TINCTURE OF CANTHARIDES AND ITS ASSAY.

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Two years ago I had the privilege of calling the attention of this Association to the onery character of cantharides and the deceitfulness of its tincture.

Further experiments on these bugs which are herein reported, again bear testimony to their bug-nature and irritating behavior and the "equally successful" results which are obtained when they are "extracted."

First let me remind you that the active principle of cantharides is cantharidin —a body of an anhydride character, and obstinate action—whose chief peculiarity, from a pharmaceutical standpoint, is its contrariness with solvents. Chloroform, acetone, benzol and acetic ether are its best solvents, but one is continually impressed with its indifference even to these. Chloroform dissolves it most readily, but while it is stated to be quite as soluble in acetone, yet it dissolves in this with exasperating slowness, and in acetic ether it is just as bad. Indeed, in any solvent which I have tried, satisfactory results are only obtained when the solvent is used warm, or the action is allowed to continue for prolonged periods.

A series of tinctures was made, representing 5 grams of drug in 100 cc. and with varying menstrua. All tinctures contained 5 cc. of glacial acetic acid in 1000 cc., which acid was used in the menstruum employed to moisten the drug, and was employed for the purpose of liberating the combined cantharidin in the drug. The drug contained 0.65 per cent. of cantharidin. Following are the results:

	Menstruum	Process	Strength	Per cent. of exhaustion
No. 1	Alcohol	Percolation	0.0092% 0.010%	30
No. 2	Acetone ..... 50 Alcohol 950	Percolation	0.012% 0.013%	38
No. 3	Chloroform ..... 50 Alcohol 950	Percolation	0.018%	54.5
No. 4	Acetone .....100 Alcohol 900	Percolation	0.014% 0.015%	45
No. 5	Chloroform .....100 Alcohol 900	Percolation	0.014% 0.016%	45
No. 6	Acetic ether.....125 Alcohol 875	Percolation	0.014% 0.016%	45

In all the above, maceration was continued 48 to 72 hours, then percolation was allowed to proceed very slowly, the final yield being obtained by pouring on alcohol. It will be noted that in the best tincture obtained the drug was but little more than half exhausted, and in this case the chloroform was all used in the preliminary maceration. But the tincture precipitated badly on standing, while No. 5, which was made with a mixed menstruum, remained clear.

No. 1 was made by first extracting the drug with petroleum ether to remove the

fatty matters, thinking that these might hinder the extraction with alcohol. The drug yielded 8.22 per cent. of fat and the fat showed a slight activity—producing a small blister on three of seven victim-volunteers after 12 hours' application, but no irritant effects in four hours in any of the seven.

Ten Per Cent. Tinctures. Nine of these were made and tested, as follows, all containing 0.5 per cent. of acetic acid by volume.

Menstruum	Process	Strength	Per cent. of exhaustion
No. 1 Alcohol	Percolation	.016 .020	27.7
No. 2 { Acetone ..... 50 Alcohol 950	Percolation	.022 .024	37.0
No. 3 { Chloroform ..... 50 Alcohol 950	Percolation	.0165 .0175	26.1
No. 4 { Acetone .....200 Alcohol 800	Percolation	.0175 .0190	27.1
No. 5 { Chloroform .....200 Alcohol 800	Percolation	.0165 .0180	26.1
No. 6 { Acetic ether.....250 Alcohol 750	Percolation	.029 .031	46.0
No. 7 { Acetic ether.....250 Alcohol 750	Digestion at 40° C.	.043 .047	66.0
No. 8 { Acetic ether.....250 Alcohol 750	Digestion at boiling	.045 .047	66.0
No. 9 { Acetic ether.....400 Alcohol 600	Digestion at boiling	.045 .050	74.0

What can one conclude from figures like these? Apparently alcohol is just as good a menstruum as mixtures of alcohol and chloroform, or alcohol and acetone, in spite of the fact that acetone and chloroform are good solvents for cantharidin, while alcohol is not. Again, it is noticeable that a quarter to a third of the cantharides is exhausted by cold percolation.

The one fact which stands out clearly in the above is that digestion is of decided advantage in extracting cantharides with alcoholic menstrua, and it is also evident that a short digestion at 40° C. is as effective as long boiling, since No. 7 was digested at 40° for 6 hours, No. 8 was boiled 6 hours, and No. 9 was boiled 12 hours.

Furthermore, this line of tinctures does not equal those reported in 1910, in which a menstruum of 10 per cent. glacial acetic acid and 90 per cent. of alcohol was used and which showed an exhaustion of 90 per cent, 76 per cent. and 88 per cent. by cold percolation, on three different drugs.

Some experiments were made with cantharidin to learn if an alkaline extraction would be likely to prove effective. It was found that alcohol containing ammonia

will dissolve cantharidin very slowly in tincture strength, requiring four to six days for solution. Potassium hydroxide will not dissolve cantharidin in alcohol in tincture strength, the potassium cantharidinate being almost insoluble in the fluid. These results did not encourage an attempt at alkaline extraction and none was made.

Furthermore, an alkaline tincture would not produce the blistering effects of cantharides when applied externally, and so would not truly represent the drug.

The first of the above tinctures were made from fat-free cantharides, obtained as before by extracting the fat with petroleum ether and drying the mass. This process undoubtedly removes a small portion of the cantharidin, but the loss is probably very small. Still, since it does not aid extraction materially, and the tincture itself shows no advantages in color, clarity or appearance, the method offers no advantages.

The different tinctures show small variations in color, but all remain practically clear, or with slight precipitation after eight months. The difference in odor is less than one might anticipate, since the heavy odor of cantharides stands out plainly in all menstrua.

With regard to the assay of the tinctures, it was found that evaporation on sawdust at a temperature not exceeding 40° C., then treating the sawdust as a drug, gave very low and erratic results. This may be due to remaining traces of acetic acid, which will not evaporate readily, or to slow volatilization of the alcohol, which may carry off some of the cantharidin. As with most volatile bodies, there is a greater loss on evaporating a solvent slowly, than with rapid evaporation. In order to test the process of assay which was finally employed, an artificial tincture was made by dissolving 0.1875 gm. of pure cantharidin in 2.5 cc. of glacial acetic acid and 10 cc. of chloroform and making up to 500 cc. with alcohol; 200 cc. of this solution should contain 0.075 gm. of cantharidin. There was actually obtained in four trials 0.077, 0.076, 0.078 and 0.077 gm. of cantharidin. Whether the excess was due to errors in measurement (ordinary graduated volumetric flasks being used), or to an obstinate occlusion of chloroform by the crystals, was not determined.

The assay of cantharides and its tincture offers one peculiar difficulty which makes it troublesome. The cantharidin must be obtained in crystals sufficiently large to permit of washing to remove fatty matters without loss, and yet the crystallization must be sufficiently rapid to avoid serious loss. Unless exposure in a warm place is very prolonged, serious loss is not likely to occur, but the container should be removed from heat soon after the last portions of solvent have disappeared.

A number of attempts were made to titrate cantharidin, treating it as an anhydride which yields a bibasic acid on hydrolysis. Solutions in alcohol, acetone and benzol, were made alkaline with an excess of semi-normal alcoholic potassium hydroxide, digested at different temperatures for varying lengths of time, then titrated with decinormal acid, using phenolphthalein as indicator. The acetone solutions gave all sorts of results,—due probably to the fact that acetone itself combines with alkalies, and alcohol solutions gave low and uneven results. The

benzene solution seemed more promising in a few instances, giving results close to 100 per cent., but constancy could not be secured, and the results were as likely to be nearly 20 per cent. high or low as to be nearer the truth, so this plan was abandoned. Other chemists have failed in attempting to estimate cantharidin by titration, yet it seems probable that if the proper conditions and solvents can be secured, it may yet prove to be a practicable method of estimation.

But thus far the gravimetric method of assay is decidedly the most satisfactory and with practice quite concordant results are obtained. The process which was adopted after trying several methods is a modification of the Self and Greenish method, and is as follows:

100 cc. of 10 per cent. tincture is distilled rapidly to near dryness under reduced pressure, and the residue is rinsed into a 250 cc. Erlenmeyer flask with small portions of distilled water, aided by a few drops of ammonia, and using 40 cc. of water in all. Ten cc. of strong hydrochloric acid is then added, a couple of capillary tubes dropped into the flask to prevent excessive bumping, and the mixture is boiled under a reflux condenser for about 15 minutes. The flask is then removed from the heat, and the hot aqueous liquid is sucked out from under its layer of fat with a pipette, taking care to remove as little of the fat as possible; 25 cc. of distilled water and a few drops of hydrochloric acid is added to the residue, boiled under the reflux condenser about 10 minutes and the aqueous liquid removed as before and added to the first. This process is repeated twice more, using 25 cc. of water each time, and obtaining about 125 cc. of combined liquid. This is cooled and shaken out with 30, 30, 20, 20 and 20 cc. portions of chloroform, and the chloroform filtered.

The combined chloroform washings are evaporated rapidly to about 10 cc. then set aside in a moderately warm place for the remainder of the chloroform to evaporate spontaneously and the crystals of cantharidin to form. When the chloroform has entirely disappeared (usually on standing over night), add a little ether and evaporate off the ether, preferably quickly. To the residue add 5 cc. of a mixture of equal volume of absolute alcohol and petroleum ether, which has been saturated with cantharidin, and rotate the container occasionally until the crystals are loosened and the fatty matters are dissolved. Pour the liquid through a small pledget of cotton, retaining the crystals in the beaker, add 2-3 cc. more of alcohol—petroleum-ether solution and repeat until the crystals are free from fat. Then dissolve the crystals in 5 cc. of warm chloroform and filter the chloroform solution through the pledget of cotton, receiving the filtrate in a clean tared beaker or flask. Wash the first flask (or beaker) and cotton with successive small portions of chloroform, then evaporate the combined chloroform washings rapidly, removing the last traces of chloroform with a little ether, dry the crystals at 40° for 30 minutes and weigh.

Conclusions: The writer has not yet succeeded in making a tincture which fully represents the drug, by any method or menstruum tried. Ordinarily, the drug is from one-quarter to one-third exhausted by percolation. By digestion from half to three-quarters exhaustion is obtained.

The use of 10 per cent. glacial acetic acid and 90 per cent. of official alcohol

(both by volume) has thus far proved the most efficient menstruum—tinctures made with this representing 80 to 90 per cent. of the drug used. Such a menstruum is, however, very strongly acid.

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DISCUSSION.

F. R. Eldred stated that in his experience the best way to dry the residue of cantharidin was in a vacuum desiccator, at room temperatures, as there was practically no loss when it was dried in this manner, and it was very easy to get rid of the solvent.

Charles Caspari, Jr., said he hoped that Mr. Scoville would continue his work with cantharides and the different solvents, and give the Association a table of his experiences. If, with the present methods, only one-third of the cantharidin was extracted by percolation, it showed the absolute necessity of some general, improved formula.

THE PHYSIOLOGICAL ACTIVITY OF CANNABIS SATIVA.

Comparison of Extracts from Indian and American-grown Drug Upon Human Subjects.

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It has been claimed by various investigators that the common hemp (*Cannabis Sativa*) grown in the United States contains the same active constituent as is found in Cannabis Indica, the name of the official drug which is grown in India. Botanists do not distinguish between the two, the plant being identical wherever grown.

The fact that the Indian grown drug was used in all the early accounts of its intoxicating action may have led to the belief that the peculiar climate of India is accountable for the presence of an active constituent not normally present in the plant.

No recorded data have been advanced, however, to substantiate the claim that drug grown elsewhere does not contain such constituents. On the other hand Wood (Proc. Am. Phil. Soc., Vol. XI, p. 226), Houghton and Hamilton (Am. Journal of Pharmacy, Jan., 1908), True and Klug (Proc. A. Ph. A., (1909), True (Am. Journ. of Pharmacy, Jan., 1912), and Hamilton (Am. Jour. Pharmacy, March, 1912), have submitted the drug to careful pharmacological tests, and report that extracts from American grown drug are no less active than those obtained from India.

Dr. H. H. Rusby raised the question whether the test for activity on dogs can be accepted to prove its activity as a therapeutic agent.

Much of our knowledge of the action of drugs is obtained by observing their effects when administered to animals. The physiological action of almost every powerful drug is so characteristic as to be almost unmistakable to an experienced observer. Any one who has observed the characteristic effect of Cannabis Indica on susceptible dogs, symptoms which almost invariably appear in an hour after administering one to two grains of an active extract, and then has observed the same effect from an equal dose of an extract from the American drug, is inclined to accept it as proved that the two are identical.