Collagen Formation During the Healing of Colonic Anastomoses*

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To study new collagen formation in the healing of anastomoses in the right colon, five male Sprague-Dawley rats had single-layer colonic anastomosis performed. Five additional rats had suture of the colon with interrupted sutures without transection of the colon. Ten animals served as controls. One week after surgery, animals were given ^{3}H-proline repeatedly to label new collagen formation during the second and third weeks. Animals were sacrificed three weeks after suturing or anastomosis. Two- or 10-mm segments were precisely excised on either side of the suturing or anastomoses. Total collagen and its total radioactivity were measured per segment. Collagen hydroxyproline and its radioactivity increased (100 per cent) only within 1 cm of the anastomosis or suture alone. No local or distant decrease in collagen was observed. Collagen formation in colonic wounds appears to be a local process.

THE VAST AMOUNT of colonic surgery done annually for a variety of diseases and trauma makes the understanding of colonic healing important. As many as 69 per cent of colonic anastomoses leak.[1] Despite the fact that 4 to 8 per cent of anastomoses cause clinically significant complications, [2,3] numerous clinical and animal studies, [4–10] have not fully elicited causes or remedies for these serious complications.

Injury to the colon is healed by scar formation with collagen. The rate of healing is very rapid, as demonstrated by recovery of bursting strength within five to seven days.[11, 12] The role of new and old collagen in this healing process has been ill-defined. Some authors[11, 13, 14] have proposed a lysis of old collagen and formation of new collagen. Others[15] have proposed an increase in total collagen with a possible decrease in collagen concentration. Although a decrease in collagen concentration during healing has been suggested in other systems,[16, 17] this has not been adequately evaluated in the colon. To further define this problem, the following study was undertaken.

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Methods

Twenty adult male Sprague-Dawley rats weighing between 220 and 250 g were divided into three groups. In the first group of five animals, right-sided colonic anastomoses were made 5 cm distal to the ileocecal valve. The colon was divided and then resutured in a single-layer, end-to-end anastomosis with interrupted 6.0 prolene sutures. In the second group of five animals, the same procedure was followed except that the right colon was not divided, but similar sutures were placed at the same location to study the effect of the suture alone without the stimulus of actual colonic transection. Ten animals served as controls; five controls were sacrificed at the beginning of the experiment and five controls at the conclusion, three weeks later.

Thirty microcuries of ^{3}H-proline were injected subcutaneously into all animals three times a week, starting one week after surgery and ending three days before sacrifice. Five injections totaling 150 microcuries were given to each rat. At three weeks after surgery, all animals were sacrified by *in situ* fixation with glutaraldehyde perfusion, and the area surrounding the anastomosis or suture was excised and divided precisely into 2-mm or 10-mm segments. Segments were hydrolyzed in 6 N HCl, and hydroxyproline[18] and radioactivity[19] were measured to reflect total collagen and new collagen formation per centimeter.

Results

By measuring hydroxyproline and radioactivity as compared with length of intestine from anastomosis, differences caused by changes in total dry weight are eliminated. Total hydroxyproline per centimeter,

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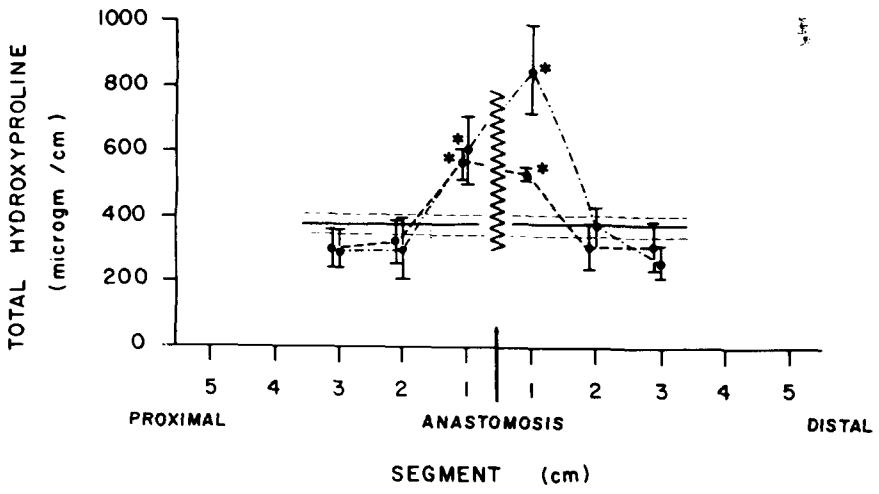

FIG. 1. Collagen mass per centimeter in proximal and distal segments of the right colon, three weeks after suturing alone or after transection and anastomosis. Data are presented as mean ± SE. Increase in hydroxyproline (collagen) immediately adjacent to colonic anastomosis. * = $P < 0.01$. Key: —— Control. — • — Anastomosis. — — Suture only.

representing total collagen, is seen in Figure 1. All increases in collagen (100 per cent) occur within a centimeter on each side of the anastomosis. Further defining the segments in millimeters, as seen in Figure 2, shows that essentially all increases occur within 2 to 4 mm ($P < 0.05$). Similarly local increases in collagen occur with suturing alone.

When the location of radioactivity is examined, a similar pattern emerges. When 1 cm segments are taken, only the segment on each side of the anastomosis shows a significant increase ($P < 0.05$) (Fig. 3). When 2 mm segments are examined (Fig. 4), all increases are within 4 mm of the anastomosis or suture. The segment within 2 mm increases more than twice ($P < 0.01$) and is significantly increased over the suture injury alone ($P < 0.05$).

Random distant segments failed to show any changes of collagen mass or uptake of radioactivity, showing the stability of distant collagen.

Discussion

Attempts to improve healing and technique of colonic repair must begin with a basic understanding of the healing process of the colon. The rate of colonic healing, as measured by bursting strength,[11,12] is much faster than would be expected if compared with healing in skin, tendon, or bone.[11,20,21] In fact, the post-anastomotic bursting strength returns to normal in eight to 12 days after injury.[11,22] This indicates an extremely rapid system of wound healing.

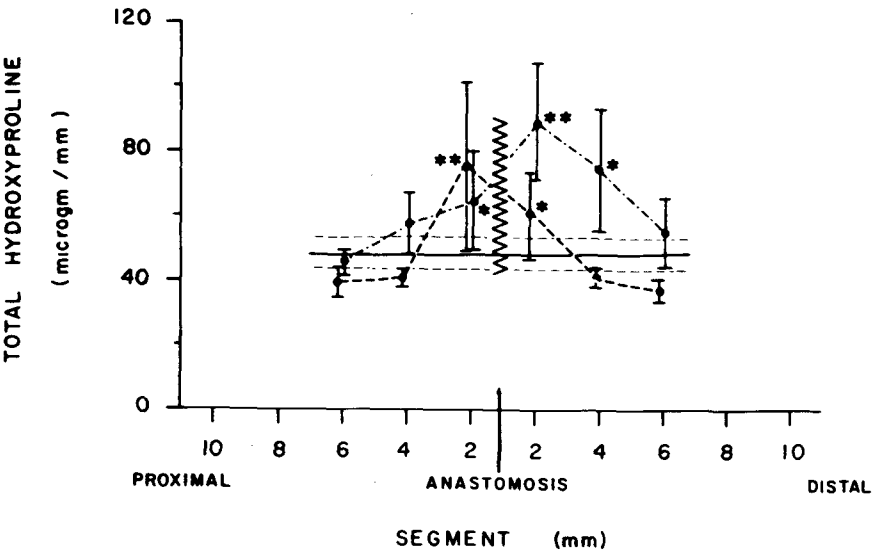

FIG. 2. Collagen mass per 2 mm in proximal and distal segments of the right colon, three weeks after suture alone or after transection and anastomosis. Data are presented as mean ± SE. Increase in hydroxyproline is seen only within the first 2 mm around the anastomosis. ** = $P < 0.01$. * = $P < 0.05$. See key to Figure 1.

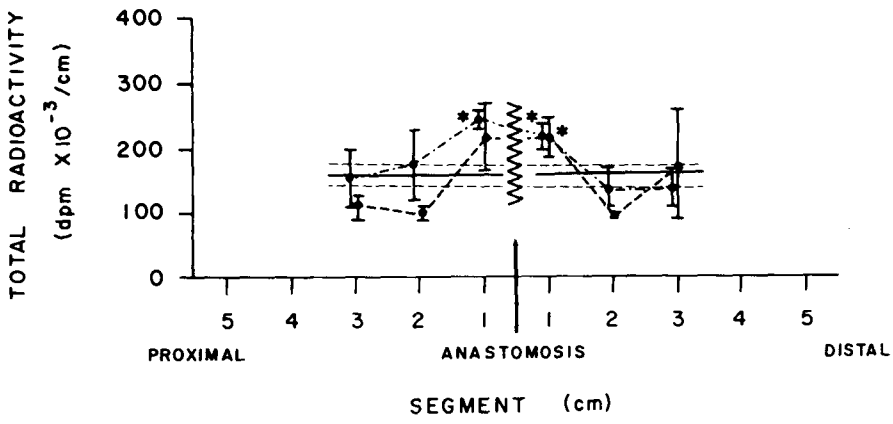

FIG. 3. Radioactive collagen mass per centimeter in proximal and distal segments of the right colon, three weeks after suture alone or after transection and anastomosis. Data are presented as mean ± SE. Increase in acutely labeled hydroxyproline (representing new collagen) is seen near the anastomosis. * = $P < 0.05$. See key to Figure 1.

It has been difficult to evaluate the amount of collagen formation because most authors have used collagen concentration (amount of collagen per dry weight) to report their data.[11,13,14,20,23,24] Factors such as edema due to injury and surgery, fluid excesses or deficiencies, malnutrition, and others may greatly affect wet weight and dry weight (and so affect concentration) without having any effect on the actual amount of collagen present. Thus, the actual collagen amount is not obtainable from concentration measurements. To follow actual collagen present, the collagen should not be reported as concentration, but rather, a more reproducible unit, such as length, should be used. Then changes in weight due to any factor other than collagen will not affect the interpretation of data.

When collagen is measured per unit length in colonic injury and repair, as in the present study, a new pattern emerges. Colonic collagen mass does not appear to be decreased because of injury. Rather, edema and its accompanying proteins may decrease the concentration, but the preexisting collagen, expressed as mass per unit volume or length, appears to be stable. New collagen, as represented by acute labeled ^{3}H-hydroxyproline, is formed in an area very close to the injury and limited to that area. Distant collagen shows no change in amount and no uptake of new radioactive collagen. Thus, there is no apparent formation or breakdown of distant collagen in response to an isolated injury. This local phenomenon of colonic healing is similar to that observed in the healing of skin wound[1] and tendon wound,[2] where healing also represented a localized phenomenon in regard to collagen dynamics.

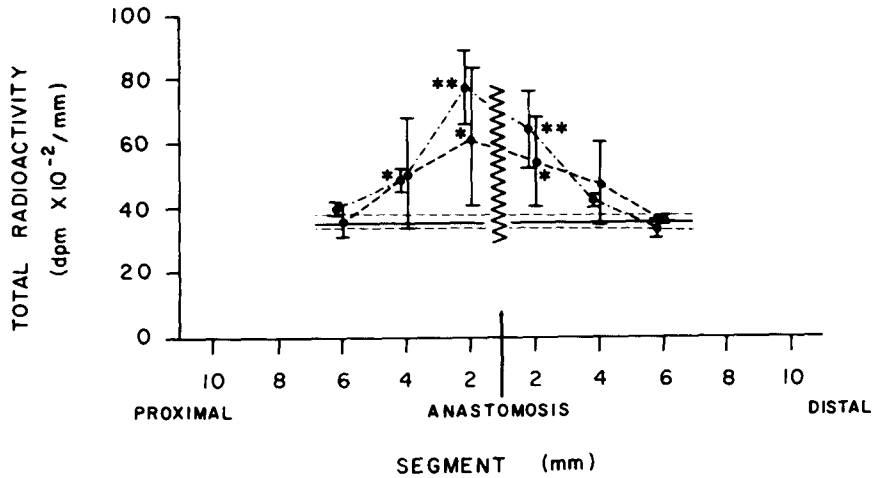

FIG. 4. Radioactive collagen mass per 2 mm in proximal and distal segments of the right colon, three weeks after suture alone or after transection and anastomosis. Data are presented as mean ± SE. New collagen is localized to the first 2 mm surrounding colonic anastomosis. ** = $P < 0.01$. * = $P < 0.05$. See key to Figure 1.

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