Absence of Wound Healing in Young Chick Embryos

During attempts to modify the development of feather tracts, Saunders had noted the fact that skin wounds in three-day chick embryos failed to heal[1]. This casual observation, which contrasted curiously with the numerous reports on smooth healing of embryonic transplants and the repair of older skin, seemed to hold a promising clue to the mechanisms of wound healing in general. We therefore undertook a more systematic study of the phenomenon, the first results of which are reported in the following.

Circular skin wounds were made on the dorsal side of the neck in chick embryos from five days of incubation age upward until hatching. The wounds measured in the younger embryos (5–9 days) about 0·1 mm. in diameter and 0·1 mm. in depth, and in the older ones (10–21 days) 1 mm. and 0·5 mm., respectively. After the operations, the segment of shell removed for access to the embryo was replaced by a glass window. The embryos were killed four to five days after the operation, fixed, sectioned, stained, and studied microscopically.

The normal healing of skin wounds is primarily a process of cell migration. The epidermal sheet moves with its free margin concentrically over the defect until the hole is closed by a thin layer of cells. Proliferation by mitotic division follows secondarily, gradually making up for the initial deficit of cells.

Wounds made in chick embryos older than twelve days and studied between the seventeenth and twenty-first days showed this same standard pattern of epidermal regeneration. However, embryos operated on at an early stage and studied prior to the twelfth day gave a radically different result. The epidermal sheet had not even started to move over the lesion, and its free margin was still at the site of the original wound edge. Mitotic activity near the margin was high, and the excess cell production had given rise to local thickenings and cysts. Despite this abundant growth along the border, the wound surface had remained raw, bounded only by the condensed outer layer of connective tissue cells. The earlier embryo thus is distinguished by the marked incapacity of its epidermis to spread over the wound surface, even though cell multiplication is stepped up much as in the older group that healed normally. An intermediate age-group, studied between the thirteenth and sixteenth days of incubation, showed signs of flattening of epidermal cells along the border and some contraction of the wound area, thus signalling presumably the initiation of the normal closure process. Fig. 1 summarizes the cases that survived the operation and could be properly evaluated.

It can be seen that there is a rather abrupt change in the embryonic conditions conducive to wound healing about the twelfth day of incubation. Since, moreover, embryos prior to that critical period neatly dissociate the migratory component of wound healing (which is absent) from the proliferative component (which is present), this object lends itself singularly well for a more penetrating analysis of the fundamentals of wound healing. The crucial change about the twelfth day may be found in the mobility of the epidermal cells, or in the constitution of the wound substratum, or in systemic changes in the embryo, or in the nature of the extra-embryonic fluid, or in the tensional stresses, or other as yet unsuspected

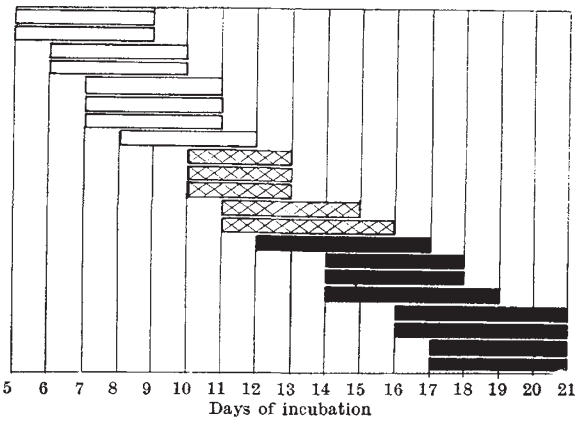

Fig. 1. Bars indicate period of observation between wounding and fixation in days after incubation. White bars, absence of wound healing; cross-hatched bars, incipient wound healing; black bars, complete wound healing

conditions. The location, shape and size of the lesion will likewise have to be taken into consideration. Experiments to pinpoint the effect are under way. One preliminary series has already led a step further in indicating that embryos operated on during the early non-reparative phase, but kept alive into advanced embryonic stages, later start to close their wounds, all just about the critical age when closure was observed in the older series. This would mean that the initiation of migration cannot possibly be related to the wound stimulus as such. It would, of course, also account for the fact that early transplants kept into later stages no longer show skin gaps, the earlier existence of which one might expect from the results reported above.

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[1] Saunders, jun., J. W., and Weiss, Paul, *Anat. Rec.*, **108**, 93 (1950).

Autoradiographic Detection of Sulphone in the Affected Tissues of Leprosy Patients

In two previous communications[1,2], the relative concentrations of diamino-diphenyl sulphone in body fluids and affected and healthy tissues of leprosy patients were compared. It was shown that the drug was preferentially concentrated in the affected tissues. In these studies the assay of radioactive drug in any tissue was made by counting macerated samples with the help of a Geiger–Müller counter.

Autoradiographic studies have now been made to determine the exact sites of localizations of the drug in the affected tissues. Positive autoradiographs of tissue sections are reproduced here.

Diamino-diphenyl sulphone labelled with sulphur-35 (supplied by the Atomic Energy Research Establishment, England) was administered to patients as a single oral dose of 4 μc./kgm. body-weight. The initial specific activity of the drug was 1·0 μc./mgm. 6–18 hr. after administration of the drug samples of tissues were obtained by biopsy. These were fixed