A COMPARATIVE STUDY OF THE MECHANISM OF WOUND HEALING.*

LEO LOEB.

(From the Department of Comparative Pathology of Washington University School of Medicine and the Department of Pathology of St. Louis University.[1])

On the basis of our earlier studies on wound healing[2] and of later comparative and quantitative studies, which have been carried out in our laboratory by Addison, Spain, Akaiwa and the writer[3] in the course of the last ten years, we intend to separate the various factors which are concerned in this process and to analyze each one separately. Wound healing is a composite process in which activities of various kinds enter and interact with each other, each showing definite quantitative variations. In order to obtain a full understanding, it is not sufficient to follow the process as a whole. The mere observation with the naked eye does not allow a separation of the various factors.

In studying, furthermore, the process in various species we will find quantitative differences in each species, and in comparing the data thus obtained we may be able to separate the essential factors underlying this process from varying conditions of a more accidental nature. While at present this analysis is still far from being complete, a survey of our findings will enable us to gain a provisional orientation which may serve as the starting point for further investigations.

1. Cell movements. — If we compare the cell movements in pigeon, rat and guinea-pig, we notice in each case certain irregularities which depend upon variable factors which enter into the individual experiments. The rapidity of the movement depends among other factors upon the character of the wound. At a corresponding period of time the epithelial tongues are much longer in smooth and shallow wounds than

* Received for publication, August 6, 1919.

in uneven wounds, in which the unevenness of the wound or the firm adherence of the scab causes an obstacle to the movements of the cells. This has been brought out with particular clearness in the measurements of Akaiwa. It is furthermore probable that other factors, as, for instance, the situation of the wound, plays a part also. Perhaps the epidermis at the tip of the ear would move more quickly than in the center of the ear. Whether the age, general state of health and nourishment of the animal plays a part needs further investigation. Notwithstanding these irregularities, which are as yet not fully understood, we may on the basis of our present data now draw certain conclusions. Thus it appears that in the pigeon the response of the epidermis to the wound stimulus is slowest during the greater part of wound healing, until between the ninth and eleventh day, just before the closure of the wound, a marked elongation of the epithelial tongues accompanied by a marked contraction of the wound takes place. Thus in the pigeon the closure of the wound takes place more slowly than in the guinea-pig and rat. The epithelial movements throughout are quickest in the guinea-pig; the results in this respect are similar in both series of experiments. In the rat, the results are somewhat different in both of our series. In the first, the movement is decidedly slower than in the guinea-pig, but faster than in the pigeon; while in the second series the rapidity of movement approaches closely that of the guinea-pig. Accordingly we find in the first series the time of closure in the rat somewhat later than in the guinea-pig, while in the second series the time of closure is similar in both species. Altogether we may state that the motor response to the wound is most energetic in the guinea-pig and weakest in the pigeon. In the rat it stands between that of the pigeon and guinea-pig, but it may approach that of the guinea-pig. A similar order we find in the activities in the normal skin of the pigeon, guinea-pig and rat as expressed in number of mitoses, thickness of stratum germinativum and cell size. The normal epidermis of the guinea-pig is most active; the normal epidermis of the pigeon is least active; the epidermis of the normal rat stands between that

of the pigeon and guinea-pig, but approaches more closely that of the pigeon. On the other hand, while in regard to the normal epidermis the rat is much more similar to the pigeon than to the guinea-pig, under the influence of the wound stimulus the rat epidermis may develop an energy of migration which almost equals that of the guinea-pig. The response to the stimulus is therefore relatively much greater in the rat than in the pigeon.

We may then conclude that a certain relation exists between the motor response to the wound stimulus and the proliferative energy in the normal epidermis. The greater the latter, the greater the motor reaction; but in addition there seems to exist a reserve power which during wound healing makes it possible for the normally more sluggish epidermis to display an energy almost equal to that of the normally more active epidermis.

2. If we compare the rate of growth of the epithelial tongue at different periods, we notice again certain irregularities which make impossible, at the present time, a definite formulation of the law underlying the process. These irregularities are especially marked in the case of the pigeon. It is probable that secondary variable factors complicate the conditions. But, making allowance for these complications, we may tentatively conclude that on the whole neither a marked retardation nor a marked acceleration in the growth of the tongue takes place during the later as compared with the earlier periods of wound healing, but that an approximate proportionality exists between the increase in the length of the tongue and the duration of time of wound healing, and that this proportionality is independent of the phase of wound healing. Thus we find in the second guinea-pig series at five days the tongue 2.55 times as long as at two days, which satisfies the theoretical demands. In the same series after seven days the tongue is 3.3 times as long as after two days. We find, then, the following relation: Length of tongue at five days is to the length of tongue at seven days as 5.1:7. Theoretically we ought to expect, instead, 5:7 The agreement is satisfactory.

In the first guinea-pig series the tongue is at five days 3.1 times as long as at two days. The increase in this case is somewhat greater than should be expected, if direct proportionality existed. After seven days the tongue in the first guinea-pig is 4.4 times as long as after five days. The relation is as follows: The length of the tongue at five days is to the length of the tongue at seven days as 4.97:7. Theoretically it ought to be 5:7. The difference is not very great.

In the first rat series we find after five days the tongue 2.6 times as long as after two days. In this case the agreement is good. After seven days the tongue is about 3.8 times as long as after two days. We find, therefore, the relation of 4.83:7 between the length of tongue at five and at seven days. Theoretically it ought to be 5:7.

In the second rat series the tongue is about 1.9 times as long after five days as after two days. In this case the growth is less than should be expected under the assumption of proportionality. At seven days the length in the second rat series is 2.5 times as great as at five days. The relation between the length at five and seven days is therefore 5.2:7, instead of 5:7 as theoretically required.

In a provisional way we may then conclude that, while variable factors complicate the result, on the whole a direct proportionality seems to exist between the length of tongue and the duration of wound healing.

Now, we must consider that several factors enter into the formation of the tongue, namely, (*a*) the amœboid migration of the epithelial cells; (*b*) the increase in the size of the epithelial cells, and (*c*) the increase in the number of cells as a result of mitotic proliferation. While the mitotic multiplication leads primarily to an increase in the number of cell rows, it contributes also to an extension of the tongue in a longitudinal direction. Both factors (*b*) and (*c*) contribute therefore to the growth of the tongue to some extent, yet the migration of the epithelial cells proper is in all probability the essential element in the enlargement of the tongue. If, as a result of the coöperation of all three factors, at best proportionality between length of tongue and duration of its growth is attained,

it is very probable that the energy of amœboid activity shows on the whole a relative decrease in the later periods of wound healing just preceding the closure. That, however, this does not apply generally may be concluded from the rapid growth of the tongue just preceding closure in the case of the pigeon.

3. In the rat as well as in the guinea-pig, the rapidity with which the tongue enlarges depends upon the size of the wound. The motor response of the epidermis to the wound stimulus is in both species greater in the case of the larger wound. In the larger wound, the area of the defect as compared to the circumference of living epithelium is greater than in the smaller wound, where the circumference is relatively greater. It seems, therefore, that this relationship determines in some way the rapidity of movement of the epithelial cells. But, while in the case of the guinea-pig the larger wound closes earlier than the smaller, in the rat the greater rapidity with which the tongue enlarges in the large wound is not sufficient to lead to an earlier closure of the larger wound. In the rat, the shorter wound closes at an earlier date notwithstanding the more rapid elongation of the tongue in the larger wound. This difference between the effect of the size of the wound in the guinea-pig and rat depends upon the coöperation of a second factor, namely, the contraction of the wound. The intensity of contraction differs in the rat and in the guinea-pig, and being more marked in the guinea-pig, this factor is added to the greater rapidity of amœboid movement in the larger wound and leads to a more rapid closure of the larger wound in the case of the guinea-pig.

4. Contraction and retraction of the wound. — A contraction of the wound occurs in all three species which we have examined so far; but it differs in strength in different species, and it differs also in the same species according to the length and probably also the depth and situation of the wound. There is one character in common to all the experiments in the various species: contraction does not set in to any considerable extent within the first few days after the defect has been made. It is not usually noticeable in the first four days

of wound healing. In many cases it seems to become very marked at the period of wound closure and at the period directly following wound closure. This is especially noticeable in the second guinea-pig series. Here the closure occurs earlier in the four-millimeter series than in the two-millimeter series. The contraction sets in in accordance with the difference in closure; it occurs earlier in the four-millimeter series, and the entire contraction throughout the whole period of wound healing is therefore much greater in the four-millimeter series. In the pigeon also the principal contraction occurs between the ninth and eleventh day, which is the time of closure.

In the rat, the main contraction takes place either at the time of closure or in the period following closure. In the first guinea-pig series contraction is also most marked in the period following closure. But in the pigeon and in the rat (second series) contraction during the essential processes of wound healing plays only a very insignificant part in our experiments. In the second rat series, contraction is not only very late but also quantitatively weak, and it merely suffices to overcome the initial retraction which took place, as is shown by Akaiwa.

In the pigeon, as well as in the rat, not only does contraction not occur in the early stages of wound healing, but on the contrary a retraction takes place which is due to the natural tension in the skin at certain places and to the relative looseness of the attachment of the skin proper to the underlying tissue. We see, then, that contraction is relatively most marked in the guinea-pig and least in the pigeon and rat. It occurs mainly in the later periods of wound healing, and is probably due to the activity of the connective tissue which proliferates in the floor of the wound. In those cases in which an active contraction coincides with the time of closure, the active contraction which takes place at this time is probably the primary factor which makes conditions favorable for the occurrence of closure at this time. The second rat series is of special interest because here Dr. Akaiwa was enabled to study the factors in wound healing almost uncomplicated by

contraction. In the guinea-pig, contraction showed the maximum effect; it was particularly strong in the four-millimeter wounds and associated with the rapid outgrowth of the epithelium it enabled the larger wound to close more rapidly than the smaller wound.

The effect of the size of the wound on the amount of contraction of the granulations has been previously studied by A. Carrel.[4] He finds that contraction is the more rapid the larger the wound. It is most rapid in the early stages of contraction, and gradually becomes slower. It becomes about zero when the edges have reached a distance of about ten or fifteen millimeters. In our experiments we find contraction even in wounds as small as four and two millimeters in diameter. Carrel believes that the wandering of the epithelium sets in only after the period of granulous retraction has come to an end. We find, on the contrary, that the amœboid wandering of the epithelium is the first response of the tissue to the wound stimulus and that the contraction sets in only at a later period. Carrel states that the rate of epidermization is inversely proportional to the dimensions of the wound. We find that within the range of wounds examined by us the rapidity of outgrowth is greater in the larger than in the smaller wounds. A quantitative determination of the various part processes which constitute wound healing is only possible through microscopic determinations.

5. Closure of the wound. — In regard to the time of closure of the wounds we find marked variations in the different species, and within the same species under different conditions. In the pigeon, in which all activities show the least intensity, the wound closes later than in the other species, namely, between the ninth and eleventh day. It closes first in the four-millimeter wound of the guinea-pig, but it may close almost as early in the two-millimeter wound of the rat, although in other cases it closes somewhat later in these. On the whole, the time of closure is slightly later in the rat than in the guinea-pig. Thus we find the fourth and eleventh days to be the extremes in the time of closure, and we also

find a certain parallelism between the energy in the cell activities in the normal and regenerating skin in the different species and the time of closure.

In the shallow wounds the closure takes place earlier than in the deeper wounds; this explains the difference between the time of closure in the razor and in the trocar series, in the rat in Dr. Akaiwa's experiments.

6. Mitotic Proliferation. — In guinea-pig, rat and pigeon the mitotic proliferation of the epithelium shows definite similarities. Everywhere we find in the first period, which usually extends over approximately four days, only very rare mitoses in the new epithelium (the tongue); indeed, here the mitoses are more rare than in the normal skin. In the old epithelium adjoining the wound, on the other hand, we find a considerable increase as early as two days after operation. However, under certain conditions the new epithelium in the tongue may show a marked increase as early as three and a half days after operation, namely, in cases in which an early closure of the wound occurs. This is the case in the four-millimeter series of the guinea-pig. Shortly before closure a marked rise takes place in the new epithelium. Usually the closure of the wound occurs at a date later than three and a half days after operation. We invariably find a rise in the number of mitoses in the new epithelium, which reaches a maximum just preceding or at the time of closure and a decided fall directly following the closure. In one series (the two-millimeter series in the rat) the fall in the number of mitoses in the new epithelium was apparently somewhat delayed after closure. From the neighborhood of the wound the increase in mitotic activity extends into the old epithelium with gradually decreasing intensity. The greater the increase near the wound, the farther back the increase extends. The effect of the wound on the mitotic proliferation in the adjoining epithelium appears to be cumulative; it increases more and more until the closure takes place. The curve of mitotic proliferation in the old epithelium varies somewhat in the different series. In the majority of cases the proliferation at

this place is most marked after two days, and then gradually decreases, while in the new epithelium it still increases during this period; so that at the time of closure or directly following it the mitotic proliferation is usually more marked in the new than in the old epithelium, while at an earlier period the condition was reversed. This holds good if we compare the number of mitoses in one half of the total area of the new epithelium with the number of mitoses in a unit area of the old epithelium. But, even if we compare the mitoses in a unit area of the new with the mitoses in a unit area of the old epithelium, the gradual increase in the mitotic proliferation in the new epithelium comes out quite clearly; and this increase continues until the maxima in both the old and new epithelium become at least equal.

But in the second rat series a gradual increase took place in the mitotic proliferation, also in the old epithelium, and the proliferation reached a maximum just previous to the time of closure, at a somewhat earlier period than in the new epithelium; after the maximum had been reached the number showed a continuous fall. In a tentative way, we may explain these facts as follows: It is primarily the defect which causes the mitotic cell proliferation. Wherever the defect adjoins directly the epithelium or is separated from it only by a short piece of tissue, the cell proliferation is marked provided the conditions are otherwise favorable for cell proliferation. Such favorable conditions depend upon the character of the circulation and the proliferation of the underlying connective tissue. Both of these factors are deficient in the wound in the first few days of wound healing. The effect of the wound is cumulative. Therefore the mitotic activity increases in the new epithelium continuously until the time when the wound is closed. The cumulative effect of the defect would also extend to the old epithelium were it not that this effect is counteracted by the constantly growing distance between defect and the margin of the old epithelium. Usually, it seems, the latter factor overbalances the former, and then the maximum is reached in the old epithelium in the early periods of wound healing. In the second rat series, however, the

second factor seems to have been relatively stronger and here a maximum was reached in the old epithelium only a little earlier than in the new. However, even after the closure has taken place and thus the defect has been eliminated, the mitotic proliferation in the new and old epithelium does not abruptly return to its normal level, but, while a decided fall takes place, the cell multiplication continues with a more than ordinary energy for some time. We must either assume that the stimulus previously exerted by the defect has an after-effect outlasting the defect, or that there exist other factors causing cell proliferation in addition to the defect. The abnormal condition of the soil on which the epithelium, especially the new epithelium, is resting even after the closure of the wound may represent such an additional factor.

If we consider the whole course of the wound healing, we find the greatest absolute cell proliferation in the new epithelium. But if we compare the cell proliferation in the unit areas in the old and new epithelium the maximum occurs in the first area of the old epithelium in the majority of experiments.

7. If we compare the mitotic proliferation in the two-millimeter and four-millimeter series we find in the rat a greater absolute and relative number of mitoses in the four-millimeter series. The larger wound causes a greater rise in cell multiplication than the smaller wound. It is different in the guinea-pig; here the total number of mitoses is considerably greater in the two-millimeter series. This difference between the guinea-pig and the rat is evidently due to the difference in the time of wound closure in guinea-pig and rat. In the rat, the four-millimeter wounds close later than the two-millimeter wounds, while in the guinea-pig the four-millimeter wounds close considerably earlier. Now the time of closure determines the mitotic activity. With the closure there is everywhere a great reduction in the number of mitoses, and thus it comes about that in the four-millimeter series in the guinea-pig the increase in mitotic proliferation extends over a much shorter period than in the two-millimeter series. The

reverse holds good in the rat. The effect of the wound being cumulative, the maximum reached in the rat is greater in the larger wounds than in the smaller. The maximum reached is greater for the same reason in the deeper than in the more superficial wounds. Correspondingly the fall in the number of mitoses in the rat after closure is steeper in the larger than in the smaller wounds, and more marked in the new than in the old epithelium.

We may, then, conclude that the effect of the size and depth of the wound on the number of mitoses is partly an indirect one. Whether in addition they have also a direct effect, comparable to that on cell movements, needs further investigation. There is, at least at the present time, no direct indication that such an influence exists. We found, however, that the cell size is greater in the case of the larger wound, also in the guinea-pig. It might therefore have been that accidentally we did not examine the larger wound at the time of maximum proliferation.

8. There seems to exist a second indirect relation between the character of the wound and the number of mitoses. In the experiments of Dr. Akaiwa the number of cells which had migrated into the defect seemed to influence the mitotic activity in the old epithelium adjoining the wound; the greater the number of migrated cells, the greater was the number of mitoses. This seemed, however, only to hold good for the early stages of wound healing, and furthermore it only applied provided we compare the character of the two tongues in the same wound. If we compare the wounds in different animals and species, such a relationship between number of emigrated cells and intensity of mitotic proliferation does not seem to exist, probably because the other factors, differences in responsiveness of the epithelium in different species, cover the other effect. This relationship between the number of cells which have grown into the defect and the number of mitoses in the adjoining area of the old epithelium might be interpreted in either of two ways: we might assume that the outgrowth of cells as such is responsible for an increase in the

number of mitoses, or that the same factor which favors a more energetic outgrowth on the one side of the wound is likewise responsible for an increase in the number of mitoses on this side. On the latter assumption, both factors would be coördinated. If we consider, however, the fact that the rapidity of outgrowth depends upon accidental conditions, such as the rigidity, shape and fixity of the attachment of the scab of the wound, we may conclude that the former assumption is more probably the correct one.

In our studies on the growth of tissues in vitro we made likewise some observations which point to the conclusion that an active outgrowth of fibroblasts causes an increase in the number of mitoses. On the other hand, mitotic proliferation can be very active without outgrowth, especially in epithelial tissues, and Walsh and ourselves have shown previously that mitotic proliferation is increased in wounds of the uterus in which a ligature has been applied at the end of the wound and in which therefore an outgrowth of cells has been prevented.[5] At best, the outgrowth of cells and subsequent pull represent a subsidiary factor in mitotic proliferation.

9. It is of great interest to compare the proliferative power of the epidermis in the guinea-pig, rat and pigeon in the normal skin as well as during regeneration. In the normal epidermis the frequency of mitoses in a unit area of 1 mm. x 50μ is approximately as follows: Guinea-pig, 12; rat, 3–4; pigeon, 3. However, the number of cells in a unit area differs in each of these three species. It is therefore necessary to determine the number of mitoses which occur in the same number of cells. An exact determination of this number is impossible at the present time. But, subject to later revision on the basis of more accurate determinations, we may provisionally conclude that if the number of mitoses in a definite number of cells in the guinea-pig is 12, it is 4.8 in the rat and 7.5 in the pigeon; or, if we take 22 as the standard of mitoses in the guinea-pig, the corresponding numbers in the rat would be 8.1, and in the pigeon 13.7. We see, then, that in the

normal skin the mitotic activity in the guinea-pig is considerably greater than in the rat or pigeon. In order to obtain comparable figures for the proliferative energy in the various species during wound healing, we selected the number of mitoses in the first unit area of the old epithelium, and in each series we determined the average of mitoses at this place throughout the various periods of wound healing. We thus obtained the following figures:

I. Guinea-pig series: average, 28 mitoses; maximum, 40. (In this series the average is somewhat higher than in the second guinea-pig series, because in the former the closure took place somewhat later.)

II. Guinea-pig series: 2-mm. series: average, 21 mitoses; maximum, 44. 4-mm. series: average, 17 mitoses; maximum, 30.

I. Rat series: average, 16 mitoses; maximum, 26.

II. Rat series: 2-mm. series: average, 12 mitoses; maximum, 22. 4-mm. series: average, 17 mitoses; maximum, 26.

Pigeon: average, 13 mitoses; maximum, 24.

If we determine in each species the average of the figures obtained in the various series of experiments, we obtain the following figures: In the

Guinea-pig: average, 22 mitoses; maximum, 38.

Rat: average, 15 mitoses; maximum, 29.

Pigeon: average, 13 mitoses; maximum, 24.

We see again that the mitotic proliferation is most active in the guinea-pig and much weaker in the rat and pigeon. The maxima run parallel to the averages.

In both the normal and regenerating epidermis the number of mitoses in a unit area is largest in the guinea-pig and smallest in the pigeon; the position of the rat is intermediate, but approaches more closely that of the pigeon than that of the guinea-pig.

These figures give the number in a unit area independent of the number of cells in each unit area. If we reduce the number of mitoses to a unit number of cells rather than to a unit area, the advantage of the guinea-pig over the other species becomes still greater; the number of mitoses in the pigeon decreases still more, and is again the lowest in all three species. In the normal skin, on the other hand, the number of mitoses in a unit number of cells is probably somewhat greater in the pigeon than in the rat. If during regeneration the number

of mitoses in a unit number cells is in the guinea-pig 22, it is 10.4 in the rat and 5.4 in the pigeon. These modifications in the relative number of mitoses are due to the fact that during regeneration the cell size is greatest in the guinea-pig and smallest in the pigeon, and that the relative difference between the cell size of guinea-pig and rat, on the one hand, and guinea-pig and pigeon, on the other, is greater during wound healing than in the normal skin.

We may then conclude that the epidermis of the guinea-pig shows the most active proliferation under normal conditions as well as during regeneration, and that in the pigeon and rat the mitotic activity is considerably weaker during wound healing; the lead which the guinea-pig has in the normal skin is maintained during wound healing. We may furthermore conclude that, just as in the pigeon the migration of cells is weaker than in the other two species during wound healing, so also the mitotic proliferation in the pigeon is weaker than in either guinea-pig or rat.

10. Size of cell and nucleus. — In the second rat series, Dr. Akaiwa determined the size of cells and nuclei at six different places, while in all the other series the measurements were made only at the place of insertion of the new epithelium. If we consider the figures found in the various series we may draw the following conclusions:

(*a*) Variations in the size of cell and nucleus follow closely the variations in the number of mitoses; both are an expression of the same kind of cell reaction.

(*b*) It appears, however, that variations in size of cell and nucleus are probably a more constant, less variable factor than the number of mitoses. The latter change more rapidly; cell size is less liable to rapid changes; it is therefore probably on the whole a somewhat safer guide than changes in mitoses. This is especially noticeable in the second guinea-pig series. Here the maximum in the number of mitoses is higher in the two-millimeter series than in the four-millimeter series. This is perhaps due to the fact that the examination in the four-millimeter series was not made at a time when the maximum

was at its height. Size of cell and nucleus, on the other hand, shows a maximum in the larger wounds of the four-millimeter series.

(*c*) In both the rat and guinea-pig series the greater maximum is reached in the larger wound. Furthermore, Dr. Akaiwa's experiments show that also in the deeper wounds the size of cells and nuclei reaches a greater maximum.

(*d*) In all the series the relation between size of cell and nucleus and the time of closure of the wound comes out very clearly. The maximum is reached everywhere either just before or at the time of closure. And just as in the case of mitotic proliferation, so also in the case of size of cell and nucleus we find that those cells in the new epithelium which are nearest the center of the wound reach their maximum somewhat later than the cells in the old epithelium. The cells intermediate between these two points show a somewhat intermediate behavior.

(*e*) The greater the increase in cell size and mitotic activity near the insertion of the tongue, the farther it extends backwards into the old epithelium.

11. It is of interest to compare the figures for size of cell and nucleus obtained in the three species of animals which we studied. However, in attempting to provide a basis for such a comparison we suffer from the difficulty that only two diameters were actually measured; in the case of the third diameter we assumed it to be equal to the mean of the two other diameters in the regenerating skin. In the normal skin of the guinea-pig we took it to be approximately intermediate between the mean of these two diameters and the smaller one of the two diameters, and in the normal skin of the rat and pigeon we took it to be intermediate between the mean of the two diameters and the larger one of the two diameters. An additional difficulty is the fact that the figures for size of the normal cell and nucleus in the rat differ somewhat in the first and in the second series, presumably owing to differences in the place where the cells were selected and also perhaps to differences in the age of the

animals used. We intend to investigate this question again. In the meantime we consider a size intermediate between the sizes found in both determinations as probably sufficiently accurate for our purposes. While our figures are therefore not exact, they probably represent an approximation which is sufficiently close to permit certain conclusions. In considering only the two diameters which were actually measured we obtained the following figures:

1. Normal cells and nuclei. — (*a*) Guinea-pig — cell 75, nucleus 49.
(*b*) I. Rat — cell 58, nucleus 31. II. Rat — cell 82, nucleus 45. Mean: cell 70, nucleus 38.
(*c*) Pigeon — cell 54, nucleus 22.
2. Cells and nuclei during wound healing. — (*a*) I. Guinea-pig — cell 132, nucleus 72. II. Guinea-pig — (1) 2-mm. series: cell 115, nucleus 69; (2) 4-mm. series: cell 126, nucleus 68.
(*b*) I. Rat — cell 92, nucleus 53. II. Rat — (1) 2-mm. series: cell 107, nucleus 69; 4-mm. series: cell 112, nucleus 68.
(*c*) Pigeon: — cell 80, nucleus 35.

(*a*) All guinea-pig series combined, average: cell 124, nucleus 69, maximum 158. (*b*) All rat series combined, average: cell 104, nucleus 63, maximum 140. (*c*) Pigeon: cell 80, nucleus 35, maximum 101.

Gain in the average and maximal size of the regenerating epidermal cell and nucleus over the corresponding size of the normal cell and nucleus:

(*a*) Guinea-pig average — cell 40 per cent, nucleus 29 per cent, maximum cell 53 per cent.
(*b*) Rat average — cell 33 per cent, nucleus 40 per cent, maximum cell 50 per cent.
(*c*) Pigeon average — cell 33 per cent, nucleus 37 per cent, maximum cell 47 per cent.

It is also of interest to determine the ratio between size of cell and nucleus in the normal epidermis and in the regenerating epidermis:

(*a*) Guinea-pig normal ratio, 1.5 : 1. During wound healing: I. series, 1.8:1; II. series, 4-mm. series, 1.9:1; 2-mm. series, 1.67:1. Average during wound healing, 1.8:1.
(*b*) Rat normal ratio, 1.85:1 During wound healing: I. series, 1.74:1; II. series, 4-mm. series, 1.65:1; 2-mm. series, 1.55:1. Average during wound healing, 1.65:1.
(*c*) Pigeon normal ratio, 2.46:1 During regeneration, 2.3:1.

From these figures we may draw the following conclusions:

1. The normal size of cells and nuclei in each of the three species show a relationship to each other similar to their respective proliferative energies; both are greatest in the guinea-pig, smallest in the pigeon.

2. The relative average gain in the size of cell and nucleus during wound healing is of a similar order in all three species. It ranges approximately between thirty and forty per cent. The guinea-pig gains relatively a little more in size of cell than rat and pigeon. The maximum gain is also of a similar order in all three species; it is approximately fifty per cent; it is largest in the guinea-pig, smallest in the pigeon.

3. The ratio between size of cell and nucleus is in the normal skin smallest in the guinea-pig and largest in the pigeon. The ratio in the rat is intermediate, but nearer that of the guinea-pig than that of the rat. During wound healing the cell gains relatively more in size than the nucleus in the guinea-pig, while in the rat and pigeon the nucleus gains relatively more than the cell. During wound healing the ratios in guinea-pig and rat are again similar to each other, and both are considerably lower than in the pigeon; but during regeneration the ratio in the guinea-pig has become somewhat higher than in the rat. If we consider three diameters, the conclusions remain the same; but now the average gain ranges between fifty-nine per cent (pigeon) and seventy per cent (guinea-pig).

The characteristic high ratio between size of cell and nucleus in the normal epidermal cell of the pigeon is maintained in the regenerating cell of the pigeon. If we consider three diameters, the figures are as follows:

Normal cells. — (*a*) Guinea-pig — cell 601, nucleus 257.

(*b*) Rat — cell 603, nucleus 206.

(*c*) Pigeon — cell 421, nucleus 90.

During wound healing. (*a*) Guinea-pig average — cell 1,364, nucleus 573, maximum in cell size 1,975.

(*b*) Rat average — cell 1,050, nucleus 498, maximum cell size 1,680.

(*c*) Pigeon average — cell 720, nucleus 210, maximum in cell size 1,010.

Relative gains during wound healing. (*a*) Guinea-pig average — cell 56 per cent, nucleus 56 per cent, maximum 70 percent.

(*b*) Rat average — cell 43 per cent, nucleus 59 per cent, maximum 64 per cent.

(*c*) Pigeon average, — cell 43 per cent, nucleus 57 per cent, maximum 59 per cent.

Ratios between cell and nucleus: guinea-pig, normal ratio 2.3:1; during regeneration, 2.38:1.

Rat, normal ratio 2.8:1; during regeneration, 2.1:1.

Pigeon, normal ratio 4.6:1; during regeneration, 3.4:1.

12. The thickness of the stratum germinativum. — A study of the changes in thickness and number of cell rows in the stratum germinativum of the epithelium during wound healing leads to the following conclusions: (*a*) the thickness of the stratum germinativum and the number of living cell rows take a parallel course. (*b*) In a way similar to cell size and proliferative activity, the changes in the thickness of stratum germinativum show a marked dependence upon the time of closure. We can very clearly see in the different series that on the whole the maximum increase is reached at the time of closure. In those series in which the closure takes place later, the maximum is likewise reached at a later date. After closure, a fall usually takes place in all of the three species. (*c*) But, just as in the case of cell size and mitotic activity, so also in the case of the thickness of the stratum germinativum the maximum is reached in the center of the wound at a somewhat later date than near the original wound border. (*d*) On the whole, the variations in the thickness of the stratum germinativum are not so sharp an indicator of the wound closure as the other factors which we have determined quantitatively. In several series the maximum in the thickness of the stratum germinativum persisted for some time even after the closure had occurred. (*e*) At the time of closure the greatest thickness is reached in all species and in all the different series at the place of the insertion of the tongue, though at the time directly following the closure the thickness in the center of the wound may exceed the maximum near the margin of the original defect. In this respect the thickness of the stratum germinativum differs from the size of the cells. The maximum for the size of cells (and probably also for mitotic activity) is in the old epithelium near the

insertion and not at the point of insertion. (*f*) In the guinea-pig the maximum reached in the larger wounds is about the same as in the smaller, although in the early periods after operation higher values are noted in the larger wounds. In the rat the maximum is higher in the larger wounds. This difference between wounds in rat and guinea-pig is in all probability due to the fact that in the guinea-pig the larger wound closes at a very early date. Therefore the time during which the wound can act on the epidermis, causing its enlargement, is very short in the guinea-pig, while in the rat the time is longer in the case of the larger wounds than in that of the smaller. Somewhat higher values are therefore reached in the larger wounds. (*g*) The depth of the wound is apparently also a factor of great importance. In the first rat experiments of Dr. Spain, the values reached are only about one half as large as in the second. This is in all probability due to the fact that the wounds of the first series are on the whole more shallow. In a similar manner Dr. Akaiwa found that in the more shallow wounds made with the razor the stratum germinativum is thinner than in the thicker wounds of the trocar. (*h*) We can explain the variations in the thickness of the stratum germinativum and in the number of cell rows best by assuming that several factors participate. In the first place, changes in the size of cells and mitotic activity: We see that, on the whole, with the few modifications mentioned previously, all these factors show identical curves; the time of maximum reached, the dependence of the maximum upon length and depth of wounds, the difference in the location of the maximum in the center of the former defect and near the margin of the wound are the same in the case of all these factors. This shows that actual growth phenomena — increase in the number as well as in the size of cells — play a part in determining the thickness of the stratum germinativum; and it is of interest in this connection that there should be an actual increase in growth in the center of the former defect after the closure of the wound. But in addition to these factors movements of cells in the direction from the old epithelium towards the center of the wound

undoubtedly take place. This explains the sudden increase in the thickness of the epidermis in the center of the wound as soon as the two tongues have met. They evidently exert pressure upon each other. It explains also why, as Dr. Akaiwa has found, in the perforated wounds the thickness in the center is greater than in the ordinary wounds. The amount of epithelial tissue moving towards each other is much greater in the case of the perforated wounds. It also explains why in the case of the thickness of the stratum germinativum the maximum is shifted more towards the new epithelium as compared with the maximum for cell growth. In the case of the former, evidently movements of cell rows in the direction from the old towards the new epithelium are an active factor in addition to the increase in size and cell multiplication.

13. In the normal skin of the guinea-pig the thickness of the stratum germinativum is not quite three times as large as in the rat, and slightly more than three times as large as in the pigeon. The number of rows of living cells in the guinea-pig is three to four times as large as in the rat and four times as large as in the pigeon. The normal epidermis of the pigeon is therefore the thinnest of the three, and the epidermis of the guinea-pig the thickest; the epidermis of the rat is only slightly thicker than that of the pigeon.

During regeneration, the maximum in the deeper (trocar) wounds in the rat becomes very similar to that of the guinea-pig; the rat gains therefore more than the guinea-pig in thickness, but even in the shallower wounds the rat gains relatively more than the guinea-pig and pigeon. In the regenerating skin of the guinea-pig the relative gain in thickness of stratum germinativum and number of cell rows over that of the normal skin is very similar to that of the pigeon.

The figures are as follows:

Maximal increase in thickness in the regenerating skin: (*a*) in the guinea-pig, about three hundred to four hundred per cent; (*b*) in the rat, about one thousand per cent; (*c*) in the pigeon, about three hundred eighty per cent.

Maximal increase in number of cell rows in the regenerating skin: (*a*) in the guinea-pig, two hundred twenty per cent; (*b*) in the rat, seven hundred per cent; (*c*) in the pigeon, three hundred per cent.

During wound healing the pigeon epidermis is therefore the thinnest of the three, just as the normal pigeon epidermis. Again the pigeon shows the least activity during regeneration.

14. At fourteen days the epidermis has nearly returned to its normal condition, although it has not yet reached it completely. Fourteen days after the operation the epidermis in the guinea-pig, in which the normal values are higher than in the rat and pigeon, has reached a condition more approximating the normal condition than in the pigeon and rat, where the figures are still somewhat higher. Thus in the guinea-pig the mitotic proliferation has become almost normal at that time, while in the rat and pigeon it is still somewhat higher than normal. Similar differences we find in the thickness of the stratum germinativum. In the guinea-pig it is at this period only about ten to fifty per cent larger than in the normal skin, while in the rat and pigeon it is still approximately one hundred per cent larger. On the other hand, the size of cells has almost returned to normal figures in all three species. The duration of the cycle of wound healing is therefore approximately the same in all the animals examined; it lasts somewhat more than fourteen days; and within the range of our experimental variations this seems to hold good, independently of the size of the wounds.

15. Number of cells. — The variations in the number of cells were determined by Dr. Akaiwa. His investigations show that such variations run parallel to the variations in cell size, proliferative activity, thickness of stratum germinativum and number of cell rows. In all, a maximum is reached at about the time of wound closure. As in the other cases, the maximum is reached somewhat earlier in the old than in the new epithelium. Inasmuch as in the rat the closure takes place earlier in the smaller wound and at fourteen days

the level reached is similar in all the series, the descent from the maximum is somewhat steeper in the larger wounds.

The variations in the number of cells in the unit area during the different periods of wound healing are relatively smaller than the variations in the number of mitoses, in the thickness of the stratum germinativum, and in the number of cell rows. The relative constancy in the number of cells is similar to that found in the case of the cell size. It should of course be expected that the variations in the size of cells are relatively less great than those in the other factors. Not only are the curves for the number of cells more shallow than those for number of mitoses and thickness of stratum germinativum, but in addition the average number of cells in the different unit areas differs less than the average number of mitoses and the average thickness of the stratum germinativum. Here again cell number and cell size show a concordant condition. Absolutely the number of cells is greatest in the new epithelium, but if we consider the number of cells in a unit area we find the number greatest in the old epithelium adjoining the wound; at the same place the size of cells and probably also the number of mitoses are likewise greatest.

In the various series the average numbers of cells in the corresponding areas agree with each other to a surprising degree; they do this to a much greater degree than any of the other factors with exception of cell size which naturally would be much less accessible to marked variations than number of mitoses, thickness of epithelium and number of cells. It almost appears as if the equalization in the number of cells in the various areas was the aim towards which the various activities were directed during wound healing.

16. Wound healing and physiological regeneration of tissues. — Various tissues show proliferation of their cells under the usual conditions of normal life. Such a proliferation is especially marked in the tissue with which we are dealing, the epidermis; but it occurs also in certain other tissues, wherever a loss of cells takes place or a liability to injury is noticeable.

This relationship between liability to injury and normal proliferative activity is particularly distinct in the case of the outer covering, the epidermis. It is evidently this relationship which induced von Hansemann to interpret the physiological regeneration in the same way as the pathological regeneration, viz., as the reparative response of the tissues towards a loss of cells, the result of the tear and wear of ordinary life. But might it not be that the relationship between "normal" injury and physiological regeneration is in reality the opposite, namely, that the physiological proliferation of cells is the primary factor which makes possible a constant desquamation of cells? We believe that our comparative investigations permit us to draw some definite conclusions in this regard.

In the first place, we found in our studies two types of skin, (1) the type represented by pigeon and rat, in which the living epidermis consists of one or two cell rows, and (2) the type represented by the guinea-pig, in which there are three to five rows of living cells. In the first type the cells are flat and, on the whole, smaller than in the second type. There is considerably more mitotic proliferation in the second type than in the first type. According to the customary interpretation we would then have to assume that in the guinea-pig, in the course of normal life, the cells are exposed to injury and destruction to a greater extent than in the rat and pigeon. As a result of the greater normal loss a greater cell proliferation occurs. If that were so, then we should expect the epidermis of these three species to show an equally strong proliferative activity in response to the same kind of injury. This, however, is not the case, inasmuch as the investigation of Addison, Spain, Akaiwa and the writer have shown that during wound healing the activity of the epidermis of the guinea-pig is greater than that of the rat and pigeon. The difference between guinea-pig and pigeon is especially marked; and while the rat epidermis displays a greater amount of reserve energy than the pigeon during wound healing, yet even in the rat the proliferative power does not equal that of the guinea-pig.

We see then that towards the same stimulus the epidermis of the guinea-pig reacts more actively than the other species and we must therefore conclude that there is inherent in each kind of epidermis a definite proliferative tendency which is characteristic of the tissue in this particular species and that the amount of normal proliferation is the result of this tendency which is primary and which determines secondarily the amount of cell desquamation. Thus the greater thickness of the stratum corneum in the guinea-pig is but the expression of the marked proliferation and subsequent desquamation of cells in this species.

We may further conclude that the difference in structure of the skin in these three species is likewise secondary to the different tendencies to mitotic cell proliferation. It is greater in the guinea-pig, and causes here a greater thickness of the epidermis, a greater number of cell rows and probably also a greater thickness of the epidermal cells. As Akaiwa has observed in the rat, different parts of the skin may differ in proliferative activity. It would be of interest to determine whether such differences in the normal structure within the same animal are accompanied by similar differences in rapidity of regenerative activity during wound healing.

While we must then conclude that the physiological tendency to mitotic cell multiplication is the primary factor, which is characteristic of the species and transmitted by heredity, and that it determines structure as well as normal regeneration and desquamation of cells and likewise the energy displayed during wound healing, still the relation between the liability to injury and physiological regeneration found in different tissues is suggestive and it is probable that phylogenetically it was the liability to injury and the actual injury which caused the normal proliferative activity of cells, but that somehow in the course of phylogenetic development this quality became fixed and independent of the factor which was responsible for its origin.

17. Concerning a theory of wound healing. — In order to explain wound healing we have to explain the various processes which enter as components into this activity. These are

mainly the amœboid movements and the mitotic proliferation of the epithelium. The retraction and contraction of the wound represent secondary, more or less accidental, factors which may, however, become of practical surgical importance. The contraction is probably due to the production of connective tissue fibrils and their subsequent shortening in the course of wound healing. It does not need to concern us in this connection. The problem of amœboid cell movement and cell proliferation is essentially the same during wound healing, in the organization of blood clots and in the growth of tissues in vitro. We have discussed these problems on several previous occasions.[6] As one of the important factors in the amœboid movements of all tissue cells enters their stereotropic response. We have demonstrated the existence of this reaction particularly in the case of the epidermal cells, but it applies to all other cells equally.[7] We showed that the epithelial cells always move in contact with solid or semi-solid bodies, particularly along fibers of fibrin.[8] Later we experimentally substituted for fibrin coagulated blood serum and coagulated agar. Other solid substances can be used equally well for this purpose, as was subsequently shown by Harrison, Carrel, Burrows and others.[9]

This stereotropic response explains the movement of the epidermis along the solid floor of the wound and especially in the interstices between scab and wound, or under certain conditions along the fibers of the coagulum. In a similar way in the organization of a clot, in the growth of tissues in culture media, in the process of atresia of ovarian follicles,[10] the cells move in all possible directions in contact with the fibers and other solid surfaces.

But in addition to this stereotropic reaction there is apparently another agency at work which directs the cells away from their own tissue and into the foreign material, particularly into the wound defect. This tendency finds expression in a centrifugal, often fan- or tree-like growth, which takes place in solid media in the absence of distinct directive fibers. This induces the various kinds of cells to move into clots and especially fibroblasts to organize blood

clots; this induces likewise epidermis gradually to fill the defect, and muscle and nerve to regenerate in a centrifugal direction.

On several previous occasions we referred in this connection to the changes which take place in the amœbocytes of arthropods, especially the amœbocytes of *Limulus* after they have left the blood channels and the body and come into contact with solid material like glass. Under these conditions the formerly elliptical plates, in which granula are distributed throughout, begin to separate into a hyaline exoplasm and a granular endoplasm. The hyaline material moves in the form of pseudopodia, and more and more spreads out over the surface of the glass. At the same time the surface of the cells becomes distinctly sticky, an agglutination takes place between neighboring cells and between the cells and the foreign body, processes of dissolution occur gradually in the cells, the granules becoming smaller and gradually disappearing. Sometimes the processes of liquefaction at the surface of the cell are more far-going and lead to the sudden inflow of granules into the hyaline pseudopods. All those experimental processes, which favor the amœboid spreading out, favor likewise the dissolution processes in the cell, especially the dissolution of granules, while all those factors which prevent or retard the former have a similar preventive effect on the latter. This correspondence in effect holds good for the various changes in the chemical as well as in the physical environment. Those solutions, which without killing the cells, prevent the amœboid activity, preserve the granules and conversely those solutions that favor amœboid activity bring about a solution of the granules. Oil which prevents the contact between the cell surface and glass prevents both changes, but it is probable that other substances may likewise exert a certain preventive effect. The contact with the solid substances or other surface changes produced through chemical action bring about alterations in the surface of the cells which call forth in the cell as a whole changes of a physical as well as of a chemical nature.

It is very probable that these changes in surface tension are preceded by metabolic changes. These latter may consist perhaps in processes of ionization, which take place first in the proteins of the cell surface and later extend into the granular endoplasm of the cell. Such cell ionization leads to a taking up of water by the cell, to an increase in viscosity, liquefaction and greater stickiness of the surface. Slight changes of this kind may perhaps be reversible; farther-going changes, which are probably accompanied by other chemical processes, are not longer reversible; and thus the amœbocyte dies in a spread-out hyaline condition. Whatever the ultimate changes underlying this process may be, how much of it may be attributed to mere changes in surface tension and to what extent such changes in surface tension may merely accompany farther-going chemical and physical changes, to which we referred above, must at present remain more or less a matter of surmise. But the changes which take place in these amœbocytes suggest to us the process which may take place in all kinds of cells when stimulated by a foreign body.

When the amœbocyte leaves the body of the animal it comes under the influence of a foreign body which affects the greater part of its surface; therefore the reaction of the cell is not confined to one particular part of its surface but is of a general character. On the contrary, in wound healing, the organization of blood clots, the growth of tissues in culture media in vitro, the effect of the foreign body is polarized; it affects only one side of the cell, viz., the one directed towards the foreign body, or at least it affects this side of the cell to a much greater extent than the other sides. Like every other stimulation of cells, this polarized stimulation is connected with differences in electrical potential on the stimulated side; and this increase in electric potential may play a part in the changes in surface tension and in the accompanying chemical and physical changes which take place on this side of the tissue. This difference in electric potential will exist as long as a cell faces on one side a foreign body, while on the other side it is in contact with cells of its own kind; the latter

contact would serve to diminish or destroy this electrical potential on this side. Thus a centrifugal movement directed towards the foreign body is brought about. The presence of oxygen is necessary for this activity. In vitro the cells show, therefore, on the whole, amœboid movements only at such places where oxygen is present in sufficient quantity. Organization in the living organisms can only become definite in the neighborhood of blood vessels. Whether in addition a gradient in the quantity of oxygen may also exert a directing influence on the cells is uncertain at the present time. Thus a centrifugal growth in the direction away from the tissue and towards the foreign body is brought about. The wandering cells send out laterial pseudopoda directed towards the foreign body at an angle which corresponds to the angle under which cells branch off from the main centrifugal direction. Thus the tree- or fan-like growth is brought about which we observe so often in the coagula in vivo as well as in vitro.

At the same time the changes in stickiness to which we referred above occur at the surface; these changes lead to the manifestation of stereoptropism. The tendency to centrifugal growth is modified by the stereotropic response, and the stereotropic reaction is stronger than the strictly centrifugal tendency as long as the growth takes place in the environment of the foreign body. Therefore the cells may deviate to a considerable degree from the centrifugal growth. But in wound healing the stereotropic response strengthens, on the whole, merely the centrifugal growth.

Thus the growth continues until the effect of the foreign body is removed; that is, until the wound is closed or the clot has been organized. This is in agreement with the results of our quantitative analysis of wound healing. All the measurements show the preponderating influence of the closure of the wound on the activities of the epidermis.

But the foreign body action may lead to further changes. We noticed that all those cells, which under the stimulus of a more or less solid foreign substance are capable of amœboid movement, are likewise capable of including such foreign

particles into their cell body. We observed this especially in the case of epidermal cells growing in culture media,[8] but it applies also to fibroblasts, carcinoma cells and various other tissues; thus the acinus cells of the thyroid may include solid foreign particles into their cell body under the same conditions under which free phagocytes exert this function. The capacity for amœboid movement varies quantitatively among different kinds of cells; a similar quantitative difference we find in the phagocytic power; it is most marked in those cells which under normal conditions of life are phagocytically active, namely, in certain leucocytes and endothelial cells. The phagocytic function is merely a further application of amœboid activity, carried out under the stimulation of the foreign particles, just as the amœboid movement of the tissue cells is produced under this influence. That in both cases the same factors are at work is further confirmed by the observations of various authors [11] who showed that phagocytic activity is accompanied by increased stickiness of the surface of the phagocyte, just as we saw such an increased stickiness under the influence of the foreign body in case of amœbocytes and stereotropically responsive tissue cells. This foreign body action finds a still more interesting illustration in the formation of giant cells. The principle underlying this action is again the same. The foreign bodies cause changed surface conditions, probably as the result of chemical and physical changes which they induce and which are combined with alterations of electrical potential. If these changes are very marked, an agglutination of adjoining cells surrounding the foreign body takes place; the cells unite to form a giant cell.

All those stimuli which cause amœboid movements may also call forth mitotic proliferation in those cells in which the structure admits of such an activity. Why, in addition to amœboid movements, these cells respond to various kinds of stimuli with cell division, we do not know at the present time. The structure of the cell seems relatively more important in this reaction than the external stimulus; yet evidently the proximity of a foreign body on a wound calls forth this reaction. Under the influence of the foreign body the cells not

only increase in size, are transformed into epithelioid cells, and become more sticky and agglutinate into giant cells, but they may also multiply. Epithelioid cells may still divide mitotically and thus resemble normal regenerating cells, but cells whose surface has undergone changes which led to the formation of giant cells are thereby prevented from dividing mitotically. They show only amitotic division of the nuclei. Their protoplasm is injured under those conditions. The same factor which leads to the agglutinative type of giant cells also leads to the amitotic type. Also in other cases in which the cell lives under unfavorable conditions, a stimulus, that otherwise would bring about mitotic division of the cell, calls forth merely amitotic division of the nuclei.

Thus those epidermal cells which are farther removed from the body fluids divide amitotically under conditions under which the basal cells divide mitotically. The near relationship between those factors which make for agglutination of cells on the one hand and amitotic division of nuclei and amœboid migration on the other hand is furthermore well exemplified in the case of the placental syncytium. Here the same cells which agglutinate under the influence of the peculiar conditions under which they live become the chorionic wander cells and form amitotically plasmodia. In this case, of course, the stimulus calling forth these reactions is of a somewhat different character from the one active in wound healing.

We see, therefore, that the same stimulus produces under favorable conditions the wandering and the mitotic division of the cells, and under certain unfavorable conditions amitotic division of nuclei and agglutination of cells. As we have shown previously it is probable that the pull of the wandering epithelial cells may also secondarily call forth mitotic division. Mitotic division is evidently not dependent merely upon one kind, but may be the result of diverse kinds of stimuli.

There remains another application of the same principle; and we have drawn attention to it on former occasions. The agglutination of amœbocytes may lead to the formation of a tissue in which, under the influence of mechanical factors, definite fibrillar structures can be produced just as in certain

normal tissues mechanical factors may determine the development and direction of fibrillar structures. It is evidently also a process of agglutination of cells that brings about the formation of syncytical structures in the embryo.[12] In this case the stimulus producing the surface changes is of an unknown character.

Thus we can consider the various reactions of tissues which we observe in wound healing as a reaction towards a foreign environment, and especially towards the stimulating effect of a solid foreign body. And we see, furthermore, that reactions which in principle are the same in all living tissues may lead under various conditions to results which appear to be of a diverse nature and unconnected with each other, such as the spreading out of the amœbocyte, the covering of a defect by epidermis, the organization of a blood clot, the inclusion of a foreign body by a phagocyte and the formaton of giant cells. And even the formation of synctia and of chorionic wander cells and plasmodia in the embryo are an expression of similar reactions.

SUMMARY AND CONCLUSIONS.

Some of the conclusions which we may draw as the result of the comparative and quantitative studies of Addison, Spain, Akaiwa and the writer are as follows:

1. In response to the wound stimulus, changes take place in the epidermis which consist in amœboid cell movements, mitotic cell multiplication, increase in cell size, increase in thickness in the stratum germinativum, in the number of cell rows and in the number of cells. All these variables with the exception of the number of cells were followed quantitatively under different conditions of depth and size of wounds and were studied comparatively in different species. The curve which represents the changes in all these variables is profoundly influenced by the closure of the wound. This is the event in wound healing towards which cell activities tend and which is the turning point in the various curves.

2. The amœboid movements of the epidermis as evidenced by the formation of the tongue seems to take place steadily

until closure is reached. The two plates of epidermis, coming from opposite sides and meeting, exert upon each other a pressure which varies in strength under different conditions. In our experiments the increment in the length of the tongue remained approximately the same during different periods of wound healing. On the whole, the amœboid movement is continuous throughout the different periods of wound healing or it may perhaps show a gradual slight decrease. Exceptions from these rules occur in certain cases.

Movements of the epithelium seem, however, not to be limited to cells adjoining the wound, but to extend from farther back towards the center of the defect; a movement of cell rows from the old epithelium towards the center of the defect seems to occur and to continue until the period directly following wound closure.

3. The wound exerts a cumulative effect on all the variables except amœboid movement, in particular therefore on those depending on cell multiplication and increase in size of cell and nucleus. But at the same time the effect of the wound decreases with increasing distance from the constantly shifting border of the defect; and lastly the condition of the soil, vascularization and proliferation of the connective tissue are of importance. These three factors combined explain the variations which we find during the course of wound healing. All the variables mentioned with exception of amœboid movements show maxima at about the time of closure of the wound; either just preceding this time (in the old epithelium) or at or directly following the closure (in the center of the defect). Then a decline takes place, with a steepness which varies somewhat in the case of the different variables.

4. The curves representing the changes in cell size and number of cells at different places of the wound are more flat and more similar to each other than the curves for other variables. This applies to the several series of experiments. As far as the cell size is concerned, this applies also to the curves in the different species of animals. It appears as if the wound healing tended towards the production of a definite, and as far as possible, equal number of cells throughout the

different periods of wound healing and at the different places of the wound, and as if all the other activities were subordinated to this aim as established by Akaiwa.

5. Increasing length and increasing depth of the wound within the ranges examined increase all the activities of the tissues. Increasing length of the wound increases directly the rapidity of amœboid movement. The greater the relation of size of defect to circumference of living epithelium, the more rapid is the amœboid movement; likewise the contraction of the wound is more marked in larger wounds. Contraction of the wound is, however, a later manifestation of wound healing than epidermal amœboid movements.

The significance of contraction varies in different species. The greater contraction combined with the more rapid amœboid movements may lead to a more rapid closure of the larger wounds in certain species. In those species in which, as in the rat, contraction plays a more subordinate rôle, the closure takes place earlier in the smaller than in the larger wounds. All this applies to the range of sizes of wounds examined. Shallow wounds close more rapidly than deeper wounds.

6. Length and depth of wounds influence also the other variables which depend on cell multiplication and changes in cell size. This effect is to a great extent an indirect one, mainly depending on the time of closure. The later the closure, the greater the increase in these variables, the higher the maxima. It is, however, possible that there exists in addition a direct effect of size and depth of wounds on these variables, the larger and deeper wounds exerting a greater effect than the more shallow and smaller wounds.

7. All the activities of the epidermis during wound healing, the amœboid cell movements as well as production of living tissue, show an intensity which on the whole is parallel to the energy displayed by the tissue in the normal state. However, the wound stimulus may lead in some species to a greater use of reserve energy than in others. The maximal as well as average increase in size of cells and nuclei during wound healing, if expressed in percentage of the normal figures, is

very similar in all the species. The ratio between size of cells and nuclei is characteristic for each species; it is, on the whole, maintained in wound healing as well as in the normal tissue, although some shifting in the ratios occurs.

8. The so-called physiological regeneration of tissues is not a regenerative process, but is the expression of a primary inherited tendency to multiply, inherent in certain tissues and characteristics for each species. This primary tendency to grow and multiply determines, on the one hand, the intensity of cell multiplication and desquamation, and, on the other, the normal structure of the tissue.

9. An attempt has been made by us to explain theoretically the various phenomena measured during wound healing. The reactions seen in wound healing are considered essentially as reactions of cells towards foreign bodies. The phenomena observed in amœbocytes are taken as the prototype of these changes. Surface changes in cells leading to phenomena of agglutination occur in response to these stimuli. Amœboid movements, as well as cell multiplication, are caused by the same environmental changes. Association of stereotropism and of a tendency towards centrigfual growth explain the extension of tissues, both reactions taking place in response to the same stimulus. The centrifugal movements might result, if the cells moved constantly in the direction towards a change in electrical potential. Amœboid movements, phagocytosis and formation of foreign body giant cells represent different manifestations and degrees of the same reaction. Intense degrees of stimulation may lead to pathological reactions, such as agglutination of cells on the one hand and amitotic division of nuclei on the other, the latter taking the place of mitotic division of cells which occurs under normal conditions. It is pointed out that such a combination of syncytial formation with intense migration and amitotic division of nuclei leading to the production of plasmodia are also found in certain other cases in which the stimulus is of a different character (chorionic wander cells).

REFERENCES.

1. Some of the work on which these studies are based was done in the Pathological Laboratory of St. Louis University, in the spring and summer of 1915. At that time Dr. R. L. Thompson and the authorities of St. Louis University Medical School very generously offered me the facilities of their laboratories.

2. Loeb, Leo. Archiv. f. Entwicklgsmech., 1898, vi, 297.

3. Loeb, Leo, and Addison, W. H. F., Archiv. f. Entwicklgsmech., 1911, xxxii, 44; Addison, W. H. F., and Loeb, Leo, Archiv. f. Entwicklgsmech., 1913, xxxvii, 635; Spain, K. C., Journ. Exper. Med., 1915, xxi, 193; Spain, K. C., and Loeb, Leo, Journ. Exper. Med., 1916, xxiii, 107; Akaiwa, Hachiro, Journ. Medical Research, 1919.

4. Carrel, Alexis. Journ. Amer. Med. Assn., 1910, 55, 2148. This paper was unknown to us at the time of our later publications, probably owing to the title chosen by the author, "The treatment of wounds," which did not give any indication of the character of the contents. Our own quantitative studies on wound healing were begun at the University of Pennsylvania in conjunction with Dr. Addison during the years 1908 and 1909.

5. Walsh, L. S. N., and Loeb, Leo. Journ. Medical Research, 1918, xxxvii, 441.

6. Loeb, Leo. Anatomical Record, 1912, vi, 109. Virchow's Arch., 1903, clxxiii, 105. Biol. Bull. 1903, 301. Folia Hæmatologica, 1907, iv, 313. Pflüger's Arch., 1910, cxxxi, 465.

7. Loeb, Leo. Arch. f. Entwicklgsmech., 1898, vi, 297.

8. Loeb, Leo. Arch. f. Entwicklgsmech., 1902, xiii, 487. Journ. Medical Research, 1902, viii, 109.

9. Harrison, R. G. Journ. Exp. Zoölogy, 1910, ix, 787; 1914, xvii, 521. Burrows, M. T., Transactions Intern. Congress of Medicine, London, 1913, Sect. III, 217.

10. Loeb, Leo. Science, 1911, xxxiv, 414.

11. Sawtchenko. Arch. Biolog. Naouk., 1909, xv (cited from Barikine) Levaditi and Mutermilch. C. R., Soc. Biol., xviii, No. 22. Barikine, W. Zeitsch. f. Immunitatsf., 1911, viii, 72.

12. Loeb, Leo. Biol. Bull., 1903, iv, 301.

[We take this opportunity to correct an error in the paper by M. S. Fleisher and ourselves on the growth of tissues in the test tube, Journal Medical Research, 1919, xl, 509. On page 518, in the third last line, it should read 1/357% instead of 1/35%.]