OXYGEN AND EPIDERMAL WOUND HEALING

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INTRODUCTION

Healing of cutaneous wounds involves regeneration of surface epidermis and repair of connective tissues by events that are largely independent of one another. If the wound is a shallow one epidermal regeneration precedes repair in the dermis, but if the injury extends the full thickness of the skin epidermal regeneration and growth of granulation tissue takes place concurrently.

In normal undamaged skin the dermis supports the epidermis mechanically and supplies it nourishment. Because the epidermis has no blood supply of its own its need for oxygen, glucose, amino-acid molecules and other metabolites are met from the dermis. These molecules diffuse through the basement membrane, a combined epidermal-dermal structure and travel in the fluid between the epidermal cells to reach the uppermost living ones in the metabolically active granular layer. If this is true for oxygen it is reasonable to expect a gradient of oxygen concentration across the epidermis and such measurements that are available confirm it. Silver (1972) reports oxygen tensions of the order os 20mmHg at the basal layer and 7mmHg at the surface in human forearm skin when the ambient temperature was 20°C.

Normal human epidermis and porcine epidermis have rather low oxygen permeabilities under resting conditions but a measurable fraction of the total respiratory exchange of CO_2 and O_2 takes place across the skin (Fitzgerald, 1957). The thin epidermis of mice, rats, and rabbits is very permeable to oxygen and human epidermis becomes oxygen permeable if the keratin layer is stripped away. Apparently the oxygen barrier is mechanical and resides in the horny layer.

Given that human and porcine epidermis normally derives most of its oxygen from the dermis, what of its situation on a wound surface when the dermal papillary layer with its rich subepidermal rete of blood vessels has been destroyed? Again we are indebted to Silver (1972) for showing that there is little oxygen available on the surface of human shallow skin wounds; measured oxygen tensions were often below 10mmHg. The regenerating epidermis, expending energy in actively moving across the wound surface and in synthetic activities associated with making new cells is confronted with an arid, oxygen depleted, inimical environment. To make matters worse the migrating epidermal cells must compete with hosts of polymorphonuclear cells, macrophage and bacteria for the little oxygen available. Adapting to this altered environment the epidermal cells at the wound margins, those about to move, and this includes cells in the outer root sheathes of hair follicles too, begin to accumulate a store of glycogen. The granules can be demonstrated histochemically. (Bradfield, 1951)

In view of the low oxygen tension on the wound surface and the observation that one of the first reactions to injury exhibited by epidermal cells is storage of the appropriate substrate it can be deduced that epidermal cells, when migrating across a wound surface, usually respire anaerobically. Support for this hypothesis comes from the work of Gibbins (1972) who showed that epidermal cell migration is inhibited by antimetabolites that block anaerobic glycolysis.

Numerous experimentalists, for more than a century past, have tried to improve the speed of wound healing with all manner of substances, to no avail. What if the epidermis were simply supplied with more oxygen, could it utilise the citric acid cycle and gain eight times more ATP? Would this increased energy production enable it to move faster and repair itself more rapidly? In other words, is the supply of oxygen the rate limiting factor in epidermal wound healing? The evidence will be reviewed.

SCAB FORMATION AND EPIDERMAL MIGRATION

The relevant observations have been made on shallow skin wounds. The base line for the study of the speed of epidermal repair is the wound exposed to the air, without a dressing, which is nature's morn, the way in which healing has evolved for land living animals. Within 24 hours the exposed dermal tissues, unprotected by an epidermis, have dehydrated to a depth of about 60μm and this desiccated tissue forms the scab together with dry blood clot, serous exudate and leucocytes. The regenerating epidermis, originating from hair follicles and the surface epidermis at the wound margins moves across the wound under the scab in an environment low in oxygen. The epidermis moves as a sheet of cells; at the moving edge the cells roll over one another and colonize the wound surface successively (Winter, 1962, 1964, 1972).

The speed of this migration can be measured by taking selected serial histological sections of standard shallow wounds in young domestic pigs at different times after wounding and measuring the area of wound repopulated by epidermis. It is found that under a scab, and it is the same if a cotton gauze or any similar ventilated dressing is used, the epidermis moves on cell diameter, about 7μm, each hour. Because cell movements do not begin until the end of the first day by which time the scab has stabilized and because the average distance between sources of epidermis is about 2.0 mm, it is seven days after the injury was inflicted before the entire wound surface is covered by a new sheet of epidermis. A burst of mitotic activity occurs in the non-migrating epidermis at the borders of the wound and mitosis is seen in the new epidermal layer about 24 hours after the migrating cells have implanted on the wound surface and become static.

HYPERBARIC OXYGEN EXPERIMENTS

To investigate whether the epidermal cells can move faster if supplied with more oxygen, Perrins and I (Winter & Perrins, 1970) performed some experiment with hyperbaric oxygen. We chose to study wounds at the end of the third day when under normal conditions about 37% (37.4% +/- 1.08% {S.E.}; n = 26) of the surface of a standard shallow wound is covered by new epidermis. Standard shallow wounds were made on the backs of three small pigs which were then treated intermittently in a "one-man" oxygen chamber using pure oxygen at a pressure of 7.5 lb/sq. in. (51.7kNm^{-2}), equivalent to 1.5 atmospheres absolute. The animals were in the chamber for a total of 24 hours during the 72 hour period. It was found that an average of 46% (45.8% +/- 1.09% {S.E.}; n = 18), of the wounded area was covered by new epidermis at the end of the third day. The epidermal regeneration was speeded up by about 22%. The difference between the means fo the treated and control groups was statistically significant; t = 4.7166, p = <0.001.

A more pronounced effect was obtained when the pressure of oxygen was doubled, (15 lb/sq.in. = 103.4 kNm^{-2}), equivalent to 2 atmospheres absolute. There was 86% more epidermis by area on the treated wounds than the controls at the end of the third day; (68.8% +/- 1.05% {S.E.}; n = 11). This is a significant difference; t = 13.3984, p = <0.001.

It is deduced from these results that is possible to speed epidermal wound healing by supplying oxygen. By implication the speed of epidermal migration on the normal wound is critically dependent on the amount of oxygen available and this is the rate-

limiting factor. It is likely that in these hyperbaric oxygen experiments the oxygen reached the epidermal cells directly, by diffusion through the scab rather than via the lungs, blood plasma and tissue fluid because it has been demonstrated that administration of oxygen centrally is not an effective way of raising the oxygen supply to a wound (Silver, 1969. Ehrlich et al, 1972).

OCCLUDED WOUNDS

Another way of investigating the effects of oxygen on the speed of epidermal repair is to cover wounds with films of plastics having widely different oxygen permeabilities and to observe the effects on the speed of epidermal regeneration. When shallow wounds are covered with films that restrict the loss of water vapour from the exposed dermis, no scab forms and the superficial fibrous tissue remains viable. Serous exudate collects on the surface of the fibrous tissue under the dressing and the migrating epidermis moves through this fluid layer, over the cut surface of the dermis.

On wounds covered by thin polyester film (0.0025 in., grade O, Melinex, I.C.I. Ltd.) having low oxygen permeability, just over half of the total wound area was covered by new epidermis at the end of the third day (52% +/- 4.4% {S.E.} (n = 19) (Winter, 1972). Using a similar polyester film Silver (1972) found that the oxygen tension on wound surfaces was only 4mm Hg under the epidermis and 21mm Hg above the epidermis.

When standard shallow wounds in the young domestic pig were covered with polypropylene film (0.005 in., T.R.B./5, Shorks Metal Box Ltd.), which had 60 times greater oxygen permeability than polyester film, 70% +/- 5.1% {S.E.} (n = 7) of the wound surface area was covered by new epidermis at the end of the third day.

Polyethylene film is highly permeable to oxygen and has a very low water vapour permeability. When Polythene (0.0015 in., natural grade, low density, British Cellophane Ltd.) is used to cover standard shallow wounds the speed of epidermal movement is increased threefold compared with that which obtains under a scab (Polythene: 3 days, 90% +/- 3.7% {S.E.}; n = 12).

The differences in the speed of epidermal regeneration under the various films are statistically significant, i.e.: polypropylene vs. polyester, $p = <0.005$; polyethylene vs. polyester, $p = <0.001$.

The measured oxygen tensions on wounds on the human forearm covered with polyethylene film were high; 123mm Hg above the epidermis and 89mm Hg below it, compared with a wound with scab formation or one covered with polyester film,

confirming that oxygen from the air had diffused through the more permeable plastics film. A detailed study of mitosis in these shallow wounds showed that when, as under polyethylene film, epidermal regeneration was most rapid, there was an earlier burst of mitotic activity and more cells entered into cell division (Winter, 1972).

The conclusions are that when wounds are covered with plastic films preventing scab formation, the mode of epidermal regeneration is completely altered. Under a scab the epidermis must dissolve a pathway through bundles of collagen fibers at the interface of the hydrated, viable dermal tissue and the overlying, dry, non-viable scab. Under an occlusive dressing the cells move unhindered through a moist exudate between the dressing and the wound surface. The maintenance of hydration alone allows of more rapid epidermal cell migration because even under polyester film there is 15% more wound area covered by new epidermis in 72 hours. But evidently even under these different, artificial conditions the supply of oxygen is rate limiting because, as the data shows, the speed of regeneration is proportional to the oxygen permeability of the plastic film used to cover the wounds.

HYPERBARIC OXYGEN AND OCCLUDED WOUNDS

Epidermal cells under the polyethylene film moved three times more rapidly than did the cells migrating under a scab. A further experiment was designed to discover whether this is the limit to which epidermis can be stimulated by oxygen or whether it is capable of even faster regeneration. The pigs, bearing wounds covered with polyethylene film, were put into a hyperbaric chamber. Because over 90% of a wounded area is covered by new epidermis at the end of the third day under polyethylene film in air at atmospheric pressure it was appropriate in this series of experiments to make the measurements sooner than this and so the biopsy specimens were obtained exactly 48 hours after injury.

The mean area of wound surface covered with new epidermis in the controls (polyethylene covered standard shallow wounds) was 49.2% +/- 1.05% {S.E.} (n = 30) at 48 hours. When animals were treated in the hyperbaric oxygen chamber for a total of 8 hours in 48 hours using pure oxygen at a pressure of 7.5 lb/sq. in., 53.4% +/- 1.17% S.E. (n = 12) of the wound surface was covered by new epidermis. The difference, + 4.2%, although small, is statistically significant ($t = 1.7588$, $p = <0.035$). Using a pressure of 2 atmospheres absolute 79.6% +/- 1.07% S.E. (n = 5) of the wound was covered by new epidermis at the end of the second day which represents a 60% acceleration compared with similar wounds covered with polyethylene film in air at atmospheric pressure. This is a significant result; $t - 10.2250$, $p = <0.001$.

The conclusions are that neither the optimum rate of epidermal cell migration nor the maximum new cell production of which the epidermis is capable is expressed during normal wound healing. The path of the migrating epidermal cells and damage to the superficial blood vessels causes an acute shortage of oxygen at the wound surface. The results of the various experiments reported here strongly suggest that the epidermis can utilise more oxygen if it is made available, by switching from anaerobic to aerobic metabolism of carbohydrates which results in more rapid epidermal regeneration. Nature can be improved upon by using dressings that prevent scab formation and are oxygen permeable.

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