Advanced age alone does not suppress anastomotic healing in the intestine

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Background. *Because retrospective clinical studies yield conflicting results and experimental data are completely absent, this study was performed to determine whether anastomotic repair in the intestine deteriorates with age.*
Methods. *Ileal and colonic anastomoses were constructed in two groups of healthy rats, ages 2 to 3 months and 27 to 30 months, respectively. Healing was assessed, both 3 and 7 days after operation, by measuring anastomotic bursting pressure, breaking strength, and collagen content, the latter both biochemically (hydroxyproline) and morphometrically. In addition, the ex vivo collagen synthetic capacities were compared.*
Results. *The development of anastomotic strength was similar in young and old rats; average strength increased from 3 to 7 days and was never lower in the older animals. This was true for both bursting pressure and breaking strength. The collagen production capacity was suppressed in the old rats, particularly in the ileum ($p < 0.05$), whereas the synthesis of noncollagenous protein remained unaltered. However, this did not result in a reduced accumulation of collagen in the anastomotic area—both anastomotic hydroxyproline content and the volume percentage of collagen in the actual wound area were unchanged.*
Conclusions. *Advanced age per se does not affect development of strength or deposition of collagen during early repair of intestinal anastomoses. (SURGERY 1996;119:15-9.)*

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LEAKAGE OF INTESTINAL ANASTOMOSES is a serious complication in surgical practice with concomitant high morbidity and mortality rates. If complicating factors are present, for example, infection or ischemia, chances for dehiscence may rise to such an extent that the surgeon will forgo construction of a primary anastomosis. Most surgeons believe that advanced age is such a complicating factor detrimental to anastomotic repair that it should weigh heavily in the decision to postpone anastomotic construction in high-risk situations.[1]

This belief is based on both the general impression that elderly patients heal more slowly than young patients and on the outcome of some retrospective clinical studies that show increased frequency of anastomotic leakage in advanced-age groups.[2-4] However, other series have failed to confirm this outcome.[5, 6] Such studies also suffer from the unavoidable drawback that they concern patients rather than healthy human beings, which complicates conclusions regarding age as an independent factor.

The question whether advanced age alone indeed impairs intestinal healing is therefore perhaps best investigated by comparing healthy young and old animals. So far, no such experimental data are available. Results of experiments on cutaneous wounds in rodents appear contradictory.[7-9] Moreover, it is best to show restraint in extrapolating results of experiments on cutaneous repair to healing of other soft tissues.[10] In view of the lack of pertinent experimental data and because of the obvious importance of the problem—the elderly representing a substantial part of the patient population needing bowel surgery—we have compared anastomotic healing in the intestines of young and old rats.

MATERIAL AND METHODS

Animals. Two groups ($n = 31$ each) of male rats of outbred strain Cpb:WisWinkelman (Winkelman, Borchen, Germany), ages 2 to 3 and 27 to 30 months, were used. The rats were housed under standardized conditions and received pelleted food (diet RMH-B; Hope Farms, Woerden, The Netherlands) and tap water ad libitum. To acclimatize them the animals were kept for at least 1 week before operation. Only rats that looked healthy and had no palpable tumors were used in the experiment.

In each group, 20 animals were used to measure anastomotic strength and anastomotic hydroxyproline at 3 ($n = 10$) and 7 ($n = 10$) days after surgery. Another

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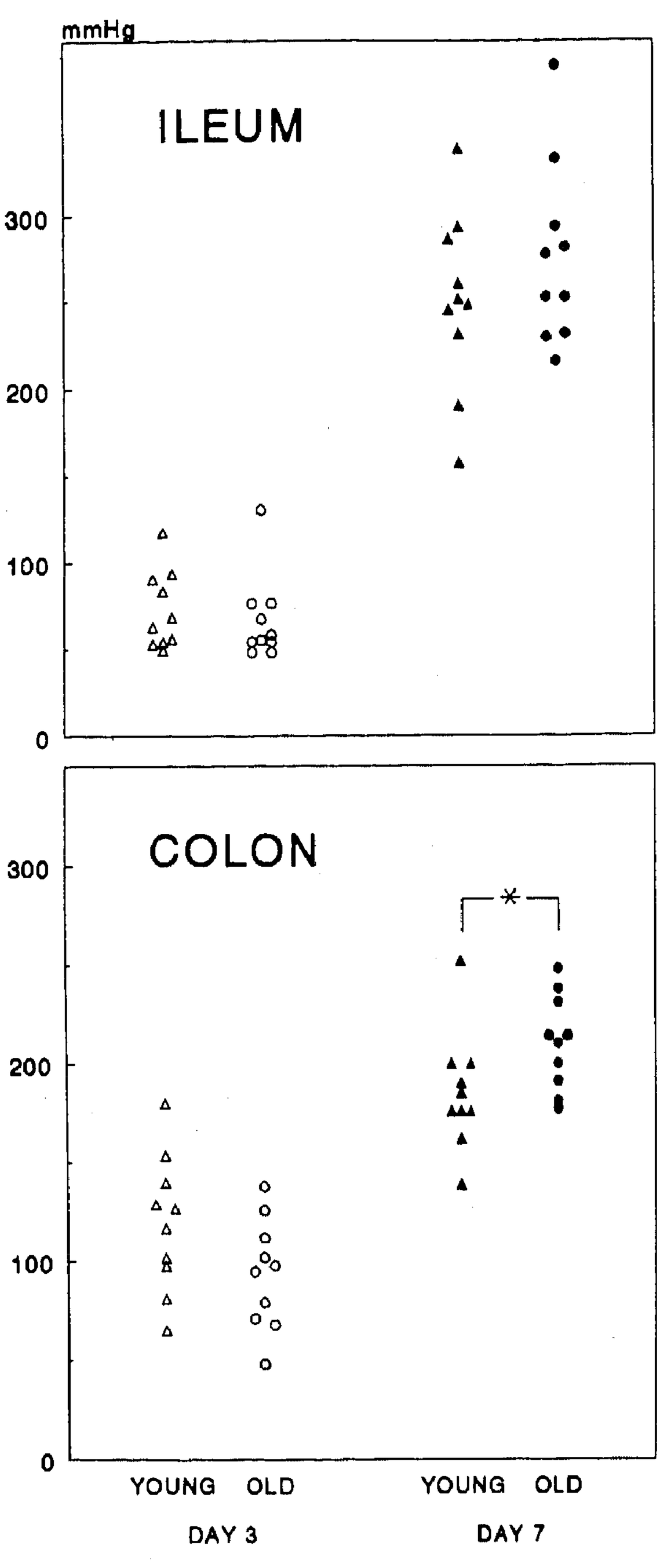

Fig. 1. Anastomotic bursting pressures in individual rats. *Triangles* represent young animals; *circles*, old animals. *Open symbols* and *filled symbols* represent bursting sites within and outside the anastomotic area, respectively. $^{*}p < 0.05$ (Wilcoxon).

six animals were killed 4 days after operation to measure the collagen synthetic capacity in anastomotic explants, and the final five rats were killed 7 days after the operation for the purpose of performing morphometry.

The study was approved by the Animal Ethics Review Committee of the Faculty of Medicine, University of Nijmegen.

Operative procedure. All rats were anesthetized with

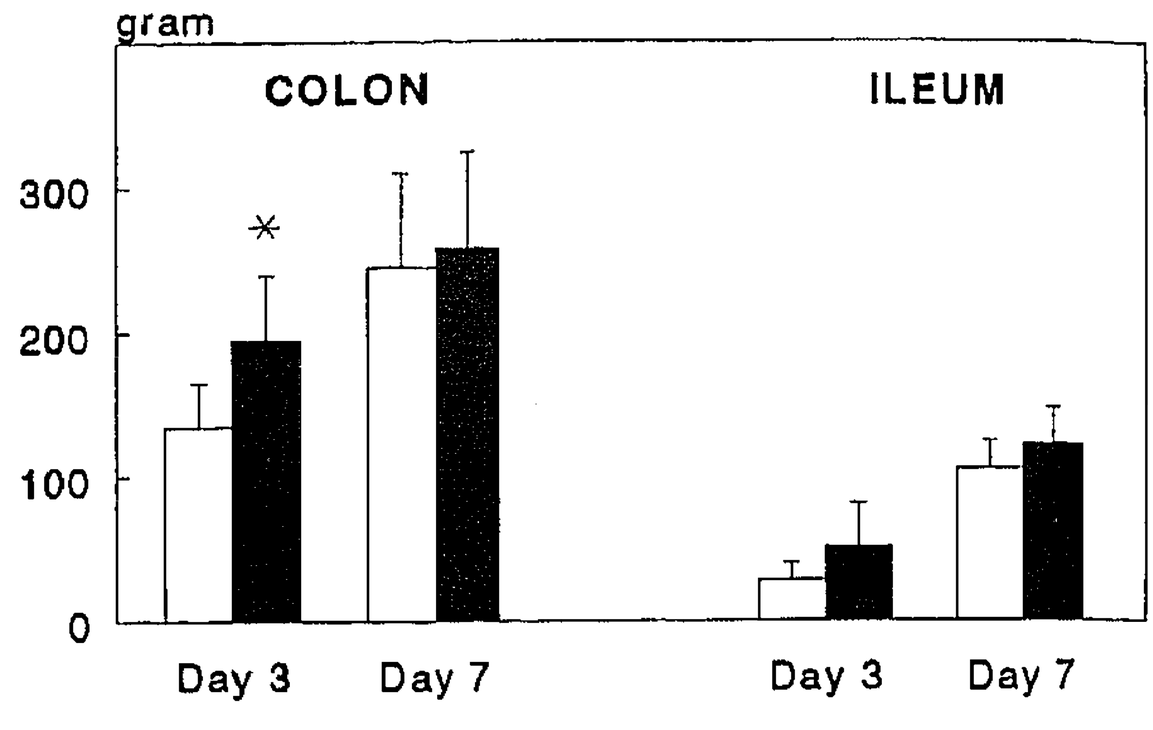

Fig. 2. Anastomotic breaking strength. Average values + SD ($n = 10$) are given. *Open bars* represent young animals; *filled bars* represent old animals. $^{*}p < 0.05$ (Wilcoxon).

isoflurane (4 vol%) administered by mask. After induction, anesthesia was maintained with isoflurane 1.5 vol% and a single dose (1 mg/kg body weight) of morphine HCl, administered subcutaneously.

Operation was performed under semisterile conditions, with an operation microscope. The abdomen was opened through a median incision of approximately 4 cm. One cm of colon was resected 3 cm proximal to the peritoneal reflection and an end-to-end anastomosis was constructed by using eight single-layer inverting interrupted 8*0 Ethilon sutures (Ethicon, Norderstedt, Germany). Subsequently, the procedure was repeated in the ileum, 15 cm proximal to the coecum. The abdominal fascia was closed with silk, and the skin was closed with staples placed 1 cm apart.

Analytic procedures. The rats were killed by cardiac puncture and bleeding. After the abdominal wound was opened and the anastomoses were identified, the adhesions were cut as far as possible without injuring the intestine. An intestinal segment with the anastomosis in the middle was removed; the sutures were left in place, and the segment was analyzed sequentially for bursting pressure and breaking strength.[11]

The anastomotic segment was then cleaned from the surrounding tissue, and a 5 mm segment with the suture line in the middle was collected. The samples were frozen immediately and stored in liquid nitrogen until processing. After weighing, the samples were pulverized and lyophilized and the hydroxyproline content was measured as described previously.[12]

Collagen synthetic capacity in control segments (removed at operation) and anastomotic tissue, collected 4 days after operation, was quantitated by measuring the incorporation of proline into collagenase digestible protein (CDP), according to a procedure previously validated for rat intestinal tissue.[13]

To perform morphometric analysis of collagen in the wound area, 3 cm segments of intestinal tissue, including the anastomosis, were collected 7 days after operation. Each specimen was opened longitudinally, pinned on a plastic grid, and immersed in 4% buffered forma-

Table. Hydroxyproline in uninjured intestine

	Ileum		*Colon*	
	Concentration μg/mg dry weight	*Content μg/cm*	*Concentration μg/mg dry weight*	*Content μg/cm*
Young	6.3 ± 1.7	132 ± 64	14.3 ± 3.5	184 ± 62
Old	6.3 ± 1.7	166 ± 74	21.4 ± 4.8	304 ± 92
p Value	NS	NS	0.0001	<0.0001

Average values (±SD, $n = 20$) measured in control segments, removed at operation. Differences between young and old animals were tested for significance with Wilcoxon test.
NS, Not significant.

line (pH 7.4). The tissue was routinely processed and embedded in Paraplast (Klinipath, Duiven, The Netherlands). Sections with a thickness of 4 μm were stained with hematoxylin-eosin or Sirius-red F3BA for collagen.[14] The collagen concentration in the actual wound area was analyzed by computer-aided morphometry by using a point-counting program and a microscope with a M42 test grid. Measurements were taken directly from microscopic images (magnification ×400) calculating the volume fraction of collagen in the tissue (V(collagen)/V(tissue)). V(collagen) was expressed in percentage of the wound tissue. For each specimen, 15 to 20 microscopic fields were chosen at random and measured.

Statistical analysis. Because the variables tested did not show a Gaussian distribution in all groups, differences between groups were tested for significance with a two-sample Wilcoxon test.

RESULTS

No premature deaths were encountered in either of the groups. The average body weight on the day of operation was 297 ± 35 gm (SD, $n = 20$, animals used for measurement of anastomotic strength) for the young rats and 462 ± 50 gm ($n = 20$) for the old rats. In both groups an average weight loss of 21 gm was observed on the first day after operation.

The results of the bursting pressure measurements in individual rats are depicted in Fig. 1. At day 3, average bursting pressures were similar in young and old rats in both the ileum (73 ± 22 versus 67 ± 25 mm Hg, respectively) and the colon (119 ± 39 versus 94 ± 28 mm Hg, respectively). The bursting site was always within the anastomosis. Between 3 and 7 days after operation the anastomoses grew stronger to such an extent that at day 7 rupture always occurred outside the suture line. Thus the significantly higher bursting pressure observed in the colonic segments of old animals in fact reflects the strength of the uninjured bowel wall. Measuring breaking strength, the breaking site was always within the suture line, even 7 days after operation. Fig. 2 shows the average anastomotic breaking strength in the two groups. Again, there was no indication of reduced strength in the older rats. On the contrary, average values in the older group were higher than in the younger group, significantly so in the 3-day-old colonic anastomoses. Also the gains in strength between days 3 and 7 were not significantly different between the groups: in the ileum 76 ± 7 (young) versus 71 ± 12 (old) gm and in the colon 109 ± 22 versus 63 ± 25 gm, respectively. As a measure for collagen, hydroxyproline was quantitated in intestinal segments. The Table gives hydroxyproline concentration and content in uninjured intestine, removed as control segments during operation. In the colon both hydroxyproline concentration and content were significantly higher in the old rats. Average values for anastomotic segments are depicted in Fig. 3. In the 3-day-old anastomoses hydroxyproline content was higher in the old animals. In the ileum the increase between days 3 and 7 appeared higher in young rats—146 ± 34 versus 58 ± 25 μg/5 mm, respectively. The same was true for the hydroxyproline concentration in colonic anastomoses, which increased by 2.8 ± 1.1 μg/mg in the young animals and decreased by 2.6 ± 1.2 μg/mg in the old rats.

The data mentioned above pertain to segments that contain uninjured intestine next to the actual wound area. To compare collagen in the true anastomotic area we performed morphometric measurements on histologic sections. There was no difference in the volume taken up by collagen fibrils in the wounds from young and old rats. The average volume percentages in the ileum were 30 ± 5 and 29 ± 6 and in the colon 26 ± 4 and 28 ± 3 for young and old animals, respectively.

Finally, collagen synthetic capacity was measured ex vivo in explants of control and anastomotic tissue. Synthesis was calculated on the basis of sample wet weight, protein content, and DNA content. Because results were essentially the same, only the data calculated on a DNA basis are shown (Fig. 4). In both young and old animals collagen synthetic capacity—expressed as dpm CDP—in the control segments was higher ($p < 0.05$) in the colon than in the ileum. Collagen synthesis was strongly stimulated in the anastomosis. Average anastomotic values were reduced in the old animals, although only significantly so in the ileum (by 50%). The increase between anastomotic and control segments was similar in young and old rats. Stimulation of protein synthesis in the

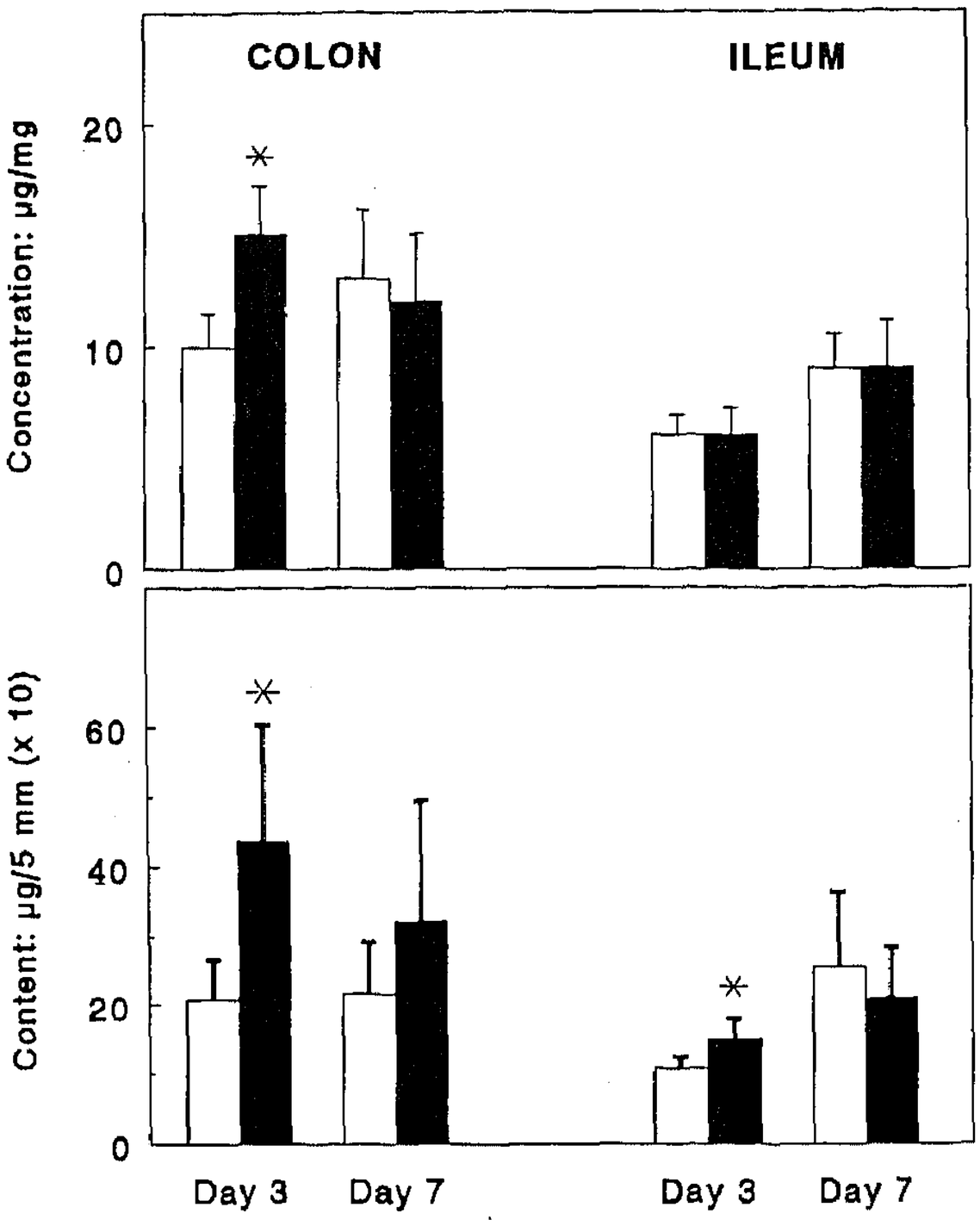

Fig. 3. Anastomotic hydroxyproline. Average values + SD ($n = 10$) are given. *Open bars* represent young animals; *filled bars* represent old animals. *$p < 0.05$ (Wilcoxon).

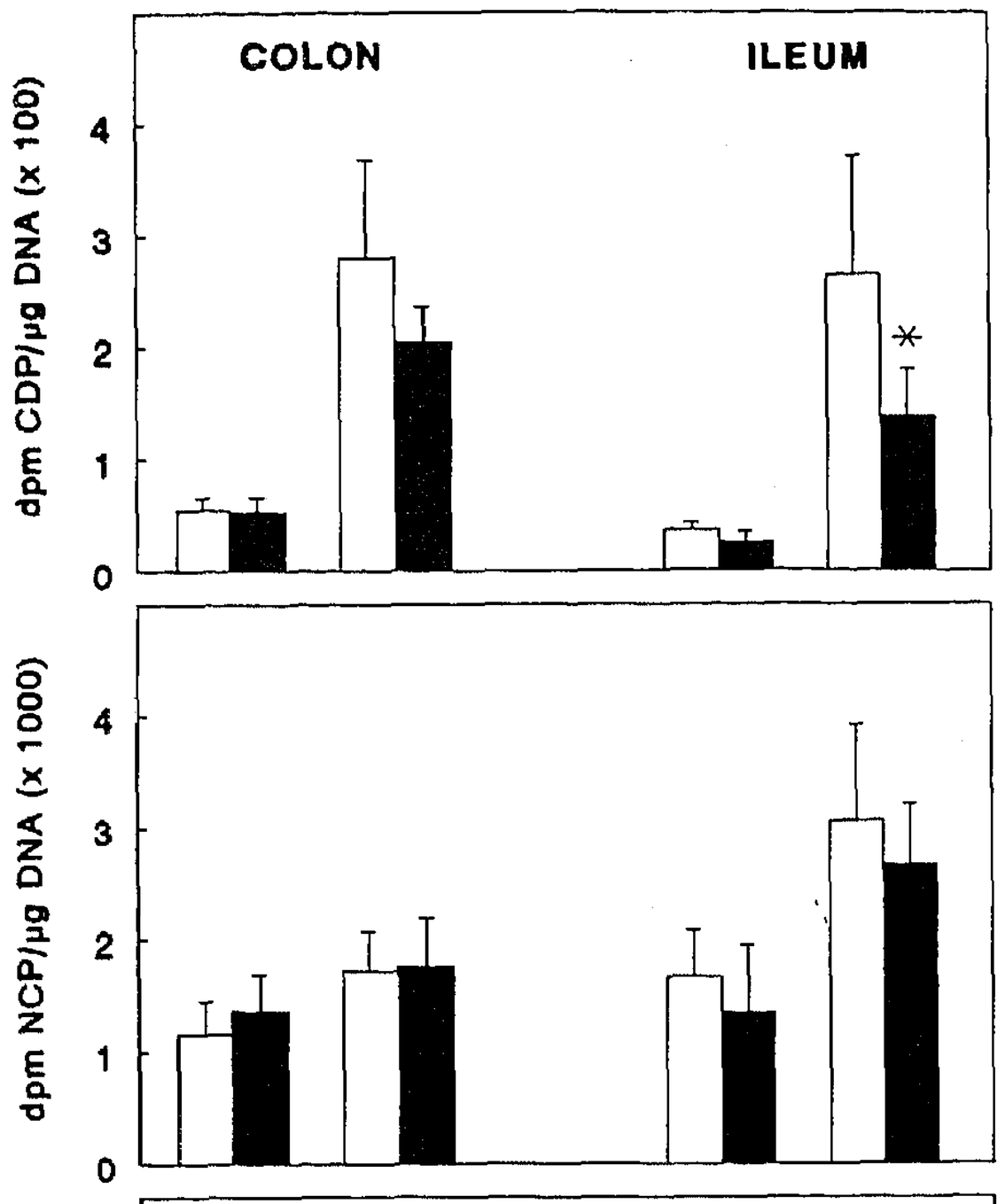

Fig. 4. Ex vivo synthetic capacity for collagen and noncollagenous protein. Average values + SD ($n = 6$) are given for absolute collagen synthesis (as dpm in CDP, *upper panel*), relative collagen synthesis (as % RCS, *lower panel*), and synthesis of noncollagenous protein (as dpm in NCP, *middle panel*) in control intestine and 4-day-old anastomoses. *Open bars* represent young animals; *filled bars* represent old animals. *$p < 0.05$ (Wilcoxon).

anastomosis was virtually specific for collagen, because the relative collagen synthesis was increased to almost the same extent as the absolute collagen synthesis. No differences between young and old animals were observed with regard to the capacity to produce noncollagenous protein.

DISCUSSION

Improvements in medical care during the last several decades are allowing people to live longer and to remain healthy longer. As a consequence, more surgical procedures will be performed in the elderly, and thus the question of if and how aging affects the wound healing sequence gains increasing interest.

Aging is a complex biologic phenomenon and the molecular mechanisms involved are little understood. Each physiologic system appears to have its own trajectory of aging, and the various organs exhibit their own particular changes with age.[15] This supports our contention[10] that conclusions regarding healing in the gastrointestinal tract should be based on experiments and/or clinical data on this particular tissue.

The present findings indicate that intestinal anastomoses in healthy, aged animals heal as well as those in young animals. This conclusion is based on the comparison of two major parameters of healing, wound strength and wound collagen content. We did not find either slower development of anastomotic strength or diminished accumulation of anastomotic collagen in the old rats, at least not in the first postoperative week, when strength is low and the risk for anastomotic dehiscence relatively high. In general, there were no indications that the old rats tolerated anesthesia and extensive surgical manipulation less well than the young rats.

Two measurements were performed sequentially on each anastomosis to assess wound strength, the bursting pressure and the breaking strength. The former is a measure of strength of the true wound area during only a few days after operation. At 7 days the bursting site is always outside the suture line. We have previously found

that if healing is impaired by, for instance, blood transfusion, 7-day-old anastomoses mostly burst within the suture line.[11] In this experiment this was not the case in the old animals, indicating unsuppressed gain of bursting strength. The fact that at this time the colonic bursting pressure was significantly higher in old rats probably means that their intact bowel walls were stronger to start with. Early breaking strength probably reflects the suture holding capacity, that is, the capacity of existing connective tissue to retain the sutures. Three days after operation the anastomotic breaking strength was also higher in the old animals, again indicating increased strength of the intestinal wall, particularly in the colon. This is probably due to the higher collagen content, which we measured in the control segments removed from the colon during operation.

Assuming that anastomotic strength is primarily determined by collagen, it is interesting to observe that the collagen content, assessed either biochemically or morphometrically, was not lowered in the old animals, despite the fact that the collagen synthetic capacity appeared somewhat reduced. Apparently in the young surgery induces an excess capacity to produce collagen. Reduction of this synthetic potential, as observed in the aged rats, does not necessarily lead to less accumulation of collagen in the wound area, as long as sufficient synthetic capacity remains. The reduced collagen synthetic capacity may be a direct result of decreased proliferation and activity of fibroblasts or may be mediated by declined macrophage function. Age-related reduction of fibroblast collagen production is well documented,[16] and it has been reported that cutaneous wound repair in old mice may be promoted by application of macrophages.[17]

The results of our experimental study on healthy old animals thus seem to support the outcome of those retrospective clinical studies that conclude that advanced age is not a single factor correlating with increased frequency of anastomotic complications.[5, 6] A recent study in healthy human volunteers reports that, although the elderly volunteers exhibited delayed epithelialization of superficial skin wounds, collagen deposition and cellular infiltration in subcutaneous catheters were similar to those observed in the young.[18]

In conclusion, we submit that advanced age per se does not suppress anastomotic healing in the intestine. Underlying illnesses or diseases may play an important role in determining the outcome of clinical studies on surgical patients that show a direct correlation of age with disturbed healing. In the absence of additional detrimental factors anastomotic repair will be essentially unimpaired. Possibly the vulnerability of the healing process in the elderly will only become evident in the presence of certain conditions that exert, on their own, a negative influence on wound repair. It remains to be established whether such conditions indeed affect healing more strongly in the elderly than in the young.

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REFERENCES

1. Hunt TK. Comment. In: Hunt TK, ed. Wound healing and wound infection. New York: Appleton-Century-Crofts, 1980: 192-3.
2. Irvin TT, Goligher JC. Aetiology of disruption of intestinal anastomoses. Br J Surg 1973;60:461-4.
3. Schrock TR, Deveney CW. Factors contributing to leakage of colonic anastomoses. Ann Surg 1973;177:513-8.
4. Montesani C, De Milito R, Chiappalone S, Narilli P, D'Amato A, Ribotta G. Critical evaluation of the anastomoses in large bowel surgery: experience gained in 533 cases. Hepatogastroenterology 1992;39:304-8.
5. Fielding LP, Stewart-Brown S. Anastomotic integrity after operations for large-bowel cancer: a multicentre study. Br Med J 1980; 9:411-4.
6. Jex RK, van Heerden JA, Wolff BG, Ready RL, Ilstrup DM. Gastrointestinal anastomoses: factors affecting early complications. Ann Surg 1987;206:138-41.
7. Holm-Pedersen P, Zederfeldt B. Strength development of skin incisions in young and old rats. Scand J Plast Reconstr Surg 1971;5:7-12.
8. Beck LS, DeGuzman L, Lee WP, Xu Y, Siegel MW, Amento EP. One systemic administration of transforming growth factor-β1 reverses age- or glucocorticoid-impaired wound healing. J Clin Invest 1993;92:2841-9.
9. Quirina A, Viidik A. The influence of age on the healing of normal and ischemic skin wounds. Mech Ageing Dev 1991;58:221-32.
10. Hendriks T, Mastboom WJB. Healing of experimental intestinal anastomoses: parameters for repair. Dis Colon Rectum 1990; 33: 891-901.
11. Tadros T, Wobbes T, Hendriks T. Opposite effects of interleukin-2 on normal and transfusion-suppressed healing of experimental intestinal anastomoses. Ann Surg 1993;218:800-8.
12. Hesp WLEM, Hendriks T, Lubbers EJC, de Boer HHM. Wound healing in the intestinal wall: a comparison between experimental ileal and colonic anastomoses. Dis Colon Rectum 1984;27:99-104.
13. Martens MFWC, de Man BM, Hendriks T, Goris RJA. Collagen synthetic capacity throughout the uninjured and anastomosed intestinal wall. Am J Surg 1992;164:354-60.
14. James J, Bosch KS, Zuyderhoudt FMJ, Houtkoper JM, van Gool J. Histophotometric estimation of volume density of collagen as an indication of fibrosis in rat liver. Histochemistry 1986;84:129-33.
15. Evers BM, Townsend CM Jr, Thompson JC. Organ physiology of aging. Surg Clin North Am 1994;74:23-39.
16. Johnson BD, Page RC, Narayanan AS, Pieters HP. Effects of donor age on protein and collagen synthesis in vitro by human diploid fibroblasts. Lab Invest 1986;55:490-6.
17. Danon D, Kowatch MA, Roth GS. Promotion of wound repair in old mice by local injection of macrophages. Proc Natl Acad Sci USA 1989;86:2018-20.
18. Holt DR, Kirk SJ, Regan MC, Hurson M, Lindblad WJ, Barbul A. Effect of age on wound healing in healthy human beings. SURGERY 1992;112:293-8.