REGENERATIVE HYPERTROPHY OF THE SKIN IN RATS

Yu. K. Man'ko

Laboratory of Growth and Development (Head – Prof. L. D. Liozner) of the Institute of Experimental Biology (Director – Prof. I. N. Maiskii) of the AMN SSSR, Moscow

(Presented by Active Member AMN SSSR V. N. Chernigovskii)

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In recent times the problem of regeneration of the mammalian skin has undergone considerable reappraisal [1-3]. It has been increasingly found that the processes taking place after the infliction of a wound cannot be dismissed simply as the drawing together of the edges of the wound and the formation of a scar. A subject that is particularly worthy of attention is that of the restorative processes taking place in the immediate surroundings of the wound.

The power of growth, shown by areas of skin of the adult animal after the infliction of a wound, was clearly demonstrated by Billingham and Medawar [5]. These workers found a considerable increase in the dimensions of small areas of skin which they left, in the form of islands, in the center of skin wounds in rabbits. The original thickness of the skin was maintained in the course of this process.

Experiments of a similar character were performed by I. V. Markelova [4] on albino rabbit. She also left islands of skin in the center of a square wound. One month after operation, the area of the islands had increased on the average threefold by comparison with their original area. I. V. Markelova further showed that reparative phenomena are observed not only in the skin of the island but also in the skin surrounding the wound.

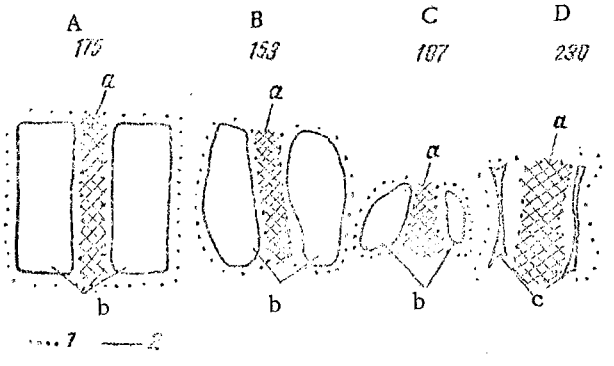

Fig. 1. Scheme of the operation in the first series of experiments and the sequence of changes in the area of the piece of skin (a) situated between the two wounds. A) Scheme of the operation; B,C,D) disposition of the wounds and ink marks in rat No. 8 at different times after infliction of the wound: B) after 24 hours; C) after 10 days; D) after 60 days. a) Measured area of skin (shaded); b) wounds; c) scars. The figures above the figures indicate the area of the piece of skin (a) in square millimeters.

In view of the inadequate study of this problem, it was important to ascertain whether these manifestations of interposed growth, observed after the infliction of a wound, are peculiar to rabbits or are also to be found in other mammals; whether, in particular, they occur in rats, in which animals it is generally accepted that the process of wound healing takes place exclusively by cicatrization and drawing together of the wound edges.

METHODS

Experiments were carried out on male white rats weighting 170 g. In order to ascertain the changes taking place in the skin adjoining the wound in the process of healing, two series of experiments were performed. In the first series we studied the changes in the piece of skin situated, in the form of strip, between two wounds inflicted on the animal's back; in the second series of experiments observations were made on the zones of skin surrounding the wound. We first tried to leave a small island of skin in the center of the wound, but we had to abandon this method for the island was always found to die. We accordingly had to resort to the modification of the experiment described above.

In the first series of experiments we used 8 animals. A tattoo mark, in the form of a square, was made on the rat's back, through a stencil, and in the center of the mark, two further rows of marks were inscribed (in ink, with the point of a needle). In the spaces between the marks, two wounds were cut, so that a strip of skin was left between them in the form of an isthmus (Fig. 1, A). The skin was excised together with the cutaneous muscle. The size of the square was 3.5 × 3.5 cm, the width of the strip of skin 0.5 cm and its length 3.5 cm. It was necessary to make a preliminary

tattoo mark because, during the healing of the wounds, their outlines of their edges and the shape of the strip underwent considerable changes. The method by which we carried out the experiments enabled us to observe the changes in the area of a definite piece of skin, delineated by ink marks (see Fig. 1, A, B, C, D-a).

The outlines of the wound and the disposition of the marks were systematically traced at various times after operation on to a celloidin film, and the tracing was then transferred to squared paper.

RESULTS

The wounds healed under a dry scab. Their dimensions decreased progressively as a result of indrawing of their edges. After removal of the scab (25-28 days after operation), scars were revealed, covered with young epidermis, considerably smaller in area than the original wounds. In the course of the first 10-20 days, the area of the strip of skin between the wounds decreased on the average by 30%, evidently on account of poor conditions of nutrition. After healing of the wounds, however, the strip began to grow. Thirty-seven days after operation the area of the strip was on the average, 124% its original area, and 4 months after operation it was 172%. In some rats the growth of the strip was particularly well marked; in one, for example, the area of the strip 4 months after the operation was 275% of the original area. This increase in the dimensions of the strip of skin left between the wounds cannot be explained purely by growth of the rats. In this space of time, the distance between marks on the back of rats of the same size, not undergoing operation, increased by only 20%.

The results of the first series of experiments thus revealed a more intensive growth of the skin situated between the two wounds; this, in our opinion, should be regarded as a restorative reaction, developing in response to the removal of an area of skin.

In order to ascertain whether the increase in the area of the skin situated between the wounds was the result of mechanical stretching, we made a histological examination of areas of skin taken at the beginning and end of the experiment. In the first case we used the piece of skin excised when the wound was made, and in the second case, pieces taken from the strip of skin itself. In the histological preparations attention was directed towards the general structure of the skin, its thickness was measured, and the number of hair groups and the number of hairs in each group were counted. The thickness of the skin and its structure in rodents are known to change considerably, depending on the phase of the growth cycle of the hair. This factor was also taken into consideration when the preparations were examined.

The results obtained showed that the skin structure was practically unchanged during the time of the experiment; its thickness also remained unchanged (Table 1).

It can be seen from Table 1 that no change was caused in the thickness of the skin by the infliction of

TABLE 1 Changes in the Thickness of the Skin of Rats during the Experiment

Rat No.	Time	Stage of hair growth	Thickness of skin (in μ)
1	Beginning of experiment (15/VII)	Rest	7.5
	End of experiment (21/XI)	Intensive growth	10
3	Beginning of experiment (15/VII)	Rest	12
	End of experiment (21/XI)	Intensive growth	10
6	Beginning of experiment (15/VII)	Rest	12.5
	End of experiment (21/XI)	Intensive growth	10
8	Beginning of experiment (15/VII)	Rest	12.5
	End of experiment (21/XI)	Intensive growth	12.5

a wound and the processes connected therewith. In those cases in which the skin was in the same stage of the growth cycle at the beginning and the end of the experiment (rat No. 8), its thickness was the same.

The number of hair groups found in one field of vision (counted in tangential sections) fell by 24% at the end of the experiment. This fall, however, was not of the same order as the increase in the area of the strip of skin.

The number of hairs in the hair groups was practically unchanged. In the original piece of skin, from 5.2 to 6.9 were counted, and in the piece of skin taken at the end of the experiment there were 5.4 to 6.6. We were unable to detect any difference in the structure of the epithelium, or in the thickness and distribution of the collagen fibers in the pieces of skin fixed at the beginning and end of the experiment. The more intensive growth of the piece of skin situated between the two wounds evidently took place as the result of processes similar to the growth processes in the undamaged skin of the animal.

The second series of experiments was carried out on 4 rats. A wound, measuring 2.5 × 2.5 cm, without an island, was produced in the middle of the animal's back. Ink marks were applied in a direction from the wound edge outwards, at intervals of 7-8 mm (Fig. 2, A). The marks were traced throughout the experiment.

The wounds decreased in size asymmetrically during healing (the transverse measurement shortened the most). The scar formed after healing of the wound was always elongated in shape. During and after the healing period, the distances between neighboring marks were observed to increase gradually; in other words, on account of growth of the skin, the marks became further

TABLE 2 Changes in the Distances Between the Marks in the Course of the Experiment, in Percentages of the Initial Distance

Zone	Before wounding	Time (in days)					
		5	10	15	26	77	182
Ia	100	111	127	139	148	174	181
Ib	100	109	116	123	126	142	139
II	100	108	109	113	128	123	127
III	100	105	102	107	119	127	116

apart from each other. This process took place irregularly. Areas of skin situated nearest the wound, especially at its sides, underwent the most intensive growth. This was in accordance with the decrease in the size of the wound mainly in the transverse direction (Fig. 2, B).

In Table 2 we give the figures showing the changes in the distances between the marks (mean values for 4 animals). The figures are given collectively for the individual zones, for in this way they reveal the characteristic trend more clearly. We designated zone I the distance between the edge of the wound and the 2nd

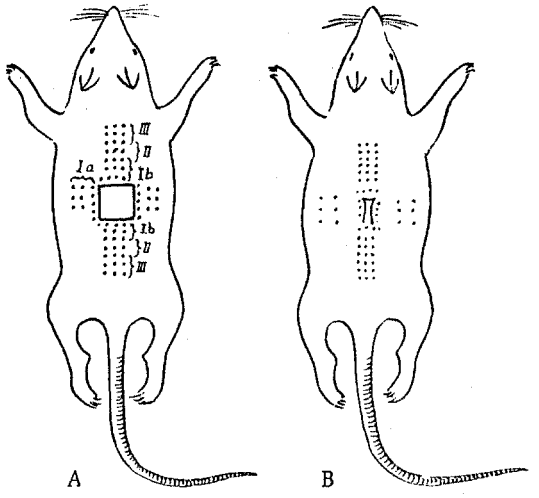

Fig. 2. Results of the second series of experiments. A) Initial disposition of the marks on the rat's back; B) disposition of the marks at end of the experiment. The Roman numerals designate the zones including the measured areas of skin.

row of marks, zone Ia including the marks situated at the sides of the wound, and zone Ib the marks anterior and posterior to the wound; zone II, the distance between the 2nd and 4th rows of marks, and zone III the distance between the 4th and 6th rows of marks (see Fig. 2).

Corresponding to the fact that the transverse measurements of the wound fell sharply, the distance between the marks of zone Ia showed a large increase; the distance between the marks in zone Ib, although it increased more than that in zones II and III, did not increase to the same extent as that in zone Ia.

In order to ascertain whether the unequal growth of the areas of skin in the rats undergoing operation was not the result of the growth of the animals themselves, we carried out the following experiment. A square was tattooed in the center of the back of 3 control rats in just the same way as in the experimental rats, and parallel rows of marks were applied in an outward direction from the square on the 4 sides; no wounds, however, were excised.

It must be mentioned that this experiment was conducted at a different time of year (November-January) and on younger animals. Measurements taken after 40 and 110 days gave the following results (Table 3).

In rats not undergoing operation, the growth of the skin in the different zones thus proceeded more or less uniformly. From this it can be concluded that the uneven growth of the areas of skin in the rats with wounds of the dorsal region was due to growth of the skin surrounding the wound, under the influence of wounding.

On the whole, the results show that in rats, just as in rabbits, the healing of extensive skin wounds is accompanied not only by mechanical drawing together of the wound edges, but also by a more intensive growth of the skin in the vicinity of the wound, leading eventually to the filling up of the defect. This reparative process may be described as regeneration, taking place by means of regenerative hypertrophy. By this term is understood the restoration of the volume and the func-

TABLE 3 Changes in the Distances Between the Marks on the Back of Rats Not Undergoing Operation (in percentages of the initial distance)

Zone	Time (in days)	
	40	110
Ia	120	132
Ib	105	135
II	118	139
III	128	128

tion of an organ, by means of processes of growth and development, taking place in the remnant of the organ, at the same time as a connective tissue scar is formed on the surface of the wound. The healing of skin wounds cannot thus be regarded simply as the formation of a scar, for, besides the drawing together of the wound edges, leading to closure of the wound, an increase is observed in the mass of the skin surrounding the wound, as a result of which the thickness of the skin remains as before.

SUMMARY

The measurement of the area of the skin located between two wounds on the back of rats and delimited by India ink marks, has demonstrated that with time it increased considerably, in 4 months time constituting 175% of the initial area. Observation of India ink marks made at a definite distance around the wounds demonstrated that the infliction of the wound intensified the growth of the areas of the skin adjacent to the wound. The initial thickness of the growing skin was preserved, and the number of the hair follicle groups was somewhat reduced. The data obtained demonstrated the development of restorative processes of the regenerative hypertrophy type in the skin surrounding the wound.

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