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ROLE OF THE LACRIMAL GLANDS IN WOUND HEALING

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Although the history of the study of wound healing goes back many centuries, the problem still reamins of the utmost medical, general biological, and social importance. Its urgency is determined by the fact that none of the many therapeutic substances suggested until now can be regarded as a sufficiently effective agent for stimulating healing. One of the most promising ways of investigation is the search for endogeneous biologically active compounds, together with a more penetrating analysis of the physiological mechanisms of healing.

We know that during strong nociceptive stimulation lacrimal gland function is intensified, with abundant tear production [1]. The biological importance of this reaction is not clear. We submit a hypothesis according to which a nociceptive stimulus, causing injury to an organism, acts at the same time as a signal for activation of healing processes.

On this basis excitation of the lacrimal glands under the influence of pain can be explained on the assumption that the lacrimal glands participate in the healing of injuries which always arise under the influence of nociceptive stimulation. The investigation described below was undertaken to study this problem.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar albino rats weighing 200-300 g (312 animals altogether). Each series consisted of control and experimental groups, each of 8-10 animals. A circular area of skin 23 or 17 mm in diameter was removed with the aid of a suitable stencil, in the dorsal region always at the same level. The time course of healing was studied by measuring the area of the wounds. In some series of experiments, this was accompanied by the photography of the wounds, followed by projection of the wound area on tracing paper (with a constant focal length), and weighing the cut-out areas. The results of planimetry and weighing did not differ significantly, and accordingly, data obtained only by planimetry will be described. The time of complete cicatrization and epithelization of the wound was taken as an indication that healing was complete. Only those animals whose wounds were uncomplicated were considered. The results of the measurements were analyzed by the Fisher—Student method, with a 95% level of significance of differences ($P \leqslant 0.05$).

The lacrimal glands were excited by nociceptive stimulation of the conjuctiva of both eyes at the boundary with the sclera with a thermocautery (70-80°C), in the form of a small local burn. To maintain the stimulation, the thermocautery was applied four times at intervals of 3 days.

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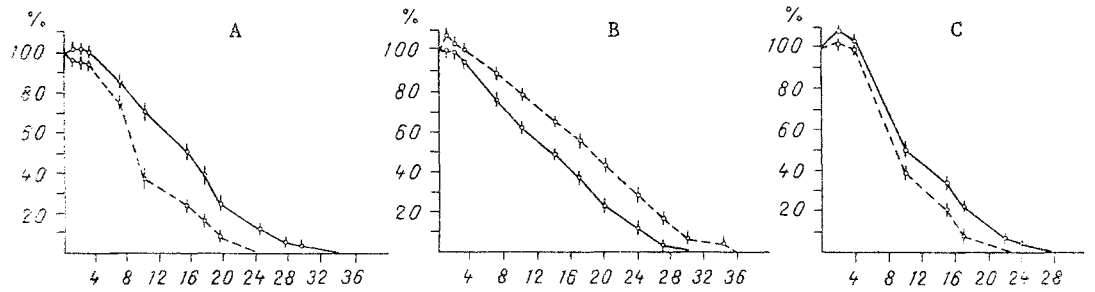

Fig. 1. Rate of wound healing in rats with excitation of lacrimal glands (A), after lacrimectomy (B), and after intraperitoneal injection of lacrimal gland extract (C). Abscissa, time (days); ordinate, changes in area of wounds (in % of original area, taken as 100). Continuous line, control; broken line, experiment.

Bilateral lacrimectomy was carried out by surgical removal of the orbital and extraorbital parts and coagulation of the palpebral parts of the glands by means of the thermocautery. The wound edges were glued together with MK-2 glue. All operations were performed under pentobarbital anesthesia (40 mg/kg, intraperitoneally), with observance of the rules of asepsis and antisepsis. Total lacrimectomy did not lead to drying of the conjunctiva and sclera of the eye. They were still moistened by the residual small glands of the eyelids and other glandular formations. To obtain extracts of the rats' lacrimal glands a homogenate of their extraorbital part was prepared in Hanks' solution buffered with HEPES (20 mM), pH 7.4, in a Polytron PT-20. After centrifugation for 60 min at 100,000g the supernatant was dialyzed and lyophilized. The residue was dissolved in Hanks' solution and centrifuged for 20 min at 30,000g, then dialyzed and lyophilized. An aliquot of the residue was used to determine total protein, and the rest of it was dissolved in 0.9% NaCl solution to the required concentrations and injected intraperitoneally twice a day for 10 days.

EXPERIMENTAL RESULTS

In the experiments of series I the effect of excitation of the lacrimal glands on healing of skin wounds was investigated. Aseptic inflammation, induced by local thermal stimulation of the conjunctiva, led to increased tear secretion. Against the background of this excitation of the lacrimal apparatus, marked and statistically significant acceleration of wound healing was observed (Fig. 1A), on average by 9-12 days, i.e., by 26-30% compared with the control. The effect of the thermal factor itself on the rate of repair was studied in a special series of experiments. Small skin burns in the region of the superciliary arches, induced with the same frequency as stimulation of the conjunctiva, did not significantly change the times of wound healing. According to some investigators [5], keeping animals together stimulates the healing of skin wounds as a result of mutual licking. Several growth-stimulating and differentiating factors have been found in the saliva of animals and, in particular, of mice and rats [2-4, 6]. In addition, the bactericidal compound lysozyme is found in saliva. In this connection the effect of excitation of the lacrimal apparatus on animals kept separately was studied. Under these conditions also, statistically significant activation of healing was invariably observed.

In the experiments of series II the effect of extirpation of the lacrimal glands was studied. Control animals underwent mock lacrimectomy and infliction of thermal burns of the skin in the region of the superciliary arches. Delayed healing of the skin wounds by 6-8 days (18-20%) compared with the control was observed in animals of the experimental groups (Fig. 1B). After lacrimectomy, an increase in width of the dorsal skin wounds was found, as early as during the first hour, and reached a maximum (26%) after 3-4 h. This fact is evidence that the lacrimal glands help to maintain normal skin tone. Neither thermal stimulation nor mock lacrimectomy had any effect on the tone of the skin wound edges. Stimulation of the conjunctiva in lacrimectomized animals did not affect the rate of healing or the phenomenon of wound widening.

In series III the effect of lacrimal gland extract on the course of healing was investigated. Doses of 0.001, 0.01, and 1 mg protein/kg body weight were used. The control animals received physiological saline. In rats with intact lacrimal glands the extract, in a dose of 0.001 mg/kg, initially accelerated wound healing statistically significantly. However, starting with the 15th day the healing process slowed down. Injection of the extract in larger doses

TABLE 1. Effect of Various Procedures on Average Times of Complete Wound Healing

Procedure	Average time of complete healing, % of control	P
Excitation of lacrimal glands		
Of animals kept together	70.1	<0.01
Separately	74.4	<0.01
Thermal stimulation of skin	96.8	>0.05
Mock lacrimectomy	94.4	>0.05
Stimulation of conjunctiva in lacrimectomized rats	102.2	>0.05
Lacrimectomy	118.0	<0.01
Injection of extract into intact rats:		
0.001 mg/kg	137.8	<0.05
0.01 mg/kg	112.6	>0.05
1.0 mg/kg	129.7	<0.05
Injection of extract into lacrimectomized rats:		
0.001 mg/kg	78.2	<0.05
0.01 mg/kg	88.4	<0.05
1.0 mg/kg	96.0	>0.05

(0.01 and 1 mg/kg) inhibited the rate of repair distinctly, evidence of a certain toxic effect. Conversely, in lacrimectomized (1 month before the experiment) rats, injection of the extract caused definite acceleration of healing (Fig. 1C). With a decrease in the dose from 1 to 0.001 mg/kg the effect was enhanced. Meanwhile injection of the extract did not block atony of the skin, which was constantly found in lacrimectomized animals. The impossibility of correcting the disturbed skin tone by means of extract of the extraorbital lacrimal glands can be explained, in our opinion, by the structural and functional heterogeneity of the different parts of the lacrimal apparatus. The appearance of definite atrophy of the skin and widening of the wounds were observed only after coagulation of the palpebral part of the glands, and they were less clearly observed after removal of the orbital and extraorbital parts. This is perhaps the reason why extract of the extraorbital glands, used in the investigation, was not sufficiently effective (Table 1).

Analysis of the experimental results indicates that the lacrimal glands produce not only substances moistening the mucous membrane of the eye and exerting a bactericidal and bacteriostatic effect, due, for example, to lysozyme [1], but also biologically active substances which enter the blood stream and participate in wound healing. The site of their formation, the mechanism of their release and action, and certain other problems require further investigation. There are also grounds for accepting as valid the hypothesis that the nociceptive afferent stimulus plays the role of triggering mechanism for activation of wound healing *in vivo*, although this problem also requires further study.

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