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A STUDY OF REFRIGERATION AND CONSTRICTING BAND FOR EARLY TREATMENT OF PIT VIPER SNAKEBITE

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ABSTRACT

The refrigeration and constricting-band treatment was studied as a method that could be adapted to a first aid procedure for poisonous snakebites. A search of the literature revealed that snakebite authorities recognize the serious limitations and possible harmful effects of the incision-and-suction first aid procedure. This study was conducted by injecting rabbits with eastern diamondback rattlesnake venom and comparing the survival time of untreated animals and animals treated with the refrigeration and constricting-band method. A significant increase in survival time of the treated animals was indicated. Recommendations are made for first aid procedures.

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A STUDY OF REFRIGERATION AND CONSTRICTING BAND FOR EARLY TREATMENT OF PIT VIPER SNAKEBITE

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SUMMARY

A method was studied that could be adapted to a first aid procedure for poisonous snakebites. A search of the literature revealed that snakebite authorities recognize the serious limitations and possible harmful effects of the popularly used incision-and-suction first aid procedure. General agreement exists on the value of immediate immobilization of the bitten individual and of the administration of specific antivenom antiserum under medical supervision.

An investigation was made of a treatment using a loosely fitted ligature and cooling to produce local hypothermia and hemostasis. Because of the availability of the necessary apparatus and the simplicity of application, the treatment is readily adaptable to a first aid procedure. Techniques developed for measuring the diffusion rates of venom into the systemic blood circulation in treated and untreated animals were not successful. The effect of treatment on survival time of rabbits injected with eastern diamondback rattlesnake (*Crotalus adamanteus*) venom was studied. Animals treated with the refrigeration and constricting-band method survived for a significantly longer time than the untreated animals.

In view of the significant increase in survival of treated versus untreated animals and of the remarkable recovery of two treated animals (sacrificed after 3 days), additional research is recommended to evaluate the treatment further and to relate the results to human use.

The following general recommendations are also made:

1. The use of incision and suction by laymen should be discouraged pending clarification of its therapeutic value.

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2. Immobilization should be emphasized to laymen as an important first aid procedure for poisonous snakebites.

3. Broad-spectrum antibiotics should be included in snakebite kits for first aid treatment.

INTRODUCTION

Statement of Problem

The purpose of the experiments was to evaluate a new type of treatment for poisonous snakebites. A first aid treatment was studied in which cold was applied to the envenomated area by spray from a bottled refrigerant gas and in which hemostasis was achieved by use of a constricting band.

Although only 15 to 20 deaths a year are attributed to snakebites in this country, Parrish (ref. 1) calculates that 6680 snakebite cases occur annually in the United States (excluding Alaska and Hawaii). The region of Arkansas, Louisiana, Oklahoma, and Texas was found to have the highest incidence with 13.30 bites per 100 000 population per year. Of all the states, Texas recorded the highest annual death rate from snakebites (ref. 2). The pit viper snakes of the family Crotalidae (rattlesnake, copperhead, and water moccasin) are responsible for almost all of the reported bites. The coral snake of the family Elapidae (the only other poisonous snake indigenous to this country) accounts for 0.4 percent of the annual cases (ref. 1).

The pit viper venom is strongly histolytic. Extensive tissue destruction and incapacitation are common symptoms of pit viper bites. Once in the tissues, the venom initiates a rapidly lethal course of events, and measures must be undertaken to prevent systemic spread. In view of the isolated habitats of poisonous snakes, a significant time lapse can be anticipated before medical attention is available. An additional delay occurs when necessary horse-serum sensitivity tests are carried out before antivenom therapy.

Adequate knowledge of the pathophysiology resulting from the bite of a venomous snake has not been developed, and frank disagreement on treatment principles exists. There is, however, general agreement on the value of immediate immobilization and subsequent administration of specific antivenom. Optimization of first aid procedures is badly needed.

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Survey of Literature

The primary first aid method for poisonous snakebites currently recommended to laymen in the United States involves incision and suction. The technique is described in the American Red Cross First Aid Textbook (ref. 3) as the standard treatment of poisonous snakebites. All commercially available snakebite first aid kits contain devices for incision and suction in spite of numerous recent reports questioning the reliability of the method.

Variations in the incision-and-suction procedure historically have been used by man for snakebites. Widespread medical acceptance in the United States followed the experiments of Jackson and Harrison (ref. 4) in 1928. Jackson and Harrison reported that incision and suction resulted in the survival of dogs injected with four minimum lethal doses of rattlesnake venom. Two animals died following injection with the extracted exudate. The incision-and-suction method was concluded to be a rational method for treating rattlesnake bites in humans, either alone or in conjunction with antiserum. The viewpoint remained essentially unchallenged until Puranananda (ref. 5) focused attention on the ineffectiveness of the method some 30 years later. His study reported that incision and suction aggravated the injury and provided additional surface area for absorption, thus increasing the danger of the systemic spread of the venom. In 1960, Leopold and Huber (ref. 6) reported experiments on dogs and rabbits following injection of lethal doses of venom from eastern diamondback rattlesnakes (Crotalus adamanteus). Death occurred earlier in the experimental animals treated with incision and suction than in the untreated control animals. Leopold and Huber concluded that the procedure was not an effective means for removing venom. Ambrose (ref. 7) reported no clinically discernible difference in two series of patients whose treatment was the same, except that the incision-and-suction method was used in one series. In 1956, Parrish (ref. 8) reported the death of all dogs treated with immediate incision and suction following the injection of six minimum lethal doses of western diamondback rattlesnake (Crotalus atrox) venom. Ya and Perry (ref. 9) reported experiments on dogs which showed that the incision-and-suction method was useless if delayed for 0.5 hour following injection of Crotalus adamanteus venom. They also reported that survival was no higher when the incision-and-suction method was immediately carried out than when the animals were given antiserum after a 4-hour delay. Gennaro (ref. 10) worked with rattlesnake venom labeled I^{131} and reported in 1963 that removal of venom by suction was effective primarily during the first 15 minutes and was ineffective after 30 minutes. Gennaro stated that subcutaneous incisions accelerate venom spread. In a 1966 publication (ref. 11), Stahnke wrote: "The layman should be discouraged from using incision and suction. The stress involved is highly undesirable, and the double potential of infection and permanent damage due to a lack of anatomical knowledge is great. Furthermore, there is doubtful evidence that the effects of venenation could be lessened; in fact, the evidence is to the contrary."

Theoretically, by slowing metabolic and circulatory rates, chilling of the snake-bitten site would depress the systemic spread and the resultant toxic activity of the venom in the tissues. In 1906, Crum (ref. 12) reported the use of ethyl chloride to freeze the area of snakebites in his patients. Crum wrote: "I am inclined to attribute some virtue to the freezing by ethyl chloride over and above the mere anesthetic effect." In 1939, Allen (ref. 13) published results of experiments on rats, rabbits, and cats with venom from Crotalus atrox and western cottonmouth moccasins (Agkistrodon pescivorus). Although condemning the prolonged use of chilling (up to 48 hours), Allen concluded that limited use of local refrigeration offers a unique means of gaining time until antivenom can be obtained and that further investigation along this line may be of interest.

The ligature and cryotherapy (L-C) treatment popularized by Stahnke (ref. 14) in 1953 included immersion of the bitten extremity in fresh-water ice for up to 6 days.

Stahnke suggested the use of a bottled refrigerant gas to produce hypothermia as a preliminary to cryotherapy if crushed ice was not available. Since 1953, numerous investigations concerning cryotherapy as a definitive treatment have been conducted and the conclusions have been conflicting. Few references to the use of a refrigerant spray for treatment and no controlled experiments have been reported.

EXPERIMENTAL METHODS

All experiments were performed with albino rabbits of both sexes, ranging from 2 to 5 months of age and weighing 1.14 to 1.5 kg. The animals were initially anesthetized either with 2 mg/kg of acepromazine maleate or with 10 mg/kg of amobarbital sodium. Supplemental doses of the anesthetic agent were given as needed. The anesthetized animals were immobilized on a table in a spread-eagle supine position (fig. 1). Following treatment, the animals were returned to the cages and given food and water. No additional medication was administered.

Figure 1. - Position of anesthetized rabbits during treatment.

The dried venom Crotalus adamanteus was reconstituted with physiological saline immediately prior to use. Subcutaneous injections of the venom were made into the shaved lateral aspect of the left thigh. A tuberculin syringe and a 23-gage needle were used (fig. 2). The treatment consisted of using a constricting band (loose tourniquet) and of spraying the injected area with a skin refrigerant. The constricting band was applied to the leg immediately after the injection and was positioned proximal to the injection site, as close to the body as possible. The band was initially adjusted to produce an indention in the thigh muscle of approximately 0.25 cm. The band was not readjusted and became embedded in edematous tissues before the end of the treatment period. Immediately after the constricting band was fitted, an area with a radius of approximately 3 cm surrounding the point of venom injection was sprayed with either ethyl chloride or dichlorotetrafluoroethane. The properties of the two products are compared in table I.

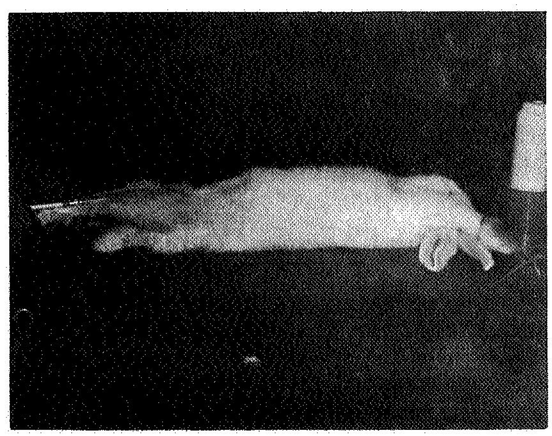

Figure 2. - Subcutaneous injection of venom into shaved, lateral aspect of left thigh.

TABLE I. - COMPARATIVE PROPERTIES OF REFRIGERANTS

Properties	Ethyl chloride	Dichlorotetrafluoroethane
Flammability.	Flammable	Nonflammable
Toxicity	Toxic	Nontoxic
Heat of vaporization, cal/g	93.7	32.7
Boiling point at 1 atmosphere, °C . . .	13.1	3.5

The area above the constricting band was not refrigerated. The spray was continued for 3 to 5 seconds, until a slight frost formation appeared on the skin (fig. 3). The procedure was repeated every 3 minutes for 2 hours. The animals after treatment are shown in figure 4. After the 2-hour treatment, the constricting bands were removed, and the animals were returned to individual cages. For convenience, the treatment using refrigeration and a constricting band will be referred to as the RCB treatment.

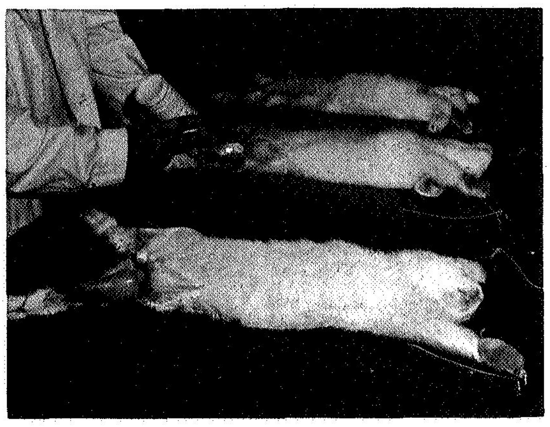

Figure 3. - Refrigeration and constricting-band treatment. Note frost formation from spray and edema distal to band. Animal in background received neither treatment nor venom injection.

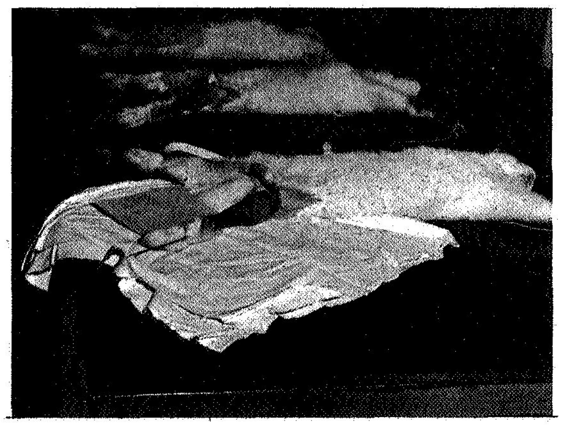

Figure 4. - Appearance of animals at end of 2-hour treatment. Animal in background received neither treatment nor venom injection.

An effort was made to measure the diffusion rate of venom from a subcutaneous site of injection into the circulating blood in treated and control animals. The techniques employed were not successful and produced no consistently reproducible results. The plasma samples of those animals injected with venom showed marked turbidity and hemolysis which made examination of the blood samples by optical methods impossible.

Animals used in evaluating the RCB treatment were divided into three groups as follows:

1. Blank animals: Three animals received only the RCB treatment to determine the effect of the treatment on the integrity of the exposed leg. Following the treatment, the animals were examined periodically for 10 days.

2. Untreated control animals: Twenty-one animals were used to determine the survival time following injection with 10 mg/kg of venom. This dosage was chosen from a preliminary study in which 10 mg/kg were found to result in a 6- to 8-hour survival time for most untreated animals. The survival times were recorded, and the mean and standard deviations were calculated.

3. RCB treated animals: Nineteen animals were used to determine the effect of the RCB treatment on the survival time. All animals were injected subcutaneously with 10 mg/kg of venom, and the RCB treatment procedures were begun immediately. The survival times were recorded, and mean and standard deviations were calculated. The Mann-Whitney U test and the Student's "t" test were used to compare the results.

RESULTS

The three animals which received only the RCB treatment (blank animals) showed no significant effects. Immediately following the treatment, the rabbit's leg distal to the constricting band was moderately edematous. The refrigerated area appeared whitish and slightly ischemic. The edema was reduced considerably in 24 hours and completely subsided after 2 or 3 days. The refrigerated areas developed petechiae and one to three small weeping lesions. At the end of 10 days, the legs appeared essentially normal, and the rabbits were moving about with no apparent discomfort or weakness.

After venom injection, 17 of the 19 RCB treated rabbits died within 72 hours, while all of the 21 untreated rabbits died in 10 hours (table II and fig. 5). Two treated rabbits were

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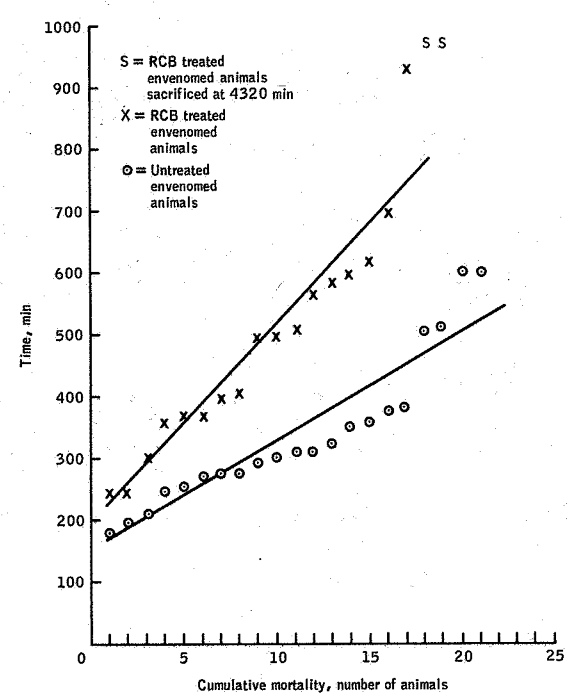

Figure 5. - Death rate of RCB treated versus untreated rabbits.

TABLE II. - THE MANN-WHITNEY U TEST[a]

RCB treated animals		Untreated control animals	
Survival time, min	Rank	Survival time, min	Rank
240	4	180	1
243	5	197	2
300	12.5	210	3
360	18.5	245	6
370	20.5	255	7
370	20.5	270	8
395	24	275	9.5
405	25	275	9.5
495	26	290	11
497	27	300	12.5
503	28	310	14.5
565	31	310	14.5
580	32	325	16
595	33	350	17
620	36	360	18.5
697	37	375	22
930	38	380	23
[b]4320	39.5	505	29
[b]4320	39.5	510	30
		600	34.5
		600	34.5

[a]$Z = 2.911$, $P < 0.0018$.
[b]Animals sacrificed after 72 hours.

sacrificed at 72 hours. These rabbits had been tested on different days and with different batches of reconstituted venom. Similar pathological sequelae were observed in each rabbit. On the second day following venomization, the rabbits that were to be sacrificed appeared to be relatively stable, in contrast to the other animals which showed generalized cyanosis. The area surrounding the site of injection of the two rabbits developed extensive tissue damage which was characterized by marked edema, hemorrhagic exudate, bulla formation, and superficial and deep ulceration. At the time of sacrifice, the animals were stuporous, and the wound had spread over the entire extremity.

The mean survival time for the 21 untreated control animals was 339 ± 117 minutes, and the mean survival time for the 17 RCB treated animals was 481 ± 176 minutes or, if the two animals that were sacrificed after 72 hours are included, 895 ± 204 minutes. The Student's "t" test indicated that the survival times for the control and treated animals were significantly different when the two groups were compared without the two surviving animals ($P < 0.01$). When the two surviving animals were included, the calculations showed unequal variances ($F < 0.011$), and the assumption for the Student's "t" test was not met. The two groups were compared using a nonparametric test, the Mann-Whitney U test (ref. 15), which does not assume equal variability. The results of the test (table II) indicated a Z value of 2.911, thereby rejecting the hypothesis that the RCB treated and untreated groups came from the same population at the $P < 0.0018$ level. The time of death of animals in the RCB treated and untreated groups is shown in figure 5.

The statistical results indicate that the data for the control and treated animals were drawn from different populations, and therefore, the mean survival time for the RCB treated animals was significantly longer than for the untreated animals.

DISCUSSION

The venom from snakes in the Crotalidae family is a poorly defined complex but contains varying proportions of proteolytic enzymes, phosphatase, neurotoxins, and hyaluronidase. The biological function of the venom is to kill the prey and rapidly digest the tissues. The effects result not only from the toxic action of the venom complex but also from the autotoxins (such as histamine and lysolecithin) formed in the tissues.

In vitro freezing of snake venom does not neutralize or alter potency, and no evidence exists to indicate that venom reacts differently when chilled in vivo. The value of the exogenous hypothermia in the treatment of poisonous snakebites appears to be in the depressing effect of chilling on the tissue reaction to the venom. Neutralization of the venom would be an indirect consequence of hypothermia and would result from the slowed absorption rate and time gained for effective mobilization of the natural body defenses against the toxins.

The absolute temperature in the tissues following local application of a refrigerant spray to the skin was not measured; however, it is reasonable to assume that if the skin surface were sprayed to the point of frost formation, the subcutaneous tissues would reach temperatures slightly above freezing, and enzymatic reactions would be slowed markedly. Because of the curvature of the pit viper fangs, venom is generally deposited in pockets at a shallow depth beneath the skin. Chilling the bitten area to the point of frost formation, therefore, substantially lowers the temperature of the venom and of the adjacent tissues and results in depression of local metabolic reactions. The low temperature in the tissues would then inhibit the systemic spread of the toxic substances by slowing perfusion and impeding circulation. The immersion of an extremity in ice water has been observed to produce a pattern of repeated peripheral vasodilation and vasoconstriction in the immersed part (ref. 16). A similar phasic vasomotor action with a longer interval of vasodilation would be expected to occur in the area treated by intermittent refrigeration as used in the experiments. The use of the constricting band in conjunction with the refrigerant spray further controls, without stopping, circulation through the area. The objectives of these measures are to obtain maximum benefit from the effects of hypothermia and slowed circulation on the venomous activity and to minimize the destructive effects of freezing and ischemia on the tissues.

The RCB treatment investigated in the experiments is not intended for definitive therapy. The treatment was studied for possible value as a first aid procedure with minimal systemic spread of the venom and minimal local tissue damage, thereby affording protection against snakebites for a significantly longer period of time than would be possible without the treatment. The data from table II and figure 5 show that the treated animals survived for a significantly longer time than did the control animals. The RCB treatment alone did not result in permanent tissue damage, which was demonstrated by the two animals receiving the treatment but not envenomed. The lesions that occurred were minor and healed rapidly with no aftereffects.

The possibility that the combined effects of the venom and the treatment could produce more necrosis than either alone must be considered. Although no evidence in the experiments supported the possibility of a combined effect, the control animals did not live long enough to allow a valid comparison of the necrotic reactions between the treated and the control animals. The only observable difference between the two groups was the discoloration of the skin following the venom injection. In the RCB treated rabbits, a slight line of demarcation developed between the bluish color of the thigh distal to the constricting band and the normal-appearing tissue proximal to the constricting band. The line became quite evident when the tourniquet was removed at the end of the 2-hour treatment (figs. 3, 4, and 6). Subsequently, the bluish discoloration spread proximally over the body of the RCB treated animals and became darker (fig. 7). The line of demarcation was still detectable in animals which lived as long as 24 hours. In the untreated animals, the discoloration spread more rapidly, showed no specific line of demarcation, and, in general, appeared darker in color. The difference in response between the treated and the untreated animals could be interpreted as an indication that the treatment slowed the local spread and the histolytic activity of the injected venom.

The two RCB treated rabbits sacrificed after 72 hours were in a morbid condition. The animals were sacrificed for pathological study and for humane consideration of the animals. Possible reasons why these animals survived so much longer than the rest of the RCB treated group could be the experimental chance, a procedural mistake, and/or the unique characteristics of the specific animals. The possibility of a procedural mistake would seem to be slight since the two rabbits were injected on different days with different solutions of reconstituted venom. Also, each of these rabbits was treated simultaneously and in an identical manner to that of two other rabbits which died within 10 hours. At the time of sacrifice, both of the rabbits were stuporous and their injected

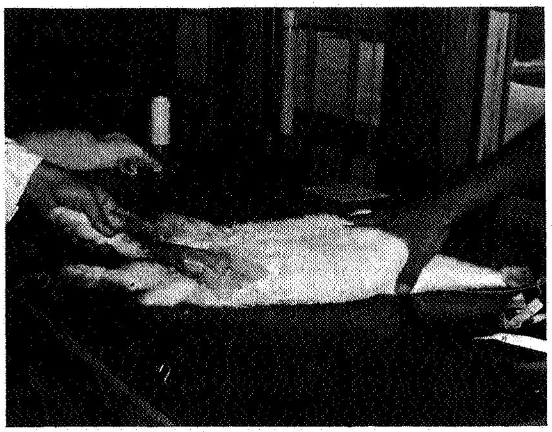

Figure 6. - Appearance of leg immediately after removal of band following 2-hour treatment.

Figure 7. - Appearance of treated animals 8 hours after venom injection. Animal in background died within 5 minutes. Animal in foreground lived approximately 1.5 hours longer.

legs showed extensive deterioration and putrefaction. Both of the animals were overwhelmed by infection. Only speculation may be made concerning the possibility of complete recovery if the animals had received antibiotic therapy.

The histolytic activity of pit viper venom creates an ideal media of necrotic and hemorrhagic tissue for bacterial growth. In the two sacrificed experimental animals, pathogenic organisms invaded the devitalized tissue subsequent to the aseptic injection of venom. In addition to this route of infection, the snakebite victim may have pathogens penetrated deep into the wound at the time of the bite. Several studies have shown the presence of pathogenic bacteria in the mouths and venom glands of poisonous snakes. Jackson (ref. 17) reported the presence of gas gangrene organisms in mouths of snakes and in snakebite wounds. Parrish (ref. 18) studied the bacterial flora in the mouths and venom glands of eastern diamondback rattlesnakes, timber rattlesnakes, and cottonmouth moccasins. Enteric and coliform organisms were found to be the most common bacterial inhabitants, and Parrish concluded that snakebites were contaminated puncture wounds. In view of the types of bacteria implicated in snakebite infections, the effectiveness of penicillin is marginal, and a broad-spectrum antibiotic is indicated for early treatment.

CONCLUSIONS

A first aid treatment for Crotalidae snakebites was investigated by using eastern diamondback rattlesnake (Crotalus adamanteus) venom. The refrigeration and constricting-band treatment consisted of producing intermittent hypothermia of the venom-injected area of immobilized albino rabbits by a refrigerant and by the use of a loosely fitted tourniquet (constricting band) for 2 hours immediately following envenomization. The refrigerant was sprayed on the skin to the point of frost formation once every 3 minutes. The animals were furnished food and water but received no medication.

Techniques for the indirect measurement of the diffusion rates of venom from a subcutaneous injection site into the systemic blood circulation were not successful.

The refrigeration and constricting-band treatment alone did not cause significant tissue damage. No observable difference was noted in tissue necrosis of venom-injected rabbits between refrigeration and constricting-band treated and untreated groups. However, a comparison of the discoloration of the skin in refrigeration and constricting-band treated and untreated animals indicated that the treatment slowed local spread and hystolytic activity of the venom.

The two refrigeration and constricting-band treated animals which survived for 72 hours had overwhelming infections. If the infections had been treated, the chances for recovery would have been greatly enhanced. Broad-spectrum antibiotics should be used in the early treatment of snakebites.

A statistically significant increase in survival times of refrigeration and constricting-band treated versus untreated animals was demonstrated ($P < 0.0018$).

The availability of the necessary apparatus and the simplicity of use make the refrigeration and constricting-band treatment readily adaptable to field use as a first aid procedure. Additional research is needed to evaluate the treatment further as a first aid procedure for pit viper snakebites and to equate the treatment in humans.

Immobilization should be emphasized to laymen as an important first aid procedure for poisonous snakebite. Incision-and-suction techniques used by laymen should be discouraged until the therapeutic value of such procedures is clarified.

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