SURGERY *and* BIOLOGY *of* WOUND REPAIR

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PREFACE

"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 beings 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,

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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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Chapter One

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 the 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, the leukocytes in the local vessels appear to become "sticky" and begin to adhere to the endothelium, particularly of the 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 the capillaries, but since platelet-fibrin thrombi do not form until later, this occlusion can be reversed.

Coincident with vasodilatation, leakage of fluid from the venules occurs. This fluid has the same composition as plasma with its full complement of macromolecules. This occurs before any cells leave the affected vessels and also occurs in the absence of obvious "gaps" in the vessel walls. Electron microscope observation of the 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 of these cells. By some not yet proved mechanism these cells 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 these channels.

After passing the blood vessel wall, the 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

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bloodstream. However, the polymorphonuclear cells are very short-lived compared to the mononuclears so that in the older inflammatory reactions, the mononuclear cells predominate.

The escape of fluid from the local vessels, combined with the migrating leukocytes and the 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, the lymphatic drainage.

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

The local vasodilatation, the leakage of fluid into the extravascular space, and the stoppage of lymphatic drainage produce 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. The discovery of the anti-inflammatory action of the corticosteroids provided a useful tool in the analysis of the many factors alleged to be responsible for the various components of the inflammatory reaction. However, although we have gained much insight into these mechanisms, a full and final explanation cannot yet be written.

It is convenient to concentrate on the two major aspects of the acute inflammatory reaction: the alteration of vascular permeability and the forces responsible for the 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. The increased vascular permeability, which is usually referred to as increased capillary permeability, but which is actually confined to the small venules, is the key to all subse-

quent 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 the increased capillary permeability was far from satisfactory.

In 1936 the isolation of a substance called leukotaxine was described; it was claimed that this substance was the agent responsible for increasing the 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 the 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 the 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, and these 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 the 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 the capillaries. This was amply demonstrated by electron microscopy. The effect is on vessels 20 to 30 μ in size on the venous side of the capillary loops. Vessels 4 to 7 μ, true capillaries, are unaffected by these amines. The action appears to result in a separation of the 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.

Kinins. Kinins are biologically active peptides that appear to be involved in the inflammatory process and are found in areas of tissue injury. The kinins (bradykinin or kallidin) are released from the α_2-globulin of plasma, which has also been termed a kininogen, by a plasma enzyme, kallikrein. Kallikrein is activated by the Hageman factor (Factor XII) which, in turn, is activated by contact with glass or by other negatively charged insoluble substances. Kallikrein was described over 30 years ago and was thought to be a blood pressure-controlling substance. A similar material was found in the pancreas and urine and subsequently in the salivary glands. It was generally overlooked until the mid-fifties when its enzymatic nature was recognized and its relation to bradykinin established. More recently, a kallikrein has been found in granulocytes, and it is suggested that release of this kallikrein initiates an inflammatory response.

A number of kinins have been described, but their separate identities are questionable. Certainly two, bradykinin and kallidin, have been shown to be nearly identical. The terminology in this field is confusing and, until the substances are better characterized, it would seem best to refer to them collectively as kinins. Bradykinin and two kallidins have been characterized as identical peptides except that the two kallidins have one and two extra terminal amino acids respectively. Their structure has been elucidated, and bradykinin contains nine amino acid residues.

The kinins appear to act on the microvasculature in a manner

quite similar to histamine and serotonin. The kinins are short-lived since they are rapidly destroyed by plasma and tissue proteases. Thus, it appears unlikely that they are involved in producing the late phase of vascular response.

Macromolecular Mediators. Several ill defined large molecule "permeability factors" have been described which are alleged to be responsible for the delayed permeability changes in the microvasculature at the site of an injury. It is difficult to assess the validity of these substances since the kinins are derived from macromolecules as is leukotaxine. Thus, some of these "factors" may merely be precursors of already known vasoactive materials. It is likely, however, that one or more of these may be responsible for the the late vascular reactions.

Chemotactic Agents. It has been shown that although many of the substances affecting vascular permeability also have mild leukotactic action, this is insignificant at concentrations present locally at the site of an injury. For example, leukotaxine, which was originally thought to be the principal substance causing leukocyte emigration, does not show any marked chemotactic activity at levels of concentration seen in lesions *in vivo.*

Three substances have been shown to have the greatest effect in promoting the influx of leukocytes into the inflammatory zone. One of these is the so-called "lymph node permeability factor" (LNPF). This is derived by extraction from lymph nodes which have been disintegrated by ultrasonic means or by freeze-thawing. White cell emigration commences immediately after injection of this material and lasts for less than 24 hours. A substance with a similar action is derived from rat serum incubated with minced rat liver. Also, an extract of leukocytes is reported to produce a similar action. None of these substances have been isolated in pure form, and their identity and mode of action are unknown. Some believe that the LNPF may be important in chronic inflammation and in inflammation of the delayed hypersensitivity type.

All of these preparations also exhibit an effect on vascular permeability.

Summary of the Acute Inflammatory Process

At the present time, the best evidence indicates that the acute inflammatory reaction comprises the following sequence of events:

Injury, either mechanical, chemical, or bacterial, causes the release of intracellular materials into the extracellular compartment of the injured tissue. These cell-derived materials now react directly upon the local microcirculation. Some of these materials, such as histamine and serotonin, directly increase the permeability of this microcirculation. Simultaneously, certain intracellular enzymes can destroy norepinephrine (normally found extracellularly) which is required to maintain the tonus of the microcirculation. Vasodilatation is the result.

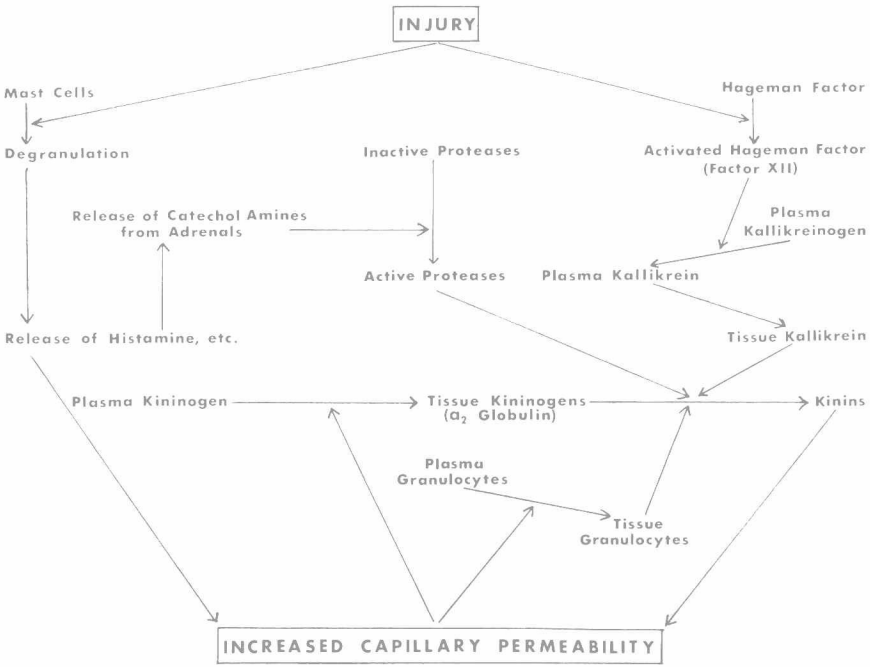

Figure 1-1. Schematic diagram of the probable mechanism of the acute inflammatory reaction. It is important to note that there are at least three pathways involved in the release of the tissue kinins, and that in the case of two of them there are postulated feedback mechanisms that act to prolong the effect. Thus, although the duration of histamine and kinin actions on capillary permeability is short, the feedback mechanism may play a role in producing the prolonged effect actually observed.

Proteolytic enzymes can attack directly the endothelial (or basement membrane) lining of the microcirculation, rendering it "leaky" with respect to circulating macromolecules and cells. Some of these released proteases may also be capable of activating kallikrein which, in turn, releases bradykinin from the α_2-globulin substrate found normally in the circulation. These proteases may also act directly in a kallikrein-like manner, to release kinins from the appropriate extracellular substrate. These kinins increase markedly the permeability of the microcirculation, causing an increase in the concentration of proteins and cells within the wound space. This is probably not a complete explanation of the events that occur, but it does illustrate the complexity of the process.

Tissue Destruction by the Inflammatory Reaction

A very mild injury will produce only a transient inflammatory reaction and may not result in further damage. However, if the trauma is extensive, resulting in the destruction of a considerable amount of

tissue, or if the wound is contaminated with either irritants or viable bacteria, the reaction may be extensive and this has certain unfavorable consequences.

Granulocytes have been shown to contain within their lysosomes a wide variety of proteolytic enzymes, including a collagenase. Normally, serum contains an inhibitor of tissue collagenase, but this inhibitor can be destroyed by released proteases. Furthermore, there is recent evidence that the granulocytic collagenase is not inhibited by serum. Granulocytes are extremely sensitive to lowering of the pH. With plugging of lymphatics, vascular stasis, and the resulting anoxia at the site of injury, glycolysis produces excessive lactic acid and the local pH drops. Granulocytes are lysed and release their enzymes, among which is the collagenase that becomes active. This collagenase acts upon the local connective tissue and solubilizes it. Proteases digest it further. Since drainage from the area is impaired, the necrotic debris, lysed granulocytes, and so forth, accumulate and become a necrotic abscess. This constitutes severe destructive inflammation.

Destructive inflammation is encouraged by the presence of dead tissue, bacteria, blood clots, and poor circulation. Thus, gentle handling of tissue, meticulous hemostasis, aseptic technique, and avoidance of tight sutures will prevent this type of inflammation in the ordinary surgical procedure.

CHRONIC INFLAMMATION

In most noncontaminated wounds, particularly those produced by the surgeon's knife, the acute inflammatory reaction subsides and recognizable repair commences in from three to five days. Unfortunately, some wounds, even those produced by the surgeons, become contaminated, or contain foreign material that cannot be removed during the acute inflammatory reaction. A condition of chronic inflammation then exists.

The predominant cell in the chronic inflammatory process is the mononuclear cell. As we pointed out, in the early stages of acute inflammation, the mononuclear cell is only rarely seen. This is not because it fails to migrate at the time the granulocytes are invading the zone of injury, but because the relative numbers in the circulation are far less than the granulocytes. As the granulocytes disappear through destruction or migration, however, the mononuclear cells, which are much more resistant to lysis than the granulocytes, persist at the site of injury. Thus, their relative number increases. For a time they are augmented by additions from the circulation.

These mononuclear cells modulate into cells we recognize as macrophages and become actively phagocytic. Some coalesce and become multinucleated giant cells, which are also phagocytic. The mono-

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