THE JOURNAL OF INVESTIGATIVE DERMATOLOGY
Vol. 48, No. 2
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Printed in U.S.A.

THE HEALING OF SKIN WOUNDS IN PRIMATES

I. THE KINETICS OF CELL PROLIFERATION*

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This study deals with the rate of epidermal proliferation and mitotic duration in the wounded skin of the rhesus monkey.

The mitotic count, often used synonymously for mitotic rate, mitotic activity or mitotic coefficient, has been employed by investigators to estimate epidermal proliferation during growth and in the healing process of surgical wounds. Whereas investigations have shown that the mitotic index is directly proportional to the rate of cell proliferation, it is not possible to interpret the results unless the mitotic duration during the experimental period is also known (1).

It is pertinent at this point to clarify certain aspects of cell proliferation. By definition, the mitotic rate is the average number of cells which complete their division in a unit of time, or mitotic rate = mitotic count/mitotic duration (2, 3). To calculate correctly the absolute rate of cell proliferation from the mitotic count from this equation it is necessary to know the mitotic duration. The duration of epidermal mitosis is variable (3, 4); therefore, any calculation of cell renewal must be based on the true measure of the mitotic rate. The mitotic rate and duration can be estimated by using colcemid. With this method data can be obtained on the percentage of dividing cells during a specific time period.

MATERIALS AND METHODS

Animals and Histological Procedures

Twelve young healthy male rhesus monkeys (*Macaca mulatta*) weighing between 3 and 4 kg were divided into two groups. Under aseptic conditions cuts 10 mm long and 2 mm deep were made with a blade on the thorax of each animal. An adjustable guard was applied to the blade in order to control the depth of the incisions and to obtain reproducible wounds (5). At specific time intervals during the ensuing 4 days the animals in one group received subcutaneous injections of 0.3 mg colcemid at the wound sites. The animals in the second group, used as controls, were injected with normal saline. Skin biopsy specimens obtained from each animal 4 hours after the injections were fixed for 12 hours in Susa fluid. The specimens were oriented so that the wound edge was cut transversely. Sections 6 μ thick were stained in Harris hematoxylin and eosin for histological examination, and with the PAS technic for the demonstration of glycogen. The monkeys, kept in individual restraining chairs in a room kept at a constant temperature of 21° ± 2° C., remained vigorous throughout the experiment. After removal of the biopsy specimen the wounds were allowed to heal by "secondary intention," and were fully recovered in about 10 days.

Mitotic Counts

Mitotic figures and the interphase nuclei of each tissue were counted in a unit length (1.0 mm) of epidermis, beginning at the edge of the wound, at 400 × magnification. The stages of mitosis counted were from late prophase to early telophase. Late prophase was identified from its chromosomes arranged into thick, often spiral strands (Fig. 1); in early telophase the small daughter nuclei are intensely stained (Fig 2). The count for each tissue was based on about 2000 epidermal cells; the results were expressed as a percentage of nuclei in division.

Mitotic Rate and Mitotic Time

The method used here is that of Dustin (6) and Leblond (7); full details and a critical evaluation have been given by Stevensens-Hooper (8).

Colcemid (n-desacetyl-methil colchicine) arrests mitosis at metaphase. Like colchicine, colcemid belongs to the spindle poisons, but its toxicity is 30 times lower than that of colchicine (9). The mitotic duration was estimated by means of the equation: mitotic duration = Mt/x, in which M is the number of phases of mitosis in the absence of colcemid, x the count of arrested mitoses, and t hours after injection of colcemid. The formula for calculating mitotic rate was given earlier.

Publication No. 196 from the Oregon Regional Primate Research Center, supported in part by grant FR-00163 of the National Institutes of Health and by funds from Public Health Service grant No. AM 08445-02, Biology of Skin of Man and Other Primates.

Received for publication February 26, 1966.

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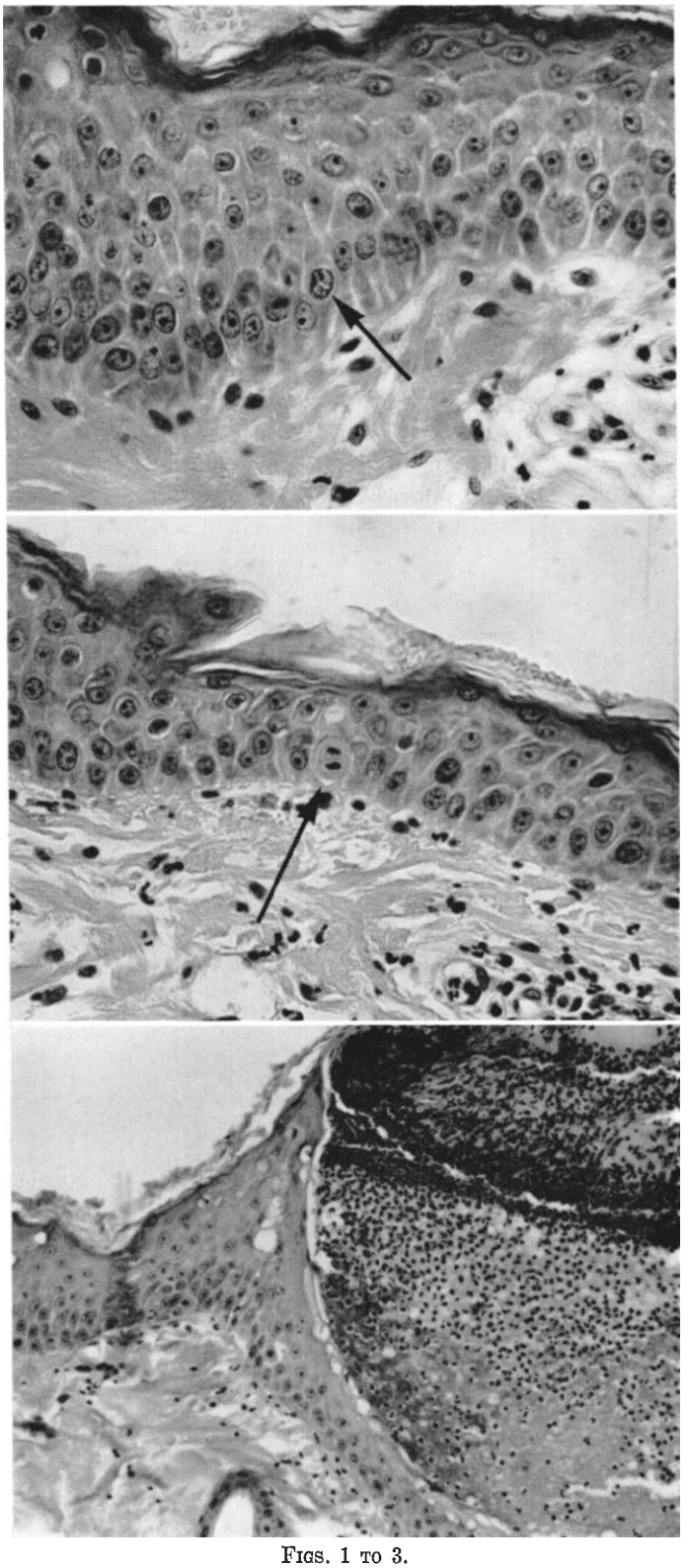

FIGS. 1 TO 3.

RESULTS

Normal Skin

For an appreciation of the changes that occur with wounding one should keep in mind the anatomical details of the normal, intact skin. A full account of the skin of the rhesus monkey has been given by Montagna *et al* (10) and only the pertinent features will be mentioned here. The epidermis of the skin of the thorax consists of a malpighian layer three cells thick and a compact stratum corneum. The dermoepidermal junction is more or less rectilinear around the hair follicles and sweat glands. The epidermis contains no glycogen and is free of melanotic melanocytes. A well-defined PAS basement membrane separates the epidermis from the dermis.

Wounds

Leukocytic infiltration occurred 4 hours after wounding. Between 16 and 18 hours a compact layer of leukocytes delineated the uninjured dermis from the wound; there was a generalized edema and vascular dilatation in the dermis, and the epidermal cells showed an accumulation of glycogen. Within 20 hours epidermal cells from the wound margin commenced to migrate inward beneath the overlying clot. After two days the epidermis had grown between the leukocytic barrier and the dermis; occasionally the advancing epithelial cells moved through the intact dermis below the leukocytic layer (Fig. 3). At the same time the connective tissue showed evidences of inflammatory exudate and a proliferation of fibroblasts. From the second to the third day the inward migration of the epithelium had advanced farther.

Experiment I

Since the estimation of the kinetic parameters must be based on combining results from different animals, we have examined the mitotic response of various animals and the degree of individual variation. Counts of the percentage of the epidermal cells undergoing division at various hour intervals and S.D. are summarized in Table I.

TABLE I

No. of Animals	Age of wound (hours)	No. of wounds studied	Average No. of mitoses S.D.
2	24	7	0.43 ± 0.08
2	36	8	0.87 ± 0.06
2	48	8	1.12 ± 0.2
2	60	8	1.23 ± 0.14
2	76	8	1.39 ± 0.25

Experiment II

The results of a number of separate experiments were combined and are presented in Table II. Although there was a noticeable increase of the mitotic rate 12 hours after wounding, the first sharp mitotic burst occurred after 32 hours. The rate remained constant for 12 hours, subsided, and after 64 hours increased to approximately 3.9%. Thereafter only minor fluctuations occurred. The intervals between the two mitotic rate maxima were about 18 hours. The pattern of fluctuation of the mitotic duration was similar to that of the mitotic rate (Table II).

The relationship between the average mitotic rate and duration is presented in Fig. 4. Mitotic duration varied inversely with mitotic rate: when the mitotic rate was high the mitotic duration was reduced, and vice versa.

DISCUSSION

The mitotic rate of epidermal cells adjacent to a wound does not remain uniformly high from hour to hour; it proceeds in a series of waves at intervals of approximately 16–18 hours. Such a fluctuating pattern of mitotic response to injury has been described recently by Hell and Cruickshank (11) in the guinea pig and by Epstein and Sullivan (12) in human epidermis. Hell and Cruickshank (11) have suggested that such a pattern of

FIG. 1. Late prophase. The chromosomes are clearly visible and arranged into thick and short strands.

FIG. 2. Early telophase. The chromosomes have coalesced; the small daughter nuclei are intensely stained.

FIG. 3. Vertical section at the edge of a 3-day wound. The epidermis is moving through the dermis tissue, much below the leukocytic layer.

response may indicate a temporary parasynchronous cell division. In wounded primate epidermis the duration of mitosis is between 1.05 and 2.51 hours. It is difficult to make a comparison between our results for mitotic duration and estimates of other workers because of the differences in the criteria used for the onset and the end of mitosis. Mitotic time has been reported to be 3.8 hours in mouse ear epidermis (13) and 2.8 hours in mouse epidermis maintained *in vitro* (14).

TABLE II

Average number of mitoses, mitoitc rate and mitotic duration in unit lengths of 1.0 mm of epidermis adjacent to a wound

Age of the wound (hours)	Mean % mitoses arrested by colcemid	Mean % of mitoses	Mitotic rate	Mitotic duration (hr)
8	0.22	0.10	0.14	1.49
12	0.86	0.25	0.64	1.18
16	0.76	0.34	0.51	1.47
20	0.79	0.24	0.70	1.12
24	0.66	0.48	0.27	2.36
28	0.78	0.53	0.32	2.43
32	1.72	1.17	0.70	2.43
36	2.97	0.87	2.70	1.10
40	2.85	0.92	2.43	1.17
44	3.50	1.20	2.86	1.22
48	3.42	1.12	2.89	1.18
52	3.56	1.81	1.76	2.02
56	3.43	1.63	2.22	1.54
60	3.05	1.23	2.24	1.36
64	4.10	1.12	3.90	1.05
68	2.06	1.44	0.83	2.47
72	2.42	1.32	1.15	2.10
76	2.78	1.39	1.39	2.00
80	3.12	1.55	1.96	1.59
84	2.10	1.45	0.85	2.45
88	2.14	1.15	1.02	2.08
92	1.76	1.26	0.70	2.51

The duration of mitosis may vary according to the condition of the epidermal cells. Our work shows that the mitotic rate is inversely related to the mitotic duration.

Several hypotheses have been proposed to explain the mechanisms that control mitotic rate. Iversen and Mercer (15, 16) offered the theory that an inhibiting substance is released by the epidermal cells during their differentiating process. Such a substance would diffuse to the basal cells and prevent them from dividing. Bullough (17) has shown that adrenaline slows down mitosis, but that by itself, adrenaline is not a mitotic inhibitor. It can act as an inhibitor if combined or in cooperation with a local inhibitor substance produced by the epidermal cells, which Bullough calls chalone (17). Evensen and Heldaas (18) found that adrenaline decreases the mitotic duration in the epidermal cells, but they did not study the possible relationship between chalone and adrenaline. The results of our investigations suggest that in wound healing an epidermal mechanism controls the rate at which cells enter division and the duration of mitosis. Adrenaline may mediate the velocity of the epidermal cells in the dividing phase, but it may have no effect on

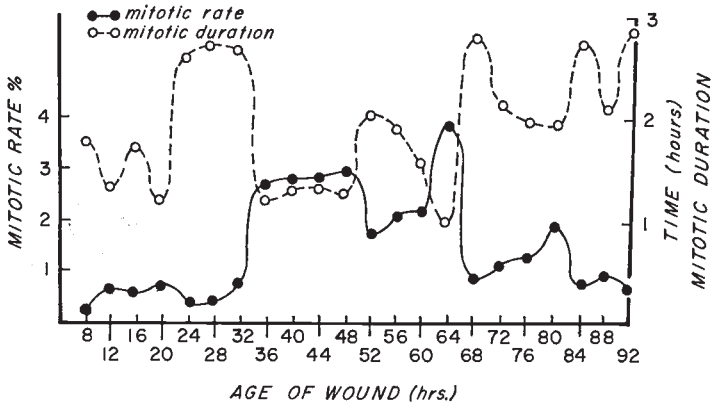

FIG. 4. Variations in mitotic duration and mitotic rate during 92 hour period after wounding. The solid line shows the percentage of mitotic rate; the broken line shows the mitotic duration.

the rate at which the cells enter into mitosis.

SUMMARY

The kinetics of epidermal cell proliferation after wounding has been investigated in rhesus monkeys. Mitotic rate and mitotic duration were determined by the colcemid technic. It was observed that the mitotic rate adjacent to the epidermal wound does not remain constant from hour to hour but proceeds in a series of waves. When the mitotic rate is high, mitotic duration is reduced, and when the mitotic rate is low, mitotic duration is prolonged.

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