1.09 ± 0.14 to 2.46 ± 0.4 μmoles (Fig. 2; $P < 0.01$). Sensitivity of acetylcholine receptors, on the other hand, was doubled. Kallikrein also caused opposite changes in the number of active receptors. Analysis of the parameter P_M showed that under the influence of kallikrein the number of actively functioning adrenoreceptors was increased by 61% whereas the number of active acetylcholine receptors was reduced by 80%.

After incubation of the isolated vessel with thrombin the number of adrenoreceptors was reduced by two-thirds, as shown by a decrease in the value of P_M from 251.6 ± 35.4 to 79.5 ± 22.7 mg ($P < 0.001$). The sensitivity of the receptors to noradrenalin also was reduced by two-thirds. This was reflected in an increase in the parameter K from 1.09 ± 0.14 to 3.22 ± 0.51 μmoles ($P < 0.001$). The number of active acetylcholine receptors was unchanged by the action of thrombin. Their sensitivity increased by 67%, as shown by a decrease in K from 0.76 ± 0.15 to 0.25 ± 0.07 μmole ($P < 0.002$).

Proteolytic enzymes of the blood thus behave as an important component of neurohumoral regulation and help to maintain vascular tone at a level which corresponds to the characteristics of the blood flowing through them.

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EFFECT OF DIBUNOL LINIMENT ON POST-TRAUMATIC REGENERATION OF THE MOUSE SKIN

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KEY WORDS: skin wounds; cell profileration; regeneration; 4-methyl-2,6-di-tert-butylphenol (dibunol).

Dibunol (4-methyl-2,6,di-tert-butylphenol) has been suggested for use in the treatment of several diseases [5, 6]. Depending on the dose used dibunol either stimulates proliferative activity of cells or has a cytotoxic action [1]. When dibunol emulsion was used to stimulate healing of skin lesions, epithelization of indolent defects was observed with the formation of a delicate and unobtrusive scar [2, 3]. The mechanism of this wound healing effect is not quite clear. When dibunol was used to treat radiation ulcers of the skin in rats, dystrophic changes in the tissue were less marked than in the control and growth of epithelium of hair follicles was stimulated [3]. Healing of full-thickness skin wounds on the dorsal region of mice, rats, and hamsters terminated nearly always with the formation of a scar both in the control and under the influence of various regeneration stimulators [4].

The investigation described below was conducted to study the action of dibunol liniment on proliferative activity of cells of the epidermis and dermis and also the rate of contraction of the wound surface during post-traumatic regeneration of the skin on the dorsal region

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TABLE 1. Effect of Dibunol Liniment on Change in Area of Defect Depending on Dose and Schedule of Application (initial area of wound 0.5 cm^2)

Experimental conditions	Area of defect			
	on 5th day		on 18th day	
	cm^2	% of control	cm^2	% of control
Control: 0.9% NaCl solution, 0.1 ml	0,43	100	0,29	100
Experiment: 5% dibunol liniment diluted in 0.9% NaCl solution, 0.1 ml of each:				
Dibunol, 50 mg/kg, 1 day	0,47	109,3	0,21	72,4
1 and 3 days	0,67	155,0	0,13	44,8
1, 3, and 15 days	0,67	155,0	0,23	79,3
Dibunol, 30 mg/kg, 1 day	0,66	153,5	0,19	65,5
1 and 3 days	0,59	137,2	0,21	72,4
1, 3, and 15 days	0,59	137,2	0,27	93,1
Dibunol, 10 mg/kg, 1 day	0,62	144,2	0,27	93,1
1 and 3 days	0,77	179,1	0,20	69,0
1, 3, and 15 days	0,77	179,1	0,15	51,7
Dibunol, 5 mg/kg, 1 day	0,69	160,5	0,24	82,8
1 and 3 days	0,71	165,4	0,24	82,8
1, 3, and 15 days	0,71	165,4	0,26	89,7

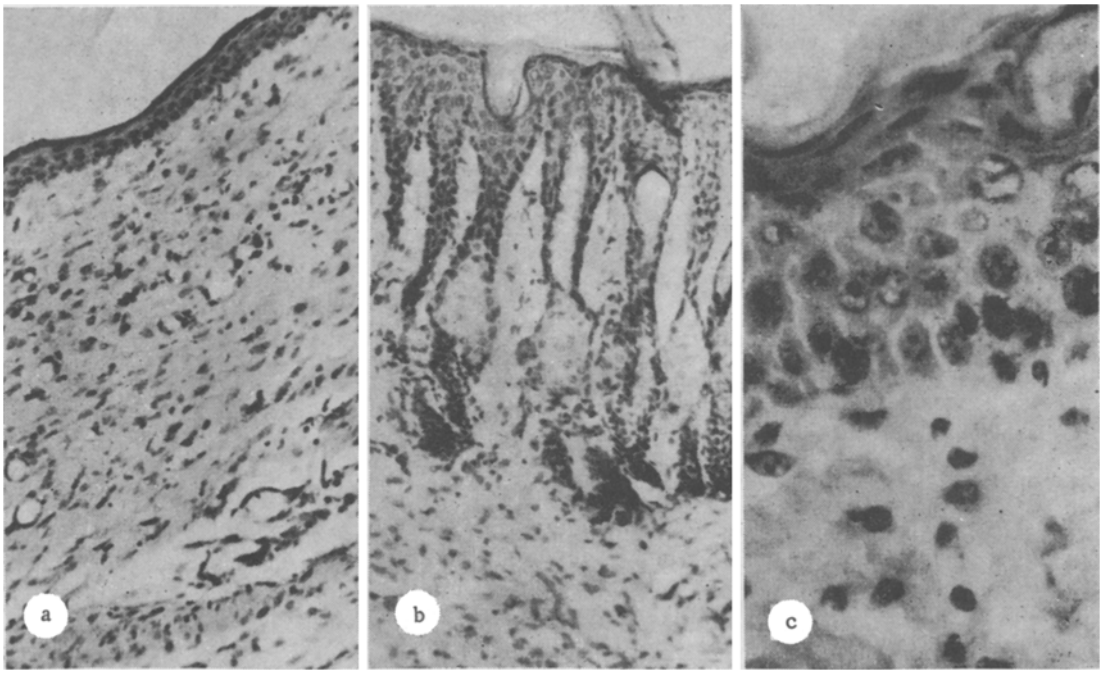

Fig. 1. Region of origin wound defect: a) regeneration of dermal type in animals of control group (24th day). Magnification 100 ×; b) normal regeneration after treatment with dibunol liniment (10th day). 100 ×; c) labeled cells in stratum basale of epidermis in skin adjacent to region of defect, when treated with dibunol liniment (3rd day). 400 ×.

TABLE 2. Effect of Dibunol Liniment on Change in Area of Defect with Large and Medium-Sized Wounds

Experimental conditions	Initial area of wound, cm^2	Preparation, dose	Schedule of application	Area of defect by 12th day	
				cm^2	percent of control
Control	3	0.9% NaCl solution, 0.1 ml	Two days before and on 1st day after wounding	0,35	100
Experiment: 5% dibunol liniment diluted in 0.9% NaCl solution, 0.1 ml	3	Dibunol, 30 mg/kg, twice	The same	0,53	151,4
Control	1	0.9% NaCl solution, 0.1 ml	On 2nd and 6th days after wounding	0,19	100
Experiment: 5% dibunol liniment dilute in 0.9% NaCl solution	1	Dibunol 20 mg/kg, twice	The same	0,25	131,6

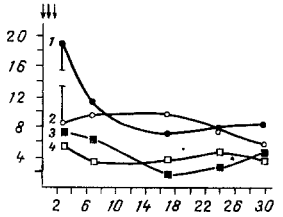

Fig. 2. Changes in LI in epidermis (1, 2) and dermis (3, 4) of animals of control group (2, 4) and mice receiving dibunol liniment externally (1, 3). Abscissa, time after wounding (in days); ordinate, LI (in percent).

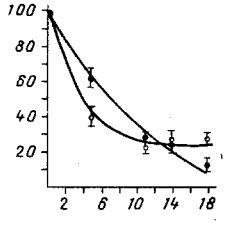

Fig. 3. Time course of reduction of area of wound defect in mice of control group (empty circles) and mice treated with dibunol liniment (filled circles). Abscissa, time after wounding (in days); ordinate, area of defect.

of mice. The quality of healing was assessed on the basis of microscopic examination of regenerating tissues.

EXPERIMENTAL METHOD

SHK mice (females and males weighing 20-22 g) were used. Under hexobarbital anesthesia (150 mg/kg) a full-thickness area of skin was removed from the dorsum of the animals in the interscapular region. The hair in the region of the defect was not epilated in order to avoid additional stimulation of healing. The area of the wound in different experiments ranged from 0.5 to 3.0 cm^2. The original preparation, a 5% liniment of dibunol, was diluted in 0.9% NaCl solution and applied once to three times to the wound in a dose of 0.1 ml of the diluted liniment, containing 1.0, 0.6, 0.2, and 0.1% of dibunol, corresponding to doses of 50, 30, 10, and 5 mg/kg. The schedule of application of dibunol is indicated in the description of the experiments and in Tables 1 and 2. Animals of the control group had their

wounds treated with 0.9% NaCl solution at the same times. The area of the wounds was measured at successive times for 18 days. Each experimental and the control group contained 6 to 10 animals.

To study proliferative activity of the cells of the epithelium and dermis, animals receiving dibunol and mice of the control group were killed by rupture of the cervical spinal cord on the 3rd, 7th, 17th, 24th, and 30th days after wounding. A single intraperitoneal injection of [^{3}H]thymidine (1 μCi/g) was given to the animals 1 h before sacrifice. Tissue from the region of the defect and adjacent skin were fixed in Carnoy's fluid and embedded in paraffin wax; serial sections 6 μ thick were cut. The sections were coated with liquid type "M" photographic emulsion, exposed for 2 weeks, developed in amidol developer, and stained with hematoxylin and eosin. The fraction of DNA-synthesizing cells was determined on autoradiographs as an indicator of proliferative activity, among 500-1000 cells separately in the epidermis and dermis, in the edges surrounding the wound in the early stages, and in the region of healing of the defect in the later stages. The fraction of DNA-synthesizing cells was expressed as a percentage of total (labeling index — LI, in percent).

EXPERIMENTAL RESULTS

During healing of full-thickness skin wounds measuring 0.5 cm^2 in area in the control animals, in 70% of cases a small epithelized connective-tissue scar was formed in the center of the original defect, and much less frequently (30% of cases) a regenerating structure of dermal type appeared, in which the collagen fibers formed a band with the characteristic structure of intact dermis, but neither hairs nor sebaceous glands were present in this regenerating tissue (Fig. 1a).

In animals receiving dibunol in a dose of 5 mg/kg daily for 3 days, in 65% of cases a normal area of regenerating tissue was present at the site of the wound, containing hairs and sebaceous glands (Fig. 1b). The connective-tissue skeleton of this regenerating tissue resembled intact dermis in the arrangement of its fibers. Scar at the site of the defect were found very rarely when dibunol was used (12%). DNA-synthesizing cells in the regenerating tissue of the control animals were found among the basal cells of the epidermis, in animals receiving dibunol, and also among cells of sebaceous glands and hair follicles (Fig. 1c). Data on changes in LI during wound healing in the regenerating epidermis in mice receiving dibunol in a dose of 5 mg/kg daily for 3 days, compared with animals of the control group, with an initial area of the wound of 0.5 cm^2, are given in Fig. 2. The use of dibunol led to considerable stimulation of proliferative activity of the epithelial cells: LI increased from 8.5 to 19% ($P < 0.05$). When the preparation was discontinued LI fell, and after 5 days it reached the control level. The proliferative activity of cells of the dermis was virtually unchanged when dibunol was used.

It can be concluded from these results that dibunol liniment has marked ability to stimulate proliferative activity of cells of the epidermis and it has an appreciable effect on the intensity of multiplication of cells of the dermis.

Data on the effect of dibunol on the rate of contraction of a wound with an initial area of 1.0 cm^2 are given in Fig. 3. Dibunol was applied in a dose of 50 mg/kg on the day of the operation and on the 3rd day. The use of dibunol led to a change in the time course of reduction of area of the defect. On the first days the area of the defect in animals receiving the preparation was greater than in the control ($P < 0.05$). By the 18th day, the defects in mice receiving the preparation were smaller in area ($P < 0.05$). Inhibition of reduction in area of the defect during the first days was observed when different doses and schedules of application of the liniment were used (Table 1), and also during treatment not only of small wounds, but also of wounds with a larger area (Table 2). This effect was dependent neither on dose nor on the schedule of application of the liniment.

The ability of dibunol liniment to inhibit contraction of the wound surface in the early stages of healing deserves special attention. Wounds in areas with mobile skin are known to be covered not only by epithelization, but also by indrawing of the wound edges toward the center, which is called contraction. Reduction in the size of wounds by contraction is linked with activity of fibroblasts, some of which acquire contractile properties and become similar to smooth-muscle cells (myofibroblasts) [7]. It can be postulated that the use of dibunol liniment leads to inhibition of the contractile function of myofibroblasts or to a decrease in their number, as a result of which contraction is inhibited.

On the basis of these results the following explanation can be put forward of the mechanism of action of dibunol liniment in the treatment of skin defects. The use of the liniment leads to selective stimulation of proliferative activity of cells of the epidermis and, at the same time, to inhibition of the process of indrawing of the wound edges. As a result, wound healing takes place without deformation of the wound surface. Under these circumstances it will be evident that conditions are created for organotypical regeneration of the skin normal in all respects.

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