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The Bioassay of Aconite*,†

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INTRODUCTION

Although *Aconitum napellus* has been official in every U. S. Pharmacopœia from 1820 to 1930, inclusive, there has never yet been devised a completely satisfactory method for its standardization. Aconitine is widely recognized as one of the most potent alkaloids known; consequently, any assay procedure which gives a product of variable toxicity is dangerous. It has been largely because of this fact that aconite has recently fallen into disuse.

It has been fairly well established that the chief active alkaloids of aconite are aconitine, benzoylaconine and aconine. Pharmacologic tests indicate that aconitine is about 500 times as toxic as benzoylaconine, and 5000 times as toxic as aconine. And it is quite generally conceded that the activity of aconite is due to aconitine, which is the chief alkaloid both qualitatively and quantitatively.

Aconite has been suggested for many and varied therapeutic uses; however, since its chief therapeutic uses depend upon its analgesic action, it seemed worth while to conduct a preliminary investigation to ascertain whether this analgesic action is due to aconitine or some other plant constituent or constituents. Having satisfactorily determined the analgesic agent in aconite a suitable assay procedure was then sought.

A great amount of work has been done on the standardization of aconite and, since none of the methods devised have proved entirely satisfactory, it was deemed worth while to attempt a different method hitherto untried rather than to try to improve on some one of the more or less unsuccessful methods.

It has already been shown (1) that aconite will produce emesis and since the pigeon emesis method of assay has recently been applied to several drugs with success, it appeared logical that aconite might be standardized by this method. Therefore the second part of this work was undertaken to determine if the pigeon emesis method was applicable to the assay of aconite.

It is recognized that a chemical method of assay is most desirable when such an assay is

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possible; however, aconite appears to be one of those drugs that does not lend itself to a chemical assay in that to date no satisfactory method of separating the active constituents has been devised. It has already been pointed out that an assay based on total alkaloidal content is useless. It therefore appears that a biological assay must be resorted to.

It is also recognized that, for a biological assay to be justified, it must be practical in that it must possess the following virtues: simplicity, rapidity, economy, a definite and unmistakable end-point and a reasonable degree of accuracy.

The chief physiological tests heretofore proposed for aconite have been mainly of two classes (2):

(1) Those based on the reaction of the tissue of a living animal.

(2) Those based on the minimum lethal dose.

Those utilizing cats and dogs have been inaccurate; those using frogs are inapplicable, because of seasonal variations, and it has been our experience that the end-point is very indefinite. Frogs that were apparently dead within fifteen minutes after injection in ventral lymph sac were found on examination to still be just alive as long as six to eight hours later. The heart beat was apparently arrested, but upon slight stimulation, such as gentle stroking with the finger, or a slight electrical shock, it would again be set into motion and beat for some time without further stimulation. Those tests using mice have not been sufficiently experimented with and there is a wide variation in the susceptibility of rats to aconitine (3). The guinea-pig method has been highly recommended because of its sensitivity and constancy.

The chief objections to the guinea-pig method are that it is expensive, and that it is a measure of the toxicity of aconite rather than of any particular pharmacological action. It has generally been assumed that a physiological reaction such as emesis is a manifestation of the pharmacological action of the drug; therefore, an assay based on such an action is a satisfactory measure of the pharmacological activity.

EXPERIMENTAL

Part I

To determine if aconitine is the essentially active analgesic agent, it was decided to compare the analgesic activity of the other alkaloids present, benzoylaconine and aconine, and of the total alkaloids of aconite, with the analgesic activity of pure aconitine. Aconitine as purchased from Merck labeled "Aconitine Potent, 10 to 15 times strength of Amorphous" which gave a sharp melting point at 192–193° which agreed with the accepted melting point of pure aconitine (195° C.) closely enough to be accepted as pure for pharmacological work was used. This gave an even higher melting point than did our sample of U. S. P. Reference Aconitine. An attempt was made to prepare from this benzoylaconine and aconine which are breakdown products of aconitine by hydrolysis. Although many hours were spent in this attempt, and although the procedures as found in the literature were followed in every detail, benzoylaconine and aconine were never obtained in anything near the pure state.

Since this procedure did not give the desired results another method was attempted. It appeared that if aconitine was the active analgesic agent, a comparison of the analgesic action could be made between pure aconitine, the aconitine breakdown products and the total alkaloids of aconite.

The breakdown products were prepared by boiling gently a solution of aconitine with dilute ammonia water until there was a slight color change indicating a breakdown of the aconitine. It was then heated to dryness on a water-bath and placed in a desiccator over phosphorous pentoxide. This product will hereafter be referred to as the "breakdown products;" however, it is in truth a mixture of the "breakdown products and aconitine."

The total alkaloids were obtained from an "Extract of Aconite" furnished for this research by Mr. John T. Lloyd of the John T. Lloyd Laboratories of Cincinnati. It was labeled "Aconite—First Extraction." The extract was shaken out with ether and chloroform by the usual method, and the ether and chloroform volatilized and the residue dried over phosphorous pentoxide.

Since there is no known method of definitely measuring analgesia, we decided to use several of the common methods rather than to rely upon the results obtained from any one method. The methods used were:

1. The effect upon corneal reflex.
2. The effect upon frog reflex.
3. The sensitivity of the cat's tail.

In the first method rabbits were used. Varying concentrations of aconitine, breakdown products and total alkaloids were tested in this manner, but all three in dilutions even as high as 1 to 10,000 produced such irritation and extreme salivation that the results could not be taken as indicative. While the

reflex was abolished in all cases, no reliable data could be obtained.

In the second method, the frogs' reflexes were tested to 0.1% HCl and to the prick of a dissecting needle. The acid was washed off well each time. Then one leg was immersed in the solution to be tested for two minutes while the other leg was likewise immersed in the control solution. The reflex of each leg was again tested with 0.1% HCl and the prick of a needle. It was soon found that, if the reflex to the acid was abolished, it was also abolished to the needle, so for the remainder of the tests only the acid was used. The results with this method were more satisfactory. They are as follows:

Table I.—Effect of Aconitine on Frog Reflex

Dilution	Reflex to 0.1% HCl	
	Control Leg	Test Leg
1:500	Sharp	Abolished
1:2000	Sharp	Abolished
1:4000	Sharp	Abolished
1:5000	Sharp	Abolished

Table II.—Effect of Breakdown Products on Frog Reflex

Dilution	Reflex to 0.1% HCl	
	Control Leg	Test Leg
1:500	Sharp	Abolished
1:2000	Sharp	Abolished
1:4000	Sharp	Very slight (delayed)
1:5000	Sharp	Good

Table III.—Effect of Total Alkaloids on Frog Reflex

Dilution	Reflex to 0.1% HCl	
	Control Leg	Test Leg
1:500	Sharp	Abolished
1:2000	Sharp	Abolished
1:4000	Sharp	Fair
1:5000	Sharp	Good

These results indicate that aconitine is the active analgesic agent, it being more active than either the breakdown product mixture or the total alkaloids. The 1:4000 dilution of the breakdown solution did not produce complete analgesia, and the 1:4000 dilution of the total alkaloids produced practically no analgesia, but complete analgesia was still obtained with the 1:5000 dilution of aconitine.

The third method was a variation of that used by Eddy (4). The weight required to produce a definite reaction on the cat, characterized by a cry of pain and an effort to pull away, was determined on the normal cat and then the weight required to produce this same reaction on the cat after its tail had been immersed in the testing solution for a period of five minutes was determined and a comparison made.

In this experiment, only the 1:500 dilutions of each the aconitine, the breakdown products and the total alkaloids were used. Alcoholic solutions were used to facilitate absorption through the skin. It was found that the aconitine solution was about twice as analgesic as were the breakdown products and the total alkaloids; in other words, the increase in weight necessary to produce the characteristic reaction after immersion in aconitine was on the average twice the increase necessary to produce the reaction after immersion in the breakdown products or the total alkaloids. The latter two solutions produced approximately the same degree of analgesia in this experiment.

Of course, the very nature of these experiments necessitated the use of a great many test animals and it was necessary to repeat the tests many times before any conclusions could be drawn. It is realized that these tests are crude and at their best are only relative, but they do appear to indicate that aconitine, if not the only, is the chief analgesic agent in aconite.

It therefore appears that an assay of aconite could be based upon aconitine content.

Part II

Varying doses of the aconitine, aconitine breakdown products and total alkaloids of aconite were injected into a series of pigeons to determine the minimum emetic dose of each of these preparations. Solutions were used containing 0.03 Gm. per 1000 cc. in physiological salt solution.

The method used was similar to the one proposed by Hanzlik (5) for the standardization of digitalis by the pigeon emesis method and the one by Christensen and McLean (6) for the assay of *Veratrum Viride*. Heretofore most of the procedures suggested that the pigeons be starved for approximately twelve hours prior to injection. However, we obtained better results by starving the pigeons for only four to six hours. It was noticed that after the pigeons had been starved for twelve hours a certain dose would cause nausea and retching with an attempt at emesis, but no actual emesis would result. But when this same dose was administered to pigeons that had been starved for only about six hours, emesis was prompt.

After injection intravenously, the birds were placed in small wire cages for observation. A period of thirty minutes was selected as the time for observation for it was found that emesis did not occur after this lapse of time. The pigeons were observed for emesis preceded by nausea. This was characterized by ruffling of the feathers, dizziness, flapping of the wings, strained swallowing, shaking of the head, downward craning movements of the neck followed by the discharge of grain and mucous secretions.

The pigeons apparently recovered from the effect of the drug within a period of a few hours; however, they were allowed to rest for a period of one week before they were again used. This period was also necessary to allow the hematoma formed by the injection to dissolve.

In order to avoid the possibility of any change in the emetic dose due to hydrolysis of the preparations, or for any other reasons, fresh preparations were made each day immediately preceding the

injections The drug was accurately weighed on an analytical balance and the dilution made in a volumetric flask to avoid any possibility of error there.

Table IV.—Showing Average Minimum Emetic Dose of Aconitine

Dose Mg./Kg.	Number of Injections	Results of Injections: Emesis	No Emesis	Death
0.030[a]	10	..	10	..
0.031	3	..	3	..
0.032	1	..	1	..
0.034	1	1	..	..
0.036	1	..	1	..
0.037	1	..	1	..
0.038	5	2	3	..
0.039[b]	7	4	1	2
0.040	1	..	..	1
0.042	1	..	..	1
0.045	1	1	..	..

[a] Including 0.030 and less than 0.030 mg. per Kg.
[b] Minimum emetic dose.

Table V.—Showing Average Minimum Emetic Dose of Aconitine Breakdown Products

Dose Mg./Kg.	Number of Injections	Results of Injections: Emesis	No Emesis	Death
0.040[a]	9	..	9	..
0.043	3	1	2	..
0.045	1	..	1	..
0.047	5	..	5	..
0.048[b]	10	7	2	1
0.049	3	1	..	2
0.050	2	..	..	2

[a] Including 0.040 and less than 0.040 mg. per Kg.
[b] Minimum emetic dose.

Table VI.—Showing Average Minimum Emetic Dose of Total Alkaloids of Aconite

Dose Mg./Kg.	Number of Injections	Results of Injections: Emesis	No Emesis	Death
0.040	1	..	1	..
0.041	2	..	2	..
0.043	3	..	2	1
0.044	2	..	2	..
0.046	5	2	3	..
0.047[a]	9	6	2	1
0.048	2	..	..	2
0.049	2	1	..	1

[a] Minimum emetic dose.

Table VII.—Showing Average Minimum Emetic Dose of Aconitine Intraperitoneally

Dose Mg./Kg.	Number of Injections	Results of Injections: Emesis	No Emesis	Death
0.040	1	..	1	..
0.060	1	..	1	..
0.065	1	..	1	..
0.075	4	1	3	..
0.076[a]	8	6	2	..
0.077	3	..	..	3

[a] Minimum emetic dose.

For the dual purpose of comparing methods of administration and to ascertain whether the emetic action of aconitine is central or peripheral, one preparation, aconitine, was administered intraperitoneally and the minimum emetic dose determined.

In this experiment, by minimum emetic dose (M. E. D.) is meant: the intravenous dose given in solution and expressed in terms of mg. per Kg. of pigeon which will just cause emesis in two out of three pigeons.

Tables IV, V, VI and VII show the data obtained in this study.

DISCUSSION AND CONCLUSIONS

The results from this work indicate that aconitine is the active analgesic agent in aconite. It is further shown that aconitine will consistently produce emesis and is in all probability the only emetic principle in aconitine.

It appears that the determination of the minimum emetic dose of an aconite preparation would be a direct measure of the aconitine content.

In this work approximately one hundred injections were made and emesis was produced in a good percentage of them. This is contrary to the conclusions drawn by Hanzlik (5) that aconite does not produce emesis when injected intravenously. It agrees, however, with Eggleston and Hatcher (1) who produced emesis upon the intravenous injection of aconitine in dogs with a dose of 0.035 mg. of aconitine per Kg. of dog.

The technique of administration is convenient and simple and does not necessitate the help of an assistant.

For the purpose of comparing methods of administration and also to ascertain whether the emesis was due to central or peripheral action, the emetic dose of one preparation, aconitine, was determined by intraperitoneal injection. This method of administration was found to require approximately twice the dose to produce emesis as did the intravenous method of administration. It also required a much longer period of time to produce this reaction.

No other attempt was made to determine the seat of emetic action; however, several of these facts taken together indicate that the emesis of aconitine is the result of a central action. *First*, intravenous injection produced emesis in from two to twenty minutes while intraperitoneal injection required from forty-five to sixty minutes. *Secondly*, the intraperitoneal dose required to produce emesis is approximately twice the intravenous dose. And since it is known that the emetic centers are located in the

medulla and that aconitine does stimulate the other medullary centers, this would seem to further substantiate the theory that the emetic action of aconitine is central.

All the pigeons upon injection reacted in the same characteristic manner whether death followed, or whether emesis followed or whether there were no other results. Immediately after injection the bird appeared to be very ill, there was a downward drooping of the wings, dizziness and very rapid breathing. The bird's sense of equilibrium appeared to be upset for it had no control of balance and would either squat clear down until its body rested on the floor of the cage or else lean against the side of the cage.

If emesis followed it occurred in a characteristic manner as before described. If death followed it too occurred in a characteristic manner. Just preceding death there would be a violent or convulsive flapping of the wings, the bird would fall over with its neck bent far backward, and gasping for breath. Death followed in a very few seconds from what appeared to be respiratory paralysis. Occasionally death occurred before the bird could be released from the operating table, and a few times even before the needle was withdrawn.

It would appear that the chief disadvantage of the pigeon emesis method of assay is that the emetic dose is entirely too close to the fatal dose, for too many times the same dose would one time cause emesis and the next time cause death. This is further seen in the fact that in each case, regardless of the method of administration, an increase of approximately 0.001 mg./Kg. over the M. E. D. would be fatal, indicating that the emetic effect is obtained only when the near lethal dose is administered.

It was noticed that the birds which were able to vomit appeared to recover from the effects much more rapidly than did the ones that did not vomit. And also if the bird did vomit it was more likely to recover from the larger doses than if it did not vomit, for in no instance did a bird die after emesis, regardless of the size of the dose.

The results of this work indicate that the pigeon emesis method of assay is applicable to aconite and offers a possible method of standardization. It is, of course, realized that this work is for the most part only preliminary and that fruther research is necessary before any definite conclusions can be drawn.

It should also be kept in mind that this work was pursued with the intent of finding a method of standardizing the *analgesic* agent in aconite and not with any reference to the cardiac or blood pressure effects.

The pigeon emesis method of assay is simple, rapid, economical, has a definite and unmistakable end-point and gives a reasonable degree of accuracy.

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Assay of Digitalis

The Use of Dogs in, and a Comparison of the International Standard (1936) and the U. S. P. Reference Powder

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In this work we present evidence to show that dogs are quite suitable animals for the standardization of digitalis; that the U. S. P. Digitalis Reference Powder is stronger than it is labeled; and to correct a measurable error in the data of a previous paper.[1]

EXPERIMENTAL

Method.—The method used is that described by McGuigan and McGuigan (1). Dogs are anes-

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[1] Paper by McGuigan and McGuigan, *J. Pharm. and Exp. Therap.*, 63 (1938), 76.