Effect of Malnutrition on Colonic Healing

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It has been suggested that colonic healing is impaired in malnourished subjects, but there have been no biochemical studies of the effect of malnutrition on colonic healing. The effects of malnutrition on the colon and the healing of colonic anastomoses were studied in rats fed a protein-free diet. Test animals were compared with control animals of similar age, and control animals of similar weight. There was a significant reduction in the body weight, total serum proteins and serum albumin of animals starved of protein. Malnutrition resulted in a reduction in the weight of the uninjured colon, and an increase in the colonic collagen *concentration.* There was a significant reduction in the collagen *content* of the colon in animals starved of protein for seven weeks, and the collagen *content* of anastomoses in these animals was significantly lower than the value in control animals. Anastomotic edema occurred during colon healing in animals starved for seven weeks. Measurements of colonic bursting pressure were an inaccurate guide to colonic healing. It is concluded that severe malnutrition resulting in 34% loss of body weight had an adverse effect on colonic healing.

CLINICAL AND EXPERIMENTAL STUDIES have shown that malnutrition has an adverse effect on the healing of wounds of skin and abdominal fascia.[16,17,21,22] Tissue collagen is removed during malnutrition,[2,3,10] and studies of experimental wounds have shown that collagen synthesis is defective in malnourished subjects.[21,24,27]

Clinical observations have suggested that the healing of colonic anastomoses may be affected by malnutrition,[12] and recent experimental investigations seem to support this contention. It has been shown that the tensile strength of healing colonic wounds and anastomoses is abnormally low in malnourished rats.[4,5,18] However, precise information regarding the biochemical features of colonic healing in malnourished subjects is lacking.

In this study, the effects of malnutrition on the colon and healing colonic anastomoses were studied in rats.

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Methods

Two-hundred and sixty male Sprague Dawley rats were studied. Control animals were fed a normal rat diet, and test animals received a protein-free diet. The composition of test and control diets was based on the recommendations of Hegstead and Chang[11] (FIG. 1). The normal rat diet contained 25% casein, and the caloric deficit in the protein-free test diet was corrected by increasing the amount of cornstarch. However, it was observed that rats consumed the test diet less avidly than the control diet, and it is likely that test animals were subjected to protein-calorie malnutrition rather than simple starvation of protein.

Test animals weighed 220–270 gm at the start of the experiment, and they were later compared with control animals of similar age (age controls), and control animals of similar weight (weight controls).

Serum Proteins

Blood was withdrawn by cardiac puncture for the measurement of serum proteins. Total protein was determined by the biuret method, using the standard Technicon autoanalytic procedure, and the serum globulin was measured by the glyoxylic-tryptophan reaction.[8] The serum albumin was determined from the difference between the total protein and globulin values.

Uninjured Colon

Standard biopsies of the left colon measuring 5 mm in width were obtained from test and control animals. The wet weights of the biopsies were determined, and the specimens were dried to constant weight in an oven at 106 C. The biopsies were hydrolyzed in 6N hydro-

CONTROL AND PROTEIN-FREE DIETS
after Hegstead and Chang. J. NUTRITION 85, 159, 1965

DIETARY CONSTITUENTS	CONTROL (NORMAL) RAT DIET (% of Total Weight)	PROTEIN-FREE (TEST) DIET (% of Total Weight)
CASEIN	25·0	—
CORNSTARCH	60·0	85·0
HYDROGENATED COTTONSEED OIL	8·0	8·0
HAWK-OSER SALT MIXTURE	4·0	4·0
VITAMIN SUPPLEMENT	1·0	1·0
CELLULOSE NON-NUTRITIVE FIBRE	2·0	2·0

FIG. 1. Constituents of test and control diets.

chloric acid, and the collagen content was determined by the measurement of hydroxyproline, using the auto-analytic procedure of Jackson and Cleary.[13]

Age controls, weight controls, and test animals were sacrificed for measurements of colonic tensile strength. Segments of the left colon measuring 8 cm in length were resected, and the colonic bursting *pressure* was determined with a mercury manometer during distention of the colon with air at a constant rate of 1 ml/minute. The colonic bursting *wall tension* was determined from the product of the bursting pressure and the radius of the bowel at the point of rupture, according to the Law of La Place.[19]

Colonic Anastomoses

The healing of colonic anastomoses was studied in age controls, weight controls, and test animals in groups of 16–20. Segments of bowel measuring 1 cm long were resected from the distal left colon, and end-to-end anastomoses were made with an inverting technique, using a single layer of interrupted 6-0 silk sutures. The animals were sacrificed immediately or on the third or seventh postoperative days, and the tensile strength and collagen content of the anastomoses were determined as described above. The anastomoses were completely excised as standard strips of tissue measuring 5 mm in width, and the total collagen content was determined.

Statistical analyses of the results were made, using the Student t-test.[7]

Results

Body Weight

The weight changes in test animals and age controls are shown in Fig. 2. Two groups of age controls, each comprising 20 animals, were studied for three weeks prior to surgery. These animals increased their weight by 34% during this period. Three groups of test animals, each comprising 20 animals were subjected to protein starvation lasting three, five and seven weeks. Animals starved for three weeks lost 25% of their body weight; weight loss after five weeks of starvation was 27%; and protein starvation for seven weeks resulted in a weight loss of 34%.

Two groups of weight controls were studied, and the weights of these animals were similar to those of test animals starved for five and seven weeks. The average body weight of one group of 20 weight controls was 164 gm (S.E. ± 2.21), and the average weight of the other group of 16 animals was 185 gm (S.E. ± 2.20).

Serum Proteins

Table 1 shows the mean values for total serum protein and serum albumin in age controls, two groups of weight controls, and six groups of test animals starved for two to seven weeks. Each group comprised seven animals. The total protein and albumin values in test animals starved for two weeks were significantly lower than the values in age controls (for total protein, $P < 0.01$; for albumin, $P < 0.001$), and the serum proteins decreased as starvation progressed.

In the weight controls, the serum protein values in the group with an average weight of 185 gm were not

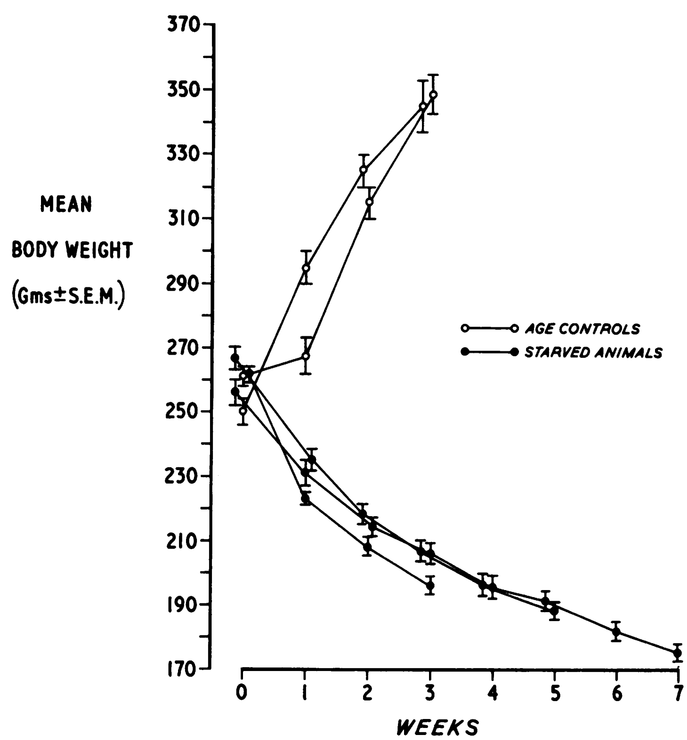

FIG. 2. Body weight measurements in age controls and three groups of test animals starved for three, five and seven weeks.

TABLE 1. *Total Serum Protein and Serum Albumin Measurements in Age Controls, Two Groups of Weight Controls, and Groups of Test Animals Starved for Two to Seven Weeks*

Animals	Mean Total Serum Protein (g/100 ml ± S.E.)	Mean Serum Albumin (g/100 ml ± S.E.)
Age Controls	6.61 ± 0.11	3.58 ± 0.08
Weight Controls (Mean Body Weight 164 g)	5.62 ± 0.07	3.28 ± 0.08
Weight Controls (Mean Body Weight 185 g)	6.35 ± 0.17	3.42 ± 0.10
Two Weeks Starvation	6.18 ± 0.12	2.92 ± 0.09
Three Weeks Starvation	5.74 ± 0.15	3.02 ± 0.07
Four Weeks Starvation	5.65 ± 0.10	2.84 ± 0.05
Five Weeks Starvation	5.46 ± 0.26	2.66 ± 0.18
Six Weeks Starvation	5.30 ± 0.05	2.57 ± 0.05
Seven Weeks Starvation	4.94 ± 0.27	2.28 ± 0.15

significantly different from those of age controls, but the values in the group with an average weight of 164 gm were significantly lower than those of age controls (for total protein, $P < 0.001$; for albumin, $P < 0.02$). However, the proteins in the latter group of weight controls were significantly higher than those of test animals starved for seven weeks (for total protein, $P < 0.05$; for albumin, $P < 0.001$).

Uninjured Colon

Table 2 shows the mean values for the wet weight, percentage of water content, total collagen content, and collagen concentration in standard biopsies of colon from age controls, the two groups of weight controls, and groups of test animals starved for three, five and seven weeks. Nineteen age controls were studied, and the two groups of weight controls comprised 16 and 20 animals. The groups of test animals starved for three, five and seven weeks comprised 18, 20 and 16 animals respectively.

The wet weights of colon biopsies from starved animals were significantly lower than those of age controls ($P < 0.005$) and weight controls ($P < 0.001$), but the relative water content of the biopsies from starved animals was significantly greater than that of age controls ($P < 0.001$). However, the relative water content of biopsies from weight controls was significantly greater than that of age controls and test animals ($P < 0.01$).

The total collagen content of colon biopsies from test animals was not significantly different from that of age controls except in the case of the test group starved for seven weeks. In this group, the total collagen content was significantly lower than the value in age controls ($P < 0.02$). The total collagen content of biopsies from weight controls was significantly lower than that of test animals and age controls ($P < 0.001$).

The collagen concentration in dry colonic tissue in test animals was significantly higher than the values in age controls and weight controls ($P < 0.01$), but there was no difference between the values in age controls and weight controls.

The collagen concentration in wet colonic tissue in test animals starved for three and five weeks was significantly higher than the value in age controls ($P < 0.005$), but there was a sharp decline in this parameter in animals starved for seven weeks. The striking feature, however, was the value in weight controls. In these animals, the collagen concentration in wet tissue was less than 50% of the value in age controls and test animals.

The tensile strength measurements in the uninjured colon of age controls, weight controls, and test animals starved for two to seven weeks are shown in Table 3. Each group comprised five animals. There was a progressive rise in the colonic bursting *pressure* in test animals as starvation progressed, and the bursting pressures in test animals and weight controls were higher than the values in age controls. However, the bursting *wall tension* results showed a different trend. The colonic bursting wall tension declined progressively in test animals as the duration of starvation increased, and the results in test animals starved for six and seven weeks were significantly lower than the value of age controls ($P < 0.05$). The results in test animals starved for seven weeks were also

TABLE 2. *Uninjured Colon: The Wet Weight, Percentage Water Content, Total Collagen Content, and Collagen Concentration in Standard Biopsies of Colon From Age Controls, Two Groups of Weight Controls, and Three Groups of Test Animals Starved for Three, Five and Seven Weeks*

Animals	Mean Wet Weight (mg ± S.E.)	Mean Water Content (% ± S.E.)	Mean Total Collagen Content (ug ± S.E.)	Mean Collagen Concentration in Dry Tissue (ug/mg ± S.E.)	Mean Collagen Concentration in Wet Tissue (ug/mg ± S.E.)
Age Controls	17.8 ± 0.61	85.7 ± 0.80	389 ± 15.29	179.6 ± 9.64	22.4 ± 1.12
Weight Controls (Mean Body Weight 164 g)	20.3 ± 0.97	93.6 ± 0.46	228 ± 7.91	186.9 ± 12.01	11.0 ± 0.44
Weight Controls (Mean Body Weight 185 g)	21.1 ± 1.12	92.8 ± 0.44	242 ± 13.26	165.8 ± 5.37	11.7 ± 0.54
Three Weeks Starvation	14.7 ± 0.67	89.4 ± 0.56	394 ± 14.22	267.4 ± 15.39	27.1 ± 0.83
Five Weeks Starvation	15.0 ± 0.55	89.4 ± 0.60	438 ± 21.74	292.9 ± 19.69	29.5 ± 1.37
Seven Weeks Starvation	14.5 ± 0.70	89.4 ± 1.11	338 ± 12.84	274.4 ± 35.73	23.6 ± 0.56

TABLE 3. *Uninjured Colon: Tensile Strength Measurements in Age Controls, Two Groups of Weight Controls, and Groups of Test Animals Starved for Two to Seven Weeks*

Animals	Mean Colonic Bursting Pressure (mm Hg ± S.E.)	Mean Colonic Bursting Wall Tension (Dynes/cm × 10^{-5} ± S.E.)
Age Controls	179 ± 13.82	0.725 ± 0.038
Weight Controls (Mean Body Weight 164 g)	230 ± 3.20	0.782 ± 0.043
Weight Controls (Mean Body Weight 185 g)	210 ± 8.94	0.761 ± 0.026
Two Weeks Starvation	192 ± 5.83	0.672 ± 0.049
Three Weeks Starvation	195 ± 8.00	0.649 ± 0.074
Four Weeks Starvation	185 ± 7.07	0.578 ± 0.073
Five Weeks Starvation	208 ± 9.80	0.642 ± 0.018
Six Weeks Starvation	208 ± 8.60	0.573 ± 0.042
Seven Weeks Starvation	211 ± 3.65	0.589 ± 0.043

significantly lower than the values in weight controls ($P < 0.01$), but there was no difference between the results in age controls and weight controls.

Colonic Anastomoses

Figure 3 shows the results of colonic tensile strength measurements on the third and seventh postoperative days in age controls and test animals starved for three, five and seven weeks. Each of the four groups comprised 20 animals, and equal numbers of animals were sacrificed on the third and seventh postoperative days.

The bursting *pressure* and bursting *wall tension* showed a similar trend. There was no difference between the results in age controls and test animals starved for three or five weeks, but the tensile strength of anastomoses in test animals starved for seven weeks was significantly lower than the value in age controls on the seventh postoperative day ($P < 0.005$).

Tensile strength measurements in age controls, two groups of weight controls, and the test group starved for seven weeks are shown in Fig. 4. The two groups of weight controls comprised 20 and 16 animals, and equal numbers of animals were sacrificed on the third and seventh postoperative day.

Again, the colonic bursting *pressure* and bursting *wall tension* results showed a similar trend. The results in the group of weight controls with an average weight of 185 gm were not significantly different from those of age controls, but the results in the group of weight controls with an average weight of 164 gm were significantly lower than the values in age controls ($P < 0.02$). The results in this group of weight controls were rather similar to those of test animals starved for seven weeks.

Figure 5 shows the total collagen content of the anastomoses on the third and seventh postoperative days in age controls and the groups of test animals starved for three, five, and seven weeks. The results in test animals starved for three weeks were not significantly different from those of age controls. The collagen content of anastomoses in test animals starved for five and seven weeks was significantly lower than that of age controls on the third postoperative day ($P < 0.001$), and the collagen content of anastomoses in the group starved for seven weeks was also significantly lower than the value in age controls on the seventh postoperative day ($P < 0.005$). The results in test animals starved for five weeks were not significantly different from those of control animals on the seventh day.

The total collagen content of anastomoses in the two groups of weight controls, age controls, and test animals starved for seven weeks is shown in Fig. 6. The collagen content of anastomoses removed on the day of surgery from six age controls, eight test animals, and six weight controls weighing an average of 164 gm is also shown.

The total collagen content of anastomoses in weight controls and test animals was significantly lower than the value in age controls on the day of surgery, and this

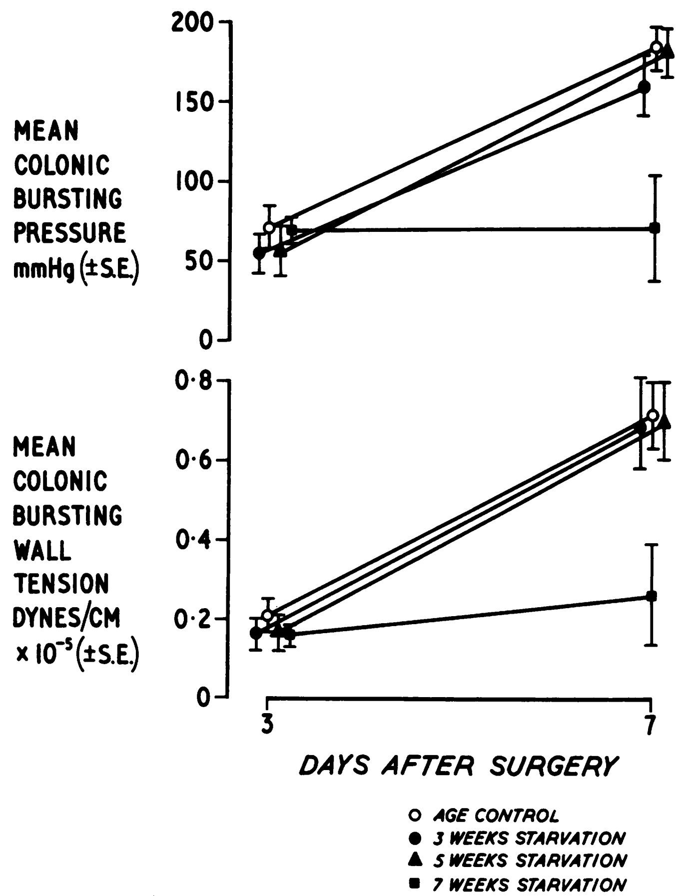

FIG. 3. Colonic anastomoses. Tensile strength measurements in age controls and groups of test animals starved for three, five, and seven weeks. Each group comprised 20 animals, and equal numbers of animals were sacrificed on the third and seventh postoperative days.

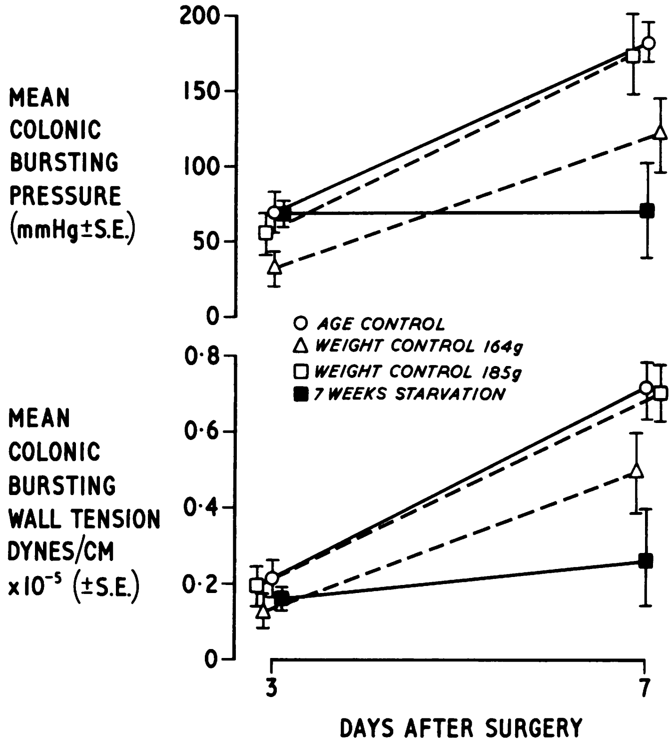

FIG. 4. Colonic anastomoses. Tensile strength measurements in age controls, two groups of weight controls, and the group of test animals starved for seven weeks.

difference was also apparent on the third postoperative day. However, active collagen synthesis occurred in the anastomoses in age controls and weight controls after the third day, and the collagen content of anastomoses in weight controls and age controls was significantly higher than that of test animals on the seventh postoperative day ($P < 0.005$).

Figure 7 shows the collagen concentration in anastomoses in age controls and the three groups of test animals starved for three, five and seven weeks.

The results do not show a close correlation with the total collagen measurements since the colonic collagen concentration in starved animals is abnormally high. However, there was a striking difference between the collagen concentration in wet and dry tissue in test animals starved for seven weeks. In this group, the collagen concentration in dry tissue increased between the third and seventh postoperative days, but there was a striking reduction in the collagen concentration in wet tissue during this period, indicating that a significant degree of edema complicated anastomotic healing in these animals.

Discussion

The biochemical factors which affect wounds in malnourished subjects may be quite complex, but defective collagen synthesis appears to be the major abnormality.[21,24,25] However, little is known of the effects of malnutrition on visceral healing. Studies in experimental animals have shown that visceral collagen is removed to a lesser extent than skin collagen in malnourished subjects,[3,10] but it is not known if visceral wound healing is less affected by malnutrition than the healing of skin wounds.

Mukerjee and colleagues[18] reported that the tensile strength of colonic wounds in malnourished rats was inferior to that of control animals, but these observations must be interpreted with caution since questionable assumptions were made in the measurement of colonic tensile strength. Daly and associates[4,5] found that there was a striking reduction in the tensile strength of colonic anastomoses in rats fed a protein-free diet, and changes were apparent even when the loss of body weight amounted to only 2%.

In the present study, colon healing was studied in rats fed a protein-free diet. It became evident that the animals responded to this diet by reducing their dietary intake, and it is likely that protein-calorie malnutrition resulted. Similar observations in animals given protein-free diets have been described previously.[23]

Malnutrition resulted in progressive loss of body weight and a reduction in the serum proteins. Changes in the serum proteins were found in test animals after two weeks of protein starvation, and the reduction in body weight and proteins was accompanied by changes in the colon. There was a reduction in colonic weight, and an increase in the colonic collagen concentration, indicating that non-collagenous materials were removed from the colon of starved animals. It seems likely that the removal of non-collagenous proteins and fat accounted for the

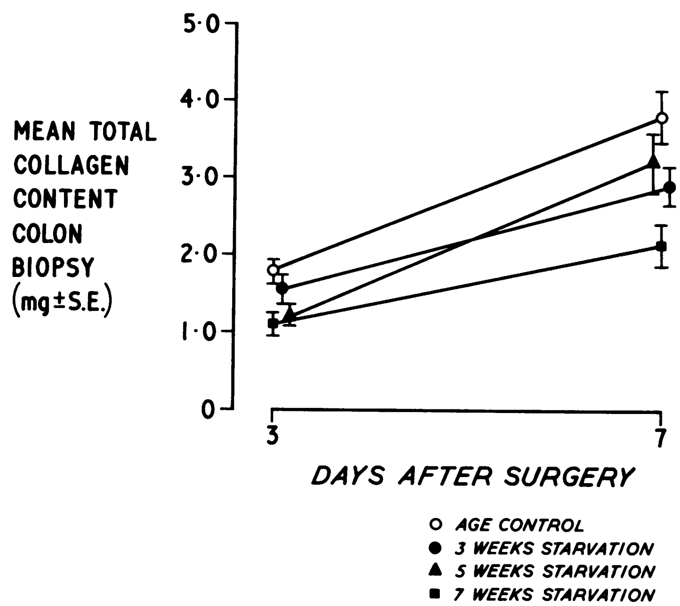

FIG. 5. Colonic anastomoses. The total collagen content of anastomoses in age controls and the groups of test animals starved for three, five, and seven weeks.

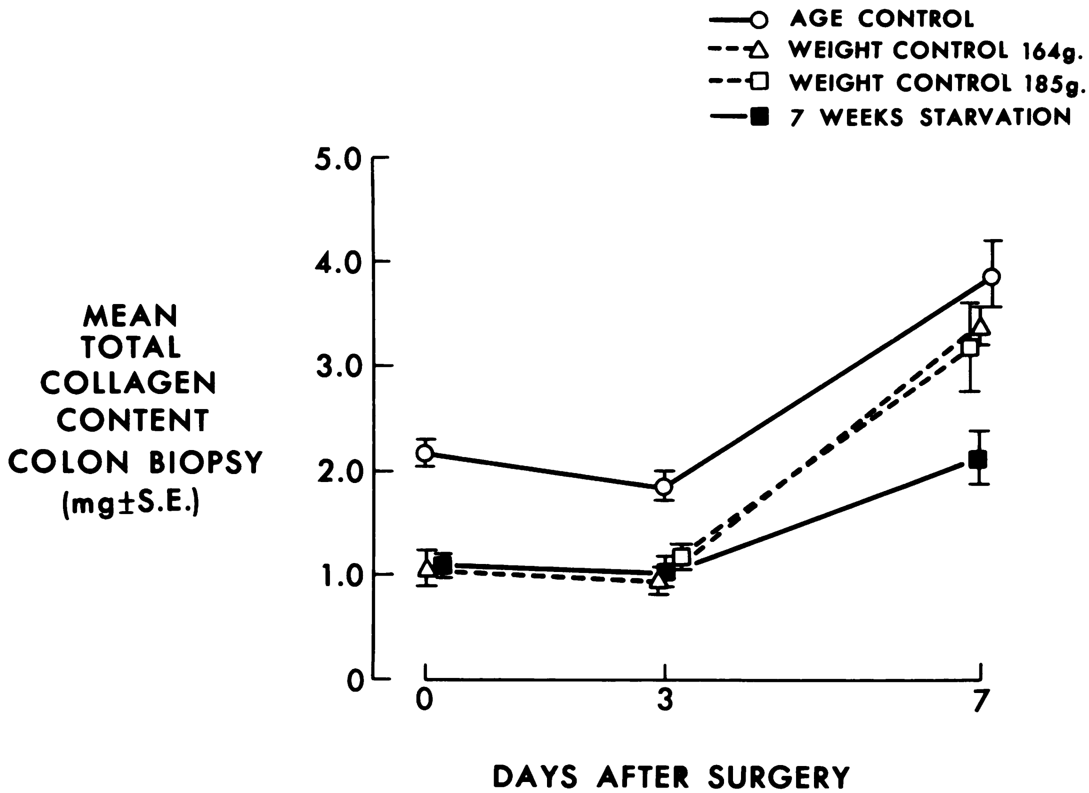

FIG. 6. Colonic anastomoses. The total collagen content of anastomoses in age controls, the two groups of weight controls, and the group of test animals starved for seven weeks. Anastomoses were removed on the day of surgery from six age controls, six weight controls, and eight test animals.

reduction in colonic weight, and these changes in the colon were accompanied by a relative increase in the colonic water content. There was little evidence of breakdown and removal of colonic collagen until the animals had been starved for seven weeks, and the loss of body weight amounted to 34%.

Test animals were compared with control animals of similar age (age controls), and control animals of similar weight (weight controls). The young weight controls differed from age controls in that they had significantly lower serum proteins, and this difference was quite striking in the case of the total protein values. According to Dimopoullos,[6] these differences between young and old animals are normal physiological features in the rat. There was also a striking difference in the collagen concentration in wet colonic tissue in age controls and weight controls due to the increased water content of the colon in young control animals. Similar differences in the collagen concentration in wet tissues in young and old rats have been described previously by Zika and Klein.[30]

The biochemical changes in the uninjured colon of starved animals were accompanied by a reduction in colonic tensile strength. This was not reflected by changes in the colonic bursting *pressure*, but this measurement is profoundly affected by the internal radius of the bowel,[19] and the radius of the colon in test animals was much less than that of control animals of similar age. The colonic bursting *wall tension*, or pressure-radius product, was a more accurate measure of colonic tensile strength in starved animals, and this parameter was significantly reduced in animals starved of protein for six or seven weeks.

Malnutrition affected the healing of colonic anastomoses only in animals starved of protein for seven weeks. These animals lost 34% of their body weight, and the tensile strength and collagen content of colonic anastomoses were significantly lower than the values in control animals. Measurements of the colonic collagen concentration in starved animals revealed the presence of edema in colonic anastomoses on the seventh postoperative day. To some extent, edema may be a normal feature of wound healing,[1] but Thompson and colleagues[25] reported that excessive edema occurred in abdominal wounds in malnourished animals. According to Thompson,[26] wound edema in malnourished subjects can be prevented by correction of the plasma colloid osmotic pressure. Daly[4,5] found no evidence of edema in colonic anastomoses in rats deprived of protein for six weeks, but his assessment was based purely on histological observations.

Clinicians have found that significant morbidity occurs in malnourished patients when weight loss exceeds 30%,[14,15] and the findings in the present study suggest that severe malnutrition has a deleterious effect on colonic healing. Daly and colleagues[4,5] found that colon healing in rats was affected by brief periods of protein deprivation, but

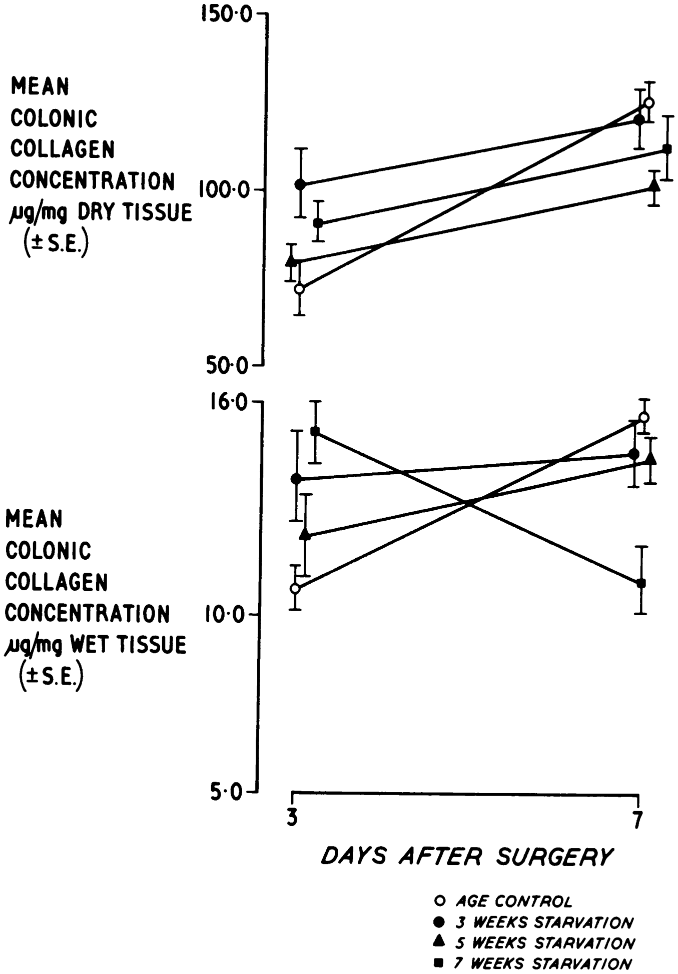

FIG. 7. Colonic anastomoses. The collagen concentration in anastomoses in age controls, and the groups of test animals starved for three, five, and seven weeks.

this assertion was based on measurements of colonic bursting pressure and the present study has indicated that measurements of colonic bursting pressure in malnourished rats may be misleading. The colon of starved animals was significantly smaller than that of control animals of similar age, and the size of the colon in starved animals resembled that of younger control animals of similar weight. The tensile strength of colonic anastomoses in these younger control animals was similar to that of starved animals, despite the presence of normal collagen synthesis in the anastomoses of control animals. It seems likely, therefore, that technical factors involved in the anastomosis of the small colon of starved rats or control animals of similar weight may result in misleading measurements of colonic tensile strength.

Clinical studies have shown that malnourished patients have an increased incidence of anastomotic dehiscence after colon surgery.[12] Various uncontrolled clinical observations[9,28,29] have suggested that improvement of the nutritional status of malnourished patients may have a favourable effect on intestinal healing, and the results of the present study imply that some form of nutritional support may be justified in severely malnourished patients undergoing colonic anastomosis.

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