the c axis length, 10.46 Å., from a single rotation photograph, a simple calculation gives v'

$$v' = \frac{33.60 \text{ Å.} \times 13.97 \text{ Å.} \times 10.46 \text{ Å.}}{\sin 95°42'} = 4934.2 \text{ Å.}^3$$

This value agrees well with the previously calculated v for the true unit cell, 4939.7 Å.^{3}. It should be noted that v' was obtained by a simpler procedure which is usually more rapid and, as noted above, the requirement of measuring only one, instead of three angles, usually results in a significant gain in accuracy.

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Effect of Drugs on Survival Time from Scorpion Envenomation

By LEO GREENBERG and JAMES W. INGALLS

Androctonus australis **venom at various dilutions was administered to matched groups of mice and the effects on survival time and number determined for various classes of therapeutic agents. Meperidine and other narcotics were consistently deleterious to envenomed animals as were alcohol and calcium acetylsalicylate carbamide. Neither corticosteroids nor muscle relaxants were beneficial. With tranquilizing agents, meprobamate tended to increase survival time, while reserpine markedly curtailed survival.**

In previous papers from our laboratories, we have investigated the interaction of tranquilizing drugs and various toxic agents in laboratory animals. These challenges have included curare (1), bacterial exotoxins (2–4), bacterial endotoxins (3, 4) and septicemias (2, 3). Presently, reports are in preparation on amoebiasis and trichinosis. It was of interest therefore to determine if the profound alterations in survival time evinced with tranquilizer treatment in the previous conditions could be demonstrated with arthropod venom. Moreover, it was felt that such a study might have more than academic interest since scorpion envenomation represents a major hazard to man and animals throughout much of the tropical and semitropical areas of the earth's surface. In some areas, principally North Africa, the danger from scorpions is greater than from venomous snakes (5).

It is known that the pharmacological actions of scorpion venom from a great variety of genera and species are quite similar (6) and that species widely separated by geography contain venom components in common (7). Symptoms of envenomation include muscular contractions, salivation, respiratory paralysis, and vasoconstriction. It is suggested that these indicate the existence of a substance or substances with strong parasympathetic activity acting in a similar manner to serotonin (8).

In the present study, in addition to tranquilizing drugs, other agents of real or imagined therapeutic significance in the envenomed state were included for comparison. The narcotics meperidine,[1] cocaine, codeine, and morphine were tested in light of the findings of Stahnke (9, 10) that both morphine and meperidine are undesirable therapeutic agents for use in envenomation from the Arizona scorpion, *Centruroides sculpturatus*. The analgesics calcium acetylsalicylate carbamide[2] and dextropropoxyphene hydrochloride[3] were included as possible substitutes for the narcotic agents in controlling pain. Methocarbamol[4] was tested on the basis of its reported value in cases of black widow spider bites (11), and two other muscle relaxants, carisoprodol[5] and phenyramidol hydrochloride,[6] were included for comparison. In view of the reported parasympathomimetic action of scorpion venoms, the antiparasympathetic scopolamine was included in this study. Alcohol was used in keeping with the long standing proscription against whisky in snake bite, and the barbi-

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[1] Marketed as Demerol by Winthrop Laboratories.
[2] Marketed as Calurin by Dorsey Laboratories.
[3] Marketed as Darvon by Eli Lilly and Co.
[4] Marketed as Robaxin by A. H. Robins Co., Inc
[5] Marketed as Soma by Wallace Laboratories.
[6] Marketed as Analexin by Irwin, Niesler & Co.

turates phenobarbital and hexobarbital were tested as possible substitutes for the narcotics as sedating agents. Finally, the corticosteroids were included in keeping with their role in the stress state, and in light of recent reports on the relation of cortisone and the effects of Egyptian scorpion venom (12) and protection against cobra venom by hydrocortisone (13).

MATERIALS AND METHODS

The animals used in this study were 20–25-Gm. CF1 male mice (Carworth Farms), caged in small groups and kept in the thermostatically controlled animal house for several days prior to use. In all experiments food was withheld after the venom challenge for at least 12 hours but water was allowed *ad libitum*.

Lyophilized venom of the North African scorpion, *Androctonus australis*[7] was diluted with sterile, isotonic saline immediately prior to each experimental series. Preliminary evaluation of this venom indicated an intraperitoneal LD_{50} of approximately 0.05 mg. for mice, which compares favorably with the published intramuscular LD_{50} of 0.091 mg. (5). In all experiments, 0.2 ml. of diluted venom was injected intraperitoneally.

All drugs were purchased locally or supplied through the courtesy of their manufacturers, diluted with sterile, isotonic saline, and injected intraperitoneally in a volume of 0.2 ml. approximately 30 minutes prior to venom challenge. Insoluble substances, such as meprobamate, were first suspended in a small volume of 5% acacia solution. Preliminary experiments established that at the doses employed none of the drugs were markedly deleterious to mice of the weight and strain used in these tests. In all experiments, matched groups of ten or more animals were used; experimental and control animals in any given series were inoculated within a few minutes of one another. Control animals all received 0.2 ml. of sterile saline solution. Death times were recorded at the cessation of respiration and heart beat.

RESULTS AND DISCUSSION

Following the establishment of an approximate LD_{50} for the venom sample at 0.2 ml. of 1:20,000 dilution, fifteen therapeutic agents were tested for their ability to alter survival time and number, the results being plotted in Fig. 1. From these data, several distinct patterns of response are evident. One group of agents, including the barbiturates, corticosteroids, the antiparasympathetic scopolamine, and the muscle relaxant methocarbamol, showed no evident difference from the control animals at the dose employed. Five of the drugs used, carisoprodol, ethanol, calcium acetylsalicylate carbamide, phenyramidol hydrochloride, and dextropropoxyphene hydrochloride markedly shortened survival time compared to control animals. Still more marked, both in shortening of survival time and in increase in mortality, were chlorpromazine, meperidine, and reserpine. On the other hand, of all of the agents tested, only meprobamate evidenced a moderate increase in survival time over control animals.

A similar group of drugs, in some cases at different dose levels, was then evaluated using an overwhelmingly lethal dilution of venom. These data are summarized in Table I. A general agreement with the previous figure is evident. Of all the agents tested, reserpine most sharply reduced survival time while increasing doses of meprobamate significantly increased survival time in the envenomed animals. At a dose of 50 mg./Kg., meperidine again shortened survival time as did calcium acetylsalicylate carbamide and ethanol while corticosteroid therapy again showed no significant alteration in survival time.

On the basis of these data, further studies were conducted with meprobamate and reserpine. With meprobamate treatment at various dose levels, time of administration and venom dilutions, a general trend toward increased survival was noted. However, this was neither of such magnitude nor consistency as to merit serious clinical consideration. With reserpine, however, a marked and consistent pattern of decreased survival time was demonstrated. Table II indicates the effect of a single dose of reserpine on the survival time and number in matched groups of animals inoculated with eight dilutions of scorpion venom. At all dilutions, mean survival time was decreased with reserpine treatment, and at or near the LD_{50}, mortality was increased.

In speculating on the mode of action by which reserpine exerts its deleterious effect in these experiments, it is tempting to implicate serotonin. Mention has already been made of the similarity in action between scorpion venom and serotonin (8).

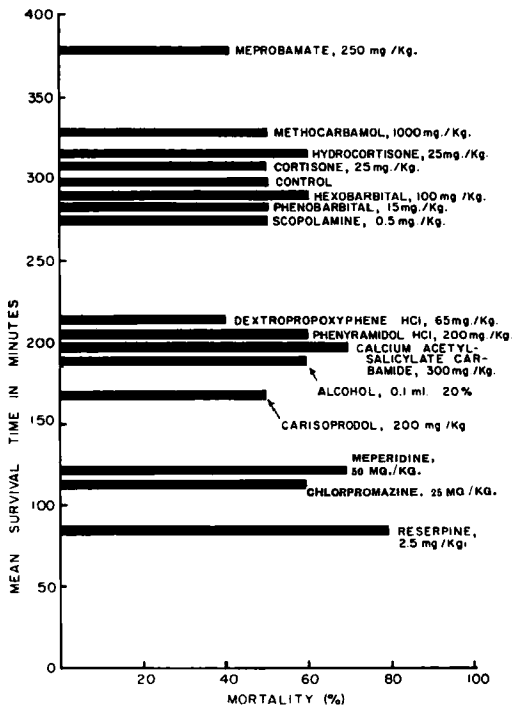

Fig. 1.—Effect of various drugs on matched groups of mice inoculated with 0.2 ml. of 1:20,000 venom (LD_{50}).

[7] Supplied through the courtesy of Dr. Lucien Balozet, Institut Pasteur d'Algérie, Algiers, French North Africa.

TABLE I.—EFFECT OF DRUGS ON SURVIVAL TIME OF MATCHED GROUPS OF MICE INOCULATED WITH A LETHAL DOSE OF SCORPION VENOM (0.2 ML., 1:2000 DILUTION)

Drug	Dose/Kg.	Survival Time, min. ± S.E.	Significance
Reserpine	2.5 mg.	14.5 ± 0.96	P = <0.01
Alcohol, 20%	0.1 ml.	14.9 ± 0.23	P = <0.001
Calcium acetylsalicylate carbamide	300 mg.	15.1 ± 1.0	P = <0.01
Hydroxyzine	25 mg.	15.2 ± 0.76	P = <0.01
Meperidine	50 mg.	15.2 ± 1.08	P = <0.02
Codeine	120 mg.	17.2 ± 0.03	P = <0.05
Cocaine	50 mg.	17.2 ± 3.0	P = >0.6
Codeine	60 mg.	17.2 ± 1.48	P = >0.3
Morphine	30 mg.	17.5 ± 1.11	P = >0.3
Meperidine	25 mg.	18.6 ± 1.12	None
Hydrocortisone	25 mg.	18.7 ± 0.76	None
Control	...	20.6 ± 1.01	...
Chlorpromazine	25 mg.	20.8 ± 0.95	None
Cortisone	25 mg.	20.8 ± 4.10	None
Morphine	15 mg.	22.5 ± 2.27	P = >0.3
Meprobamate	200 mg.	24.1 ± 1.76	P = <0.01
Meprobamate	250 mg.	26.0 ± 0.58	P = <0.001
Meprobamate	300 mg.	28.1 ± 1.08	P = <0.001

TABLE II.—EFFECT OF INCREASING DILUTION OF VENOM ON SURVIVAL TIME OF MATCHED GROUPS OF MICE WITH AND WITHOUT RESERPINE TREATMENT (2.5 mg./Kg., i.p.)

	Dilution	Mortality, %	Mean Survival Time, min.
Control	1:4,000	100	29.5
Reserpine		100	15.0
Control	1:6,000	100	34.0
Reserpine		100	24.0
Control	1:8,000	100	39.4
Reserpine		100	28.5
Control	1:12,000	100	67.5
Reserpine		100	33.0
Control	1:16,000	100	226
Reserpine		100	163
Control	1:20,000	50	425[a]
Reserpine		100	300
Control	1:40,000	0	...
Reserpine		20	...
Control	1:80,000	0	...
Reserpine		0	...

[a] Mean survival time of those animals which died.

More recently, it has been reported that serotonin is present in the venom of all species of scorpions studied, and that this ubiquitous finding cannot be fortuitous (14). Since it is well known that reserpine administration results in the release of considerable serotonin into the circulation, it is indeed possible that the overwhelming combination of exogenous serotonin in scorpion venom and endogenous serotonin from reserpine injection sharply curtails the survival time of such animals.

SUMMARY AND CONCLUSIONS

1. Venom of the North African scorpion, *Androctonus australis* was administered intraperitoneally to mice in dilutions ranging from the LD_{50} to the overwhelmingly lethal. Numerous drugs of possible significance in scorpion envenomation were tested to determine their effect on survival time.

2. Of the drugs tested, only meprobamate evidenced a trend toward increased survival in envenomed animals although this was not of sufficient magnitude to merit clinical trial.

3. Our results support previous studies demonstrating the profoundly deleterious effects of meperidine in scorpion envenomation and indicate a similar response, although not as marked, with other narcotic drugs.

4. Ethanol and calcium acetylsalicylate carbamide consistently reduced survival time while corticosteroid and barbiturate treatment showed little effect. Antiparasympathetic and muscle relaxant drugs proved of little significance or were deleterious.

5. Reserpine treatment consistently and markedly reduced survival time at all venom dilutions tested, and the likelihood that this effect is related to serotonin release has been discussed.

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