

The alcohol in the toxic dose of the tincture was about 0.00280 mil per gram of body weight.

Since the amounts of alcohol injected in these experiments are so near the minimum fatal dose of alcohol, further experiments must be made to determine the relation of the alcohol present to the minimum fatal dose of the tincture of digitalis.

My thanks are due the Powers-Weightman-Rosengarten Company for the presentation of the rats used in these experiments.

I am also indebted to Dr. William A. Pearson for his valuable assistance and suggestions, and for his kindness in granting the use of the John Clifford English Laboratories of Physics and Chemistry for these experiments.

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TOXICITY OF STROPHANTHUS TO WHITE RATS.*

BY DAVID W. BEDDOW, JR.

An attempt was made, in the following investigation, to ascertain the toxicity of *Strophanthus* to white rats. The preparation used in this experimentation was the official preparation, *Tinctura Strophanthi*, U. S. P., each one hundred mils of the tincture representing ten grams *strophanthus* in approximately ninety-two per cent of alcohol.

Before beginning the experimental work, it was deemed advisable to standardize the particular tincture which was to be used. A biological assay of the tincture was made by Grant Favorite, according to the one-hour frog method, as described in the United States Pharmacopœia, IX (2). As a result of his determinations, it was found that the minimum lethal dose of this tincture was 0.00006 cc. for each gram of body weight of the frog.

White rats, ranging in weight from one hundred and fifty to two hundred grams, were obtained and prepared for injection in a manner similar to the method adopted by the Hygienic Laboratory at Washington, D. C. (1). The animals were fed twelve to fifteen hours before injection of the drug in order to insure accuracy. Just prior to injection each animal was weighed, marked, and the amount of the drug

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to be administered calculated in proportion to the body weight of the rat. The animal was then bound to a suitable operating board. The skin over the anterior surface of the thigh was cleaned with alcohol, the hair shaven, and a small incision made over the saphenous vein, exposing the vein for a distance of about one centimeter. The syringe was filled with the calculated amount of the drug, and, after the vein was compressed, the needle of the syringe was inserted into the vein, and the contents emptied. Particular care was taken in every case to administer the contents of the syringe, at a definite rate of flow. The rate adopted was one mil per sixty seconds, as this rate proved most efficient in the majority of cases.

Early in the experimental procedure it was observed that the white rat succumbed to comparatively small doses of the tincture and, since the tincture was first administered in undiluted form, it was deemed advisable to determine the toxicity of alcohol on the white rats. A number of preliminary experiments were performed using various dilutions of alcohol with physiological saline solution. The results of these observations proved that the most satisfactory dilutions for determining the toxicity of the alcohol was a dilution containing sixty parts of alcohol diluted with forty parts of physiological saline solution. Varying doses, of this solution, in proportion to the body weight of the animal were injected intravenously, care being taken in this case also to inject at the rate of one mil per sixty seconds. The results of these determinations are tabulated below.¹

It was found, as seen by the results of the above tables, that the toxic dose of alcohol for the rat is 0.00345 mil of ninety-five per cent alcohol per gram of body weight. Advantage of this determination was taken and a solution containing fifty parts of tincture strophanthus to fifty parts physiological saline solution was used for determining the toxicity of the tincture. The results obtained from these observations are tabulated below:

TABLE III.—FATAL DOSES OF STROPHANTHUS.

Rat.	Weight.	Mil injected.	Mil of tincture per Gm. body wt.
1	240 Gm.	1.0 mil	0.0020833
2	230 Gm.	0.95 mil	0.0020652
3	205 Gm.	0.84 mil	0.0020540
4	220 Gm.	0.90 mil	0.0020454
5	200 Gm.	0.80 mil	0.0020000
6	150 Gm.	0.60 mil	0.0020000
7	178 Gm.	0.70 mil	0.0019662
8	178 Gm.	0.70 mil	0.0019662
9	173 Gm.	0.65 mil	0.0018786
10	173 Gm.	0.65 mil	0.0018786
11	200 Gm.	0.69 mil	0.0017250
12	174 Gm.	0.60 mil	0.0017241
13	170 Gm.	0.58 mil	0.0017200
14	170 Gm.	0.58 mil	0.0017200
15	145 Gm.	0.49 mil	0.0017195

Thus by progressively decreasing the dosage, in proportion to the body weight of the animal, there was found to be a certain point, beyond which any decrease in the dosage failed to prove toxic to the animal. This is evidenced in the following table:

¹ For toxicity of alcohol tables see preceding paper.

TABLE IV.—NON-FATAL DOSES OF STROPHANTHUS.

Rat.	Weight.	Mil injected.	Mil of tincture per Gm. body wt.
1	170 Gm.	0.5 mil	0.0014705
2	170 Gm.	0.5 mil	0.0014705
3	174 Gm.	0.55 mil	0.0015804
4	174 Gm.	0.55 mil	0.0015804
5	174 Gm.	0.55 mil	0.0015804
6	169 Gm.	0.57 mil	0.0016863
7	169 Gm.	0.57 mil	0.0016863
8	227 Gm.	0.77 mil	0.0017048
9	227 Gm.	0.77 mil	0.0017048
10	147 Gm.	0.50 mil	0.0017142
11	175 Gm.	0.60 mil	0.0017142
12	147 Gm.	0.50 mil	0.0017142
13	147 Gm.	0.60 mil	0.0017142
14	175 Gm.	0.60 mil	0.0017142
15	175 Gm.	0.60 mil	0.0017142

From results of these determinations, it was found that the toxic dose of Tincture of Strophanthus is 0.0017195 mil per gram body weight of the white rat. However, since the tincture contains ninety-two per cent alcohol, it is found by calculation that the amount of alcohol in the minimum fatal dose of tincture of strophanthus is only about 50% of the minimum fatal dose of alcohol.

CONCLUSIONS.

1. Since the amount of alcohol in the minimum fatal dose of the Tincture of Strophanthus is only about fifty per cent of the minimum fatal dose of alcohol, as was shown by the tables, it may be assumed that the toxicity of Strophanthus must be due to the active principles of the tincture and not to the toxic effect of the alcohol contained in it.

2. The toxic action of Strophanthus acts comparatively more quickly in the case of the rat than in other animals, since it was found that after administering a fatal dose, the rat died within a few minutes after injection of the drug; and in every case in which the rat survived ten minutes after injection, the dose proved non-fatal.

3. The Tincture of Strophanthus is about twenty-five to thirty times as toxic to the frog as it is to the white rat; and is also about thirty times as toxic to the rabbit as it is to the rat. The latter has been assigned to the congenital tolerance which the rat has to strophanthus (3).

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DETERMINATION OF MORPHINE SULPHATE IN TABLETS AND GRANULATIONS.*

BY R. E. SCHOETZOW.

The estimation of morphine has always been of peculiar interest to the pharmaceutical analyst. This alkaloid with its solubilities in the various solvents so different from those of the other alkaloids has required analytical methods especially adapted to it. Probably the average chemist when he first attempts to assay the sulphate of this alkaloid tries the U. S. P. method given under opium. But, if he is working with tablets or granulations that contain, for example, milk sugar, he will not be satisfied with the results obtained. It is the writer's opinion that the presence of sugars render this assay inaccurate.

Quite a number of methods for the determination of morphine sulphate in tablets have been proposed and used, but those most widely employed appear to be of two classes—one based on the chloroform-alcohol separation of the alkaloid from its aqueous solution, made slightly alkaline with ammonia water, and another based on the ammonia precipitation of morphine from aqueous solution. These methods or variations of them have both been used by the writer in the past, but in my hands and by those working with me these methods did not give consistent results. For three or four successive times the methods might work very satisfactorily, then the next time the results would fall far short of theory. So that finally we came to the conclusion that we could not trust either of them. Some time ago H. A. Osborne and the writer, in considering the various methods of estimation, concluded that we could not hope to attain satisfactory results as long as water was present in the separation stage of morphine from the other ingredients of the mixture. We decided that the solubility of morphine in water, the possibility of reversible reactions, the presence at times of morphine salts instead of free morphine in the alkaloid obtained for titration, indicated that we should try to separate morphine in a non-aqueous media. The best way to do this seemed to be as follows:

ASSAY FOR MORPHINE IN GRANULATIONS OR TABLETS CONTAINING MORPHINE SULPHATE AS THE ONLY ALKALOIDAL INGREDIENT.

Place an accurately weighed portion of granulation, theoretically equivalent to 0.260 Gm. Morphine Sulphate, U. S. P., or in the case of tablets, as many tablets as are theoretically equivalent to four grains (0.260 Gm.) of Morphine Sulphate, U. S. P., in a small, glazed, porcelain, evaporating dish. Dissolve in as small a quantity of water as possible. Add sufficient sodium bicarbonate—in about one gram portions—mixing well with a small stirring rod to make a stiff mass. Place

* Scientific Section, A. Ph. A., Des Moines meeting, 1925.