STUDIES ON WOUND HEALING, WITH SPECIAL REFERENCE TO EPITHELIAL HYPERPLASIA AND ITS RÔLE IN THE PROCESS

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We have been interested in the healing of simple cutaneous wounds for a considerable period of time. Burrows has studied the nature of the healing process in sterile wounds of this type in the tissue culture (1). He and others in the laboratory have studied a large series of simple wounds in the laboratory animal. In some of these experiments precautions were taken in keeping the wound clean or sterile. The great majority of these wounds were made by marking the surface of the skin on the dorsal or ventral surface of the animal by means of a cork borer of one centimeter or less in diameter and removing the skin with sharp curved scissors. Depending upon the nature of the skin folds and its relation to stress and strain the wound would remain round in contour or become somewhat eliptical in outline after the animal became active following the operative procedure. All of these wounds were made with the animal under anaesthesia. In addition to observations and measurements of these wounds in the gross, from day to day, many of them were sectioned and studied microscopically.

In the tissue culture studies Burrows noted that epithelial cells do not separate from each other in the culture while the connective tissue cells separate and disperse. Epithelial membranes appear only from firmly anchored fragments of skin in these cultures. In the embryo, the connective tissue or mesenchymal syncitium underlying the epithelium grows best. In the adult, however, the epithelium grows best; it apparently grows at the expense of the connective tissue. Barta (2) showed that this epithelial growth could be stimulated in the culture by the

addition of embryonic juice. Thus, in any slowly healing wound that is not infected this stimulus added to the wound should accentuate healing in that the rate of epithelial growth would be enhanced thereby. We have tried this in a few clinical cases, the observations of which will be reported at a later date.

In the infected wounds conditions are different. The connective tissue becomes exuberant. The epithelial edges grow over the wound only as the granulation tissue is prevented from formation or increase in accumulation. As the granulation tissue increases the epithelial growth is inhibited and as the formation of granulation tissue is inhibited, the epithelial growth is increased. The difference between the type of healing in the adult and embryonic wound is not due to the peculiarities of the cell that make up the wound and its environs. It is due to differences in the whole area undergoing healing. In the adult the epithelial cells are in a definite layer free from blood vessels and the connective tissue is sparse and vascular. In the embryo the one or two celled layer of epithelium is closely connected with the underlying syncitium. As greater growth occurs in the area of greater cell density and greater stagnation, the areas grow in the above stated proportions.

Most wounds go on to healing, growth ceasing because the blood vessels grow with the fibroblasts. In the ordinary wound the blood supply is never reduced nor the cellular constituents increased to the extent that one may prey on the other without eventual loss to itself. In the malignant ulcer one of the latter conditions has resulted. The epithelium undergoes unrestricted growth in that it has been stimulated to such growth by conditions which do not occur in the simple granulating wound. The blood supply also has been reduced to a state that allows for the accumulation of the growth stimulus to a degree commensurate with unrestricted growth.

It is interesting to note that we have no clinical or histological criteria whereby we can establish definitely whether a wound is a simple one which during its slow healing process shows an unusual amount of hyperplasia. All wounds present a certain

amount of epithelial hyperplasia. A recent communication by White and Weidman (3) has brought up anew the problem of pseudoepitheliomatous hyperplasia and its relationship to precancerous and cancerous lesions. It is of course in the border line case that it is necessary to use all possible knowledge to make a definite decision. This resume prompted me to review our experimental data on wounds, simple ulcers and precancerous lesions, as well as sections from various types of ulcers treated in our clinic during the past few years.

In a recent series of 150 wounds induced by the aforementioned technic in the white rat, I have had opportunity to study the various phases in the period of healing. There are a number of findings which warrant emphasis. The more recent studies by Lane and me (4) on the precancerous hyalinizations induced by X-rays and lipoid solvents, has given us a different basis for the study of precancerous lesions in general. The correlary deduced from these studies has not presented any fallacies in the study of a goodly number of precancerous and cancerous lesions, as well as cutaneous ulcers in man.

The striking factor observed in the healing of a simple wound in the laboratory animal is the tendency of even somewhat linear wounds to acquire a rounded contour. This rounded contour allows more rapid healing because the greatest force in the healing of a non-infected wound at least, is the pull of the epithelial edge from the circumference to the central point of the wound. We have attempted to hinder this process by repeatedly stretching open the wound manually, by making new wounds over the same area and by the application of various agents.

A wound one centimeter in diameter contracts down practically to a point within a period of 7 to 10 days. At this time it can be spread open manually to an average size of 5 millimeters. The edges are held together by delicate threads of fibrin underlying the thin sheet of epithelium, as well as the epithelium itself. With repeated manipulations of this sort complete healing is hindered to some extent.

In a large series of wounds Glenn and I (5) applied various

agents once or twice a day after the initial bleeding had subsided. We used mazola oil, mineral oil, neats foot oil, vaseline, mercurochrome and vaseline with 10 per cent arsenic. Grossly and microscopically our findings were negative insofar as any striking difference between the rate and nature of healing of these wounds and the normal controls. They all healed within 2 weeks time. The wounds treated with vaseline showed a much greater percentage of infection however. One of our purposes in the experiment was to determine depth of penetration of the oil applied to the wound. This was unsuccessful as we could not differentiate between the edema, fat vacuoles and oil droplets in the wound. At any rate the amount of vacuolization in the control series was not strikingly less in degree than in the experimental group.

In all of these wounds there is a definite amount of epithelial hyperplasia, but that per se is no index of the rate of healing. By making new wounds every 10 to 15 days or earlier, we attempted to slow the healing process also and note changes in degree of hyperplasia. This was unsuccessful, because the stretching of the skin toward the center of the wound caused the new wound to occur beyond the bounds of the older wound. Thus, the healing process was the same. Repeated excisions gave the same result. As mentioned earlier in this paper, wounds produced by X-rays and lesions produced by repeated paintings with lipoid solvents gave the most information in regard to the factors taking part in a healing or non-healing process.

There is a certain amount of hyalinization in the wound that goes on to healing. Small blood capillaries become obliterated and the fibrinous exudate becomes hyalinized. In this type of wound the mature fibrous tissue does not undergo hyalinization. Former studies from this laboratory have shown that lipoid solvents induce hyalinization in tissue. They remove the fat soluble fraction, ergusia. This process in this type of wound is a restorative process; the hyperangiomatous tissue is altered to the more normal type with adequate blood supply.

Apparently the repeated application of lipoid solvents to the

surface of these wounds did not alter the healing process because the exudation of fibrin and other tissue juices probably washed away the lipoid solvent from the surface, as well as the depth of the wound. In the animals we used, rats and mice, it was impractical to apply cloth or collodion dressings to the wound. The lipoid solvents inhibited crust formation and probably that too added to the washing away of the solvent by the tissue exudates.

Even though there is a considerable amount of epithelial hyperplasia present in these wounds, with epithelial pegs extending deep down into the depths of the wound, especially in the infected wound, the picture is different from that seen in the wounds or burns produced by X-rays, or more drastic lipoid solvents. In the latter type of wound we may have the same amount of epithelial hyperplasia, but in addition there is marked hyalinization of the mature connective tissue underlying the skin as well as the connective tissue of the corium. Areas of intact cells undergo proliferation in such an area. Burrows has shown that such hyalinized tissue is low in ergusia (6), the lipoids containing the ergusia having been removed, leaving the proteins to precipitate as a hyalin mass. Such areas lower the ergusia content about them also and if areas of intact cells remain they become relatively high in archusia or the growth stimulant as a result and proliferation ensues. Such conditions lead to independently growing systems. This tissue has a stagnant blood supply in that capillaries and small blood vessels have been destroyed in the process of hyalinization. Different from the wound described above, the circulation was only adequate to care for the needs of the local area prior to this destruction. In the simple wound, however, it is the immature fibrous tissue and capillary sprouts that are destroyed. The destruction takes place so that the area may return to its normal circulatory exchange.

Thus, in the atrophic ulcer of long standing malignancy is most liable to follow. Here the circulation does not become increased much, if any, above that of normal, due either to arterio-sclerosis or generalized atrophy of all tissues. Coinci-

dent with hyalinization or loss of ergusia in parts of this tissue, malignancy may result as during healing the normal hyperfunctionate processes have not taken place.

Considerable clinical as well as experimental data must be accumulated before this question is solved. However, these studies emphasize a feature which seems to be an important one. The type of wound produced by repeated doses of X-rays is histologically of the precancerous type. The condition which makes it so is the hyalinization of tissue. The picture is different from that observed in slowly healing wounds, especially when this delay is due to a low grade infection. Thus, these studies indicate that the degree and nature of hyaline change observed about hyperplastic cell nests is an important factor to bear in mind from the standpoint of diagnosis in these "borderline" wounds.

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