SECOND EDITION

WOUND REPAIR

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PREFACE to the Second Edition

The first edition of *Surgery and Biology of Wound Repair* was intended to bridge, where possible, many of the basic biological disciplines contributing to knowledge of wound healing and regeneration and the clinical discipline of surgery. There are some reasons to suspect that the words Biology and Surgery in the title of the first edition may have retarded rather than accelerated progress toward that goal. The use of such words seems to stake out claims and define territorial rights rather than meld interest and information. Biology and Surgery have been dropped from the title of the second edition, therefore; it was the strongest action we could devise to signify our belief that in the decade ahead the healer must come to the fore in this field if the brilliant accomplishments by fundamental biologists are to be translated into health and happiness for human beings recovering from wounds. Stated more explicitly, our greatest expectations in 1971 have been fulfilled, in determination of the final sequencing of the collagen molecule; much has been accomplished in understanding relationships within different structures. In addition, most if not all of the important cross-links in biological systems have been identified and located. Interrelations between various collagens and other substances such as mucopolysaccharides, macrophages, and endothelial cells, always a difficult problem, still are not clearly understood but considerable progress has been made. Ability to type collagens by biochemical and immunological means has provided still another tool for study of the behavior of these extraordinary proteins. Identification of procollagen peptidases and discovery of dermatosparaxis and identification of the biochemical defects in various types of Ehlers-Danlos syndrome are other examples of the wealth which has been added to our understanding of synthesis and assembly of connective tissue. But where is it all taking us?

As stated in the preface to the first edition, it seems to the authors that ultimately the road must lead to the control of scar tissue in human beings. Animals do not have serious health problems because of

a scar, but people are beleaguered by and sometimes even die because of the presence of unwanted scar tissue or the abnormal physical properties that scar tissue imparts. Control of scar tissue, therefore, is still the treasure at the end of the rainbow for those who are concerned primarily with the health of human beings. The goal is within our grasp but the physician must assume more responsibility in the years ahead if the terribly difficult step between Phase II (animal testing) and Phase III (human testing) is to be taken. Encouraging as basic contributions have been, the pall which currently hangs over human biology because of present attitudes concerning even legitimate research on human disease cannot be denied. Difficult as such attitudes may make research in human disease, the possibility of expanding connective tissue research to include control of scar formation in human beings need not be retarded or halted in the next few years. Progress, however, depends to a great extent upon interest as well as the continued support of productive basic scientists. Speculation as to the direction such "good things" may take is easy.

The area that seems most ready to send a penetrating phalanx into human biology is the control of physical properties of scar tissue. After all, scar tissue causes many of the problems injurious to human health by virtue of physical properties. Thus the applied scientist, for whom this book is directed now, can take advantage of a great deal of basic information related to the control of physical properties of collagen without interfering with or controlling synthesis and deposition of scar tissue. For example, now that a preparation of β-aminopropionitrile, apparently without toxicity for human beings other than upon newly synthesized connective tissue, is available, clinical testing in surgical patients is possible and indeed has already started in several centers. Other similar lathyrogenic agents are becoming and will continue to become available. Such agents will require individual as well as combination testing in human trials. Although Phase II studies on the use of three proline analogs to inhibit synthesis and deposition of collagen have not been encouraging from the standpoint of supporting clinical trial in human beings, other analogs and rate-limiting agents either are available or can be developed in the immediate future. Such investigations undoubtedly will include different approaches such as the development and utilization of anti-ascorbic acid agents as well as control of enzyme kinetics and use of trace metal inhibitors. Extremely toxic substances and strong teratogenic agents must be avoided at first; what is needed now is some modicum of success—even though it be relatively small—in improving the health of a human being through manipulation of even a small property of unwanted scar tissue. It appears now that the principle of controlled induced lathyrism (relatively selective for newly synthesized scar tissue because of the rapid kinetics of wound healing compared to normal tissue turnover) offers the best possibility for controlling physical properties of wound collagen without damag-

ing mature connective tissue in the immediate future. Hence, the spotlight is on the surgeon and the change in title for the second edition of the book is the signal to accelerate the pace. It is in surgical patients that the healing wound can best be manipulated selectively without damage to the rest of the body. Biologists have shown at least one way that such a manipulation is possible in this decade; the final step must be taken by those for whom the title of the second edition was selected.

Finally, the greatest pleasure in writing a book, acknowledging gratitude to all whom gratitude is due, is not such a pleasure in the preface to the second edition as it was five years ago. There simply are too many now and the debt is too great to be paid in this way. Because of seemingly unprecedented conditions which have consumed so much of the authors' time and energy during the last three years, preparation of the second edition of *Wound Repair* has been dependent literally upon a host of individuals—more than can possibly be named. As an example, in addition to our renowned colleagues in the wound healing field, Dr. Milos Chvapil, Dr. John Madden, Dr. Edward Carlson, Dr. Ron Misiorowski, and Dr. Arnold Arem and their brilliant students and assistants, the loyalty and raw courage of the magnificent men and women comprising many of the faculty and literally all of the house staff in the Department of Surgery at the University of Arizona must, for reasons known only to them, be acknowledged with devotion as well as deep appreciation. The second edition of *Wound Repair* could not have been produced without them. Miss Elizabeth Taylor of the W. B. Saunders Company has continued to provide part-time authors with technical and professional support needed to bring out a second edition. As before, the final price for whatever contribution the second edition may make was paid mostly by Mary Peacock and Frankie Van Winkle. To all these individuals and to so many others too numerous to identify here, we are deeply grateful.

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PREFACE to the First Edition

"A definitive book on wound healing is needed," said our advisers. "By all means write one as soon as possible." "You should be able to finish the job in two years," said our publishers.

These statements were made more than ten years ago, when silver nitrate was the topical agent of choice for preventing infection in burned tissue, lathyrism was primarily a disease of poultry, and zinc was of no more concern to wound healing biologists than as a material used in the battery-powered spectrophotometer. The naïveté with which such advice was given and received reflects the fact that a study of the biology of wound healing seemed a dull and nonproductive venture in those days, when so little of modern cellular and subcellular biology had found its way into surgical practice.

The concept that the goal of applied research in healing and regeneration is the "spot weld" of disrupted tissues is responsible for much of the apathy engendered in the past: most papers published in surgical journals have been directed at shortening the time required for surgically repaired tissue to regain tensile strength.

But the decade during which we have worked on this book has come to be dominated by a larger concept. It has not been only that major breakthroughs in basic research have achieved broader practical application (although this is occurring too). Rather, there has been the generation and acceptance of the idea that there are rewards in the field of applied wound healing research every bit as spectacular and possibly even more useful than "spot welding" of injured tissues. As our horizons have widened to encompass the numerous influences on health that healing exerts aside from the time it takes to occur, research in wound healing has become one of the most exciting and, in our judgment, promising fields in surgical biology.

And why should this not be so? To limit our interest in healing to the speed of its various reactions is almost as restrictive as it would be to continue embryological investigation in the hope that a baby can be

produced in less than nine months. Wound healing can in fact be accelerated, but analysis of surgical problems at the moment strongly suggests that, with the exception of bone, the length of time required to reestablish the physical integrity of an injured tissue is not a major problem. The alteration of function in a vital organ that results when a simple scar replaces complex tissue is of paramount importance to the future health and welfare of the patient. Scar tissue is a real killer disguised by its appearance as a product of a valuable homeostatic mechanism.

The changes in our plans, objectives, and thoughts about this book can be summed up by the panic we have sometimes felt as the world of healing and regeneration spun by while we were trying to write about it. Publication has become almost an act of desperation, brought about in the end by the feeling that we had too much in the book to abandon it, and that a page was inadequate literally before we had lifted our pens. By our initial standards, the manuscript is woefully incomplete; we have had to realize that we cannot produce a definitive work on healing and regeneration in human begins at this time.

We did agree, however, to terminate our work in an orderly fashion by November 15, 1969, as suggested by an unbelievably patient, although practical, publisher. To do so has meant a painful but, we believe, wise change in basic objectives. We ask that the book be judged against the goal we have now accepted, which is to bring together some of the work, thoughts, investigations, and clinical experiences of an exciting decade of biological research. It is dedicated to the proposition that healing by synthesis of scar, although undoubtedly important as a pristine function (perhaps even pivotal in such vital processes as natural selection) is no more than a second best solution to the problem created by interrupting tissue integrity.

The importance of such a concept becomes readily apparent. In so many patients it is not the lye burn of the esophagus, the inflammation of the heart valve, or the injury to the liver that kills; unappreciated though it may be, it is the scar that forms during healing that impairs health and, in some patients, may even cause death. Paré's old concept "I dressed the wound; God healed it" is simply not one under which we are willing to live.

The process of healing is the result of cell movement, cell division, and cellular synthesis of various proteins—basic biological processes which are under intensive study in laboratories throughout the world. The end product is primarily a crystalline fibrous protein which behaves predictably and which can be manipulated according to presently understood basic principles of crystalline protein chemistry.

Control of synthesis and degradation of collagen and manipulation of the physical properties which it imparts to scar are not science fiction. Such manipulations are already possible under controlled conditions, both in laboratory animals and in human beings. The therapeutic

implications are enormous. In addition, although it is pure speculation at this time, complete control of fibrous scar production might open an entirely new approach to tissue regeneration—particularly in the liver and bladder, where unusual kinds of regenerative potential appear to be expressed in various ways.

We would point out to all who are responsible for the care of human wounds that there are rewards in modern biological research that can put such work on a higher plane than it has ever been in the past. In 1970 wounds do not have to be treated solely on the empirical basis of dogmatic teaching by master surgeons. To understand *why* and *how* certain therapeutic regimens work is to make a great advance in the ability to utilize more fully some of the lessons that have been learned by trial and error.

Moreover, some of our past teaching has been erroneous, and many principles have been taught as fact even though they are simply based upon the attempt of surgeons to explain and understand clinical observations. Research has exposed some of these errors in a way that is refreshing to the inquisitive student with far better preparation than most of his teachers.

Finally, in certain areas, such as deformity caused by wound contraction, neoplasia initiated by wound healing, and complications caused by reopening a wound at an inopportune time, recent data have provided knowledge which is useful in caring for patients today. This book attempts to focus on these areas in particular, with the thought that the information presented may help practicing surgeons to treat wounds more intelligently and thus more successfully.

Just as important, we also have attempted to demonstrate to basic scientists that there is a real discipline known as human biology, and that wound healing is an area of investigation in which the best of scientific thought and practice can be truly utilized with gratifying results. As in most scientific disciplines, there is a sickening gap between brilliant research accomplishments and practical clinical applications. Perhaps it is in human biology, more than in any other discipline, that the gap between laboratory and bedside needs to be bridged. It is our fondest hope that this book will begin the work on that bridge.

Authors, particularly those who have full time occupations, need a great deal of help. One of the real privileges accorded us, therefore, is the right to acknowledge our debt to those who have made this book possible. Appreciation is due first to the faculty and house staff of the University of North Carolina School of Medicine and to Dr. Nathan Womack and Dr. Richard Peters in particular. Because the most time-consuming portion of the task was completed during the first year of development of a new Department of Surgery, a word of special thanks must be said to the surgical faculty and residents of the University of Arizona, not only to our brilliant colleagues in the wound healing field, Dr. John Madden, Dr. Milos Chvapil, and Miss M. F. Thompson,

but also to Dr. William Trier, Dr. Charles Witte, Dr. Charles Zukoski, Dr. Scott Clark, and Dr. Leonard Weiner, who helped in so many ways to make our book a reality. Similar thanks are due to our colleagues at Ethicon, including Mr. Richard B. Sellars, who gave one of us the freedom to undertake this project, to Dr. Richard Kronenthal and Dr. Irving Oneson, who helped with scientific advice, and to Dr. Emil Borysko, who provided photographs and electron micrographs.

As novice authors we are especially indebted to Miss Gloria Fitz, Mrs. Evelyn Brady, and Mrs. Jean Szymborski who cheerfully and skillfully typed countless pages of manuscript. Additional thanks are due Dr. Eddy Martin and Dr. Sam Barnes for invaluable aid in library research and editorial advice. The patience and professional skill of Mr. Robert Rowan and Miss Elizabeth J. Taylor of the W. B. Saunders Company are appreciated, as is the generosity of many authors who have allowed us to reprint illustrations from their work. Finally, to Mary Peacock, Frankie Van Winkle, and our children, the people who really paid the price for whatever contribution our book may make, we express gratitude for patience and understanding.

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CONTENTS

Chapter 1

INFLAMMATION AND THE CELLULAR RESPONSE TO INJURY

It is almost axiomatic that injury is followed by inflammation. Inflammation can be characterized as a vascular and cellular response designed to defend the body against alien substances and to dispose of dead and dying tissue preparatory to the repair process. The quantitative extent of inflammation depends upon the severity of the injury. Within limits, the inflammatory response shows a typical "dose-response" curve when related to the severity of the trauma. The qualitative nature of the inflammatory response may vary with the kind of injury produced. However, these qualitative differences are more readily discernible in chronic than in acute inflammatory reactions.

An understanding of the nature, mechanisms, and consequences of inflammation is important to the surgeon. Every surgical procedure results in an inflammatory reaction. The surgeon who understands the nature and mechanism of this reaction to injury has within his power the ability to minimize the adverse consequences and to utilize the reaction to the benefit of his patient.

THE ACUTE INFLAMMATORY REACTION

Inflammation resulting from trauma may initially appear to differ from that resulting from bacterial infection or from physical agents such as heat, cold, and radiant energy. This is only apparent; the basic

response is the same regardless of the inciting cause. However, in the case of most trauma, there is physical interruption of blood vessels with immediate hemorrhage, more or less extensive cell destruction at the immediate site of injury, and in many instances a path to the external environment permitting body fluids, including blood, to drain off and allowing bacteria and other foreign substances to gain access to the wound. The response of the body to injury, regardless of its nature, is basically the same, and the most significant element of that response is seen in the local vasculature.

THE VASCULAR REACTION. The immediate response of small vessels in the area of injury is vasoconstriction. At the point of injury, actual vascular occlusion may occur, which combats the tendency to hemorrhage. This vasoconstriction usually lasts only five to ten minutes at the most, and is followed by an active vasodilatation. All elements of the local vasculature appear to be involved in this dilatation.

Almost immediately after injury, leukocytes in the local vessels appear to become "sticky" and begin to adhere to endothelium, particularly of venules. Within 30 minutes to one hour, the entire endothelium of the local venules may be covered with adherent leukocytes. At the same time there is a lesser, but definite adhesion of erythrocytes and platelets. The erythrocytes also tend to adhere to each other and form rouleaux. These tend to plug capillaries, but since platelet-fibrin thrombi do not form until later, this occlusion can be reversed.

Coincident with vasodilatation, leakage of fluid from venules occurs. This fluid has the same composition as plasma with its full complement of macromolecules. This occurs before any cells leave affected vessels and also occurs in the absence of obvious "gaps" in the vessel walls. Electron microscope observation of vessels, however, indicates that there is a separation of endothelial cells so that they are no longer in direct contact with each other. The basement membrane is now exposed to the luminal contents of these vessels.

MOVEMENT OF CELLS. Soon after the onset of leukocyte sticking, these cells are seen to move through the vessel wall by a process of diapedesis. This phenomenon involves active motion; by some not yet proved mechanism leukocytes force their way through the basement membrane to the extravascular space. There is a visible indication that at least a temporary defect is produced in the vascular wall since often a second cell will follow in the path of the first one and erythrocytes, which move only passively, appear to escape through the same channels.

After passing the blood vessel wall, leukocytes exhibit a positive, but somewhat random, motion. Eventually, by one means or another, they concentrate at the site of injury. The predominant cell form is the polymorphonuclear leukocyte, and at one time it was thought that these cells migrated first and were followed at a later time by the mononuclear cells. However, careful studies have shown that the

migration of cells is in the same proportion that they occur in the bloodstream. However, the polymorphonuclear cells are very short-lived compared to the mononuclear cells so that in the older inflammatory reactions, the mononuclear cells predominate.

The escape of fluid from local vessels, combined with migrating leukocytes and dead tissue at the site of injury, constitutes the inflammatory exudate. As the polymorphonuclear cells die and are lysed, the exudate assumes the character of pus. It is important to realize that pus can occur in nonbacterial inflammations.

LOCALIZATION OF THE INFLAMMATORY REACTION. The major factors determining whether an area of inflammation will produce enough pus to constitute what is termed an abscess are (1) the extent of injury to normal tissue, (2) the extent of the cellular reaction, and (3) the extent to which polymorphonuclear cells accumulate and die. These last two factors are, in turn, determined by the state of the local circulation and, in particular, lymphatic drainage.

Lymphatics are more fragile than blood vessels. Thus, in any injury, damage to local lymphatics is usually greater than to the vasculature. Furthermore, leakage of fluid from venules provides fibrinogen and other elements of the blood clotting system. Fibrin plugs quickly form in damaged lymphatics, effectively stopping any drainage from the injured area. Thus, the inflammatory reaction is localized to an area immediately surrounding the injury. Eventually, of course, activation of fibrinolysin relieves the stoppage and drainage can again take place.

Local vasodilatation, leakage of fluid into the extravascular space, and stoppage of lymphatic drainage produces the classic signs of inflammation—redness, swelling, and heat. Pressure, and perhaps chemical stimulation, produces the fourth sign—pain.

The Mechanism of Acute Inflammation

A vast amount of literature in the last 20 or 30 years has dealt with the mechanism of inflammation. Discovery of the anti-inflammatory action of corticosteroids provided a useful tool in the analysis of the many factors alleged to be responsible for the various components of the inflammatory reaction. More recently, the development of antisera against each of the cell types involved in the inflammatory reaction has given insight into the specific functions of each cell and has provided a basis for study of interactions of these cells with one another. For instance, it was shown that certain cells important in defense against infectious agents are of no significance in healing of noninfected wounds. It is also suggested that one cell type may initiate or aid in migration, differentiation, and functional activity of another cell type. These in-

triguing observations will be dealt with when we discuss the cellular response to inflammation.

It is convenient to concentrate on two major aspects of the acute inflammatory reaction: alteration of vascular permeability and forces responsible for movement of leukocytes into the injured area. In a subsequent section, the consequences of inflammation and its role in repair will be considered.

THE VASCULAR RESPONSE. Increased vascular permeability, which is usually referred to as increased capillary permeability, but which is actually confined to small venules, is the key to all subsequent events in inflammation. In 1924 it was postulated that this was brought about by locally released histamine, emanating from destroyed cells, or by a closely related chemical termed H-substance. The proof that histamine or H-substance was present or responsible for increased capillary permeability was far from satisfactory.

In 1936 isolation of a substance called leukotaxine was described; it was claimed that this substance was the agent responsible for increasing capillary permeability, and also acted as a chemotactic agent, attracting leukocytes into the injured area. Leukotaxine appears to be a polypeptide and is formed in damaged tissue by enzymatic destruction of albumin. Cortisone was reported to prevent the permeability and chemotactic action of leukotaxine. However, the precise role of leukotaxine in the inflammatory process is still a matter of debate.

That there might be more than one factor involved in the induction of increased capillary permeability was suggested when some careful observations of graded thermal injury showed that increased capillary permeability occurred in two phases: the immediate reaction and a delayed reaction which occurred one to two hours after injury. These reactions appeared to be separate and independent phenomena suggesting separate mediators. The biphasic reaction was also observed in carefully controlled experimental bacterial inflammation.

It has been demonstrated that the permeability effect and the chemotactic effect can be clearly separated and are probably not due to the same substance. There is increasing evidence that the initial, short-lived increase in vascular permeability may be a result of histamine action. A number of endogenous and exogenous compounds will release histamine or cause histamine to be formed through the action of histidine decarboxylase on intracellular histidine. Local increases in capillary permeability are seen following injection of these substances. In addition, wound tissue fluid shows appreciable histamine content and blood histamine rises immediately after injury.

Since about 1955 a large number of substances have been isolated which can cause increases in capillary permeability. The precise role of these substances is as yet not clearly elucidated. The more important ones are discussed individually.

Histamine. As has been mentioned, it appears probable that the

earliest change in vascular permeability following injury is brought about by histamine. The major but not the sole source of histamine is the mast cell. These cells lose their characteristic granules at the time of injury; granules contain a wide variety of active materials including 5-hydroxytryptamine (serotonin), heparin, and histamine. Histamine is also found in platelets, but there is wide species variation in platelet histamine content. In the rat, the platelet may be a major source, but in the human the histamine content of platelets is low. Other sources are granulocytes and possibly other white cells.

Histamine action is very short-lived, probably lasting not longer than 30 minutes. Furthermore, injury will deplete local sources of histamine so that considerable time is required before sufficient endogenous histamine can be synthesized to bring about further reactions. However, since the vascular permeability increase lasts long beyond the time of histamine action, other permeability-increasing factors have been sought.

Serotonin. Serotonin, or 5-hydroxytryptamine, has an action almost indistinguishable from that of histamine. It also is discharged from mast cells. In some species, such as the rat, it is the dominant vascular amine, rather than histamine. Although it is found in inflammatory exudates, its role in species other than the rat is questionable. In humans and many other animals, serotonin has a negligible effect on vascular permeability.

It is important to emphasize that the effect of these amines, histamine and serotonin, is *not* on capillaries. This was amply demonstrated by electron microscopy. The effect is on vessels 20 to 30 μ in size on the venous side of capillary loops. Vessels 4 to 7 μ, true capillaries, are unaffected by these amines. The action appears to result in a separation of contacts between endothelial cells, possibly owing to a swelling and "rounding" of these cells. The basement membrane is not visibly affected, but acts as a filter at the points of exposure.

Serotonin also has been found to stimulate DNA synthesis in granuloma cells in the late phase of cell population growth. Evidence obtained in polyvinyl sponge-induced granulomas suggests that the principal effect is on the fibroblast population. Although synthesis of collagen appears to be suppressed, but not abolished, there is an increase in the ratio of insoluble to soluble collagen, suggesting that cross-linking of collagen is enhanced. More direct evidence that this may be the case has recently been obtained. Serotonin in concentrations of 10^{-6} M has been shown to induce a marked increase of lysyl oxidase activity in tissues and in fibroblast cultures. As will be discussed in Chapter 4, this enzyme is responsible for initiating reactions leading to collagen cross-linking.

The question arises whether serotonin is involved in later phases of wound healing *in vivo*. Certainly, in many fibrotic lesions such as cirrhosis of the liver and lung fibrosis, the association of large numbers of