RECENT STUDIES IN WOUND HEALING

EDWARD L. HOWES AND SAMUEL C. HARVEY

The proliferative response in a healing wound to a constant amount of injury offers an opportunity to study the factors that influence the velocity of growth of the injured cells. An attempt will be made to review here some of the studies on sterile wounds with reference to those factors which have been found to influence cellular proliferation, and to present the substantiating investigations on tissue cultures. The literature on the subject may be divided into a description of methods for mensurating the tissues regenerated; studies in the physiology of cells entering into the process of repair; and, lastly, the factors themselves that directly affect the regeneration of these cells.

Mensuration. The quantitative studies in wound healing have largely been carried out on skin wounds where their areas could be traced on a sterile sheet of cellophane. The measurements are then transferred to bond paper, and these areas measured by the planimeter or weighed after the method of Douglas [12], a method which was developed in this laboratory. Carrel [4, 5], using the planimeter method, mensurated the sterile skin wound both on the experimental animal and on man, and was able to construct a graph showing the diminution in area during the process of repair. This curve, Chart I, was divided into four distinct periods:

(1) A quiescent or latent period which lasts for the first four or five days and during which time the wound remains practically the same size.

(2) A period of contraction which interrupts the end of the latent period, and in which the rate of contraction is proportional to the size of the wound, i.e., the greatest contraction occurs in the larger wounds.

(3) A period of epidermization during which the epithelium begins to regenerate. The process may be continued by epidermization alone or by epidermization and contraction together.

(4) A cicatricial period during which the scar spreads and wherein pigmentation takes place.

In general, the rate of repair was found to be greatest at the beginning of the healing period and gradually diminished as the

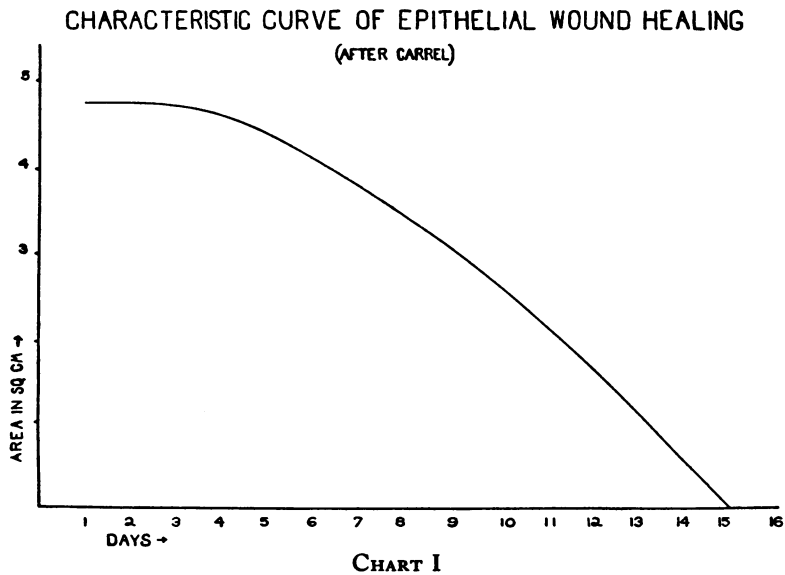

CHART I

end of the process was approached. If an infection intervened in any of the periods, the tendency was to prolong that period and the subsequent ones, unless the tissue developed sufficient resistance to overcome the invasion before the next period was reached. Du Noüy [36], evolved a mathematical expression for this curve of Carrel's, and noted [37] a difference of rate depending on the age of the individual; the elderly individual had a slower rate than the younger.

Another method of mensurating the wound has been to cut two wounds of similar size on the same portion of the animal, and to use one for the experimental wound and the other for purposes of comparison as a control.

Harvey, Sooy and Howes[25] have attempted to mensurate the process of repair in both sutured internal and external wounds by measuring the return of tensile strength. (Chart II) The sutures employed disappeared in a minimal length of time. This tensile strength they have described as being proportionate to the process of fibroplasia. This method has the advantage of measuring the velocity of growth of only one type of cell, the fibroblast, while the skin method of mensuration previously described must deal

with two factors: (*1*) contraction, and (*2*) growth of two kinds of tissue—the fibroblast and the epithelial cell.

Physiology of Cells. Investigations into the function of cells entering into the process of epithelial healing were conducted by Kate Spain and Leo Loeb[46]. They found that both the outgrowing tongues of epithelium and contraction were concerned in the closing of the defect, and that the rates of growth and contraction were greater in the larger wounds. Contraction started on the fourth or fifth day and continued with diminished intensity as the healing advanced. This finding was identical with that of Carrel. The

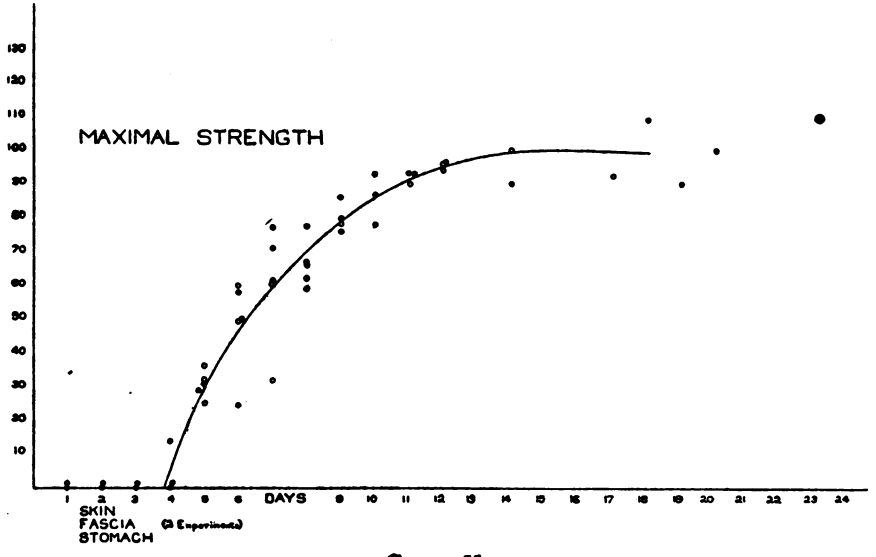

CHART II

formation and size of the epithelial tongues depended on (*a*) an amoeboid immigration, (*b*) an increase in the size of the cells, and (*c*) cell division. The amoeboid movements leading to the closure started in the old epithelium and extended toward the wound. These movements, Spain and Loeb believed, were the primary process in the healing of epithelium, and they were carried out with greater velocity in the case of the larger wound. They postulated that this initial motion of cells towards the center of the wound afforded a "pull" which brought forth mitotic cell division,

and that as soon as the cells filled in the defect and were crowded together the pressure exerted by the proximity of the cells on each other led to a diminution of mitotic proliferation. Soon after the wound was made, the epithelial cells and their nuclei increased in size. The mitotic figures occurred first and became more numerous in the old epithelium, but later the greater number of mitoses was seen in the new tongues. As the excised area diminished, there was a decrease of mitoses, both in the amoeboid cells and in the old epithelium. At the closure of the wound, there was an increase in the number of rows of cells over the defect, and both the cells and their nuclei again decreased in size during this crowding.

A dissimilar theory in regard to the initiation of proliferation is expressed by Burrows[3]. Because his ordinary tissue cultures of epithelium failed to grow when they were continuously washed with nutrient media, he postulated that stagnation and crowding lead to an accumulation of certain soluble blood products which stimulated the synthesis and migration of the cells. These same epithelial cultures grew when they were allowed to stand in the media. Akaiwa[1], working with rats, emphasized the importance of the surface over which the epithelium moved and that the character of this surface depended on fibroplasia. The epithelium moved more quickly over the shallow wounds. He stressed, also, the retraction of the edges immediately after the cutting. Hartwell[21], is still working on the rôle of the epithelial cell in wound healing, and has called attention to the function of the prickle cell layer in the process of repair.

Differing from the concept of mitotic proliferation of fibroblasts from the adult fibrous tissue is the work of Baitsell[2]. In experimental wounds made by removing various-sized pieces of skin from the frog there was a rapid coagulation of the blood plasma and lymph to form a coagulum which filled the wound cavity. This coagulum became more and more resistant and was generally of sufficient strength to hold the cut edges of the wound in place and to retain its position in the cavity. It served, at least temporarily, as a connective tissue and as a base for the epithelial cells which rapidly moved in from all the cut edges and covered the wound. The study of the prepared section of wound tissue showed this coagulum to be composed of a typical fibrin network. Later this fibrin network was transformed into a new fibrous tissue con-

taining bundles of wavy fibers in which, in many instances, individual fibrils could be noticed. The cells which later moved into the new fibrous tissue in large numbers from the surrounding areas did not digest the fibers, but apparently by their movements caused a division of the large bundles into smaller ones. These cells, when they first appeared in the fibrous tissue, were rounded, but later they assumed the typical elongated, spindle shape of the fibroblast. The staining reactions of the new fibrous tissue appeared to be identical with the staining reaction of the connective tissue in the frog's skin.

The reaction of the endothelium in wound healing has been investigated by Foote[15]. He found that the proliferation of the capillary endothelium in the aseptic wound results in the production of new vessels, phagocytic endotheliocytes, and of cells which appear to take on the function of fibroblasts. Cellular proliferation is almost equally as abundant in or near the vessels as it is in the free cells some distance from them. He, as well as other investigators, have conceived of the origin of the fibroblasts in a different light than they considered the origin of the epithelial cells. They believed that the fibroblast may be derived from wandering cells which take on a new function in filling the wound. Carrel and Ebeling[9], working with tissue cultures, have noted the transformation of monocytes into fibroblasts, and of fibroblasts into clasmatocytes. Maximov[30], has also observed that round endothelial cells tended to elongate and to take on the appearance of fibroblasts when they became sessile in connective tissue.

The question, then, as to whether the proliferation of fibroblasts takes place from pre-existing fibrous tissue or from a "fill" of undifferentiated monocytes, or both, is still undecided. Nevertheless, whatever their origin, the curve of the manifestation of their tensile strength is a true growth curve as has been pointed out by Harvey[22].

The Effect of Metabolism on Healing. Clark[10], working with Carrel's method and Du Noüy's formula, studied the effect of diets on the various periods of healing. He cut large and small geometric wounds upon the backs of dogs. His high protein diet consisted of meat and liver; the high carbohydrate diet of bread; the high fat of lard or butter; and the mixed diet of a combina-

tion of these ingredients. He found that there was no latent period on the high protein diet, but that the period was prolonged for six days on the high fat diet, while the carbohydrate and the mixed diets were intermediary, with a latent period of four days for the mixed and three days for the carbohydrate. The diets had no effect on the periods of contraction or epidermization, but they did influence the total length of time required for healing in the same proportion as they affected the latent periods. It is unfor-

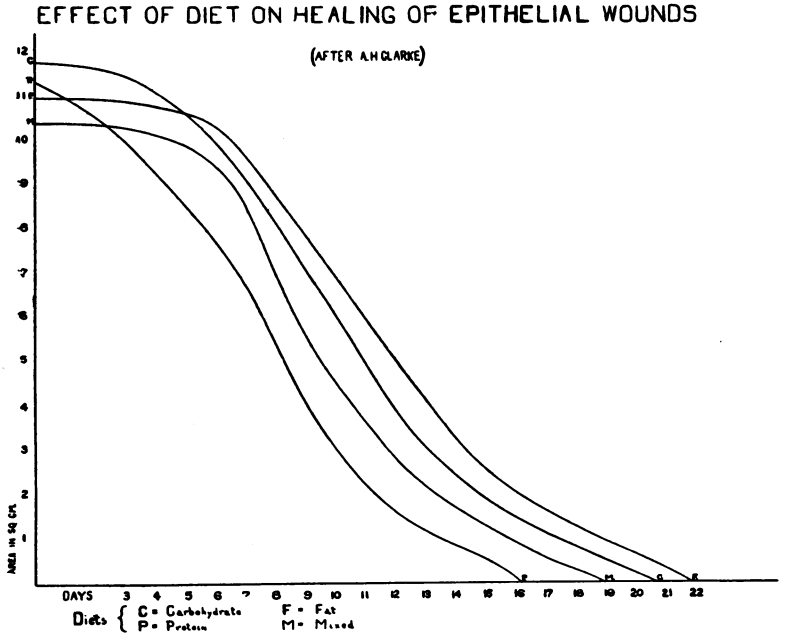

CHART III

tunate, however, that he used liver in his diets because of the pronounced effect that this substance has since been found to exert on the hemopoietic system. Carrel[6] has delayed the initiation of contraction and epidermization as long as twenty days by protecting the skin wound from "external irritation" with a sheet of fascia sutured over it. In this connection it must be remembered that Koontz[27, 28] and many others have demonstrated that fascia is an easily transplantable tissue and that it will grow on any matrix.

Buried transplants months after implantation have, both grossly and microscopically, the architecture of the original tissue, save for the ingrowth of blood vessels into them. Carrel has also shortened the latent period by adding such an external irritant as turpentine.

The factor of external stimulation is largely avoided in internal wounds. Howes and Harvey[24], using synthetic diets modified after Moïse and Smith, tested the tensile strength of sutured stomach wounds and found that there was a latent period of four days before tensile strength was manifested either on a mixed diet or a high protein diet. However, the rate of increase of the tensile strength was greater with the high protein diet and a maximum strength was reached two days sooner than was obtained with the standard diet.

The fact that proteins stimulate cellular proliferation and that fat inhibits has been demonstrated by many investigators in other ways. Carrel and Baker[8] found that the growth-promoting substances for the multiplication of the fibroblasts in tissue cultures were the proteins or the higher split-products, such as proteoses and peptones, and that the lipoids were growth-inhibiting. Smith and Moïse[45] also demonstrated that a high fat diet inhibited the reparation of liver cells in rats after chloroform necrosis and that a mixed diet gave a slightly better regeneration than a high protein diet. Their diets were adequate, well-constructed, and normal growth was obtained with them. However, they also noted that there was greater damage to the liver on the high protein diet. When they changed the protein, casein, to an inadequate one, gelatin, there was hardly any repair, while if another adequate one was employed, gliadin, good repair was obtained. The stimulating factor, then, was the protein even in the mixed diet. They also obtained hypertrophy of the remaining kidney after unilateral nephrectomy when the animals were placed on a high protein diet[34]. The fact that protein is necessary for growth has long been understood, but that an abundance of protein will lead to a more rapid rate of growth is a comparatively new concept. Mendel and Osborne[31] have but recently obtained a maximum growth curve from rats by feeding them a high protein, high vitamin diet.

Sauerbruch[43] and Herrmannsdorfer[23], reasoning from a clinical study, believed that the secretion of wounds could be lessened and their hydrogen ion concentration lowered by feeding a diet

high in fat, proteins and acid phosphates. The rate of diminution of the wound area was increased by this method.

Morgan[35] has demonstrated that an external source of food is not necessary to maintain the average rate of regeneration in an injured tissue, for the amputated legs of his starved salamanders regenerated as rapidly as those of the well-fed animals. The starved ones had an atrophy of all of the muscles and organs of the body.

Lastly, diseases that affect metabolism also affect the processes of wound healing. The slow rate of healing and the tendency toward infection are notorious in diabetes, and in malnourished infants.

Factors that Influence Healing. The principal factor that delays healing is infection, a subject outside the scope of this review. It is interesting to note, however, that both Carrel[7] and Kiaer[26] have reported retarded healing as due to distant abscesses. Syphilis as an inhibiting factor has been mentioned by Darnell[11] and Milian[33]. Reports of other systemic diseases and circulatory changes as inhibiting factors are numerous.

Factors that increase the rate of healing are manifold. As soon as it was apparent that food factors were stimulating to wound healing and that cells in tissue cultures would increase their rate of growth by changing the various nutrient media, these facts were taken over by the clinicians. Roulet[41], Carrel[6] and Kiaer[26] have all reported increased rates of healing by the appliance of chick embryo juice directly to the wound. They have also used Witte's peptone solution and proteoses with the same result.

Experiments directed at the stimulation of the healing wound by changing the circulatory reaction have been of two general kinds. Ebeling[13] has increased the rate of healing by raising the temperature ten degrees in a thermolabile animal, the alligator. Gianotti[16] has demonstrated a similar result by the use of light therapy. The other changes on the circulation have been effected by cutting the sympathetic nerve branches to the vessels of the injured area, a procedure which has also increased the rate of repair[40].

Believing that a stimulating substance may be obtained from the nuclei of the cells themselves, Hemmett[18, 19, 20] has manufactured thioglucose, a compound containing the SH group, to apply directly to the wound. He cut two wounds of the same size in the

skin of rats. The one of these that he treated with thioglucose showed more epithelium and healthier granulations than did the control which was treated with plain glucose. With this compound he has also obtained increased proliferation of the cells of plant roots, and has stimulated the epithelium of sluggish ulcers of the leg to grow.

The Italians have been interested in applying the pulp of ductless glands directly to external wounds. Pozzali[38], Siciliani[44], and Merlini[32] have all accelerated the rate of repair by employing testicular, ovarian, and thyroid tissues.

Haberland[17] obtained rapid proliferation of epithelium of skin grafts with Roentgen ray exposure.

Increased rates of healing have been obtained by Fontaine[14] and also by Leupold[29], by modifying the hydrogen ion concentration of surface wounds to a lower level. Rhodes[39] has shown that necrotic material has a lower pH than has living tissue.

It is difficult to evaluate the true efficacy of many of these substances which have been applied directly to the external wound. For, as has been mentioned previously, Carrel[6] increased the rate of healing by the application of turpentine. He believed that external irritation initiated regeneration. The fact that Ruth[42] stimulated the healing of wounds in frogs by injecting distilled water about the injured area supports this theory.

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