

Role of malnutrition in intestinal anastomosis collagenization: an analysis of procollagen (PINP) and carboxyterminal telopeptide (ICTP) by radioimmunoassay

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RESUMEN

Introducción: diversos factores influyen en la cicatrización correcta de las suturas intestinales tras la práctica de una resección intestinal. Uno de los factores más implicados es el estado nutricional del paciente.

Objetivos: evaluar la influencia de la desnutrición inducida sobre la viabilidad de una anastomosis intestinal primaria mediante el análisis del procolágeno (PINP) como marcador de la síntesis de colágeno I, y del telopéptido carboxiterminal del colágeno I (ICTP) como marcador de la destrucción del mismo.

Métodos: 40 ratas Wistar y material de radioinmunoensayo. Métodos: diseñamos 2 grupos de ratas, 20 animales por cada grupo: grupo control (A) y grupo "desnutrición" (B). Se analiza PINP e ICTP mediante RIA sobre tejido colónico homogeneizado, preanastomótico y anastomótico.

Resultados: existen unos niveles menores de PINP en el colon de las ratas del grupo B comparado con el colon del grupo A (0,3620 y 0,4340 µg/g respectivamente) ($p = 0,032$). Hay un mayor nivel de ICTP analizado en el colon del grupo B (0,9545 en contraposición a 0,8460 µg/g en el grupo A) ($p = 0,875$). En las anastomosis del grupo B existe una menor síntesis de PINP en comparación con el grupo A (0,376 y 0,468 µg/g respectivamente, $p = 0,002$).

Conclusiones: la anastomosis colónica incrementa los niveles de PINP e ICTP en el tejido cicatricial ($p = 0,000$); la malnutrición reduce la colagenización de las anastomosis ($p = 0,000$).

Palabras clave: Anastomosis intestinal. Procolágeno. Telopéptido carboxiterminal. Albúmina.

ABSTRACT

Introduction: some clinical, anatomic-pathological, and technical factors influence the correct healing of intestinal suture following an intestinal resection. One of the most influential factors is patient nutritional status.

Objectives: to evaluate the influence of malnutrition on the viability of primary intestinal anastomosis by the analysis of collagen I deposition.

Methods: 40 Wistar rats, radioimmunoassay material. We used 2 groups of rats, 20 animals in each group: a control group (A) and a "malnutrition" group (B).

Results: there was a decrease in PINP (procollagen) deposition in the colon of group B rats as compared to the colon of group A (0.3620 and 0.4340 µg/g respectively) ($p = 0.032$). There is an increase in ICTP (carboxyterminal telopeptide) in the colon of group B (0.9545 as against 0.8460 µg/g in group A) ($p = 0.875$). In anastomoses of group B there was a decrease in PINP synthesis as compared to group A (0.376 and 0.468 mg/g respectively, $p = 0.002$). As regards ICTP, there was an increase in group B ($p = 0.330$). In relation to the control group no differences were observed in ICTP increases in group B ($p = 1$).

Conclusions: colonic anastomosis increases the levels of PINP and ICTP in healed tissue ($p = 0.000$); malnutrition reduces collagenization in anastomoses ($p = 0.000$).

Key words: Intestinal anastomosis. Procollagen. Telopeptide. Albumin.

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INTRODUCTION

It is clear that factors such as the presence of peritonitis or a poor clinical status can have an adverse effect on intestinal healing. However, there are also other clinical, technical, and anatomic-pathological factors whose role in the viability of an intestinal suture is less clear. One of

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these factors is malnutrition. In this study we set out to evaluate the influence of malnutrition on collagen metabolism in intestinal anastomoses. To achieve this aim we used, just as Bode (1) and Karttunen (2) did, procollagen (PINP) and carboxyterminal telopeptide (ICTP) as markers for the synthesis and destruction, respectively, of type-I collagen in colonic tissue.

Collagen (collagen in adults is predominantly type-I) is the most common protein in the animal world, and constitutes the principal component of fibrous connective tissue, basal membranes, cartilage, cornea, cardiac valves, and other specialized tissues. The fundamental unit of collagen is tropocollagen. In the cisternae of rough endoplasmic reticulum, the α chains undergo hydroxylation of the proline in position α , which gives the collagen molecule its characteristically high hydroxyproline content. In the interior of the fibroblast the procollagen molecule continues to be soluble due to that fact that it possesses an extra carboxyterminal polypeptide fragment, which it loses in the extracellular spaces due to the action of procollagen peptidases. This procollagen, in the case of type-I collagen, is called PINP, a substance that we will utilize in this study as a marker for collagen synthesis. The carboxyterminal telopeptide, joined by trivalent bonds, is liberated by metalloproteinases during collagen degradation. It is this telopeptide of type-I collagen (ICTP) which is the other molecule that we will analyze in colonic tissue as a marker for collagen destruction.

The following working hypothesis is put forward: malnutrition enhances failure of intestinal sutures. The objective of the study is aimed at the evaluation of how this supposition influences the viability of intestinal anastomoses by analyzing the collagen deposited in said anastomoses.

Consequently, the objective pursued is to demonstrate whether hypoproteinemia affects the deposition of collagen in intestinal anastomoses.

MATERIAL AND METHODS

The materials used to achieve this objective are the following: anesthetics (ketamine and xylacine), surgical material and polypropylene, lactomer and silk threads, processing and storage of tissue samples material (precision balance, cryotubes, liquid nitrogen and freezer), processing and storage of blood sample material (tubes, Z serum Sep., Clot Activator, centrifuge, PS-tubes and freezer), material for the processing of tissue samples (liquid nitrogen, pulverizer, PBS/Tween-20 pad, C-peptide irrigation pad, precision balance, glass stirrer, centrifuge and PS tubes), and material for radioimmunoanalysis ("ICTP-RIA[®]" and "Intact PINP[®]" commercial radioimmunoassay kits, micropipettes, test tubes, absorbent paper, distilled water and gamma counter).

We designed an experiment with Wistar rats using 2 groups of rats:

1. Control group (A): 20 healthy, well nourished rats maintained in good condition.

2. "Malnutrition" group (B): 20 healthy rats subjected to fasting, with the exception of water, for 72 hours. To check nutritional status weight loss was analyzed, as well as the concentration of plasma albumin before and after fasting (by means of blood extraction by aortic puncture), and a qualitative analysis was made of mesenteric fat.

Each group, as previously stated, comprised 20 rats, a sample that we considered large enough to obtain significant data following the analysis of initial data for the preliminary group and control group. All experiments were validated from a statistical viewpoint.

The anesthesia of animals was carried out by intraperitoneal injection of ketamine and xylacine. A colonic resection and primary colonic termino-terminal anastomosis with synthetic monofilament suture was performed on each animal following colonic irrigation, extracting anastomotic tissue on the fourth day for a subsequent analysis of collagen behavior both in the "healthy colon" (collected in the first intervention) and the "anastomotic colon" (collected in the second). The slaughter was carried out by intracardiac injection of potassium chloride.

In order to analyze the modifications produced in collagen deposition we focused on the detection in anastomotic tissue of the carboxyterminal group of type-I collagen molecules (ICTP) and procollagen (PINP). PINP, as previously stated, is a viable marker for collagen synthesis, while ICTP is a direct marker for its destruction. Thus, we were able to analyze the amount of synthesis and degradation of the collagen molecule deposited in anastomotic tissue under the different conditions created. These molecules were detected using radioimmunoanalysis techniques, a method which is much more up-to-date and viable than the measurement of proline and hydroxyproline, markers used in other similar studies but providing a lower sensitivity and specificity when compared to PINP and ICTP.

For the radioimmunological analysis of samples, since these substances are detected in standard clinical practice in biological fluids such as plasma or urine, we prepared the collected samples in an appropriate manner. Thus, following the recommended methodology, we crushed and homogenized the extracted tissue in order to analyze it using a gamma camera. Earlier, with initial samples, we produced a radioactivity curve to facilitate the reading of later samples.

The results gathered were statistically analyzed by performing in the first place descriptive statistics for quantitative variables. In order to compare these variables according to study groups, Student's t-test was used as a test of statistical significance for random data when comparing independent samples, and ANOVA with Bonferroni's correction was used for repeated measurements. Pearson's correlation coefficient was used to evaluate the association between quantitative variables used. The level of statistical significance was established as $p < 0.05$.

RESULTS

In order to validate group B (malnutrition) we used 3 parameters: weight of animal, albumin plasma concentration, and qualitative variation in mesenteric fat. The results obtained included: a) weight of animal in fasting 93% of initial weight: decreasing from 350.25 to 325.75 g on average ($p = 0.032$); b) decrease in albumin plasma concentration to 81.57%: from 982 mg/dl in the control group to 801 mg/dl in group B ($p = 0.000$) (Fig. 1); and c) decrease in quantity of mesenteric fat in all group B rats in comparison with the control group ($p = 0.000$)

Having proved the validity of the experiments used, we proceeded to evaluate the behavior of collagen in the colon and in the anastomoses (Tables I and II). It must be remembered that PINP (procollagen) acts as a viable marker for collagen synthesis in tissues, just as ICTP (telopeptide) reflects destruction of the collagen molecule. Figure 2 shows the average PINP and ICTP obtained in each study group in overview form ($\mu\text{g/g}$).

We were able to observe that there was a decrease in PINP deposition in the colon of rats in group B vs. the control group (0.3620 and 0.4340 $\mu\text{g/g}$, respectively), and that these data were statistically significant ($p = 0.032$). Similarly, there was an increase in ICTP in the colon of group B animals (0.9545 as against 0.8460 $\mu\text{g/g}$ in the control group), which was not statistically significant ($p = 0.875$) (Fig. 3).

Comparing the levels of PINP as isolated data in the anastomoses of both groups, it was noted that in the anastomosis of group B there was also a decreased synthesis vs. the control group (0.376 and 0.468 $\mu\text{g/g}$, respectively, $p = 0.002$). As regards ICTP (Fig. 4), there was an increase in group B ($p = 0.330$).

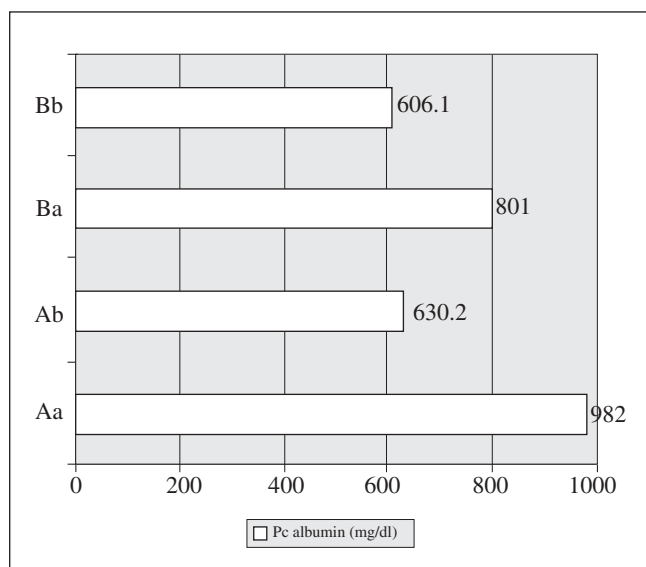


Fig. 1. Variation in the albuminemia of rats in groups A and B (Cp: plasma concentration; Aa: group A colon tissue prior to anastomosis; Ab: colon tissue after anastomoses; Ba: group B colon tissue prior to anastomosis; Bb: colon tissue after anastomoses).

Table I. PINP plasma concentration ($\mu\text{g/g}$)

	Aa	Ab	Ba	Bb
1	0.48	0.49	0.43	0.44
2	0.52	0.53	0.41	0.44
3	0.41	0.42	0.42	0.42
4	0.42	0.47	0.44	0.45
5	0.39	0.48	0.48	0.51
6	0.34	0.39	0.24	0.30
7	0.49	0.53	0.27	0.29
8	0.39	0.47	0.22	0.23
9	0.44	0.46	0.45	0.46
10	0.47	0.46	0.36	0.38
11	0.48	0.54	0.30	0.31
12	0.51	0.55	0.42	0.43
13	0.44	0.47	0.37	0.39
14	0.31	0.33	0.43	0.45
15	0.49	0.50	0.42	0.43
16	0.36	0.38	0.34	0.37
17	0.52	0.58	0.39	0.27
18	0.44	0.48	0.25	0.27
19	0.39	0.43	0.33	0.37
20	0.39	0.44	0.27	0.31
Mean	0.434	0.468	0.362	0.376
Median	0.44	0.47	0.38	0.385
Range	0.34-0.52	0.33-0.58	0.22-0.48	0.23-0.51

a: colon tissue prior to anastomosis; b: anastomotic colon.

Table II. ICTP plasma concentration ($\mu\text{g/g}$)

	Aa	Ab	Ba	Bb
1	0.41	1.31	0.74	2.41
2	0.83	1.25	0.84	2.16
3	1.09	1.75	1.04	1.74
4	0.88	1.15	1.04	2.03
5	0.71	1.16	0.99	1.32
6	0.94	1.61	0.32	1.12
7	0.50	1.28	0.86	0.75
8	0.43	1.57	0.95	2.03
9	1.06	1.17	1.59	1.57
10	0.76	1.60	1.05	2.17
11	0.97	1.32	1.18	1.71
12	1.04	1.49	1.03	0.76
13	1.01	1.25	0.84	0.94
14	1.26	1.56	0.70	1.20
15	0.99	2.14	0.81	2.23
16	1.08	1.42	1.26	1.76
17	1.03	0.71	0.86	1.54
18	0.28	0.82	0.85	1.80
19	0.96	1.75	0.98	2.21
20	0.69	2.19	1.16	1.35
Mean	0.846	1.425	0.954	1.640
Median	0.95	1.37	0.965	1.725
Range	0.28-1.26	0.71-2.19	0.32-1.26	0.75-2.23

a: colon tissue prior to anastomosis; b: anastomotic colon.

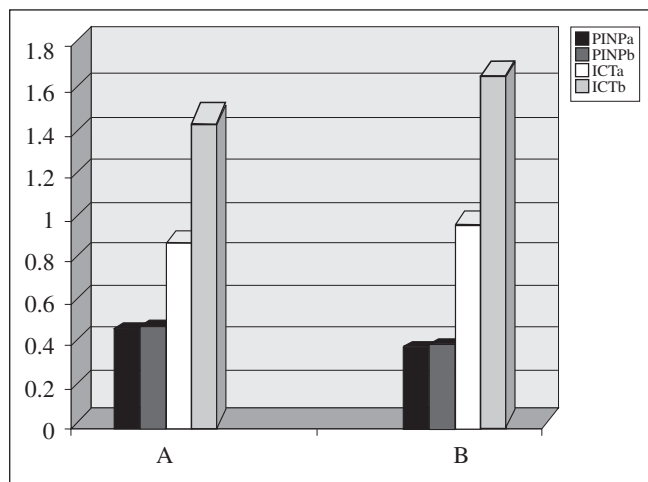


Fig. 2. Average PINP and ICTP (µg/g) for study groups (a: colon tissue prior to anastomoses; b: anastomotic colon).

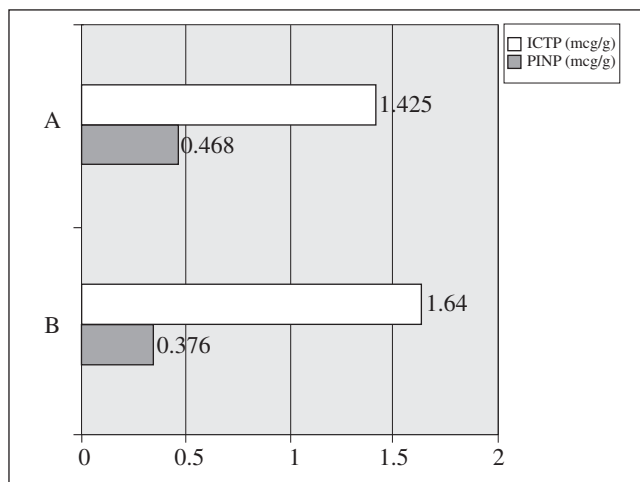


Fig. 4. Average PINP and ICTP (µg/g) in the anastomotic tissue of each group.

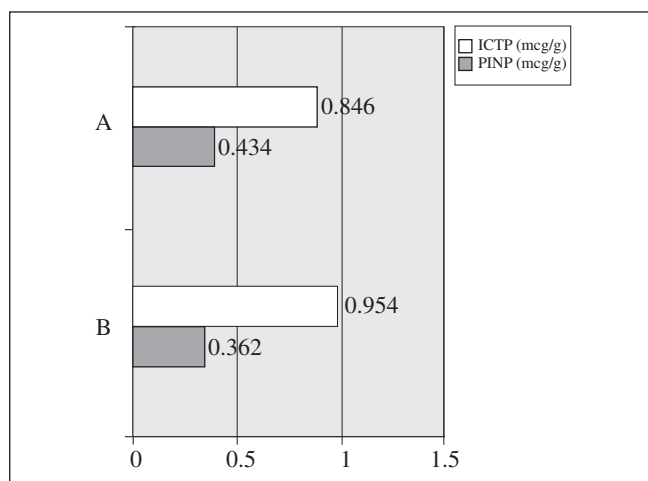


Fig. 3. Average PINP and ICTP (µg/g) analyzed in the non-anastomotic colon of each study group.

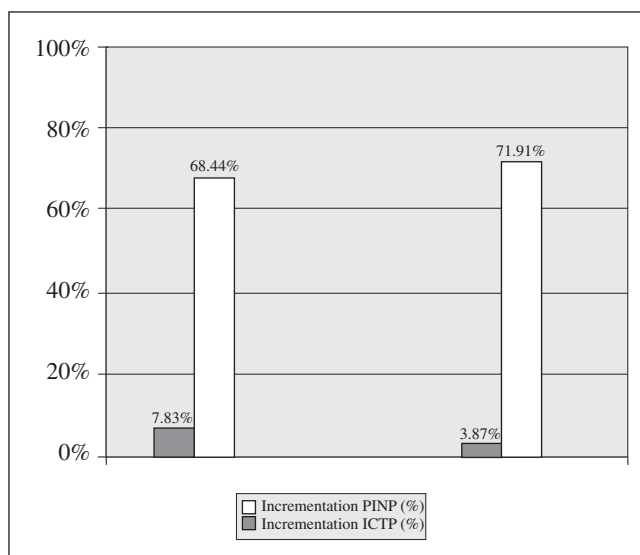


Fig. 5. Increase in PINP and ICTP (%) in the anastomotic tissue at 4 days after anastomosis.

Comparing the levels of PINP deposited in the colonic tissue before and after the anastomosis it can be seen that in the control group there was an increase in PINP at the 4th day after anastomosis of 7.83% (0.034 µg/g) (p = 0.000). In group B, the increase was only 3.87% (p = 0.086). In addition, when comparing these increments with those of control group A, increased PINP in the anastomoses of group B was significant (p = 0.356) (Fig. 5).

Although neither suture dehiscence nor complications appeared in the control group, we noted two anastomotic leakages in group B. Furthermore, one animal in group B suffered from partial evisceration on the 4th day of the postoperative period.

DISCUSSION

Numerous authors have evaluated the quality of intestinal suture as performed following emergency colonic

resections by means of analyzing the incidence of suture dehiscence. Nevertheless, these studies do not take into account the significant bias imposed by the technique used, thus being unable to guarantee that these dehiscences do not result from a flaw in surgical technique. The cited studies present figures for anastomotic dehiscence on series of patients, which are for the most part not randomized and, in many cases, do not take into account clinical factors such as advanced age or concomitant pathology. Consequently, it is very difficult to draw any significant conclusion from these series of patients regarding the incidence of suture dehiscence following primary anastomosis. It is for this reason that in order to study the effect of the various associated factors on in-

testinal suture it appears more valid to carry out an animal experiment, where the surgical technique can be more closely controlled.

As we will see later, the majority of published experimental studies use two techniques for the evaluation of suture quality:

1. Mechanical methods, such as the measurement of suture rupture pressure

2. Methods of collagen detection such as the analysis of proline and/or hydroxyproline, and metalloproteases, thus evaluating the samples by means of light microscopy.

Experimental studies using suture rupture pressure as a variable directly associated with risk of failure measure it by means of a Foley catheter connected to a manometer, by inflating the balloon in the catheter until it causes the dehiscence of the intestinal suture (3-5). This method would appear to be neither particularly viable nor sensitive enough for the measurement of quality of anastomosis, as in all probability the resistance of suture to distension may not be directly related to dehiscence. Furthermore, the pressure required to rupture the suture performed on an animal during the experiment, even when manometrically controlled, appears subjected to significant mechanical variance. Mansson (6) publishes an experimental study in which it is demonstrated, with radiological tests based on contrast extravasation, that the measurement of anastomotic pressure in rats is not a viable method for the study of resistance in these sutures.

Other experimental studies use the analysis of proline and hydroxyproline (7), metabolites of collagen, to evaluate its metabolism, but these substances are not specific to collagen and can appear in tissue as a result of metabolism of other protein molecules, so that their measurement is not necessarily directly linked with alterations induced in collagen metabolism.

Savage (8) publishes a study with rabbits in which he evaluates, by means of indirect immunofluorescence, the presence of metalloproteases (MMPs), the enzymes responsible for collagen catabolism, and the tissue inhibitor of MMPs (TIMP) in the colonic mucosa. He demonstrates that at 24 hours there is as much deposition of these substances in the rabbits subjected to anastomosis as in those on which a colostomy was performed. However, at 72 hours they only appear in the colon of the former, and on the 7th day only in the anastomosis, which furthermore links their concentration in the colonic mucosa to the development of dehiscence. The detection of MMPs as markers for collagen metabolism has been used by other authors in diverse experimental studies (9,10). Nevertheless, MMPs and their inhibitor, despite being associated in these studies with tissue regeneration and the appearance of dehiscence in intestinal sutures, are parameters that are linked only in an indirect manner with collagen catabolism.

Other studies have used histological criteria to evaluate anastomotic healing (11) using a score based on the

degree of sample coloration, cellularity, etc. This method has the drawback of not being quantifiable in an exact manner, and is subject to a degree of subjectivity on the part of the evaluator.

In the current study we were able, by means of radioimmunoanalysis techniques, to isolate molecules of procollagen (PINP) and terminal telopeptide of type-I collagen (ICTP) as markers for the formation and degradation, respectively, of type-I collagen deposited in the colonic tissue, and to link them from the base of a control group, with animal models of malnutrition, intestinal distension and intraoperative colonic irrigation. We thus avoided using the incidence of suture dehiscence, as discussed, in series of patients, as we believe this to be a factor that depends greatly on the surgical technique. We do not use the measurement of anastomotic rupture pressure, measured by means of manometry, as we consider this to be a technique with low sensitivity for the quantification of suture quality. We also prefer not to use the detection of proline and hydroxyproline or metalloproteases, as in our opinion they are less specific markers and have a more indirect link to the synthesis and destruction of collagen than do procollagen and carboxyterminal telopeptide of type-I collagen. We believe PINP and ICTP to be markers with a higher degree of specificity and sensitivity, thus providing a higher viability as indicators of collagen metabolism, as they are molecules that are directly related to this.

As previously detailed, type-I collagen is the most abundant type in the body but, depending on the connective tissue, can be encountered to a greater or lesser extent together with types III, V and VI. In the current study we will use type-I collagen as a marker for colonic tissue healing, as it is the most abundant collagen in this location. These peptides are found intact in the plasma and urine following their liberation from connective tissue. In clinical practice these markers are widely used in the study of pathologies associated with bone metabolism, since their direct link to bone resorption has been proved. Their determination by means of radioimmunoassay or enzymeimmunoassay in these patients has been a routine test for years. Furthermore, numerous studies on subjects other than bone metabolism have been published on these molecules, such as cancer of the esophagus (12) or breast cancer (13), where they are proposed as prognostic markers; in thyroid cancer (14), where they may be used as markers for the suppressive effect of levothyroxine, or associated with treatments such as isotretinoin (15). The modifications of collagen types I and III have also been studied using radioimmunoassay on the layers of atherosclerosis in the carotid arteries, common femoral artery, and the aorta (1), with a low degree of collagenization being noted in these.

Bode and Karttunen (2), at the University of Oulu in Finland, used the measurement of carboxyterminal telopeptide of type-I and type-III collagen on colon samples to evaluate its concentration in cancerous colonic tis-

sue affected by diverticulosis. The method used in this study for the preparation of designated samples in soluble extracts is that used as the basis for the present experimental study.

The determination of PINP and ICTP has been directly analyzed in anastomotic and "healthy" colonic tissue, so that their variations are directly related to the associated experiment, and their tissue concentration indicative of the *in situ* metabolism of the collagen molecule and the modifications induced for each animal model.

The modifications encountered in collagen synthesis and destruction in each study group, with respect to the control group, are related to the viability of colonic anastomoses. In other words, an increase in collagen synthesis (PINP) or a lower level of its destruction (ICTP) implies greater suture viability. In contrast, a decrease in its synthesis or an increase in its destruction increases the risk for dehiscence.

In the colonic tissue of healthy animals (group A) we encountered baseline PINP levels of 0.434 µg per gram of tissue (µg/g), and ICTP levels of 0.846 µg/g. At the fourth day after performing the termino-terminal anastomosis, under normal anatomic and metabolic conditions, PINP levels were 0.468 µg/g and ICTP levels were 1,425 µg/g. In other words, there is an increase in PINP of 7.83% ($p = 0,000$), and an increase in ICTP of 68.44% ($p = 0,000$). It is thus proved that performing a colonic suture produces an increase in collagen metabolism that is reflected in a statistically significant increase in synthesis levels (PINP) by 7.83%, as well as in destruction levels (ICTP) by 68.44%. This greater increase in ICTP is probably due to the fact that, after four days of healing, the usual process of reconstruction of the collagen network is encountered in the suture, as well as a continued synthesis of collagen fibers by cellular fibroblasts.

The malnutrition experiment was completed following 72 hours of fasting. In order to validate this study group we analyzed three parameters: weight loss, reduction in albumin plasma levels, and reduction of mesenteric fat.

Following three days of fasting there was an average weight loss from 350.3 to 325.75 g, in other words, a decrease in weight by 7% ($p = 0.032$), which suggests –according to the established standards in clinical practice– a high degree of malnutrition (93% of the initial weight). In addition, average plasma levels of albumin as obtained from arterial blood had decreased after 72 hours of fasting from 982 mg/dl in the control group (A) to 801 mg/dl in the group of malnourished rats (B); or in other words, a decrease in the albumin plasma concentration of 18.43% ($p = 0,000$), which confirms the findings with regard to animal weight. With respect to the reduction in mesenteric fat, this is a qualitative rather than quantitative parameter, and one which, although not initially foreseen, we decided to include due to its occurrence in all animals of group B. A reduction in quality of mesenteric fat was noted in 100% of animals ($p = 0.000$). These figures led us to conclude in a statistically significant manner that the

animals in group B were in a state of protein-caloric malnutrition, thus establishing its validity as a malnutrition study group.

We proved that malnutrition reduces PINP levels in the colon of the rat prior to anastomosis in 16.59%, and detected 0.362 µg/g of PINP in the colon of malnourished rats as opposed to 0.434 µg/g in the control group ($p = 0.032$). Not only this, but the anastomotic tissue also exhibited a decrease by 19.69%: 0.376 µg/g of PINP detected in group B as opposed to 0.468 µg/g in the control group ($p = 0.002$). In other words, the colonic tissue of malnourished rats presented a smaller secondary collagen deposition, probably due to a lower capacity for synthesis in the habitual regeneration of tissue collagen and, moreover, this fact is seen to be reflected in a reduction in collagen synthesis at the anastomosis. All of these are statistically significant figures.

We detected an increase in ICTP by 12.77% in the colon of malnourished rats in group B, as compared to the control group: 0.954 µg/g *versus* 0.846 µg/g in the control group ($p = 0.875$). Also in the anastomosis, levels of ICTP increased to 1.640 µg/g (1.425 µg/g in the control group), or in other words 15.09% ($p = 0.330$). These figures approached the statistical significance established in the present study, and reflected an increased destruction of collagen in malnourished animals both in the preanastomotic colon and the anastomosis at the fourth day, without there being an adequate physiological explanation for this.

Although the levels of both PINP and ICTP are found to be increased in the anastomotic tissue of malnourished rats, by 3.87 and 71.91%, respectively, *versus* the levels detected in the preanastomotic colon of this group, the increase in PINP is smaller than in the control group (7.83%) ($p = 0.356$), with no difference regarding the increase in ICTP (68.44%).

All these figures indicate the existence of a statistically significant fall in procollagen (PINP) in the colon and in the anastomoses of malnourished rats when compared to the control group, which suggests that malnutrition and hypoalbuminemia result in a reduction in collagen synthesis in intestinal anastomoses, thus promoting the development of suture dehiscence. Collagen destruction is increased in the colon and the anastomoses of malnourished rats, although not to a statistically significant level. We can find no plausible explanation for this.

The nutritional status of patients is one of the most studied potential risk factors for dehiscence in intestinal suture. Albuminemia, and in particular prealbumin plasma concentration, is the most viable marker for the detection of potential nutritional deficiency in patients. The literature consulted included studies on the clinical status and more significantly malnutrition and hypoalbuminemia as prognostic factors for primary anastomosis viability (16). Consequently, Aliev (17) and Brislin (18), using series of 242 and 263 patients with colon cancer, respectively, advocate for decompression and the resolution of

acute symptoms such as malnutrition prior to surgery as a means for reducing anastomosis failure rates. Koperna (19) believes that besides malnutrition increased age may also influence the prognosis of intestinal anastomoses.

Testini (20) has already demonstrated in a retrospective study of 200 patients undergoing colonic resection and termino-terminal anastomosis that hypoalbuminemia is associated with anastomotic leakage. The series by Longo (21) is more extensive, includes 4,711 patients, and demonstrates that decreased albumin is a predictive factor of mortality. The study published by Longo is, in terms of the large sample size presented, significant from a statistical viewpoint in demonstrating that hypoalbuminemia increases the risk of anastomotic failure and postoperative complications. Also, Ceriati (22) suggests hypoalbuminemia as a substantial clinical risk factor for failed intestinal anastomosis following chronic renal insufficiency, based on a retrospective study in an extensive series of patients.

Our experimental results, by which we demonstrate a fall in procollagen levels in the anastomoses of malnourished rats *versus* the control group ($p = 0.002$), corroborate the conclusions drawn by these authors following the observation that malnutrition has a negative influence on the viability of intestinal anastomoses. We can also corroborate data already provided by Testini (20), Longo (21) and Ceriati (22) by means of retrospective studies in series of patients, who presented hypoalbuminemia as a prognostic indicator of anastomotic failure, since in our study we demonstrate a procollagen decrease ($p = 0.000$) in the anastomoses of malnourished rats, with a reduction in albumin by 18.43%. In addition, we provide figures which suggest that malnutrition increases collagen destruction in anastomotic tissues ($p = 0.330$).

As far as the published experimental studies are concerned, there are several articles referring to malnutrition as an influential factor on the viability of intestinal anastomoses. Ward (23) publishes an experimental study where three groups of rats are compared—without food, with a hypoproteic diet, and with normal diet. On determining the pressure of anastomotic rupture in the postoperative period a lower colonic rupture pressure (statistically significant) is found in malnourished rats. Law (24) publishes an experimental study with two groups of rats that were subjected to normal parenteral and hypoproteic nutrition, respectively, proving how the latter has a negative effect on proper intestinal anastomosis and laparotomy wound healing. Delemarre (25) demonstrates by means of the measurement of rupture pressure in anastomoses performed on rats that parenteral nutrition improves suture resistance. In another experimental study carried out by Domínguez Jiménez (26) two groups of rats are used, nourished and malnourished, and results show a greater incidence of anastomotic dehiscence in the group of malnourished rats, thus confirming that nutritional status affects colonic healing and causes immunodepression, which encourages the abscess formation.

Kiyama (27) carried out an experimental study where intestinal suture tension was measured in two groups of rats—a control group and a group with precocious feeding; the author concluded that in the second group anastomoses were of a higher quality, with increased suture pressure, *versus* the control group ($p < 0.05$). The experimental studies presented show a correlation between albumin plasma concentration and manometrically measured anastomotic resistance, or the presence of suture dehiscence. However, there is some bias in these techniques, as already commented upon in the discussion of methods.

Our results suggest, as do the aforementioned studies, by means of an experiment with statistical significance, that malnutrition encourages intestinal suture dehiscence, and we believe our method of PINP and ICTP detection to be more valid than the use of rupture pressure as in other studies.

In conclusion, we can establish that: a) colonic anastomoses increase tissue levels of procollagen and ICTP ($p = 0.000$); b) fasting for 72 hours reduces both weight ($p = 0.032$) and plasma albumin ($p = 0.000$); and c) malnutrition reduces collagenization in colonic anastomosis ($p = 0.0000$).

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