CICATRIZATION OF WOUNDS IN VITRO.*

By EDWARD S. RUTH.

(From the Laboratories of the Rockefeller Institute for Medical Research, New York.)

PLATES LVIII AND LIX.

For the study of the reparation of the skin and its modifications under the influence of certain substances, it became necessary to develop a method permitting observation of the cicatrization in a medium of known composition. Last October, Carrel and Burrows cultivated *in vitro* in a drop of plasma several fragments of skin of the adult frog. The present work was begun by making experiments of the same character. It was found that fragments of skin, placed less than 0.3 millimeter apart were rapidly united by an epithelial bridge. In some cases they were attracted towards each other and their edges directly united. This process is somewhat analogous to normal cicatrization.

Then I attempted, at the suggestion of Dr. Carrel, to develop a technique which would be of practical use in the study of the healing of wounds.

The method consisted of making an incision or a rectangular wound in the middle of a small fragment of skin and cultivating it outside the body in a drop of plasma (figure 15). Skin of frogs and guinea pigs was used. The skin of the guinea pig produced a very luxuriant growth of connective tissue cells, but the epidermization could not be observed easily. The skin of adult frogs generated almost exclusively epithelial cells (figure 16). Therefore it was very easy to observe the different stages of the cicatrization of the wound and to record them by camera lucida drawings.

Immediately after the preparation of the cultures, the edges of the wound appear sharply cut and the open space is free of cells (figure 1). The reparation begins very soon. It consists of three

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mechanisms; the sliding of the epithelium from the edges of the wound into the open space, the epidermization, and the progressive contraction of the original edges of the wound.

The first phenomenon, the sliding of the epithelium towards the center of the wound (figure 2), occurs during the first six or twelve hours. It may be observed about the entire periphery of the wound or only a part of it. Then the second mechanism comes into play: from the edges of the shifted epithelium, epithelial cells begin to wander out either as a thin continuous layer (figure 3) or as isolated cells. This movement is rarely uniform. Very soon the opposing edges are united by a bridge of epithelial cells. They grow towards each other at the rate of about 0.06 millimeter per hour, and very soon the open space is completely covered by epithelial cells. A wound of about 0.82 by 0.32 millimeter may be completely covered by epithelium about ten hours after the preparation of the culture (figure 4). During that period, there is generally no change in the relations of the primitive edges of the wound.

When the epidermization is completed, the distance between the opposite edges of the original wound begins to grow smaller. The rate is at first very slow (figures 5, 6, 7) and at the same time the epithelium covering the wound becomes more dense (figures 8 to 13). Progressively the space between the edges diminishes and finally the epidermized surface, which may be considered as being the scar of the wound, may be less than one quarter of the original area of the wound (figure 14).

Thus the process of the healing of a wound outside the body has been observed and recorded by camera lucida drawings in five experiments. The contraction of the edges of the wound may occur before the epidermization is completed, and the wandering of the epithelial cells may also be different. But the process, in a general way, is identical with the process of cicatrization diagrammatically represented.

In conclusion, it may be stated that it is possible to produce the cicatrization of a wound *in vitro* and to observe microscopically all stages of the process.

EXPLANATION OF PLATES.

PLATE LVIII.

Camera lucida drawings diagrammatically representing the stages of cicatrization in a rectangular wound.

FIG. 1. The outline of a wound made in a piece of frog skin. The clear central area represents the portion that was completely excised. The black border represents the edges of the wound and the immediately adjacent parts of the skin.

FIG. 2. The same wound, six hours later. The parallel lined area represents the epidermis which has moved in mass into the wound. The irregular central outlined portion represents the wandering epithelial cells.

FIG. 3. The same wound, one hour and fifteen minutes later. The wandering epithelial cells have covered the entire center except for three small open areas.

FIG. 4. One hour and five minutes later the entire central portion is covered by wandering epithelial cells. There has been a slight shortening of the short diameter of the wound at the central portion.

FIGS. 5, 6, 7, 8. The central cell layer is now several cells in thickness; the epidermis, represented by parallel lines, has retracted.

FIG. 9. The wound has shortened considerably in its long, and to some extent in its short dimension by the moving together of the connective tissue mass.

FIGS. 10, 11, 12, 13, 14. These figures show a constant increase in the thickness of the epithelial layer in the center, and a constant moving together of the mass of connective tissue which forms the border of the wound.

PLATE LIX.

FIG. 15. The dark mass is a piece of frog skin, the clear center representing the wound.

FIG. 16. The dark mass is the original piece of frog skin. The adjacent V-shaped area is the epidermis, which has moved out in mass. The extensive outer cellular area is a single layer of new-grown epithelial cells.

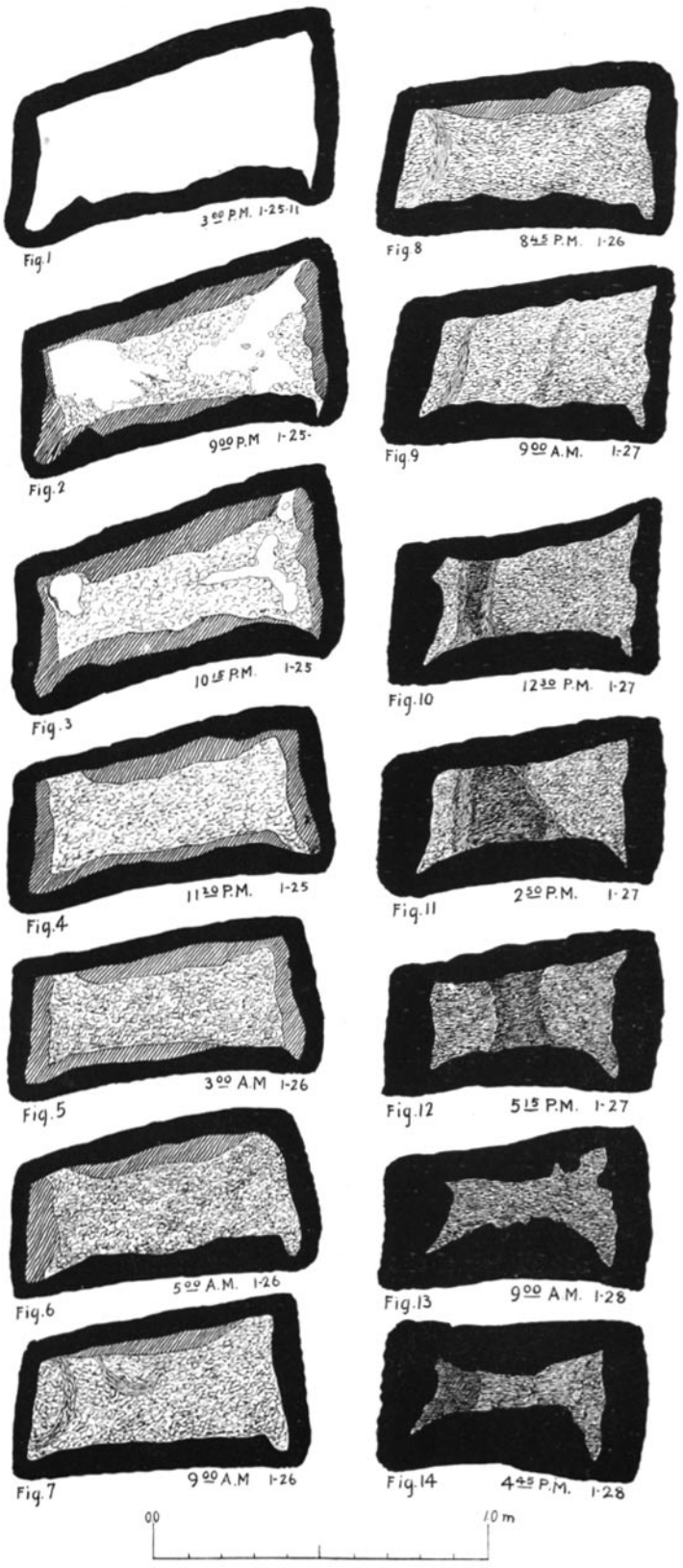
3 00 P.M. 1-25-11
Fig. 1
9 00 P.M 1-25-
Fig. 2
10 15 P.M. 1-25
Fig. 3
11 30 P.M. 1-25
Fig. 4
3 00 A.M 1-26
Fig. 5
5 00 A.M. 1-26
Fig. 6
9 00 A.M 1-26
Fig. 7
8 45 P.M. 1-26
Fig. 8
9 00 A.M. 1-27
Fig. 9
12 30 P.M. 1-27
Fig. 10
2 30 P.M. 1-27
Fig. 11
5 15 P.M. 1-27
Fig. 12
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Fig. 13
4 45 P.M. 1-28
Fig. 14
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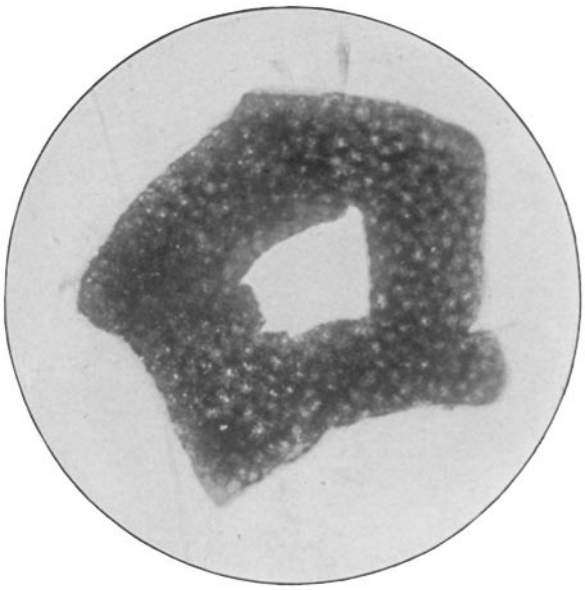

FIG 15.

FIG. 16.