40 mg./kg. dose.

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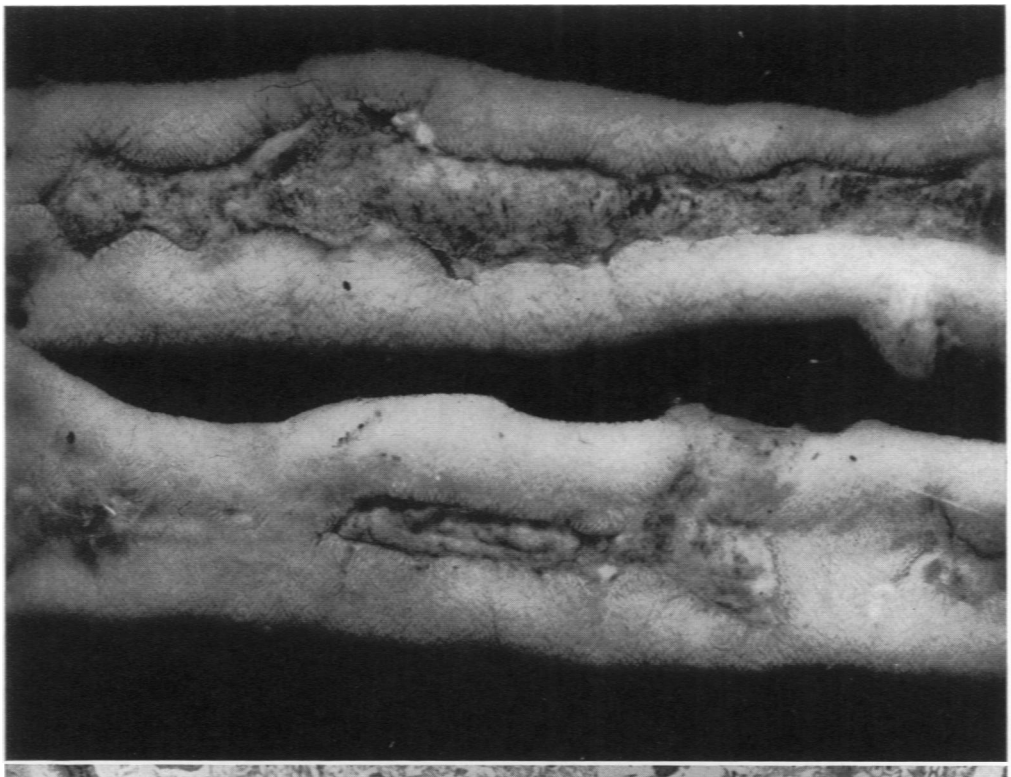
[*Illustrations follow*]

Legends for Figures

Fig. 1. Gross appearance of longitudinal ulcers in rat small intestine 3 days after indomethacin, 20 mg./kg. Grossly, the nonulcerated areas have normal-appearing villi. × approx. 3.

Fig. 2. Rat small intestine 12 hr. after indomethacin, 20 mg./kg., demonstrating early necrosis of mucosa, mild inflammatory reaction in mesentery, and fibrinopurulent exudate on serosa *(right)*. Hematoxylin and eosin. × 50.



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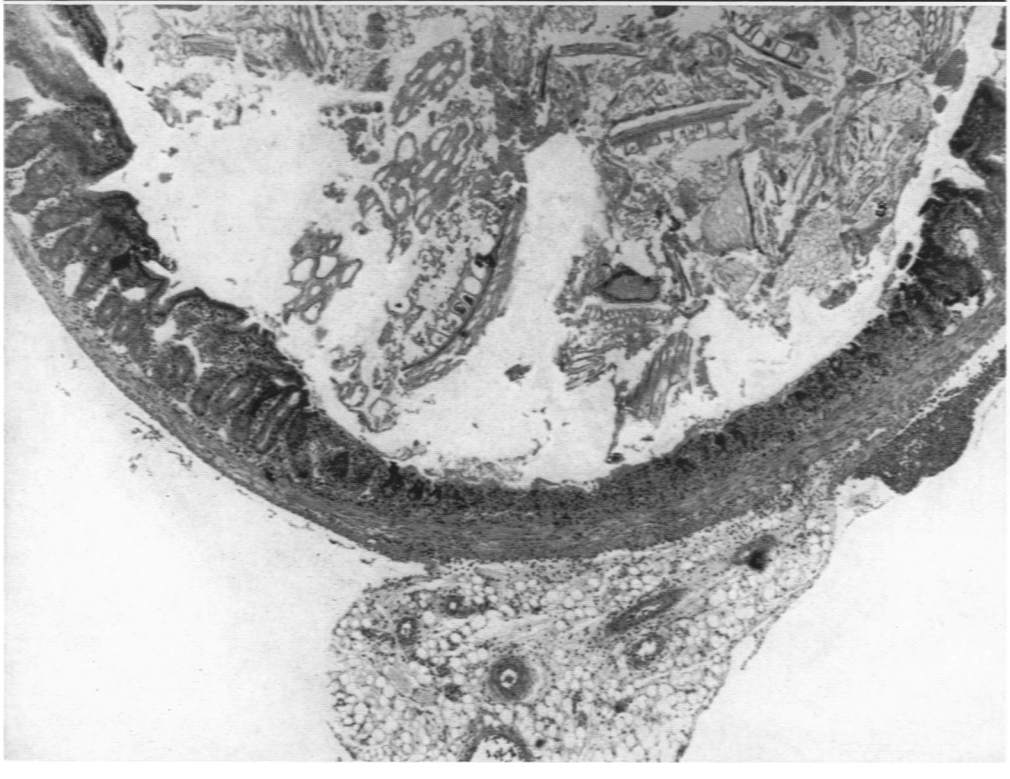

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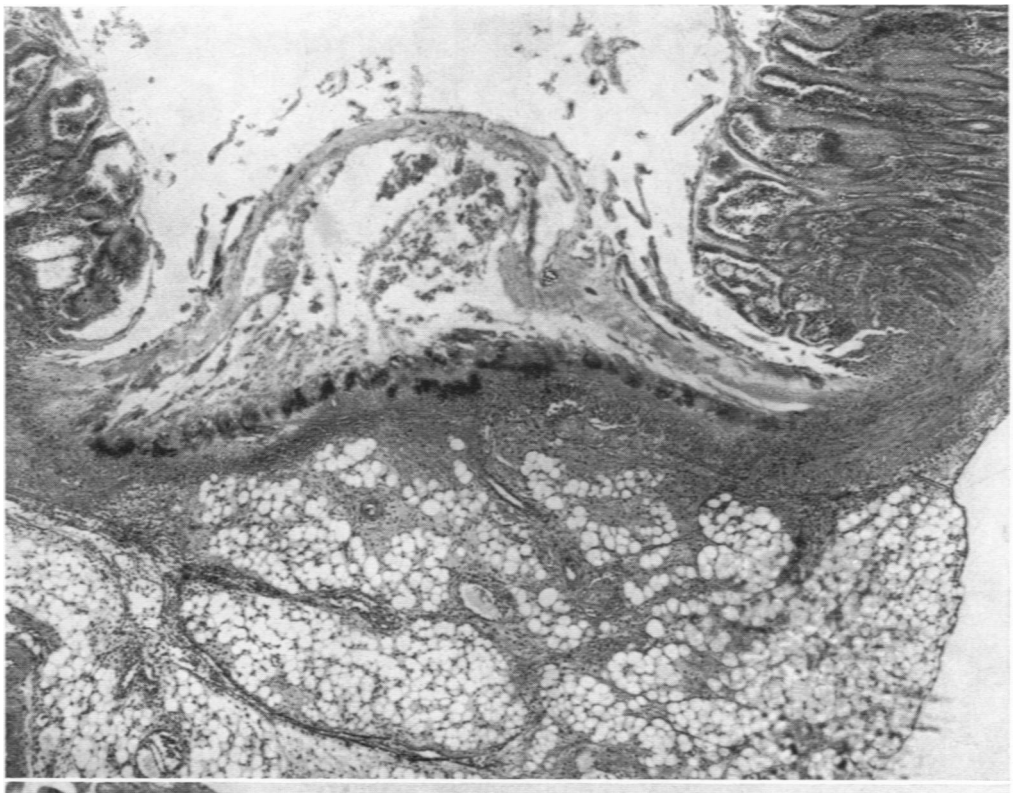
Fig. 3. Rat small intestine 3 days after indomethacin, 20 mg./kg. Ulcer extends through wall of intestine into the severely inflamed mesentery. The dark masses along ulcer surface are colonies of bacteria. This histologic appearance corresponds with gross appearance of ulcers in Fig. 1. Hematoxylin and eosin. × 50.

Fig. 4. Rat colon 3 days after indomethacin, 20 mg./kg. Focal necrotizing lesions such as this were observed histologically in about one-third of the animals. Hematoxylin and eosin. × 80.



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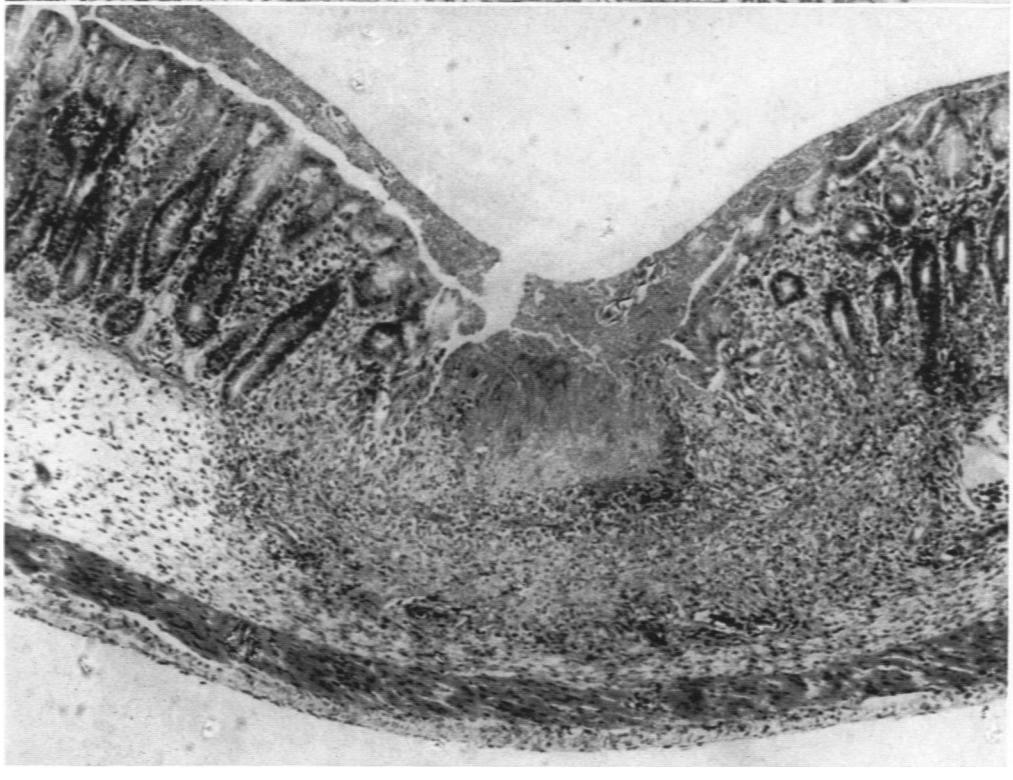

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