Historical foundations of wound healing and its potential

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INTRODUCTION

The idea that the wound healing process may be accelerated seems to have originated in the first decade of the 20th century with Alexis Carrel. In fact, the future Nobel Prize recipient redirected his extraordinary surgical research in 1907 to the goal of finding how to enhance the wound healing process.1 The scientific stimulation that triggered this transforming thought is credited to the research of Jacob Loeb, which showed that a change in the concentration of hydrogen ions in the aqueous environment of the sea urchin could enhance its replication. Carrel took this idea and applied it to the process of wound healing, which he saw as involving, at least in part, cell proliferation. While there was certain logic to Carrel’s thinking, the chiasm between Loeb’s research and wound healing was indeed substantial. However, the Carrel story on wound healing acceleration is more complicated, being part historical and personal. In 1902, Carrel was an attending physician on a pilgrimage to the religious shrine at Lourdes, France. While attending to a female patient with a very advanced and life-threatening case of tubercular peritonitis, he claimed to have observed what amounted to an instantaneous, full, and nonrelapsing recovery of his patient. Carrel went on to write a book about this event and did so under the pseudonym of Larrec, being afraid to use his own name for fear of professional reprisals. It was the profound rapidity of the healing at Lourdes that most likely initiated his interest in the potential for highly accelerated wound healing.2 Carrel was not just interested in modestly speeding up the wound healing process as he expressed the desire to enhance it by a factor of 10 or even more, something tending in the direction of his reported Lourdes experience. Thus, Carrel was prepared to see an intellectual opportunity in the rather remote data of Loeb, probably due to his “preconditioning” event at Lourdes some 5 years before.

While the initiation of wound healing acceleration started with Carrel, there were other independent research activities in the first half of the 20th century that were also focused on the acceleration of wound healing. Despite these areas of research and substantial supportive publications in scientific and medical journals, there was a strong belief, and possibly even a general consensus of the leading mainstream biomedical researchers of wound healing, that all attempts at acceleration had failed despite the pleas of some investigators that their process or agent enhanced wound healing. In fact, whatever claims that emerged during the first half of the 20th century were not the seemingly excessive claims of Carrel’s factor of 10-fold or greater. These researchers were claiming successes in the 20–40% acceleration range, just beyond the control group variation.

This article will provide an integrative assessment of the seven principal areas of attempted wound healing acceleration of the early decades of the 20th century (Table 1), including the basis of the underlying theoretical foundation, the methods by which the acceleration hypothesis was tested, how successful the researchers were in accelerating the healing process, the nature of the dose response, and possible underlying mechanisms.

TISSUE EXTRACTS

Tissue culture experiments

Historical foundations

The use of embryo and adult organ homogenates in the treatment of wounds originated with the work of Alexis Carrel at the Rockefeller Institute in the first decade of the 20th century. Inspired by the research of Jacob Loeb,3 which showed that
slight changes in the composition of the sea water (i.e., hydroxyl or hydrogen ions) in which sea urchin’s eggs were reared could induce cell division, Carrel1 generalized this concept to the goal of accelerating the process of wound healing and nerve regeneration. Thus, in 1907, Carrel initiated investigations into whether chemical agents may enhance the repair of small cutaneous wounds. Some successes were noted by Carrel using connective and epithelial tissues, displaying enhanced cell proliferation in a variety of experimental in vivo models. However, the in vivo studies of wound healing had certain methodological problems especially with respect to excessive variability and difficulty in obtaining reproducible findings.

During this period, Carrel became aware that Ross Harrison, having recently moved from Johns Hopkins University to Yale University, had demonstrated that tissues could be cultivated outside the organism. While at Johns Hopkins, Harrison noted that embryonic frog tissue could be grown in coaguable lymph and in the process revealed that nerve fibers develop as outgrowths from a central neuron. This work was of significance for both the identification of the origin of nerve fibers but even more importantly for Carrel was that tissues could be grown outside the body. These research accomplishments would come to be recognized for their biological significance with Harrison being recommended to receive the Nobel Prize in 1917. However, due to the ongoing world war in 1917, the Karolinska Institute declined to grant the award that year, thereby denying Harrison of his most significant recognition of scientific achievement.

Carrel arranged for his assistant, Montrose Burrows, to work with Harrison during the spring of 1910 at Yale. Burrows transformed Harrison’s methods, making them applicable to mammalian cells. Of significance was cultivation of tissue using plasma from the blood rather than lymph and then later using the procedure to grow fibroblasts from chick embryonic tissues. This new in vitro methodology was then applied to numerous embryonic and adult mammalian (e.g., cat, dog, rat, guinea pig) tissues and multiple tumor cell types. By January 1911, a colleague of Carrel (i.e., Dr. Ruth) developed an in vitro technique for the assessment of wound healing and its possible application for wound acceleration.3 These efforts culminated in a publication by Carrel1 indicating that tissue extracts and juice could accelerate connective tissue (fibroblasts) growth by 3–40-fold. Carrel noted that if the rate of repair could be enhanced by only “10-fold,” then a broken leg might heal in only 4–5 days. Newspapers such as The New York Times ran major stories based on the paper with the conclusion that Carrel “leads us to believe that in the future we will be exempt from all bodily injuries” (see January 5, 1913; September 14, 1913).

While this 1913 article focused on the concept of wound healing, Carrel’s focus took a decided turn toward the development of techniques for the improvement of tissue culture, becoming fascinated with the possibility that fibroblasts could grow and live indefinitely in the in vitro state. Carrel attempted to advance both concepts as he was not only interested in repairing wounds of all types but prolonging the lifespan as well.

Carrel’s research took a dramatically altered course as he volunteered to direct a Rockefeller-funded experimental hospital in France near the war zone during World War I. From 1914 to 1918, he directed research on wound healing with military applications. He was also embroiled with the issue of how best to treat wounds, a debate which pitted anti-antiseptic physicians against the antiseptic physician perspective. Working with the British chemist Richard Drysdale Dakin,6 Carrel helped to refine an optimal antiseptic product and an efficient and practical delivery system to disinfect a broad spectrum of infected wounds. Of further significance is that Carrel placed high priority on attempting to clarify the efficacy and efficiency of their procedures by massive objective documentation. Carrel’s team also published findings on the process of skin wound healing and factors that could modulate the rate of such healing. During this time period, Carrel tried to improve the four-part Carrel-Dakin wound treatment procedure (i.e., debridement and hemostasis, application of Dakin’s solution, bacteriological counting, and wound closure). In addition to the use of this systematic clinical method, Carrel undertook extensive experimental research with animal models, especially guinea pigs, providing a fundamental quantitative characterization of the wound healing process and factors that could affect it. Guinea pigs were selected because the skin of their abdomen wall is more adherent to the aponeurosis (broad tendons that join muscle to the body parts, affecting muscle action) than the cat or dog. Human wound healing times were described in mathematical terms by du Nouy.7,8 A model based on such findings became widely employed in the evaluation of agents or processes that were tested for the capacity to affect the time course of wound healing.

According to Selcer,7,8 Carrel tested numerous chemicals and biological agents for their capacity to accelerate wound healing. However, none were shown to enhance the process to his surprise and profound disappointment. This statement by Selcer is of great interest, but the source of the statement is not documented. A check of PubMed and Web of Science databases provides no article by Carrel either during or after World War I that provides data for such wound healing screening tests. Selcer7,8 goes on to state that Carrel “experienced his work in the war as a failure because he was unable to accelerate wound healing.” If this is the case, it may explain why Carrel did not publish any article directly on these efforts during the war to accelerate wound healing. Despite his apparent major disappointment on wound healing acceleration, Carrel inspired Albert Fisher to assess the role of embryo extracts on wound healing. Fisher, who studied with Carrel from 1920–1922, became an expert on tissue culture, later writing several books9,10 on the topic and developing a tissue extract product for enhancing wound healing as discussed below.

Table 1. Areas of research to accelerate wound healing during the first half of the 20th century

<table>
<thead>
<tr>
<th>Area of Research</th>
<th>Species</th>
<th>Tissue Type</th>
<th>Carrel’s Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organ/embryo tissue extracts</td>
<td>Multiple</td>
<td>Connective and epithelial</td>
<td>Enhanced cell proliferation</td>
</tr>
<tr>
<td>Sulphydryl compounds</td>
<td>Rat, mouse</td>
<td>Fibroblasts</td>
<td>3–40-fold growth</td>
</tr>
<tr>
<td>Wound hormones/vwound fluid</td>
<td>Rat</td>
<td>Connective and epithelial</td>
<td>Accelerated healing</td>
</tr>
<tr>
<td>Carcinogens and wound healing (chemical carcinogens—hydrocarbon carcinogens)</td>
<td>Rat</td>
<td>Connective and epithelial</td>
<td>Enhanced healing</td>
</tr>
<tr>
<td>Cartilage—wound healing</td>
<td>Guinea pig</td>
<td>Cartilage</td>
<td>Improved healing</td>
</tr>
<tr>
<td>Multiple chemical agents</td>
<td>Various</td>
<td>Various</td>
<td>Reproducible findings</td>
</tr>
<tr>
<td>Radiation</td>
<td>Various</td>
<td>Various</td>
<td>Accelerated healing</td>
</tr>
</tbody>
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Historical foundations of wound healing
Magnitude of healing acceleration

In his 1913 paper, Carrel emphasized the capacity of various tissue extracts, including embryonic juice, to enhance the growth of chick heart fibroblasts in vitro by some 3–40-fold. As noted previously, these observations then led to widespread publicity in newspapers that he hoped are able to accelerate wound healing in people.

According to Witkowski,1 the estimates of cell growth initially published by Carrel were problematic showing 20–50% variability (e.g., split samples) after 48 hours, for multiple studies. Of greater importance is that his method of quantification did not measure what he said, i.e., cell multiplication. What Carrel did measure was the area of outgrowth around an explanted tissue sample in culture. The explanted tissue was cut into two parts as “equal” as possible, one serving as a treatment group, the other as a control. They were transferred to two separate hollow-grooved slides. An identical volume of plasma was applied to each slide. The fundamental problem to two separate hollow-grooved slides. An identical volume of plasma was applied to each slide. The fundamental problem was that this method was not a true measure of cell multiplication. The area of cell outgrowth around the explants depended on outward migration rather than on the rate of cell growth.11 This limitation was addressed by the Carrel group but not until 1921 by Eberling.12 Furthermore, experienced researchers such as Carleton13 noted that the quantification method is extremely difficult with Carrel stating that “technique is delicate and in untrained hands the experimental errors are of such a magnitude as to render the result worthless.” Thus, in the early decades of the 20th century, Carrel employed a method that was difficult to accurately conduct, had high experimental variability, and did not precisely measure cell growth.

An assessment of the 1913 seminal paper by Carrel reveals that it was long on conclusions but short on data. Furthermore, the methodology was not written as specifically as needed to enable accurate replication. With respect to statistical analysis, hypothesis testing was just being introduced into biological testing. Consequently, neither was it applied to the data nor even a simple display of response variability provided. Furthermore, the increase of 3–40-fold in cell growth was not supported in subsequent papers by the Carrel group. Experiments that showed dose-response data for fibroblast growth displayed increases in the percentile to several-fold range.15 As with other studies, there was inadequate reporting of variability and reproducibility of findings, along with sample size limitations. For example, in one dermal wound healing study, four dogs were employed to assess the effects of *Staphylococcus* infection on the wound latency period.16 However, even in this case, there was only one single dog for each different dilution of the bacterial suspension.15 Only one dog was used to assess the effects of irritants (i.e., turpentine) on the wound healing latency period.16 Furthermore, the wounds were biologically induced with treatment and control groups in the same animal without first knowing if the response was local or systemic. Despite these methodological limitations, Carrel concluded that “the local application of certain irritants such as turpentine, chick embryo pulp, and *Staphylococci* reduces the duration of the latent period.” Despite the apparent uncertainty of the Carrel16 paper, a reanalysis of his dog wound healing data lend some support to a possible treatment effect. If all control and treatment data are separately combined across days, there is a decrease in wound area at two time points in the treated wounds. The percent wound healing enhancement was about 30–50%. In the four experiments with dogs (one dog/experiment), the percent remaining wound area was 56.0 (control) vs. 45.0% (treatment), 70.0 (control) vs. 45.9% (treatment), 54 (control) vs. 25.0% (treatment), and 57.5 (control) vs. 0.0% (treatment).

A 1911 paper by Carrel and Burrows17 assessed the effect of altered plasma tonicity on the growth of chick skin, heart, liver, and spleen cells in chicken plasma. Similar experiments were conducted with the skin of adult frogs. Eleven different plasma concentrations were assessed; five with progressively less tonicity, normal plasma, and five concentrations with progressively greater tonicity. The data were provided only for the five hypotonic concentrations. This was because the growth response for all hypertonic concentrations was zero. Figure 1 displays the results for the fetal spleen P3 study. All experiments displayed biphasic dose responses along with acceleration of growth at a concentration less than normal. This was the case for each tissue. However, according to the authors, the optimum concentration varied according to the tissue and species. Unfortunately, the data were only provided for the fetal and adult chicken spleen experiments.

**Section summary**

The research of Carrel was profound in its leadership over nearly a century, creating the future of wound healing and tissue culture. However, despite these enormous conceptual developments, the data that he published on wound healing are too limited to offer general conclusions. Where he did have intriguing dose-response findings such as the influence of hypotonicity on tissue growth in multiple species, he only published a limited representative sample.

**In vivo**

This section explores the capacity of embryo and tissue extracts to accelerate wound healing within in vivo animal studies. During the first half of the 20th century, researchers focused on three general areas. These included the use of
embryo, heart, and skin extracts. Both the embryonic and heart tissue extracts were developed into commercial products. In the case of the embryo extract, this was derived from the calf by Fischer following his years with Carrel. The commercial product was named Epicutan. Only a few papers in the peer-reviewed literature exist on Epicutan. For example, Nielsen\textsuperscript{27} demonstrated 32\% shortening of wound healing time in dogs. These findings were consistent with several earlier studies in which wound healing was enhanced by the injection of extracts of embryonic tissues.\textsuperscript{18–21} In contrast, using a Wistar rat model Dann et al.\textsuperscript{22} reporting no enhanced healing of dermal wounds.

The first study assessing the effects of heart extract preparation (HEP) on wound healing in an animal model was by Hoffman et al.,\textsuperscript{23} wherein they reported a 40\% acceleration of skin wound healing in dogs serving as their own control. This study resulted from the efforts of Doljanski and Hoffman starting in 1939,\textsuperscript{24} who wanted to apply embryonic tissue extracts to enhance wound healing following the suggestions of Carrel and Burrows\textsuperscript{5} and Carrel.\textsuperscript{1} However, the use of embryonic extracts was problematic: it could not be produced in large quantities and was unstable. So Doljanski and Hoffman\textsuperscript{26} explored adult organ extracts, and to their surprise found that they were even more active in growth stimulation than the embryonic extracts during in vitro testing. Further testing also revealed that adult tissue extracts affect both connective and epithelial tissues.\textsuperscript{25} Finally, Werner and Doljanski\textsuperscript{26} produced a powder preparation of sheep’s heart, and this set the stage for the test of Hoffman et al.\textsuperscript{23} as noted previously. The healing criterion was based on the complete closure of the wound area.

While the use of adult heart tissue extract (HEP) to facilitate wound healing became a well-studied clinical area in the 1940s, there were several complementary experimental studies with animal models. In the first animal experiment attempt, Auerbach and Doljanski\textsuperscript{27} assessed the application of adult chicken heart extract on induced dermal wounds in rats. These authors may have been the first research group in the area of animal model wound healing in which the animal did not serve as its own control. Ever since the early experimental days of wound healing by Carrel, each animal served as its own control. However, these authors\textsuperscript{28,29} had earlier demonstrated that the local application of adult and embryonic tissue extracts may have systemic effects, thereby speeding up healing in both controls and treated wounds of the same animal. Such a condition would preclude detecting a treatment effect. Thus, these authors initiated a study with independent control animals; with 30 treatment and 30 control rats wound closure was accelerated by 31\% in the treated rats at the only dose tested.

Studies by Young et al.\textsuperscript{30,31} challenged these findings, being unable to detect HEP-enhanced healing of skin wounds in rabbits treated with HEP or embry tissue extracts regardless of whether the testing was directed toward local or systemic effects. Despite the failure of Young et al.\textsuperscript{30,31} to confirm the accelerated healing of Auerbach and Doljanski,\textsuperscript{27} these studies had many differences (e.g., animal models [guinea pig, rabbit vs. rat], source of adult heart extract and the number of adult heart extract treatments [2 vs. 6–10]).

In skin extract experiments, there is some agreement among the several published studies. McJunkin and Matsu\textsuperscript{32} reported that both rat embryonic and adult skin extracts accelerated dermal wound healing in adult rats based on histologic evaluations they believed were superior to measuring wound surface area. The number of rats in the adult extract study was five, whereas it was three for the embryo extract study with each animal serving as its own control. This study showed that Kakalin, paraffin, liver, and connective tissue did not enhance dermal wound healing. Several papers by Teir et al.\textsuperscript{33,34} and Teir and Nystrom\textsuperscript{35} showed that rat embryo extract injected intraperitoneal (IP) enhanced skin epithelial cell mitosis. Other studies showed that embryo and adult skin extracts also accelerated the healing time of dermal wounds in adult rats, while extracts from multiple other tissues failed to do.

Based on the in vivo studies, the data up to the early 1960s indicated that skin extracts, and especially embryo skin extracts, could enhance adult skin mitosis and wound healing. Experimental data concerning Epicutan and HEP were less convincing but suggestive and in need of follow-up investigations. Unfortunately, the impressive findings of Teir and Nystrom\textsuperscript{35} were not significantly extended, being cited only four times over the past 50 years. Of note, however, is that the 1951 study of Teir et al. reported a clear biphasic dose response for the embryo skin extract to induce mitosis in the adult skin. These and several other studies\textsuperscript{36,37} led Teir and Nystrom\textsuperscript{37} to suggest that “contradictory” findings may be due to the dosage. Small doses of a certain substance can fail to produce any effect, higher doses can have a stimulating effect, while even higher doses an inhibitory effect on the healing process.

**Clinical studies**

Six clinical papers have been published on the effects of tissue extracts on the healing of wounds, typically indolent wounds in humans.\textsuperscript{23,38–42} These papers consist of a brief description of each case, summarizing past failed traditional treatments, the use of HEP, and the apparent degree of wound healing acceleration. Cases are similarly described when the HEP was without apparent effect. The results are striking as the tissue extract preparations were frequently observed to activate a healing process that had been quiescent for a prolonged time. Kerr and Werner\textsuperscript{41} summarized 36 cases of treated indolent wounds of soldiers in the Middle East during World War II (see Supporting Information Appendix S1). The lesions consisted of ulcers without preceding trauma or acute inflammation and wounds from projectiles and burns. The vast majority of the indolent patients were initially treated via standard practices at the time. Such treatments typically utilized one or more of the following treatments: occlusive elastic dressings, saline baths, sulphanilamide powder with tulle grass, silver nitrate, sodium sulphate dressings, glycerin, acrifavine dressings in oil, eucol (mixture of chlorine and boric acid), and skin grafting. During this time all patients were given diets high in protein and vitamins. Only after such treatments had failed for at least 3 weeks, but usually much longer, was the HEP treatment used. In the subsequent paper by Werner,\textsuperscript{42} the indolent period was extended to a minimum of 6 weeks. Despite the striking successes described in these papers, no additional clinical papers were published using this preparation on wound healing in the clinical setting, as well as in animal model studies. Why would this be the case? While a possible explanation may be the rapid onset of other antibiotics and better patient management affecting an improved wound healing strategy, it should be emphasized that even 60
years later there are over 5 million patients in the US on a yearly basis with life-threatening indolent wounds.

**EARLY PRODUCT TESTING AND APPLICATIONS**

**Sulfhydryl (SH) compounds**

The first SH compound tested for wound healing in a clinical setting was thiocresol. A preliminary note was reported in 1929 by Reimann and Hammett with a more detailed subsequent report given by Reimann. When a wound (i.e., skin cut) occurs, Reimann stated that cells soon initiate division and the process continues until the wound is healed. However, he argued that the law of mass action infers that a chemical reaction will be slowed and eventually halted by products of the reaction. As SH radicals can stimulate cell division, he proposed that the products of these processes will accumulate and stop mitosis once the wound is healed. Reimann therefore suggested that SH compounds might be of value in facilitating the healing of recalcitrant or stubborn wounds. However, concern was raised that the SH compounds may also enhance the growth of bacteria at the wound site. Based on this concern, thiocresol was used as the first SH compound to be tested in a clinical setting because the cresol part of the molecule would be liberated and could be effective as a bactericidal agent. In a pilot study, they reported that seven cases of obstinate cutaneous ulcers were successfully treated with thiocresol. The dressings were saturated with a solution of thiocresol, which was diluted to 1/10,000 and applied to wounds for a few days with alternating no treatment for a similar period. The alternating treatment was a means to slow down the rate of cell proliferation. Of the seven cases, only two were reported in detail. In both cases, the healing process appeared to be markedly accelerated. The authors noted the problem that granulations grew faster than the epithelium, necessitating the removal of excessive granulation tissue on the edge of the wound by silver nitrate, then dissolving the debris with pepsin. The thiocresol was chemically labile and had to be fresh because it can quickly oxidize, losing its bactericidal and cell proliferative effects, contributing to the enhanced healing process.

Powell et al. also compared the response in nine normal subjects given 39 scraping wounds (about four to five wounds/person) (1/8 × 1/2 inch area and to a depth that would elicit capillary bleeding). The same subjects also received 32 similar scraping wounds that were treated with iodine as a control. The merthiolate was reported to accelerate the healing process by 25–35%. These findings are complementary to the earlier reports which dealt only with stubborn wounds regardless of the SH agent.

Several papers assessed the capacity of various SH compounds to enhance wound healing in rats and rabbits. These studies used thiocresol, merthiolate, and thioglycerol. All were modest studies with Hammett and Reimann displaying accelerated healing in 20 of 25 wounds. Similar findings were reported with rats and rabbits with Powell et al. In neither case was a quantitative measure of healing provided. A representative picture was demonstrated by Hammett and Reimann from which an estimation of healing could be made. From the picture, one can discern greater wound healing in the treated rats. Acceleration of wound healing was demonstrated by Sutton for both the rat and rabbit studies, with it being 18 and 21%, respectively. A similar study by Riley with rabbits demonstrated that cysteine gave a 30–40% accelerated wound healing as determined by a wound surface area calculation.

These collective studies provide limited but consistent evidence that various SH compounds can accelerate wound healing in male rats and rabbits. Despite the general consistency of response, no research has independently confirmed a healing response on the same SH compound and in the same animal model. The sample sizes of the reported studies are 25, 12 and 20, and 38 rats. These data are consistent with the clinical findings and thereby support an overall conclusion that some SH compounds can enhance dermal wound healing in several animal models and humans. Of note was that the human data were strongly oriented toward the healing of recalcitrant wounds, while the animal studies dealt with wound healing in healthy animals.

These findings were, in part, designed to provide a link back to the research of Carrel with the stimulatory effects of embryo juice on wounds. According to Riley, embryo juice is too difficult to prepare and loses its biological effectivity quickly. There was therefore the desire to find active agents within the juice that could be tested separately. Thus, as the field was entering the 1940s, there was evidence to support
the hypothesis that some SH compounds could enhance the wound healing process. However, none of these studies dealing with SH groups addressed the issue of the dose response.

Wound hormone/wound fluid

The concept of a wound hormone (i.e., substances released by injured cells which stimulate cell proliferation near or at the sight of injury) was proposed in 1892 by Wiesner. It has been a theme that attracted researchers from a broad cross section of biological disciplines. A leading group in this area was directed by George Sperti and his colleagues John Loofbourow and Elton Cook at the Institutum Divi Thomae in Cincinnati. These researchers explored the role of cell damage and division and their relationship to carcinogens. At the core of their research were the consistent observations that when tissue is injured, the cells surrounding the wound become activated and initiate cell proliferation on an accelerated scale. This enhanced rate of cell division would continue until the damaged/destroyed tissue was replaced and the wound healed. It was speculated that the activation message was mediated by so-called wound hormones, possibly released by the wounded cells. Within this framework cancer was the product of uncontrolled cell division. Sperti claimed that an attractive approach for understanding cancer would be the isolation and characterization of the wound hormone(s).

A key initial step in this research plan was to determine how to damage cells without killing them or at least not killing them too quickly. Not knowing what specific course of action to take, they explored the responses of fish, lizards, and chickens, focusing on tissues such as the liver and kidney as well as embryos. Finally, they struck upon the use of yeast because of some important cellular similarities to human cells and the practical ease of growing yeast in the laboratory. Once the yeast was adopted as the experimental model, ultraviolet (UV) was used to induce injury. Over time they determined levels of UV, including intensity and duration of exposure, which would injure the yeast for the purpose of their experimentation.

With the exposure methodology in place, Sperti et al. assessed whether the injured yeast cells released the so-called wound hormone within the growth medium. After the UV exposure, the yeast cells were removed by a filtering process, leaving a cell-free filtrate. If the residual filtrate contained wound hormones, then this filtrate medium would be expected to stimulate the growth of normal yeast cells. In fact, this enhanced growth is what the investigators observed. Other findings revealed that the growth stimulation response to injury was not unique to yeasts nor was it only inducible by UV. Later investigations showed comparable stimulation from heat and mechanical stress. Furthermore, similar findings were also noted in mammalian liver and kidney cells as well as rodent embryos.

As a result of the potential broad implications of these findings for cancer biology, growth-promotion, and wound healing, Sperti recast the wound hormone term to that of “biodynes” from the Greek meaning “life-force.” Because their prime focus remained on the biology of cancer cells, they exposed yeast cells to suspected chemical carcinogens. While UV is a human skin carcinogen, this had yet to be accepted during the 1940s. Thus, their focus was to use cancer-inducing chemical irritants. Similar to their research with UV, heat, and mechanistic stresses, the chemical carcinogens also induced injury to the yeast, enhancing growth, presumably via release of biodynes. The investigators believed that these findings supported the then popular hypothesis that chronic irritation can cause cancer. Sperti later suggested that chemical irritants can injure normal cells, keeping them in prolonged state of injury, with the resultant chronic secretion of biodynes leading to tumor development.

Extensive chemical investigations by Loofbourow et al. revealed that the damaged cells secreted a complex mix of biodynes that displayed a broad range of potential biological activities, affecting cell division, wound healing, cellular respiration, glucose metabolism, and other functions. According to Sperti, these diverse biodynes may be at the foundation of abnormal cell division. Sperti would subsequently develop a biodyne-containing ointment for use as a wound healing agent for minor burns and abrasions, a product that remains on the market.

As a result of such findings, several widely circulated general lay magazines reported on a product called biodyne ointment that was alleged to have striking wound healing potential when applied to burns. The advertisements indicated that the ointment accelerated the rate of healing and epithelization. A review of the literature reveals that the commercial product has rarely been the object of independent testing with publication in the peer-review literature. A report by Hirshfeld et al. tested the biodyne product on skin grafts on humans and experimental animal models. No evidence was presented to support the wound healing function. However, Goodson et al. reported that a proprietary component of the Sperti hemorrhagic product enhanced several aspects of wound healing (e.g., collagen synthesis in human skin in vitro, oxygen consumption in human fibroblasts, new tissue formation in wounded skin in rat models, and wound healing in the ear of the rabbit). The skin respiratory factor (SRF) was typically tested at two dose levels. This study showed that extracts of SRF stimulated oxygen utilization by human fibroblasts and collagen synthesis in human skin specimens. Further, the whole Preparation H product (Sperti’s biodyne product) enhanced the epithelization of moist open wounds. The authors stated that they had been encouraged by a Food and Drug Administration review panel to undertake this study. Despite strong skepticism at the start of the experimentation, these authors indicated that the overall findings supported further research on the wound healing potential of this commercial product. The degree of stimulation was in the 20–40% range with statistical significance typically being showed.

Of particular interest to the present assessment were reports that different concentrations of the same cellular extract can produce opposite effects on yeast and bacterial growth. The biphasic dose response was not the focus of their research, but the results were so striking as to gain considerable emphasis in their evaluations. The most striking biphasic dose response was seen when the effects of Staphylococcus aureus filtrate were reassessed for its effects on the growth of yeast. The magnitude of the stimulatory response was 100% greater than the control, while the width of the stimulation was somewhat greater than fivefold (Figure 2).

Carcinogens and wound healing

The regenerative capacity of the epidermis displays considerable interspecies variability, much of which seems to depend
upon the number of cells in the original epithelial layer. Within this context, the rat and pigeon display much slower wound healing rates than the guinea pig. Silberberg and Silberberg \cite{65} related this to observations that the guinea pig epidermis has more cells per area than either of these two less capable species. Based on such interspecies differences, Silberberg and Silberberg \cite{65} hypothesized that hyperplastic skin within the same species may heal more quickly than normal skin. Because it is well known that certain carcinogens may induce epithelial hyperplasia, they undertook an assessment of the capacity of such hydrocarbons to accelerate wound healing in the mouse model.

Sixty-six C57 BL mice (6- to 8-week old) were divided into three groups, an unexposed control, a vehicle control treated with benzene, and the treatment group administered 3,4-benpyrene (BP) dissolved in benzene. The shaved mice were administered the chemical treatment three times per week for periods of 2 weeks, 1, 2, or 3 months, respectively. Following each of these time periods, the investigators cut a circular piece of skin and subcutaneous tissue (3 mm in diameter). Regeneration was followed over 3, 5, 8, and 10 days. The authors measured a number of histological end points, centering on epithelial outgrowths, tissue thickness, and mitoses. The benzene vehicle-treated mice displayed accelerated wound repair. After 10 days, the site was difficult to recognize in the treated mice due to the complete healing, whereas a small deficit with a scab remained in the control group. In the 3,4-BP-treated mice wound repair was also accelerated after 2 weeks and occasionally after 1 month of treatment. However, following 3 months of treatment with 3,4-BP, the closing of the wound was delayed by as much as 10 days. \cite{65} Histological evaluation revealed that the chemical treatments enhanced cell migration and cell proliferation. In the case of the benzene vehicle, the repair processes peaked at 1 month, while wound repair remained at this elevated level. In the case of the 3,4-BP, a clear biphasic duration (dose) response occurred.

According to the authors, benzene accelerated growth processes and wound repair; this appeared to occur with a distinct maxima or ceiling point. \cite{65} This stimulation was observed not only in the excised tissue location but also at a distance from the excised area. The maximum stimulation in the 3,4-BP-treated group was similar to that seen with the benzene vehicle controls. The authors speculated that this stimulation might be due, at least in part, to the presence of the benzene solvent. Finally, it should be noted that the 3,4-BP-treated mice developed four papillomas; one of these developed into a carcinoma, while one mouse developed a carcinoma without a prior papilloma.

**Cartilage and wound healing**

That cartilage could enhance the occurrence of wound healing was a principal focus of Professor John Prudden from the mid-1950s to the early part of the 1970s. Based on extensive research on the topic, he came to believe that cartilage had the capacity to accelerate the healing of a variety of wounds including surgical incisions as well as broad skin cuts and abrasions in normal and diabetic individuals. His interest in this topic grew out of the observations of colleagues at the Columbia University Medical School that cartilage treatment could prevent the well-known capacity of cortisone to inhibit normal healing processes in male Wistar rats. \cite{70,71} Prudden \cite{23} followed up with experiments which showed that bovine cartilage (but not bone flour, methionine, talcum, or gelatin) was able to increase the tensile strength of a 2-in. incision wound in female albino Sherman rats some 7 days after the incision and stitching. Why Prudden decided to use a different strain of rats and gender than the earlier studies of Lattes et al. was never mentioned. He was insistent that the best measure of wound healing was tensile strength, which he determined via the insertion of a condom into the peritoneum and pumping it until it pushed through the repaired wound. The initial increase in tensile strength was approximately 20%, and this occurred by about day 7 in the wound healing process. This tensile strength differential was maintained throughout the remainder of the experiment. While the statistical analysis strongly supported his conclusion of an increase in wound healing, the control population data had a tensile strength range of 6.3-fold (30–192 mmHg), while the treated rat group had a range of 3.6-fold (60–222 mmHg). Such variability would continue to present challenges in so far as it would affect sample size in order to have sufficient statistical power. Prudden replicated and extended these initial findings to include multiple species such as mice, guinea pig, and dogs, using different routes of exposure and using complementary biochemical indices, normal animals, and those with chemically induced diabetes. In addition to this broad range of experimental evaluations were time-related treatments, which included administering the cartilage prior to the surgical procedure, on the day of the incision, and on subsequent days after the incision. In these cases, administration prior to the surgery or soon after it resulted in the acceleration of healing. However, treatment 4–5 days after the surgery resulted in a decrease in tensile strength.

While most of the papers published concerned the capacity of cartilage to enhance wound healing in animal models, some data were presented on human responses as well. For example, Prudden and Allen \cite{70} noted studies in which the cartilage material was administered to the surface of more than 60 patients with indolent open wounds. In all but one case they observed the successful occurrence of granulation. These wounds were of a chronic nature, with none less than 2 months old with some ranging up to 7 years. According to
the authors, these clinical cases were convincing demonstrations of the material’s potential efficacy. To complement this uncontrolled set of case study examples, Prudden and Allen\textsuperscript{74} reported on a study with 15 human volunteers serving as their own control in a dermal incision experiment. In this study, two nearly 2-in. incisions were made on each volunteer with one wound receiving the cartilage treatment at the time of the incision. After 7 days, the incised wound was removed via an elliptical cut and tested for tensile strength. The cartilage-treated wounds displayed an average increase in tensile strength of 42\% over the control group. In all the testing, the amount of administered product was 25–30 mg. No data were presented that were designed to formally assess a dose optimisation question nor information on how the dose of 25–30 mg per animal was initially selected.

Of concern to Prudden et al.\textsuperscript{75} was whether the modest increase of 20\% would be enough to generate clinical interest. He cited Dunphy\textsuperscript{76} who noted that the 20\% increase in tensile strength at 7 days was only a small increase in terms of the ultimate strength of the wound and therefore of no practical consequence. Prudden tried to counter these comments by first noting that this perspective neglects the key point that (at least in his opinion) this is the first instance in which an agent had conclusively been shown to accelerate the healing of normal wounds. Second, he noted that it may be possible to increase the rate of healing once the mechanism can be determined.

### Multiple chemicals

**Chemicals assessed for their capacity to accelerate wound healing from approximately 1930–1950**

Prior to the 1960s, efforts were made to assess whether healing could be accelerated by the use of SH compounds as well as mammalian tissue extracts (sheep/calf embryo, adult sheep heart, and skin). These were used in both animal models and human clinical studies (i.e., case studies). Research was also directed to assess the concept of wound hormones/wound fluid and their possible application to healing. X-rays were also used from the 1920s to the 1950s to treat a broad range of infectious conditions, some of which included dermal wounding. Finally, other investigators selected a broad range of commonly available agents, many of which had been used in the treatment of burns, especially in industrial settings\textsuperscript{77} to be tested within an experimental framework\textsuperscript{78,79} using guinea pigs\textsuperscript{80,82} or rats.\textsuperscript{83} The present section addresses such experimental evaluations.

In the two most widely cited human case studies that surveyed chemical wound healing capacity, neither paper concluded that a locally applied agent accelerated the healing process. In the Smelo\textsuperscript{78} paper, healing was evaluated in 16 human subjects for numerous agents by comparison with healing rate predictions given by the mathematical formula du Nouy\textsuperscript{78} made that was based on a large number of carefully measured human wounds of various sizes. The formula was employed in lieu of a traditional control group, providing a standard reference of comparison. Despite the fact that the Smelo\textsuperscript{78} paper was well regarded in the 1930–1940s, it was incapable of assessing the issue of healing acceleration due to inadequate sample size, methodological confounding, and the limitations of the healing time estimations of the du Nouy method. For example, most agents were assessed on five or fewer subjects, while most subjects were administered two or more treatments. Because these treatments were often in sequence, it is not possible to address possible crossover effects either positive or negative. The only agent of potential interest was merthiolate ointment (1 : 2,000) where there was suggestive evidence of more rapid healing.

The study of Anderson\textsuperscript{80} was similarly unable to assess the hypothesis of a treatment related accelerated healing effect. Of some 32 clinical cases, 20 were assessed for accelerated healing. The other 12 cases were dropped because of factors that impeded the healing processes. Of the 20 cases, 14 were considered to have healed at a normal rate based on the du Nouy formula. A significant problem with this study was that multiple treatments were typically administered to patients making it impossible to judge possible treatment effects.

In the case of animal studies, Bruschi et al. employed the guinea pig model in five publications (Table 2), all of which followed the same methodology of Carrel and Hartman.\textsuperscript{84} More specifically, in their experimental studies, each agent was tested on 12 guinea pigs with abdominal bilateral cutaneous wounds of equal size. One wound served for the treatment, the other as the control response. Once the wounds were made, they were traced on stencil cellophane and the test substance was placed on the wound site. The wounds were dressed every 2 days. At this time, more test substance was applied and additional wound area tracings were made. The area outlined by the tracings was quantified. The end point was the days required for healing. In their 1942, 1946, and 1949 studies,\textsuperscript{80,82} 22 test substances were evaluated (Table 3). The authors concluded that none of the agents consistently enhanced wound healing. The only candidate agent that provided suggestive evidence was chlorophyll ointment. In fact, chlorophyll became the object of a follow-up wound healing study.\textsuperscript{85} Once again, the experimental findings revealed a modest enhancement of wound healing. For example, 8 of 23 (39.1\%) cases displayed at least 2-day acceleration for healing. Four of the eight had ≥4-day acceleration (20–40\% acceleration rate). It should be noted that for 16 of 22 substances healing was ostensibly delayed. This suggests that the doses selected for treatment were too high, possibly being slightly toxic.

These studies by Brush and Lam can be criticized because they used only one dose, having no follow-up experiments when the findings were to delay healing. The use of the animal as its own control is also potentially problematic especially if the treatment effect is more systemic than local, a point illustrated by Auerbach and Dolijanski.\textsuperscript{77}

Williams and Bissell\textsuperscript{83} conducted four experiments in male and female rats to assess whether a broad range of vitamins and essential oils enhanced the dermal wound healing process. The experimental protocols were considerably different than those of Brush et al. In Williams and Bissell’s\textsuperscript{83} experiments, there were three wounds of progressively greater depth, each across the back. The chemicals were applied each day. Histological assessments were conducted at days 4, 12, and 16 for the progressively deeper wounds. The vitamins were administered with sesame oil to delay the dermal absorption in their first experiment. Other experiments used water as the solvent, whereas the subcutaneous (SC) route of administration was used in the female rat study, which also tested the same vitamin set as in experiment #1. Furthermore, in the final experiment, all wounds were of a similar size/...
The sample size was six control rats and three rats for each chemical agent. Despite all the experimental permutations, the authors did not report any treatment effect.

One agent that displayed evidence of enhancing dermal wound healing was 2,4-DNP. This agent, a metabolic stimulant, was tested for its wound healing ability as thyroid extracts had proven capable of enhancing healing rates. In the Barclay et al. study, doses of 2,4-DNP were administered (0.12 and 0.9 gm) to young adult male Albino rats. Each rat received two circular wounds on the outer thighs, with one wound serving as the treatment, the other as the control. There were two low-dose groups (both at 0.12 gm/rat) and a single high-dose group (0.9 gm/rat). The low-dose groups displayed accelerated healing by 15–27%, while the high-dose group showed no treatment effect. Unfortunately, their study has never been followed up.

Oxalic acid has also been claimed to accelerate wound healing. This was achieved initially in an animal model with noninfected wounds and also in combination with sulfathiazole where the healing time was reduced by 20%. Dube et al. attempted to confirm the animal study findings by using human subjects who were skin donors in grafting procedures. Of eight subjects in one study, four had treatment and four had control wounds. The sulfathiazole—oxalic acid treatment resulted in a several day faster healing time than just the antibiotic alone. The authors also claimed an accelerated

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**Table 2. Chemical wound healing survey—study methods**

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Animal model</th>
<th>Age</th>
<th>Gender</th>
<th>Total # Control animals</th>
<th>Controls</th>
<th>Treatment</th>
<th>Measurement frequency</th>
<th>Duration</th>
<th>End point</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 different agents</td>
<td>Guinea pig</td>
<td>N/A</td>
<td>N/A</td>
<td>12/exp</td>
<td>Y</td>
<td>Each animal served as own control</td>
<td>Every 2 days, new treatment application</td>
<td>Until healed (16–18 days)</td>
<td>Wound size</td>
<td>Same as above</td>
</tr>
<tr>
<td>Mostly vitamins</td>
<td>Rat (exp #1)</td>
<td>Mostly male</td>
<td>45</td>
<td>6 controls</td>
<td>Y</td>
<td>6 controls</td>
<td>Once per type of wound</td>
<td>4, 12, 16 days</td>
<td>Treatment dissolved in Wound size, histology, thickness, mitosis, epithelialization</td>
<td></td>
</tr>
<tr>
<td>Same chemicals</td>
<td>Rat (exp #2)</td>
<td>Mostly male</td>
<td>48</td>
<td>6 controls</td>
<td>Y</td>
<td>6 controls</td>
<td>Every 2 days</td>
<td>0.05 cc SC new wounds</td>
<td>Same as above</td>
<td>83</td>
</tr>
<tr>
<td>Peanut oil, olive oil, cottonseed oil, glycerin, sesame oil</td>
<td>Rat (exp #3)</td>
<td>Mostly male</td>
<td>87</td>
<td>9 controls</td>
<td>Y</td>
<td>9 controls</td>
<td>Once per type of wound</td>
<td>10 days, 17 days</td>
<td>Same as above</td>
<td>83</td>
</tr>
</tbody>
</table>

**Table 3. Agents tested for healing acceleration**

<table>
<thead>
<tr>
<th>Agents</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petrolatum gauze</td>
<td>Smelo, 1936</td>
</tr>
<tr>
<td>Physiological saline</td>
<td>Anderson, 1938</td>
</tr>
<tr>
<td>Dry gauze dressing</td>
<td>Agents</td>
</tr>
<tr>
<td>Alcohol dressing</td>
<td>Dry gauze dressing</td>
</tr>
<tr>
<td>Iodoform gauze</td>
<td>Moist saline dressing</td>
</tr>
<tr>
<td>Merthiolate ointment (1 : 2,000)</td>
<td>Azachloramide</td>
</tr>
<tr>
<td>Merthiolate solution (1 : 1,000)</td>
<td>Merthiolate</td>
</tr>
<tr>
<td>Mercurochrome</td>
<td>Katadyne silver</td>
</tr>
<tr>
<td>Zinc peroxide paste</td>
<td>Dakin’s solution</td>
</tr>
<tr>
<td>Zinc oxide paste boot</td>
<td>Zinc peroxide cream</td>
</tr>
<tr>
<td>Thiocresol in solution</td>
<td></td>
</tr>
<tr>
<td>Thiocresol in ointment</td>
<td></td>
</tr>
<tr>
<td>Irradiated petrolatum</td>
<td></td>
</tr>
<tr>
<td>Allentoin</td>
<td></td>
</tr>
<tr>
<td>Peruvian balsam</td>
<td></td>
</tr>
<tr>
<td>UV</td>
<td></td>
</tr>
<tr>
<td>UV and petrolatum gauze</td>
<td></td>
</tr>
<tr>
<td>UV and merthiolate ointment</td>
<td></td>
</tr>
</tbody>
</table>

**2,4-dinitrophenol (DNP)**

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healing as compared with control values in other subjects, although this attempt was less convincing.

Radiation

The effects of ionizing radiation (e.g., x-rays, gamma rays) on wound healing has been explored by a number of investigators. In these investigations, wound healing was reported as being accelerated in several animal models (e.g., rats, dogs, rabbits) and humans. However, several studies have revealed that radiation treatments only inhibited the rate of wound healing. These findings lead Nathanson to suggest that the differences in response may be accounted for by the differences in total dosage administered to the wounds. Nathanson noted that the data suggested that at low to moderate doses healing was enhanced, whereas at higher doses it was depressed. In follow-up studies, Nathanson induced wounds in 15 dogs and treated them with differing doses of gamma rays. In general, he observed an enhanced healing in those with exposure in the low to moderate dose range immediately after the incision. Retardation of healing occurred in wounds treated with high doses of radiation. Nathanson noted that the observations of enhanced wound healing followed the predictions of the Arndt–Schulz Law. The most impressive assessment of the effects of x-rays on wound healing was published by Dobbs. In his study, 409 adult male albino rats were exposed to three doses of x-rays with interim sacrificing over 8 days (5, 10, 15, 20, 30, 40, 50, and 80 days). Each rat served as its own control. Two ventral skin incisions (4 cm) were made on each rat. Tensile strength was enhanced in a statistically significant manner at the 300 r (3.0 G) dose, while it was decreased at the higher doses. The enhancement of tensile strength became apparent starting after 15 days and continued throughout the remaining period (80 days) of the investigation (Figure 3).

BIPHASIC DOSE RESPONSES (HORMESIS) AND WOUND HEALING

Introduction

During the early decades of wound healing research, much emphasis was placed on how this process would be accelerated especially via the application of various tissue extracts, sulphydryl compounds, vitamins, x-rays, or any of a large number of chemical agents. Despite the prolonged interest in chemically induced acceleration of wound healing, the overwhelming number of experiments conducted used a single dose, without providing a documented justification for dose selection. Even though the concept of dose response did not have a significant impact on the early development of the wound healing field, the hormetic-biphasic dose response was reported in six different areas: embryo extracts, skin extracts, wound hormones, blood plasma concentrations, radiation, and chemical carcinogens. In each of the six cases, the authors acknowledged the biphasic dose response. However, despite the discussion of the biphasic dose response, only the discussion with the skin tissue extract and radiation related these observations to a broader range of similar reports in the literature. For example, in the case of radiation, Nathanson related his findings back to the Arndt–Schulz Law. The six areas of hormetic-biphasic dose responses are described in the following.

Plasma tonicity gradients and tissue stimulation

In 1911 Carrel, along with his colleague Montrose Burrows, reported on the effects of alterations in the tonicity of plasma on the growth of chick fetal and adult spleen tissue. A slight dilution of the plasma enhanced the growth of the spleen tissue (Figure 1). They also observed that in diluted plasma a similar enhancement of the growth of the skin, the heart, and the liver of chickens. This was also the case for frog skin that grew more in the diluted plasma. The optimal dilution varied according to the tissue and the animal model. Carrel and Burrows suggested that knowledge of the optimal concentration could lead to the discovery of mechanisms that regulate the development of the organs and how those mechanisms facilitate the overall morphological plan of the organism.

Tissue extract-induced growth stimulation

Parker reported that fibroblasts from subcutaneous connective tissue, cross-striated muscles, and periosteum respond to increased concentrations of growth-inducing substances by proceeding through a growth optimum, then followed by a decrease in growth as the concentration increases. Fischer suggested that if a substance therefore inhibits growth it might be because the concentration is too high. This suggested the need to conduct additional experiments with several dilutions.

Wound hormones

Hormetic dose responses were reported by Loofbourou and Dwyer and Loofbourou and Morgan in research concerning wound hormones. Growth media that were collected from microorganisms that had been slightly damaged by various types of chemical, physical, and mechanical stressors enhanced the growth of undamaged cells and did so with a biphasic dose response. This biphasic dose-response phenomenon occurred in a range of experimental situations. For example, a nonirradiated mixture filtrate collected from damaged yeast cells enhanced the growth of Aerobacter aerogenes and Escherichia coli at low concentrations but was...
A similar biphasic dose response was reported concerning the effect of \textit{S. aureus} filtrate from damaged cells on healthy cells for the same species. Figure 2 reveals a biphasic dose response of the concentrate “A” from the irradiated \textit{S. aureus} filtrate on the growth of undamaged \textit{S. aureus}. The increased growth was about twice that of the control group with the stimulatory dose extending over about 10-fold range (Figure 2), a response consistent with the quantitative features of a hormetic dose response.

**Skin extract and hormesis**

Teir et al.\textsuperscript{33} reported that IP injections of extracts of finely ground skin of newborn rats caused an increase in the mitotic frequency in the epithelium of the skin of 6-week-old rats. The low concentrations of the extract enhanced the mitotic frequency, while higher doses were not effective. The authors noted that failure to achieve wound healing with tissue may be related to issues of dose, suggesting that at low doses a stimulatory response is typically seen, whereas at higher concentrations inhibition usually prevails.\textsuperscript{35}

**Chemical carcinogens and wound healing**

In the 1940s, Silberberg and Silberberg\textsuperscript{65,66} and Silberberg et al.\textsuperscript{69} proposed that wound healing may be enhanced by exposure to chemical carcinogens that act via the induction of hyperplasia, whether or not the treatment eventually lead to the development of a tumor. This hypothesis was tested in several detailed experimental studies with mice and carcinogenic hydrocarbons. The findings were striking in that there was a clear enhancement of the healing process and that the results displayed a biphasic dose response, supporting their hypothesis. While this finding provided a type of proof concept no follow-up research has since been published, most likely because of the further risk of developing chemically induced skin cancer.

**Radiation**

As noted in the radiation section, Dobbs\textsuperscript{93} demonstrated an x-ray induced biphasic dose response for tensile strength, a finding consistently showed over five time periods.

**DISCUSSION**

The present assessment revealed that attempts to accelerate wound healing have had a long history, starting with Carrel in the first decades of the 20th century. However, in order to evaluate specific hypotheses in this area, it was also necessary to develop bioassay systems, especially tissue and cell culture techniques, methods, and biological models. It was also important to develop, identify, and evaluate in vivo biological models and experimental protocols. The history of wound healing therefore must be seen within a process that was highly dependent on the progress of related but independent biological subdisciplines such as the nutritional sciences as well.\textsuperscript{105,106} The history of wound healing also involves the process of clinical evaluation of large numbers of candidate agents and published via the use of numerous case studies.

With this interdisciplinary framework for assessing how the wound healing process could be enhanced, dose-response concepts also emerged, including the hormetic-biphasic dose response.\textsuperscript{100,101,107–109} The concept of wound healing following an hormetic-like biphasic dose response was reported broadly in the first half of the 20th century. Such a dose response was first reported in 1911 by Carrel and Burrows who assessed the effects of altered blood plasma tonicity on the growth of multiple tissues from multiple species. These findings suggested that the biphasic dose response was general and integrated into species developmental and growth processes. These findings were extended by a colleague of Carrel, Parker,\textsuperscript{102} who reported that tissue extracts could enhance the proliferation of fibroblasts with the process following a biphasic dose-response relationship. These findings provided a scientific framework for later investigations by Teir et al.\textsuperscript{33–35} concerning skin tissue extracts and the findings of Loofbourow and Dwyer\textsuperscript{55} and Loofbourow and Morgan\textsuperscript{98} that focused on the release of wound hormones from slightly damaged bacteria and yeasts and their biphasic effects on undamaged microorganisms. While x-irradiation was widely used in the treatment of inflammatory disease in the middle decades of the 20th century within the US in the treatment of gas gangrene\textsuperscript{10} and other inflammatory conditions,\textsuperscript{111} it was explored only in a preliminary fashion at the same time with respect to wound healing. A number of publications indicated that low doses of x-rays and gamma rays appeared to enhance the wound healing process in several animal models and humans.\textsuperscript{93} However, as in the case of Parker noted previously, the capacity to enhance healing was a function of dose, with low doses enhancing the healing process, while higher doses affected an opposite response. A final area of research in which hormetic responses occurred with wound healing emerged out of studies concerned with the effect of chemical carcinogens. In general, it was shown that agents, such as chemical carcinogens, which can induce hyperplasia in the skin, can also significantly increase the wound healing process.\textsuperscript{95}

Despite the broad and consistent findings, these observations were not central to the field of wound healing during the first half of the 20th century. If it were, then, there would be evidence in the nature of the study design. In fact, in the section on diverse chemicals being tested for the capacity to enhance wound healing they were all essentially single dose experiments. The area where low doses were emphasized and central was in the area of x-ray induced therapeutic effects.\textsuperscript{112,113} The general theme of an extensive clinical literature was that the beneficial effects of the x-ray treatments were seen at lower rather than high doses. Low doses by these investigators were considered a fraction of the erythema dose (600 r). The fraction recommended varied by author, disease, and severity of the disease. However, the range of recommended doses was from 0.05 to 0.8 of the erythema dose. While the authors clearly emphasized the need to use low doses, there were no experimental findings presented which supported this highly consistent conclusion. Despite these limitations, the historical foundations of wound healing, especially within the context of healing acceleration, provided a type of historical “preconditioning” that has provided a strong experimental foundation to assess the potential for wound healing to be accelerated and its mechanistic foundation.
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REFERENCES

Historical foundations of wound healing


**Supporting Information**

Additional supporting information may be found in the online version of this article.

**Appendix S1.** Effect of heart extract preparation (HEP) on soldiers in World War II (Source: Kerr and Werner, 1944).41