

THE ACTION OF TOAD VENOM ON WOUND HEALING

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There is evidence from published reports [1, 3, 4, 6, 7] and from our own observations that toad venom restores disordered function in pathological processes, and we have therefore attempted to determine its influence on wound healing.

METHODS

The experiments were carried out on 20 guinea pigs of both sexes, weighing 450-650 g. They were used for the experiment after having been kept for two days under the same living and feeding conditions. An area of hair was removed from the left side of the body near the spine; the skin was treated with 96% alcohol, and smeared with a 3% tincture of iodine. Under local anesthesia, produced by 5 ml of a 0.5% solution of novocaine injected subcutaneously, a skin wound was inflicted having an area of 350 mm². To maintain standard size of wound, a 12-bore cartridge case with sharpened edges was used to mark an outline on the skin, and a scissor cut was made along this line down to the muscles, to cause damage to the fascia. There was then a small hemorrhage, which was arrested by pressure with a sterile tampon. The tampon was then removed, and the wound was left open for the whole period of observation. The animals were then divided into two groups. The first was the control, and consisted of eight animals which received daily for eight days an intramuscular injection of 0.1 ml per 100 g weight of physiological saline. The remaining 12 guinea pigs formed the main group; they were used for a study of the therapeutic effect of toad venom. They received daily for eight days 0.1 ml per 100 g weight of 1 : 1,000 toad venom. The dose was determined by the sensitivity of the guinea pigs to the venom. In our experiments, 0.2-0.3 ml per 100 g weight of the venom diluted by 1 : 1,000 caused convulsions and death in asphyxia. Vulpian [7] obtained the same result. A dose of 0.1 ml produced no side effects.

As tests, we used the body temperature, the leucocyte count, and the area of the wound. The outline of the wound was traced in India ink on x-ray film, whence it was transferred on to millimeter graph paper. Studies were continued for 19 days. The wound area was measured every 2-3 days by Carrel's method [5], and the body temperature was measured and the leucocyte count made on the 2nd, 8th and 19th days after inflicting the wound. The results were treated statistically.

RESULTS

Table 1 shows the body temperature and leucocyte counts in both groups.

It can be seen from Table 1 that in the experimental group, after wounding, there was some increase in body temperature. However, the difference between the initial and subsequent values was not statistically significant.

The animals which received the toxin showed a greater temperature response to wounding. On the second day, the body temperature had risen by 1°. On the 8th day it had fallen somewhat, but was still 0.6° higher than the original value. The results obtained were statistically significant. On the 19th day, the temperature had returned to normal. Therefore toad venom caused a stronger response of the organism to the tissue damage.

The number of leucocytes in the control and experimental groups changed differently. In the control group, on the 2nd day after the infliction of the damage, the increase above normal was 3,800 per mm³, on the 8th day 3,500 per mm³, and on the 19th day 3,100 per mm³ above normal. The level was therefore maintained during the whole period of observation, and did not return to normal. In the group which received the toad venom, there was a considerable increase in the number of leucocytes during the first few days after the infliction of the wound. Thus, on the 2nd day, the increase in numbers was 7,500 per mm³, i.e., twice as great as in the control group; on the 8th

day the number was reduced, and exceeded the normal number by 5,800 per mm³, and on the 19th day, it had dropped to 500 per mm³ below the initial count.

TABLE 1. Changes in Body Temperature and Leucocyte Counts in Experimental and Control Animals (M±m)

Group of animals	Initial results	Day after wounding		
		2nd day	8th day	19th day
Body temperature				
Control	37.8 ± 0.2	$38.3 \pm 0.15^\circ$ $P < 0.1$	$38.2 \pm 0.25^\circ$ $P < 0.2$	$38.0 \pm 0.15^\circ$ $P < 0.5$
Experimental	37.6 ± 0.1	$38.6 \pm 0.08^\circ$ $P < 0.001$	$38.2 \pm 0.2^\circ$ $P < 0.01$	$37.6 \pm 0.03^\circ$ $P < 0.01$
No. of leucocytes per mm ³				
Control	9.2 ± 0.6	13.0 ± 1.3 $P < 0.001$	12.7 ± 0.6 $P < 0.001$	12.3 ± 0.519 $P < 0.001$
Experimental	8.6 ± 0.6	16.1 ± 1.9 $P < 0.001$	14.4 ± 1.6 $P < 0.001$	8.1 ± 0.1 $P < 0.05$

Thus, toad venom applied to injured animals induces a temperature increase and stimulates a leucocytic response, as was shown by the increased number of leucocytes and the raised body temperature, and the reduced time for the leucocyte number to return to normal. The effect is evidently due to a reduction of the inflammatory process through an increase in thickness of capillary walls, as has been described by V. I. Zakharov and I. P. Krichevskaya [2].

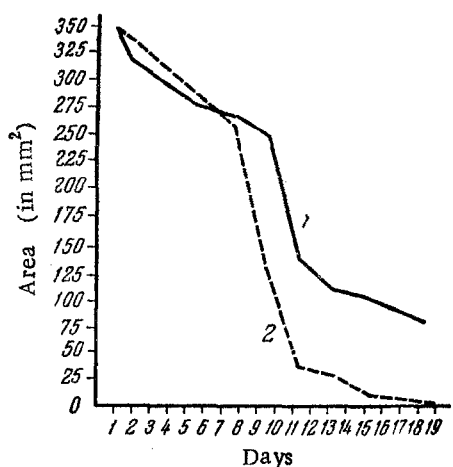
Under the influence of toad venom, the wound healing time was reduced (Table 2, see figure).

TABLE 2. Changes in the Wound Area in the Control and in the Experimental Animals (mean figures)

Group of animals	Time of measurement of wound area (in mm ²) after wounding			
	6th day	12th day	16th day	19th day
Control	325±2	147±2.6	113±4.6	89±3.3
Experimental	300±1.2 P<0.001	44±1.2 P<0.001	13±1.0 P<0.001	5±0.5 P<0.001

During the first five days in both the control and experimental animals the wound area was reduced by about the same amount. From the 6th day onwards, there was a considerable difference. In animals which had received the toad venom, the wound area was progressively reduced, until by the 12th day it was 44 mm², while in the control animals the repair process was arrested—by the 12th day the wound area was 147 mm². On the 16th day, in the

experimental group the wound area was 13 mm², and in the controls—113 mm². On the 19th day, the wound area of the experimental group was 5 mm², i.e., 1.4% of the original area, while in the controls it was 89 mm², or 25.4%



Change in the area of the skin wound inflicted in the control (1) and in the experimental (2) animals.

Thus, in the experimental group wound healing was accelerated by toad venom. At the end of the 19-day investigation, the wound area was reduced by 98.6% (average), as against 74.6% for the controls.

SUMMARY

An investigation was made into the effect of toad poisoning on the healing of skin wounds measuring 350 mm² inflicted on guinea pigs which weighed from 450 to 650 g. Injection of 0.1 ml per 100 g of toad venom daily for eight days increased the response, which by itself consisted of a rise of body temperature and of the number of leucocytes in the blood; also, these indices were more rapidly restored to the normal level. Toad venom reduced the time of skin wound healing.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. Some or all of this periodical literature may well be available in English translation. A complete list of the cover-to-cover English translations appears at the back of this issue.