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Colonic Mucosectomy Using Laser Photodynamic Therapy

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Photodynamic therapy (PDT) involves photosensitizing tissue and then activating it with monochromatic light, causing necrosis. Precise control of the extent of injury should be possible by varying the energy density of the light applied to the target tissue. We tested the sensitivity of colonic tissue to PDT by injecting 10 mg/kg Photofrin II intraperitoneally in 10 rats. After 24 hr the left colon was opened and cleansed. A 1.0-cm² area of mucosa was exposed to 630 nm (red) light produced by an argon-pumped dye laser. Pairs of rats were treated with energy densities of either 10, 20, 40, 60, or 80 J/cm², controlled by varying exposure times. After 48 hr, we sacrificed the rats and fixed, sectioned, and stained the left colons. The depth of injury was measured with an ocular micrometer and expressed as a percentage of normal bowel wall thickness. A curve was fit to the data points by computerized nonlinear regression. The relationship between depth of injury (Y) and energy density (X) was found to fit the equation $Y = 1 - ae^{bx}$, where constants $a = 1.15$ and $b = -0.0353$, ($R^2 = 0.93$, $P < 0.001$). The relationship between injury and energy density is biphasic, rising rapidly from 0 to 40 J/cm² and more slowly after this point, suggesting that colonic mucosa is more sensitive to PDT than muscularis, providing a margin of safety against perforation. Bowel perforation did not occur in this study but is predicted by extrapolation for energy densities of 100 J/cm² or greater. These data indicate that photodynamic colonic mucosectomy is possible.

INTRODUCTION

There is a need for a simple, inexpensive, and efficient means of ablating rectal mucosa in preparation for pouch-anal operations and other pull-through procedures. Various surgical and nonsurgical methods have been used in the past. Surgical removal of the mucosa is tedious and often frustrating and carries the risk of major hemorrhage. Various technical methods have aided in removing the rectal mucosa including injection of epinephrine submucosally, electrocautery, ultrasonic scalpel, and eversion of the stump [1]. Another method has been to remove the mucosa preoperatively. In a unique approach to the problem, Kojima *et al.* recommended instilling caustics into the rectum [2]. In this study, we investigated laser photodynamic therapy using a drug under investigation for cancer therapy, Photofrin II.

Hematoporphyrin derivatives such as Photofrin II initiate cytodestruction when activated by monochromatic light in the 630-nm (red) wavelength [3]. An intracellular oxidation reaction is initiated producing cytotoxic singlet oxygen and resulting in cell destruction [4]. Previous studies have confirmed the preferential uptake of Photofrin II in dysplastic and malignant cells and this has led to applications directed at eradicating malignancy [5]. However, preferential uptake of Photofrin II also occurs in some normal tissues. Colonic mucosa is an example of a normal body tissue that concentrates Photofrin II [6]. We hoped that we could use this property for colonic mucosectomy. We hypothesized that by pretreating animals with Photofrin II, we could precisely control the extent of mucosal damage by varying the light energy applied to the colon. This study reports our initial steps in studying laser–tissue interactions and the dosimetry of Photofrin II in the colon.

MATERIALS AND METHODS

Ten disease-free male Sprague–Dawley rats weighing between 300 and 325 g. were given only water orally for 48 hr before the experiment. They were each injected intraperitoneally with 10 mg/kg Photofrin II (Photofrin, Inc., Cheektowaga, NY). The rats were randomly divided into five groups of two rats each and the tails of each group were uniquely labeled. Twenty-four hours after injection, the rats were anesthetized with ketamine HCl and acepromazine malate [7]. After shaving and prepping the abdomen with alcohol, a midline incision was made. The sigmoid colon was isolated and a 1-cm longitudinal enterotomy was made. The bowel wall was flattened with a cotton swab and a 1-cm² area of mucosa was irrigated clean of fecal particles. Laser irradiations were performed with a Cooper Lasersonics (Santa Clara, CA) 770-DL argon-pumped dye laser. The dye used was DCM-premixed laser dye (Cooper Lasersonics) with a tuning range from

0022-4804/89 $1.50

610–690 n*M*. The dye laser was tuned to emit radiation at 630 n*M* for the entire experiment. The wavelength was verified to ±1 n*M* using a Jobin Yvon No. 5/354 ultraviolet monochromator (Longjuneau, France). The radiation was coupled into a 400-μm fused silica fiberoptic using a Spectra Physics (Mountain View, CA) model 316 fiberoptic coupler. The output end of the fiber was terminated with a microlens that focused the laser irradiation into a circular field of uniform light intensity. Laser irradiation emanating from the fiber was monitored with a Coherent (Palo Alto, CA) Model 210 power meter before, during, and after treatment. Rats were placed underneath an aperture that controlled the area of light illumination on the colon. The area of illumination was 1 cm^2. Total laser energy density was 10 to 80 J/cm^2 with a power density of 100 mW/cm^2.

The five pairs of rats were treated with energy densities of 10, 20, 40, 60, or 80 J/cm^2 respectively, controlled by varying exposure times. The colons were then closed with running 5-O proline and the abdominal incisions were closed with silk sutures. After 48 hr the rats were sacrificed with Euthasix injection. The laser-treated segments of colon were excised and placed in randomly numbered vials of saline. As a result of a laboratory accident, one colon specimen treated with 40 J/cm^2 was lost, so nine specimens were available for examination. The specimens were examined grossly and were then stained with hematoxylin and eosin. A pathologist, blinded as to the dose of laser energy applied to the bowel, examined the mounted specimens for both gross and microscopic changes. In addition, using an ocular micrometer, the depth of injury was measured as well as the full-thickness of adjacent intact bowel wall. The degree of injury was expressed as a percentage of the full thickness wall, allowing for comparisons between rats.

The depth of injury was plotted against the energy density delivered to the bowel in joules per square centimeter. The curve was analyzed on an IBM-AT computer using nonlinear regression (NONLIN program, Statgraphics Software, STSC, Inc., Rockville, MD). An equation relating depth of injury to energy density was generated by the program. An analysis of variance (ANOVA) for the full regression is performed with an associated P value and R^2 statistic.

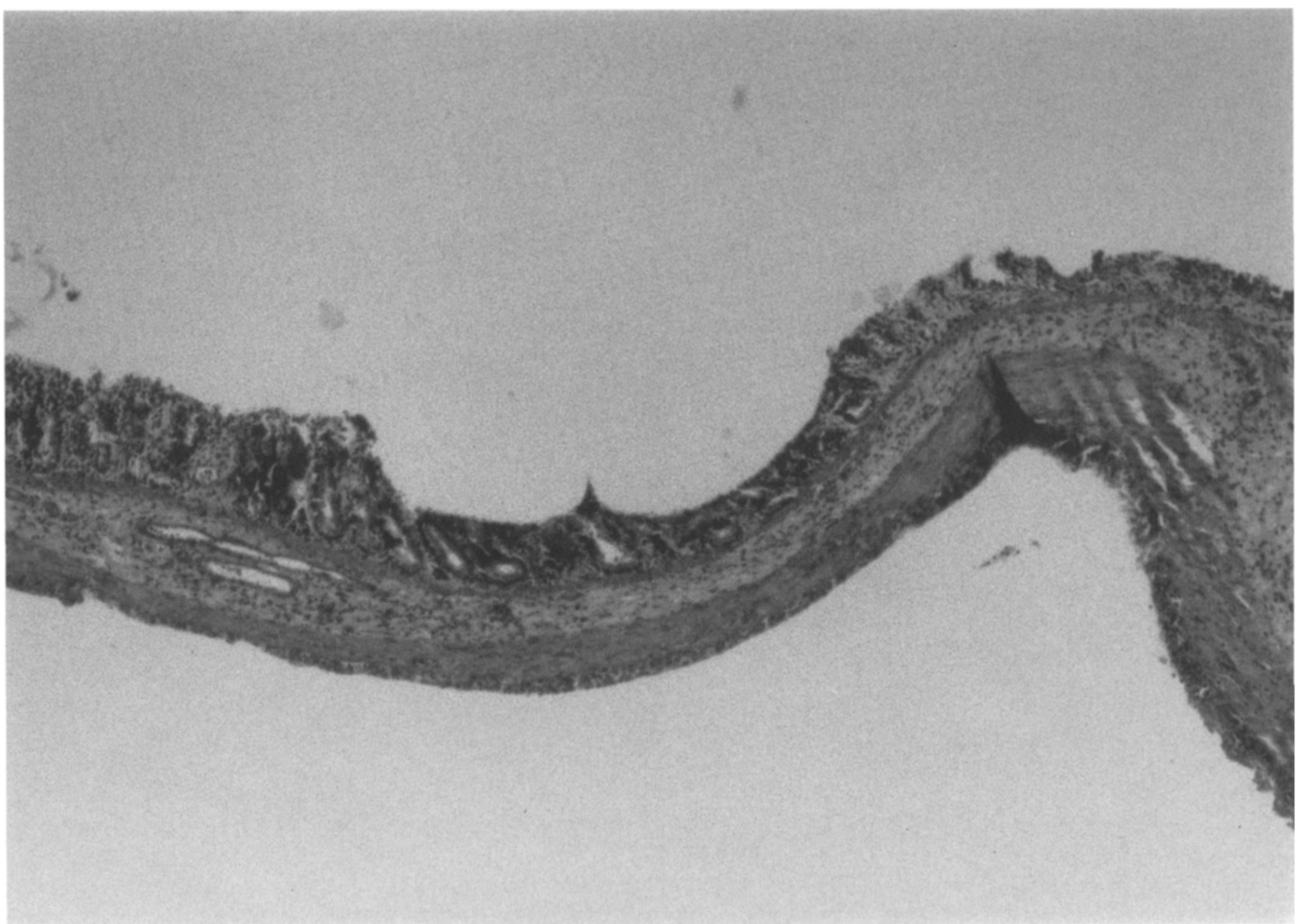

FIG. 1. Photomicrograph (50×) of colon wall after exposure to 10 J/cm^2 laser energy. The injury is about 25% of bowel wall thickness or 50% of the mucosal thickness.

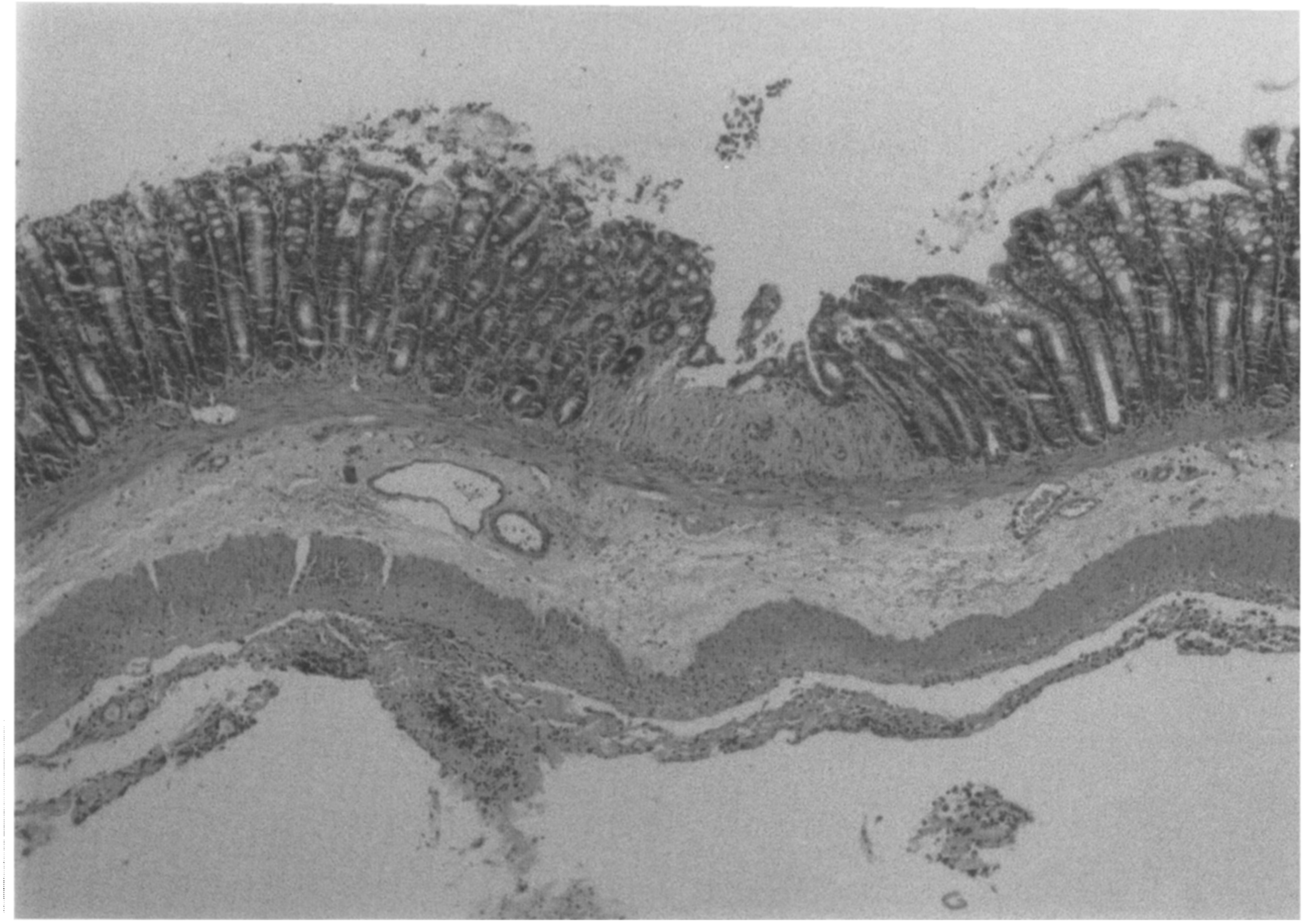

FIG. 2. Photomicrograph of colon wall after exposure to 20 J/cm^2. The entire mucosa is ablated with mild fibrosis and preservation of the muscularis mucosa. Mild to moderate inflammation was present in the submucosa.

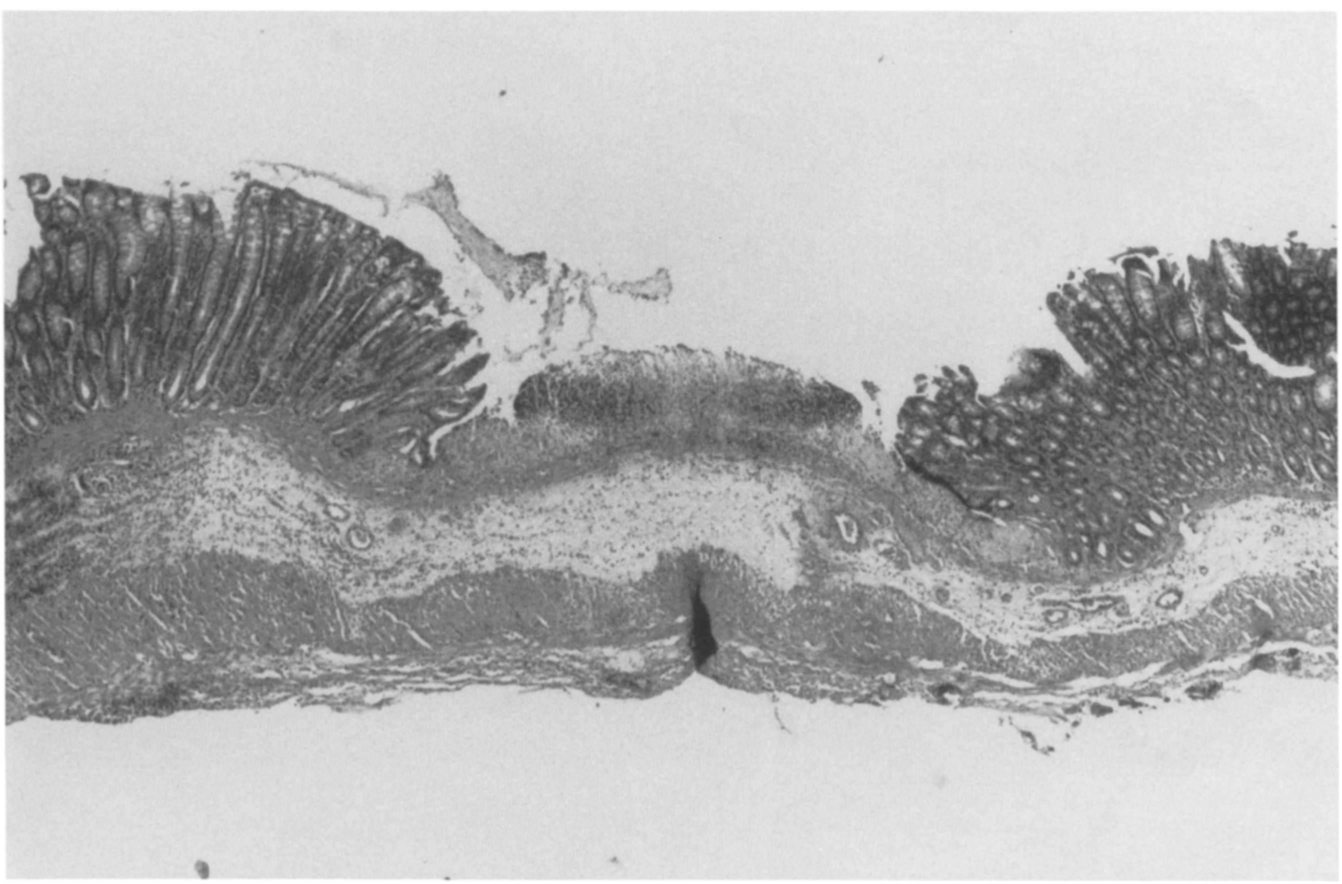

FIG. 3. Photomicrograph showing injury to colon exposed to 40 J/cm^2. There is full thickness loss of mucosa and muscularis mucosa with inflammation and edema of the submucosa.

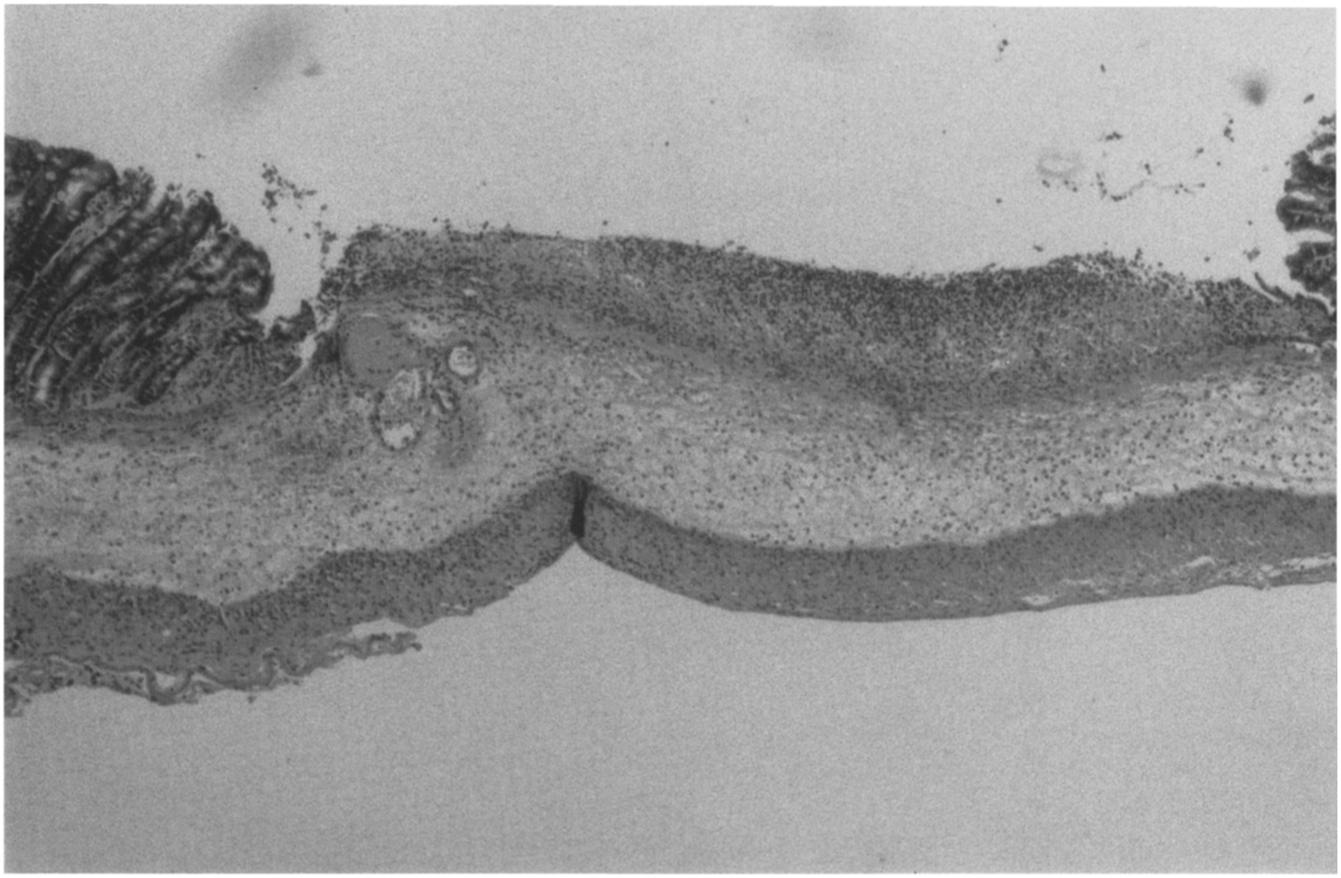

FIG. 4. Photomicrograph showing injury to colon exposed to 60 J/cm². Besides the findings described for the energy density of 40 J/cm², there is also thinning and focal loss of the submucosa.

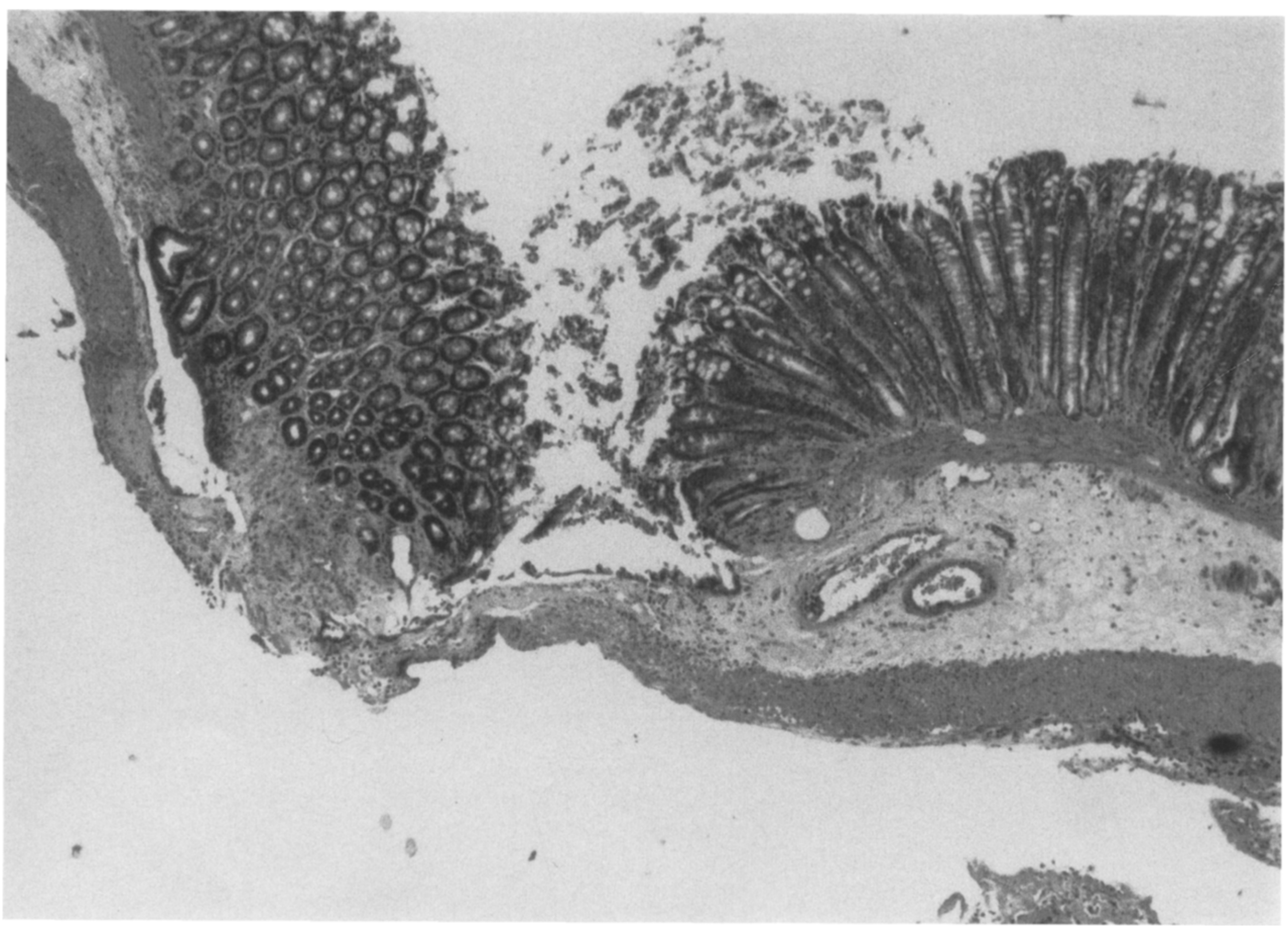

FIG. 5. This photomicrograph shows the extensive injury found after exposure to 80 J/cm². There is total absence of mucosa, muscularis mucosa, and submucosa. The muscularis propria is thinned and focally absent. The ulcer base extends to the serosa but perforation is not seen.

RESULTS

Sections of colon receiving 10 to 40 J/cm^2 were grossly normal in appearance with retention of normal mucosal folds. Colons treated with 60 and 80 J/cm^2 showed obvious mucosal ulceration. Microscopically, injury to the mucosa was evident at the lowest energy density studied. An energy density of 10 J/cm^2 ablated 50% of the mucosa thickness (Fig. 1). There was minimal lymphocyte infiltration of the underlying lamina propria. Sections of colon receiving 20 J/cm^2 developed full thickness loss of the mucosa (Fig. 2) but the muscularis mucosa remained intact. There was mild to moderate acute inflammation throughout the adjacent submucosa. In the section of colon receiving 40 J/cm^2, there was total loss of the mucosa and muscularis mucosa (Fig. 3). The submucosa was moderately edematous and infiltrated with acute and chronic inflammatory cells. Bowel receiving 60 J/cm^2 was ulcerated, with absence of mucosa and muscularis mucosa (Fig. 4) and thinning of the submucosa. There was a moderate amount of fibrosis and acute inflammation with fibrinopurulent exudate throughout the submucosa. Colons exposed to 80 J/cm^2 (Fig. 5) were more deeply ulcerated with total absence of mucosa, muscularis mucosa, and submucosa extending to, and focally through, the muscularis propria. The serosa remained intact across the ulcer base. Throughout the muscularis propria and subserosa there was marked inflammation and a fibropurulent exudate. However, free perforation of the bowel did not occur.

The depth of injury related to the energy density is shown graphically in Fig. 6. The nonlinear relationship between injury and energy density is readily apparent. This relationship is biphasic, rising rapidly from 0 to 40 J/cm^2 (through the mucosa) and more slowly after this point. The curve best fit an exponential equation, $Y = 1 - ae^{bX}$ ($R^2 = .93$), where Y is the depth of injury, a and b are constants 1.15 and −0.0353, respectively, and X is the energy density in joules per square centimeter.

DISCUSSION

Photodynamic therapy uses a photosensitizing dye that is selectively absorbed by target tissues. The dye is activated by monochromatic light where it causes an intracellular photochemical reaction that liberates singlet oxygen and kills the cell. Unlike most laser methods familiar to surgeons, this method does not use heat to ablate tissue and so avoids much of the risk commonly associated with laser destruction of tissue.

Normal colonic mucosa selectively absorbs Photofrin II, hindering its usefulness for treating colon carcinoma [4]. However, in using Photofrin II for sensitizing normal colon mucosa, we have exploited this property for a different end, ablating the normal mucosa. Is photodynamic therapy practical and safe for mucosectomy? The biphasic nature of the injury–energy curve indicates that colonic mucosa is more sensitive to phototherapy than muscularis. This finding suggests a margin of safety against perforation. While bowel perforation did not occur in this study. The curve predicts by extrapolation that perforation will occur for energy densities greater than 100 J/cm^2. This study shows that laser photodynamic therapy could possibly be a practical way of ablating colonic mucosa.

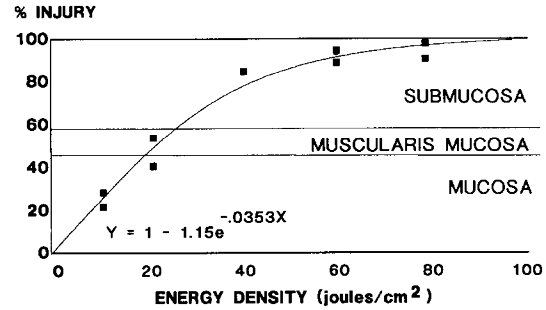

FIG. 6. This graph shows the degree of injury expressed as a percentage of bowel wall thickness plotted against the energy density in J/cm^2. The curve that fits the experimental data is nonlinear and best fits the equation $Y = 1 - ae^{bx}$ ($R^2 = 0.93$, $P < 0.001$), where Y is the depth of injury, a and b are constants 1.15 and −.0353, respectively, and X is the energy density in J/cm^2.

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