TABLE II.—RESULTS OF ANALYSIS OF THE FRACTION OBTAINED BY DISTILLING METHYL ESTERS OF SATURATED ACIDS OF OIL FROM "GUSANOS DE MAGUEY"

Temperature, °C.	162–170
Pressure, mm.	4
Iodine number	4.3
Saponification values of esters of saturated acids	205.3
Esters of unsaturated acids, %	2.99
Esters of saturated acids, %	97.01
Mean mol. wt. of esters of saturated acids	273.3
Composition of methyl esters of saturated acids, %:	
Palmitate	89.3
Stearate	10.7

The iodine numbers and saponification values of the distilled fraction were determined, and the mean molecular weight of the esters calculated according to Baughman and Jamieson (5). These results are also given in Table II.

To confirm the data in Table II, the acids were isolated from the distillate and crystallized from 59% ethyl alcohol. An acid melting at 63–64° C. was obtained, which was considered to be evidence of palmitic acid.

Using the thiocyanogen number together with the Hanus iodine number a calculation of the percentages of the glycerides in the oil was made:

Acid	Glyceride, %
Linoleic	4.3
Oleic	60.1
Palmitic	30.0
Stearic	3.6

SUMMARY

A study has been made of the composition of the glycerides of the oil from "gusanos de maguey." The oil consists of the glycerides of linoleic acid (4.3%), oleic acid (60.1%), palmitic acid (30.0%), stearic acid (3.6%). The unsaponifiable matter amounts to 2.0%.

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Pharmacological Interactions of Cobra Venom and Thiamine*

By David I. Macht and Elizabeth C. Spencer

The present investigation was stimulated by long consideration of the profound effect which cobra venom and vitamin B_1, respectively, exert on the central nervous system. The use of cobra venom in clinical therapeutics has been developed in the last eight years by French investigators (1, 2, 3) in Europe, in the United States by Macht (4, 5, 6, 7) and his collaborators, by Chopra and Chowhan (8, 9) in India, and Bullrich (10) and his school in Argentina. Injected in small doses, cobra venom produces marked analgesia and suitable preparations of the drug were first employed for relief of intractable pains of patients in the last stages of malignant disease. Macht (11) and his co-workers have shown experimentally that this relief of pain is not due to a peripheral effect on nerve endings or nerve fibers but to a direct action of the drug on the brain and that the hypothalamus is the center of cobra venom analgesia. Neurotoxin is the constituent of cobra venom which is responsible for the relief of pain. As compared with venoms of vipers, cobra venom is peculiarly rich in neurotoxin and the preparation of this drug now used in the United States is manufactured by these laboratories. Virtually it is a solution of the neurotoxic principle of cobra venom, free from the hematoxins, proteins and other harmful constituents of the crude secretion. The latest work on the chemistry of cobra neurotoxin indicates that it is not a protein and not an alkaloid but is apparently closely related to the alkaloids

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(12). In the last few years the therapeutic applications of cobra venom have been extended beyond the sphere of malignancy to relief of intractable pain of other chronic pathological conditions, especially that of severe neurological diseases. The drug has proved efficacious in trigeminal neuralgia or tic douleureux, in sciatica, multiple neuritis and other severe neuralgias. Rottmann (13) and others have employed cobra venom successfully in treatment of the crises of locomotor ataxia. Fabbri (14), Kirschen (15), Körbler (16) and Brüner-Ornstein (17) have found it effective in tic douleureux. Macht (18) and McDowell (19) have noted its efficacy in herpes zoster. Thomas (20) describes favorable results with cobra venom in acute attacks of migraine. On the other hand, the drug has been employed in treating certain nervous affections such as Parkinson's disease (21, 22) and the Canadians, Barbeau and Laurendeau (23), have reported striking results with it in several cases of epilepsy.

Thiamine hydrochloride or vitamin B_1 has also been associated with profound neurological changes and employed therapeutically in the treatment of certain nervous diseases. Indeed this vitamin is known as the antineuritic vitamin (24, 25, 26). Deficiency in vitamin B_1 is responsible for cases of multiple neuritis and other manifestations of beri-beri in both lower animals and human beings. Vitamin B_1 has proved efficacious in all kinds of polyneuritides and diseased functional affections of specific nerves such as the trigeminal and eighth nerves (27, 28, 29, 30). In such affections as the last named it has been found to relieve deafness (31, 32). It has been employed not only for the prevention and cure of beri-beri but also in treatment of alcoholic neuritis and neuralgias associated with beri-beri and pellagra. In brief, this antineuritic vitamin appears to be necessary for the integrity and normal functioning of nerve tissues (33, 34, 35, 36, 37, 38).

In view of the far-reaching and profound effects exerted upon nerve tissue and neurophysiological phenomena by two widely different compounds, cobra neurotoxin and thiamine, the writers deemed it worth while from the standpoint of general pharmacology to study the possibly antagonistic or synergistic interaction of combinations of the two drugs when administered to animals. The present investigation deals almost exclusively with experiments performed on white mice and to a lesser degree on white rats.

EXPERIMENTAL

Approximately five hundred mice have been employed in this study, which is still in progress. Half the mice were used as controls while the effect of vitamin B_1 or thiamine in relation to the toxicity of cobra venom was studied on the other half. Some of these mice were subjected to active treatment or injected with thiamine and others to passive treatment, *i. e.*, they were depleted of their normal B_1 content by means of special rations. The standard solution of thiamine in ampuls employed in all the experiments was diluted with physiological saline so that convenient concentrations of from 1 to 20 International Units could be injected into mice. Most of the injections were intraperitoneal. Results derived from special tests with subcutaneous or intramuscular injections did not differ from those obtained with the usual intraperitoneal procedure.

The results obtained in five different series of experiments may be summarized as follows:

1. The first series was made to determine the effect on mice of injections of a mixture of thiamine and a standardized solution of cobra neurotoxin. Doses of from 4 to 10 International Units of thiamine, injected simultaneously with a minimal lethal dose of cobra neurotoxin, while not altering the toxicity of the venom, appeared to exert a slightly antagonistic or protective action against it. In other words, the mortality rate in this set of mice was less than it was among the controls. Table I illustrates this antagonism strikingly. It will be seen that injection of 10 International Units of thiamine simultaneously with a lethal dose of cobra venom reduced the mortality of these mice as compared with the controls.

TABLE I.—EXPERIMENT OF JUNE 23-24

Group A
Each of 10 mice, weighing 14, 18, 22, 18, 20, 22, 19, 21, 20 and 20 Gm., was intraperitoneally injected with lethal dose of cobra venom (H., W. & D.) plus 0.5 cc. of saline.
9 dead in 24 hrs.; mortality, 90%
Group B
Each of 10 mice, weighing 21, 19, 20, 14, 21, 25, 20, 29, 20 and 19 Gm., was intraperitoneally injected with lethal dose of cobra venom plus 10 I. U. of thiamine in 0.5 cc. of saline.
6 dead in 24 hrs.; mortality, 60%

2. In the second series of experiments, standard albino mice maintained on Purina Chow were daily

injected with doses of from 2 to 4, or even 8, International Units of thiamine for two or three successive days, and on the next day the animals were injected with a standardized solution of cobra neurotoxin. In such cases the effect usually exhibited was a definite antagonism between thiamine and cobra venom; in other words, the toxicity of the venom was markedly diminished by previous saturation of the animals with vitamin B_1. This is shown by Table II, where it will be seen that the mortality effected by cobra neurotoxin was reduced one half.

TABLE II.—EXPERIMENT OF APRIL 15–17

Group A

Each of 40 mice, intraperitoneally injected for three successive days with 5 I. U. of vitamin B_1, on afternoon of third day was injected with lethal dose of cobra venom (H., W. & D.).

14 dead in 24 hrs.; mortality, 35%

Group B

Each of 40 mice, intraperitoneally injected for three successive days with physiological saline, on afternoon of third day was injected with lethal dose of cobra venom (H., W. & D.).

28 dead in 24 hrs.; mortality, 70%

3. The third group of experiments was made to determine the influence of still larger doses of thiamine on subsequent injections of cobra neurotoxin. In these animals two or three successive daily injections of 10 International Units of thiamine were followed the next day by an injection of a standard solution of cobra neurotoxin and the results of such excessive dosage with vitamin B_1 were found to be the converse of those obtained with small doses. The mice exhibited a definite toxicity and their mortality was greater than that of the controls. These findings are set forth in Table III, which elucidates this synergistic action with potentiation of toxicity.

TABLE III.—EXPERIMENT OF JUNE 18–20

Group A

Each of 12 mice, daily injected intraperitoneally with 1 cc. of physiological saline, on third day was given minimum lethal dose of cobra venom.

8 dead in 24 hrs.; mortality, 66%

Group B

Each of 12 mice, daily injected intraperitoneally with 8 I. U. of vitamin B_1, on third day was given minimum lethal dose of cobra venom.

10 dead in 24 hrs.; mortality, 83%

4. In the fourth series a study was made not of the active treatment of mice with thiamine but of the effect of the depletion of the diet of such animals of its normal B_1 content. Two sets of mice were used, one as a control being fed on Purina Chow and the other on a diet consisting of unleavened bread and water (matzoth without salt, which is definitely deficient in vitamin B_1). In ten days some of the mice showed the effect of the latter diet, developed avitaminosis and died. When the two sets of mice were compared with regard to their resistance to cobra venom, it was found that the mortality of the group fed with matzoth was much greater than that of the controls, a finding which was confirmed by use of sublethal doses of a standardized solution of cobra venom. This is illustrated by Table IV.

TABLE IV.—EXPERIMENT OF APRIL 18–28

Group A

Each of 20 control mice (fed on Purina Chow) was injected with a a sublethal dose of cobra venom, 10% less than minimum lethal dose.

Mortality, 25%

Group B

Each of 20 mice (fed for 10 days on matzoth and water, a diet partly deficient in vitamin B_1) was injected with sublethal dose of cobra venom, 10% less than minimum lethal dose.

Mortality, 45%

5. Finally, a series of tests was made on mice placed on a rigid thiamine-free diet. The formula used in preparing this ration was that of Evans and Lepkovsky (39) and consisted of casein, sucrose, autoclaved yeast and the McCollum–Simmonds salt mixture. Mice on this diet developed avitaminosis in ten days, their mortality for lack of B_1 being very great. When tested for their resistance to cobra venom, such animals revealed a higher mortality than the controls. Table V illustrates such an experiment.

TABLE V.—EXPERIMENT OF APRIL 28–MAY 8

Group A

Of 20 mice put on diet deficient in vitamin B_1, 10 with typical deficiency symptoms died after 9 days. Surviving 10 mice were injected with sublethal dose of cobra venom.

7 dead in 24 hrs.; mortality, 70%

Group B

Each of 20 control mice was injected with sublethal dose of some of the same solution of cobra venom (H., W. & D.).

6 dead in 24 hrs.; mortality, 30%

Comment.—In animals to which the two drugs were given simultaneously there thus appeared to be a definite antagonism between thiamine and cobra neurotoxin. This was especially true when two or three successive doses of thiamine were administered to mice prior to their injection with cobra neurotoxin. Indirectly this antagonism was demonstrated by deficiency experiments in which it was shown that animals kept on a diet lacking thiamine are less resistant to cobra venom than normal controls on a diet containing it. It may be remarked in passing that the authors have repeatedly observed

in connection with their assay of therapeutic solutions of cobra venom that mice obtained from dealers who are careless about their animals' feed showed a greater percentage of mortality than those supplied by reliable houses. Therefore for the assay of therapeutic solutions of cobra venom it is the writers' custom to employ mice which have been kept in the laboratory for some time and maintained on a rich standard diet such as is provided by Purina Chow.

Studies on Rats.—The greater or less resistance of mice to cobra venom in proportion to their vitamin B_1 intake suggested the possibility of thiamine deficiency playing a role in connection with the analgesic potency of cobra neurotoxin. Special tests were therefore made to obtain some light on this question. Four large healthy rats were maintained on a diet full of vitamins and the pain threshold was determined by a method described elsewhere by Macht and Macht (40). Three of the rats were injected with various doses of cobra venom and one with moccasin venom. Table VI shows that moccasin venom (very poor in neurotoxin) had little or no analgesic effect. Cobra venom injections were definitely analgesic, as pain thresholds determined on May 3 plainly indicated. All four rats were then placed on the rigid thiamine-free diet described above and their weight, as well as pain threshold, before and after injections of the two venoms was determined from time to time. The findings obtained are exhibited in Table VI. Fully a month was required to produce avitaminosis in these rats or definite loss in weight and other signs of vitamin B_1 deficiency. After six weeks, however, a marked change was noted. By that time all the animals had lost weight and the pain threshold, particularly in those which had been injected with cobra venom, was lowered, *i. e.*, less energy in volts than had been employed at the beginning was required to elicit a sensation of pain from these rats. Even more interesting was the discovery that the analgesic property of cobra venom had not been reduced; on the contrary, there was some indication at the end of the experiment that it was greater than it had been at first.

DISCUSSION

The authors have found that both cobra neurotoxin and thiamine profoundly affect the central nervous system. They therefore made tests to determine whether combinations of these drugs exerted a synergistic or antagonistic pharmacological action. It was found that suitable doses of vitamin B_1 given to mice for two or three days prior to their injection with cobra neurotoxin rendered them more resistant to the venom as their lowered mortality indicated. Much larger doses of thiamine, however, produced a hypervitaminotic condition which, though not apparent under ordinary conditions, was much enhanced and thus brought to light by administration of cobra venom. The animals succumbed to the drug in greater numbers. Other experiments with mice deprived of thiamine confirmed these findings; that is, they also proved to be less resistant to cobra venom. This interesting relation between thiamine and cobra neurotoxin indicates no lessening of the latter's analgesia.

The present investigation is of interest as it stresses the necessity of maintaining stock animals on a proper diet if they are to serve as suitable material for pharmacological assay. These findings oppose the popular notion that thiamine exerts no toxicity in large doses and show that excessive amounts of this vitamin may so affect the constitution of mice as to render them more susceptible to a poison like cobra venom. Lastly, the therapeutic implications of the findings obtained in this study are too obvious to require further comment.

SUMMARY

1. Administration of suitable amounts of vitamin B_1 to mice makes them more re-

TABLE VI.—EXPERIMENTS ON PAIN THRESHOLD OF RATS

Date, 1941	Rat No.	Normal Threshold, Volts	Venom Injected	Pain Threshold in Volts after 45 Min.	1½ Hrs.	2 Hrs.	Weight, Gm.
May 3	1	368	Moccasin, 1 Gm.	325	300	325	280
	2	368	Cobra, 1 m. u.[a]	770	630	715	230
	3	325	Cobra, 1.5 m. u.	400	630	715	240
	4	350	Cobra, 2 m. u.	445	890	953	225
May 14	All rats put on diet deficient in vitamin B_1						
May 26	1	368	Moccasin 1 Gm.	350	368	...	280
	2	368	Cobra, 1 m. u.	575	575	...	226
	3	325	Cobra, 1.5 m. u.	575	715	...	236
	4	350	Cobra, 2 m. u.	400	953	...	220
June 9	1	368	Moccasin, 1 Gm.	400	368	...	260
	2	368	Cobra, 1 m. u.	665	575	...	210
	3	265	Cobra, 1.5 m. u.	776	715	...	200
	4	350	Cobra, 2 m. u.	715	715	...	210
June 20	1	218	Cobra, 1 m. u.	300	368	...	200
	2	280	Cobra, 1 m. u.	350	715	715	160
	3	180	Cobra, 1.5 m. u.	300	715	715	170
	4	180	Cobra, 2 m. u.	300	715	890	200

[a] Mouse unit, the quantity required to kill a white mouse weighing 22 Gm. within 18 hrs. of intraperitoneal injection of the drug.

sistant to cobra neurotoxin while excessive doses of thiamine are toxic in this respect.

2. Mice maintained on diets deficient in vitamin B_1 are less resistant to cobra venom.

3. Such a deficiency of thiamine in rats does not affect the analgesic potency of cobra neurotoxin.

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