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Wound healing

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Summary

An account is given of the methods used and the results obtained in a series of experimental studies, carried out over the past 15 years, of the effects of various factors on the healing of abdominal wounds in animals. The factors examined include uraemia, jaundice, infections, and the technique of wound closure. The preliminary findings in a comparison now in progress of the results of one- and two-layer closure of laparotomy wounds in human patients are also given. The relative neglect of the study of wound healing in the past is emphasized and some aspects that are in urgent need of further investigation are mentioned.

Introduction

Tissue healing is a topic of intense importance in every branch of surgery. Without this remarkable living phenomenon surgery as we know it would, of course, be impossible. The factor that differentiates the carpenter from the orthopaedic surgeon is that the former relies entirely on his mortice joints and screws to hold his work together while the latter depends on his intimate knowledge of the healing of bone and of soft tissues in planning treatment. The same difference distinguishes the darning of a sock from the repair of a perforated peptic ulcer.

To the abdominal surgeon wound healing is of enormous interest in two respects. First, in the safe healing of the laparotomy wound, without either early rupture or late herniation, and second, in the sound union of anastomoses in the alimentary canal, failure of which would result at best in fistula formation and at worst in overwhelming peritonitis and death.

The number of possible factors affecting healing are legion, both locally at the wound site (blood supply, infection, tension, etc.) and general (vitamin C deficiency, jaundice, uraemia, anaemia, and so on). These are summarized in Table I. One might imagine that because of their great importance all these factors would have been studied in the greatest depth, and it may come as a surprise to realize that the very opposite is true and that vast unexplored territories still await the interested researcher.

The clinical study of wound healing is complicated by the fact that it is uncommon for any one factor to exist in isolation. Consider, for example, the patient who undergoes laparotomy for a carcinoma of the pancreas. He may be elderly, intensely jaundiced, anaemic, protein-depleted, and of course he is suffering from what is often advanced malignant disease. Immediately after his operation he may develop uraemia and put additional stress on his wound by going into post-operative ileus as well as developing a severe pulmonary collapse, and perhaps he may by now have been placed on cytotoxic drugs. Which factor or factors do we blame if his abdominal wound bursts? And what part, if any, does the choice of incision, of suture material, or technique of closure play in this disaster? Clinical studies are important and are the ultimate proof of our laboratory investigations, but the great advantage of the

TABLE I *Wound healing*

Local factors	*Possible general factors*
Blood supply	Age
Infection—local	Anaemia
Apposition	Malignant disease
Absence of movement	Diabetes
Absence of tension	Infection—systemic
X-irradiation	Jaundice
Technique of wound closure	Uraemia
	Steroids
	Cytotoxic drugs
	Protein deficiency
	Vitamin C deficiency

Arnott Demonstration presented on 4th November 1976

laboratory is that it enables us to dissect out each of these multiple factors and try to determine what significance they may have in wound healing.

In this demonstration I should like to discuss some of the more important factors that my colleagues and I have investigated in our laboratory over the past 15 years and then to present some preliminary results of our ongoing prospective clinical study of the healing of laparotomy incisions.

Techniques of study

Three principal experimental models have been used.

First, the parietal peritoneal defect. At laparotomy a 1-cm square of parietal peritoneum is excised, extending just into the underlying muscle, and the abdomen then closed. Here is a convenient method of studying fibroblast regeneration and peritoneal reformation in a closed system, free from infection, without the complications of sutures and without the animal being able to rub this particular wound in droppings and sawdust. In the normal rat these defects at 4 days exhibit marked fibroblast proliferation and early collagen formation. By 8 days dense collagen and flattened mature fibroblasts are present. Peritoneal serosal healing is well established by the 5th day[1]. This is a simple, convenient model which will demonstrate qualitatively gross factors affecting fibroblast proliferation such as local X-irradiation[2], jaundice[3], uraemia[4], and protein deficiency[5].

Second, a standard midline laparotomy incision in the rat sutured by a standardized 2-layer technique can be tested for bursting strength at the time of sacrifice by distending a condom inserted into the peritoneal cavity through a defect made in the apex of the vagina. The balloon is connected to a cylinder of oxygen and the pressure raised at a constant rate of 2 mm Hg/s. Bursting strength is read on the mercury column at the time the wound splits and the balloon extrudes itself. This reproduces the clinical situation well and gives a very definite end point as the wound splits from end to end. The abdominal wound studies are supplemented by conventional histological examination and tensile-strength measurements with a tensionmeter.

Third, the bursting strength of a standard gastric incision, closed in a carefully standardized manner, can be measured by inflating the stomach with air at a constant rate of 1.5 ml/min through a catheter placed in the pylorus, leaving the stomach tied at the cardia. This method reveals the weakest point of the anastomosis. Again this study is supplemented by histological as well as angiographic studies to assess fibroblast proliferation, collagen formation, and angiogenesis.

Uraemia

Any experienced surgeon will have the 'clinical impression' that uraemia inhibits healing, and certainly this was our observation in the early days of renal dialysis and renal transplantation before modern technique enabled us to maintain our patients postoperatively at a relatively low level of blood urea. Surprisingly, very few papers dealing with wound healing and abdominal dehiscence even mention the incidence of uraemia.

In 1965 Nayman and McDermott[6] produced uraemia in dogs with uranyl nitrate and showed that abdominal wound breakdown occurred in these uraemic animals which could be prevented by adequate renal dialysis. Uranyl nitrate has the disadvantage that it produces intense nephritis with proteinuria, which may well deplete the serum protein level and thus indirectly affect wound healing. Measurements of serum protein were not made by these authors. We were able to produce uraemia in rabbits by unilateral nephrectomy combined with contralateral clamping of the ureter[4] without proteinuria or alteration in the serum protein level and we were able to demonstrate marked inhibition of fibroplasia in peritoneal defects in these animals.

Colin[7] has now greatly enlarged on this investigation. He has demonstrated that a reliable and reproducible uraemic model can be prepared in the rat by unilateral nephrectomy with diathermy resection of the upper and lower poles of the opposite kidney. The animals survive for a week with a blood urea level running at a daily mean of between 17 and 83 mmol/l (100 and 500 mg/100 ml). With this model he has shown significant diminution in the bursting strength of the

laparotomy wound and of a standard small-bowel anastomosis compared with controls. Using rat skin fibroblasts in culture, he has also demonstrated marked inhibition of fibroblast growth when either urea or uraemic rat serum was added to the culture medium. Autoradiographs of skin wounds using tritiated thymidine to label actively dividing cells confirm quite gross inhibition of cellular activity in uraemic animals. Regrettably, surprisingly little prospective clinical information has been collected as yet on wound healing in uraemic patients, a subject that merits much greater attention on the part of the surgeon.

Jaundice

Ligation and division of the common bile duct in the rat provides a useful model for wound-healing studies in jaundice. Icterus is obvious by the 2nd day and the serum bilirubin concentration then remains at a fairly steady plateau at around 170 mmol/l (10 mg/100 ml) over the next 5 weeks. Death eventually occurs from rupture of the dilated common bile duct. During the crucial early days the jaundice is uncomplicated by alterations in blood count, prothrombin time, serum electrolytes and total protein, and blood urea. We were able to demonstrate a marked decrease in the bursting strength of the abdominal incision, delay in fibroplasia in peritoneal defects, and decreased angiogenesis and delayed healing on microscopic examination of gastric incisions in the jaundiced animals compared with controls. Interestingly enough, however, the sutured stomach in the jaundiced animals gave the same bursting-strength results as the controls, but this may be accounted for by the dense adhesions which developed to the gastric incision in both groups, these adhesions not being disturbed in the gastric bursting experiments[3].

In a further series of jaundiced rats Dr Konstantinou (in unpublished work) has shown a striking difference in the incidence of burst abdomen and incisional hernia in laparotomy incisions between jaundiced and normal animals. In 60 jaundiced rats there were 10 examples of burst abdomen compared with no burst abdomens in 23 control animals.

In our prospective clinical study of wound healing in laparotomy incisions 274 patients have been studied to date. Eleven of these were jaundiced at the time of surgery and 2 (18%) had complete rupture of the abdominal wound. In the 263 patients free from jaundice there were 4 examples of dehiscence (1.5%). Although the jaundiced group was small, these early results are highly suggestive. Unfortunately and surprisingly, we have been unable to trace any other study of wound healing in jaundiced patients and further careful clinical investigations are obviously required. The particular factor in jaundice which is responsible for this inhibition of healing is unknown, but we are hoping to investigate this more fully in tissue culture experiments.

It is interesting that Professor Blumgart and his team in Glasgow[8] have recently been able to demonstrate a considerable reduction of prolyl hydroxylase activity in the skin of jaundiced patients compared with normal controls. The activity of this enzyme usually parallels the rate of collagen synthesis, and this reflects collagen turnover.

Infection

It is interesting that, in their early phases, infection and wound healing are remarkably similar processes. Infection leads to a cellular and vascular response to the bacterial injury and repair then follows the destruction of bacteria and removal of necrotic tissue. Wound healing demonstrates a comparable cellular and vascular response to the mechanical trauma of wounding.

It is a common clinical observation, of course, that an infected wound breaks down and heals slowly by granulation tissue. Segree and his colleagues[9] demonstrated delay in healing of skin defects in mice whose wounds were inoculated with bacteria and Smith and Enquist[10] showed decreased tensile strength of staphylococcus-contaminated laparotomy wounds in rats from the 6th postoperative day through the remaining 7 weeks of observation. Irvin and Hunt[11] have recently adduced experimental evidence that infection increases the risk of disruption of intestinal anastomoses.

Equally, the clinician notes that patients with serious infection away from the actual wound site (for example, a subphrenic abscess) have impaired healing and it is interesting that Carrel[12] more than 50 years ago demonstrated

that an aseptic abscess delayed the healing of a distant skin wound in the dog. This effect could be transferred to normal animals by injecting the pus obtained from an aseptic abscess and Carrel[13] assumed that some systemic substance could inhibit fibroblast proliferation.

We were interested to investigate the effects of distant inflammation on wound healing rather more closely. We studied the healing of our standard laparotomy incisions, gastric wounds, and peritoneal defects in rats in which an acute aseptic inflammation away from the wound site was induced by a subcutaneous injection of turpentine. In addition, we studied the effects of local and distant infections and transient bacteraemia with *Pseudomonas aeruginosa,* an organism which does not occur as a saprophyte in our laboratory rat colony. We were able to demonstrate that distant sterile inflammation, distant bacterial infection, and transient bacteraemia all had a marked inhibitory effect on the early healing of skin, muscle, stomach wall, and peritoneal defects. Surprisingly, abdominal wounds into which the organism had been inoculated locally did not show a significantly lower bursting strength than those of controls even though macroscopic pus was visible. A group of rats in which the sterile turpentine abscess was incised and the pus evacuated immediately after operation did not show a significant decrease in wound-strength measurements, but in contrast animals injected with the pus obtained from the previous group did have a significant lowering of the bursting strength of both abdominal and gastric wounds[14].

The exact mechanism of this inhibitory process remains unknown. In our study haemoglobin, blood urea, and plasma electrolyte levels were not affected by the inflammatory process. Plasma protein values were significantly lowered, but this degree of hypoproteinaemia in a group of infection-free rats maintained on a low-protein diet, although lowering the bursting strength of the abdominal and stomach wounds, did not do so to the extent seen in infected rats. Other factors might be an increased collagenolytic activity or a hypersensitivity response mediated against some products of tissue breakdown, but much work remains to be done in this field. In clinical practice, however, the importance of the eradication of pus collections in order to obtain optimal healing of intestinal anastomoses and laparotomy wounds is supported by our laboratory findings.

Technical aspects of wound management

Surgeons are physicians who are doomed to operate, and as such we are naturally intensely interested in techniques; these include the techniques of how to inflict surgical wounds and how to repair them. One of the greatest contributions of the USA to surgery was the introduction of the electrocautery for both cutting and haemostasis. Yet many surgeons, especially the Americans themselves, are hesitant in its use because they fear impairment of wound healing. We have therefore investigated the healing of diathermy incisions in our gastric model, comparing a standard scalpel-inflicted incision with that produced by the diathermy knife. Healing was assessed by bursting and tensile-strength measurements, angiography, and histology. All incisions were soundly healed by the 10th day and the only significant difference discovered was that histologically there was a definite lag in healing up to the 5th day in the diathermy incisions. This seemed to be due to the mucosa having been dragged into the wound edges by adherence to the diathermy knife in the thin-walled rat stomach, a situation that may well not obtain in the human organ. We feel reassured in using diathermy in routine surgery, but where wound healing might be in doubt, as in irradiated tissues, the scalpel would be preferable to ensure marginally safer healing.

The technique of wound closure is still the subject of much debate and a great deal remains to be learnt. To the abdominal surgeon it is the laparotomy incision which is most intriguing, and regrettably wound failure, by which I mean both burst abdomen and subsequent development of incisional hernia, remains a significant problem. Most surgeons believe that suture of the peritoneum is an essential step in laparotomy wound closure, yet we have shown that peritoneal defects heal smoothly and with remarkable

TABLE II *One- and two-layer closures*

Total patients in series	274
Patients followed up ⩾3 months	202
99 patients 1-layer—5 hernias (5%)	
103 patients 2-layer—5 hernias (4.8%)	
Burst abdomens = 6, 3 in each group	
2 bursts in 11 jaundiced (18%)	
4 bursts in 263 non-jaundiced (1.6%)	

speed[16]. In our laboratory a series of rabbits with paramedian laparotomy incisions was divided at random into one group in which repair was by individual suture of the peritoneum and anterior rectus sheath and a second group in which the peritoneal layer was omitted. No difference was demonstrated in the histological and tensile-strength findings between the two groups[17]. We are at present studying a comparable series of laparotomy wounds, both paramedian and midline, in patients divided at random into a group treated with a double-layer closure of catgut continuous to the peritoneum and double nylon to the sheath and a second group in which the peritoneal layer is omitted and only the sheath closed with continuous double nylon. Up to October 1976 274 patients have been entered into the trial, equal numbers in each group. There have been six examples of complete dehiscence of the abdominal wound. As I have already mentioned, 2 of these patients were deeply jaundiced out of a total of 11 jaundiced patients in this series. One of these had had a two-layer and the other a one-layer closure. Of the remaining 4 non-jaundiced patients with burst abdomens, 2 were in the two-layer and 2 in the one-layer group (see Table II). In the 202 patients who to date have been followed up from 3 months to 1 year from the time of operation the incidence of incisional hernia has been 5% in the one-layer and 4.8% in the two-layer group.

Other factors

I have not the time or space to detail the interesting work of others of my colleagues, both present and past, in a wide variety of aspects of wound healing. Among these are included the effects of antitumour agents on anastomotic healing[18], of local antibiotics on wound infection[19], and of the noxious effects of starch in the wound[20, 21]. Stewart[22, 23] was able to show marked inhibition of healing of colonic anastomoses in the rat with either a local inoculation of tumour cells or with a higher concentration of tumour cells inoculated subcutaneously. Local implantation probably resulted in simple interposition of tumour cells between the wound edges but might also have resulted in direct inhibition of neighbouring wound fibroblasts and epithelial cells. The distant inoculation of tumour suspension was related to a fall in plasma protein and this systemic effect might alone be sufficient to explain the inhibition of wound healing in this group of animals.

Conclusion

Much other work remains to be done. What are the effects of modern cytotoxic drugs, alone and in combination, on wound healing? How can we reduce the breakdown of intestinal anastomoses to a bare minimum? How can we eliminate starch granuloma formation? What really is the best way of closing the abdomen and how can we prevent wound failure in desperately ill, jaundiced, uraemic, or protein-depleted patients on whom we are forced to operate, often in situations of desperate emergency? The field of wound healing, exciting though it is, has been sadly neglected. Unsolved problems abound; the doors of the operating theatre, the surgical ward, and the laboratory are wide open for those of you with an enquiring mind and a thirst for knowledge who wish to enter.

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