THE BIOLOGICAL ASSAY OF ACONITE.

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The use of aconite, at one time so largely employed as a therapeutic agent, has in recent years been almost abandoned. One reason for this is probably due to the opinion voiced by Sollman (1) that "Effective doses are practically toxic; safe doses are practically ineffective." In addition to this objection there is the further one, that the alkaloid aconitine is quite unstable in solution, and that preparations of aconite show very great variations in potency. As it is highly desirable that there be available a therapeutic agent whose main effect is vagus stimulation, further study of aconite is needed before it is wholly cast aside. The first step in this study is obviously the development of a method for assuring standard preparations of the drug.

Work in this direction began with the isolation of the pure principles from the root. The extraction of an alkaloid, aconitine, from *Aconitum napellus* was first announced by Brandes and by Peschier (2), and was afterwards confirmed by Pallas, in 1825 (3), and by Geiger and Hesse, in 1832 (4). It was not until 1866, however, that Groves (5) obtained the alkaloid in crystalline form and assigned to it an empirical chemical formula. Alder Wright, in a series of painstaking articles (6) studied this substance, gave it its present formula and brought about its decomposition into benzaconine and aconine. From the time of his work until recently a chemical method of determining the alkaloidal content of aconite has been employed. This method has been official in the United States Pharmacopœia up to, but not including, the present issue (1926).

There are, however, several factors which combine to make the chemical assay of aconite preparations of doubtful or even of negative value. Not the least of these is the rather rapid decomposition of aconitine, the most active alkaloid in aconite, into its hydrolytic products, benzaconine and aconine (Swanson and Walters (7)). Since aconitine is 200 times as toxic as benzaconine, and 1200–2000 or more times as toxic as aconine, it can easily happen that preparations of enormously varied strength may be claimed to have the same potency on the basis of chemical assay. Thus, commercial tinctures were found (Haskell *et al.*, 1915 (8)) to vary 1000% in potency while commercial solid extracts differ even more widely, some being 5000 times more active than others (A. R. L. Dohme, 1918 (9)).

Not only do these decomposition products form, but it is impossible to tell in what proportion they are present in any solution, since the chemical methods of assay determine only the amount of ether-soluble alkaloids present. The ether-soluble alkaloids are not all aconitine, therefore, but represent a mixture of the three alkaloids in varying proportions, and precipitation and solubility tests have failed (Dohme, 1922 (10)) to show any way of separating them chemically. Although Swanson and Walters in 1923 have shown that deterioration can largely be obviated by the addition of 2% acetic acid to the menstruum in making tinctures, the impossibility of chemically measuring alkaloids actually present renders the chemical method of assay useless.

Even before the attempt was made to standardize aconite by its alkaloidal content, Squibb (11), suggested a physiological method of standardization. This

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consisted in the holding of 4 cc. of a 1:600 dilution of a tincture of aconite in the mouth for one minute, and after ejection and rinsing the mouth, obtaining the characteristic tingling sensation in 15–30 minutes. Although the method is too crude to give accurate results, it is of historic interest as being one of the first attempts at the physiological standardization of a drug.

In more recent years a number of other methods of bio-assay have been proposed. Of these, the method of determining the minimum lethal dose for guinea-pigs has most evidence in its favor, and has been adopted as the official test in the 10th revision of the U. S. P. This method was first described by Githens and Vanderkleed in 1910 (12).

In 1913 Roth (13) subjected the various methods proposed to a critical analysis. He concluded that the Squibb method and the various frog methods (one-hour frog method, minimum lethal dose for frogs, perfused heart of the frog) were untrustworthy, as was also the method based on changes in the blood pressure of cats and dogs. He found that the guinea-pig method gave the most satisfactory results in his hands. It may be pointed out, however, that his cat and dog experiments were not carried out in a uniform manner. The vagi were cut in the middle of the experiments; there was no constant rate of flow of the drug; and besides unequal intervals of time between injections, different amounts of tincture were allowed to enter the jugular vein at each injection, rendering the comparison of end-points unreliable.

Dohme in 1922 (10) reported the findings of a committee on aconite standardization. He showed that chemical analysis was entirely useless in determining the strength of any preparation. On the other hand, he gives figures showing remarkable uniformity obtained by the guinea-pig method.

Swanson and Walters, 1923 (7) confirm the value of the guinea-pig method for the determination of aconitine toxicity and give comparative figures showing the reliability of the biological method. They applied the method in testing the toxicity of aconite preparations, an extract of the root, a fluidextract and a tincture and interpret their figures as showing the superiority of the bio-assay over the chemical assay. This conclusion is based on an average figure obtained from many tests. The extremes used in obtaining this average, however, show variations of 50 to 200% or more. They studied especially the question of deterioration. They found pronounced deterioration of both fluidextract and tincture as commonly prepared, when they were kept over a period of time. For example, the fluidextract gradually lost potency and became practically inert in 1–3 years. Aconitine crystals in 70% alcohol deteriorated to 1/10 of their potency in 1 year and to 1/100 in 3 years. When aconitine is kept in 2% acetic acid solution, the deterioration is reduced to less than one-half of this.

Haskell (1916) (14) on inconclusive data, using few animals, decided that the cat method described by Hatcher for digitalis standardization is worthy of a more extended trial, and that the guinea-pig method is unreliable since these animals are subject to marked seasonal variations in their response to aconite intoxication.

Swanson (1924) (15) in a later paper discusses again the guinea-pig method, and mentions certain disturbing factors which should be properly controlled. Thus he insists on a standard weight of pig, allowance for seasonal variations, acclimation

to laboratory surroundings and food, constant dilutions, equal amounts of fluid injected, etc.

Not all the reports on the guinea-pig test are favorable, however. Robinson (16) could not obtain any uniformity in his minimum lethal dose, even when using aconitine crystals. He found that some pigs survived relatively large doses (.000000075 to .0000001), while the same preparation killed other pigs in much smaller amounts (less than .00000005). The number of animals used in this work, however, was too small to invalidate the method. Even when Roth's favorable report is studied we find his figures running uniformly about twice as high as those cited in the Dohme report.

Rowe (17) has substituted the mouse for the guinea-pig from considerations of expense, and finds that his results are comparable with those obtained with pigs, if he uses an insusceptibility factor of 6.25, the mouse being relatively more resistant. His figures are not convincing.

Impressed by the Hatcher cat unit method for the standardization of digitalis preparations (18), a number of workers have applied the same technique for aconite.

Ford, Ford and Wine (19) determined the minimum lethal dose for cats (anæsthetic used not stated) injecting the aconite preparations intravenously and taking as an end-point stoppage of the heart. Although they concluded that this method compares favorably with the guinea-pig method, their figures show such wide variations that their conclusions seem unwarranted. Haskell (14), Haskell and Zircle (8), and Haskell and Thomas (20), because they found wide variations in their results with the guinea-pig method, due, they think, to seasonal variation, believe that the Hatcher method is worthy of a more extended trial.

From this brief review of the literature, it would seem that none of the methods suggested for aconite standardization is strictly dependable when used by different workers, and that further work on the subject would be useful. I have accordingly tested out the pharmacopœial guinea-pig method and in addition carried out a large number of experiments to determine the minimum lethal dose for cats and dogs by intravenous injection.

In all my experiments, aconitine itself was used, as it seemed useless to work with unknown and probably changing solutions before the method was proved accurate by means of a known substance. In fact, with any method the main point is to establish a standard. The aconitine used was supplied by Merck, and was labelled "Aconitine potent, crystals." The Pharmacopœia gives a melting point of 195° for aconitine crystals on rapid heating. On heating at a rate of 3° increase per minute I found the crystals melted at 185.3° to 185.8°. On more rapid heating the melting point was 196°. Roth quotes a melting point of 188°. It is possible that slight alterations in the structure of the aconitine molecule may account for these differences, without there being any appreciable difference in activity. For each day's experiment, a stock solution of 100 mg. aconitine in 100 cc. 2% acetic acid was prepared. Desired dilutions with normal saline were made from this stock solution.

I first repeated the pharmacopœial guinea-pig method. The guinea-pigs ranged in weight from 260 to 320 Gm. and the aconitine in dilution of 1–50,000 was carefully injected subcutaneously into the abdominal tissue by means of an

accurately graduated tuberculin syringe, using a fine needle. The experiments were done in the early Spring and a few in June. No differentiation of sex was made, nor in fact, any special selection of any kind. There was no preliminary fasting. In short, close attention was given only to the preparation of the solution and the accuracy of the injection.

The results were as follows:

Protocol 1.—EXPERIMENTS ON GUINEA-PIGS. SUBCUTANEOUS INJECTION OF ACONITINE.

No. of g.-p.	Dose per Gm. wt.	Died.
3	0.000,000,05	1
3	0.000,000,055	0
9	0.000,000,06	5
3	0.000,000,065	3
5	0.000,000,07	5

The minimum lethal dose per Gm. weight of guinea-pig was therefore between .000,000,06 and .000,000,065. This agrees with the pharmacopœial standard. It may be pointed out here that death occurs usually within one hour and that the symptoms are chiefly those of respiratory failure. In other words, the toxicity of this drug is closely connected with its action on respiration and is no measure of therapeutic effectiveness. While I have made no examination of seasonal variations in the animals tested, which according to both Haskell and Swanson may influence the fatal dose, in general the method is extremely simple, and gave in my hands remarkably uniform figures.

Whether the minimum fatal dose figure for guinea-pigs can be applied generally to other species of animals is questionable. Rowe states that mice are much less susceptible. I have attempted to determine the minimum fatal dose for cats, giving the aconitine in 1–5000 dilution by subcutaneous injection with the following results:

Protocol 2.—EXPERIMENTS ON CATS. SUBCUTANEOUS INJECTION OF ACONITINE.

No. of cats.	Dose per Gm. wt.	Died.
2	0.000,000,06	1
8	0.000,000,07	3
3	0.000,000,075	3

According to these results the dose lies between .000,000,07 and .000,000,075 Gm. per Gm. wt. One dog was given an injection of .000,000,07 Gm. per Gm. wt. and died within half an hour. It would appear therefore that the toxic dose for both cats and dogs is very close to that for guinea-pigs.

The succeeding series of experiments were carried out with the idea of determining the feasibility of the method of intravenous injection recommended by Ford, Ford and Wine and others.

In the first series dogs were used. For anæsthesia, each animal was given 1/3 cc. of a 1% morphine sulphate solution subcutaneously, followed by 1/2 cc. of a 40% alcoholic solution of chloretone intra-abdominally, per Kg. weight. A blood pressure record was taken from the carotid artery, and a respiratory record obtained by means of a tambour system attached to the chest. Aconitine in 1 to 100,000 solution was injected from a warmed burette into the femoral vein at the rate of 1 cc. per minute. The weights of the animals ranged from

4.7 to 10.5 kilos.   Stoppage of the heart was taken as the end-point.   Protocol 3 gives the minimum lethal dose of aconitine per Gm. of animal weight.

Protocol 3.—Experiments on Dogs. Morphine and Chloretone Anæsthesia.
Intravenous Injection of Aconitine.

Weight in kg.	Min. lethal dose in Gm. per Gm. weight.	Weight in kg.	Min. lethal dose in Gm. per Gm. weight.
4.7	0.000,000,039	6.3	0.000,000,062
9.75	0.000,000,046	6.27	0.000,000,063
6.65	0.000,000,046	6.0	0.000,000,093
10.5	0.000,000,056	7.9	0.000,000,094
7.8	0.000,000,057	5.75	0.000,000,108
8.1	0.000,000,060		

This table shows that of 11 dogs tested, five were killed by doses ranging between .000,000,056 and .000,000,065, which correspond to the subcutaneous lethal doses for guinea-pigs.   Three of the dogs, however, were killed by doses considerably less than these, and three by ones much in excess.   After death, the hearts of some of the animals were weighed, in order to see whether there was a definite relationship between heart weight and lethal dose.   No such relationship was found.   It is evident then that this method on dogs fails to give satisfactory results.

To determine a possible effect of the vagus in modifying the lethal dose, 5 more dogs were tested in the manner described above, with the exception that both vagi nerves were cut.   The lethal doses in these animals ranged from .000,000,046 to .000,000,076 Gm. aconitine per Gm. weight of animal.   Cutting of the vagus therefore, does not improve the accuracy of the method.

In the experiments on cats, the technic followed that described by Hatcher for digitalis standardization.   Food was withheld for 24 hours preceding the experiment.   The animals were anæsthetized by ether (drop method), and the aconitine solution in 1 to 100,000 dilution was injected into the femoral vein at the rate of 1 cc. per minute.   Blood pressure and respiration records were taken as in the dogs.   Protocol 4 gives the results of these experiments.

Protocol 4.—Experiments on Cats. Ether Anæsthesia, Drop Method. Intravenous
Injection of Aconitine.

Weight in Kg.	Min. lethal dose in Gm. per Gm. weight.	Weight in Kg.	Min. lethal dose in Gm. per Gm. weight.
3.15	0.000,000,023	2.45	0.000,000,069
2.4	0.000,000,033	5.2	0.000,000,071
2.8	0.000,000,036	3.35	0.000,000,077
2.7	0.000,000,042	2.07	0.000,000,080
2.5	0.000,000,044	2.7	0.000,000,085
2.5	0.000,000,048	2.87	0.000,000,107
4.35	0.000,000,052	3.35	0.000,000,125
2.5	0.000,000,060		

As seen in this protocol, the figures for the lethal dose show even larger variations than in the set of dog experiments.   In both sets no conclusions could be drawn as to a fairly fixed lethal dose, and the intravenous method, as commonly applied, may be dismissed as wholly unreliable.

If we look now for possible reasons for the divergence between the results by the guinea-pig and the intravenous methods, we notice that the latter method

involves the use of a new factor, namely, an anæsthetic. In the dog experiment this factor was controlled to a certain extent by using a definite dose of anæsthetic. In the cats, there was no way of correspondingly measuring the dose of the ether. Since aconite in toxic doses affects the respiration as much as, if not more than, it does the heart, and since the anæsthetics employed all have a depressant effect on the respiratory center, it is obvious that the use of anæsthetics whose effects cannot be accurately measured must be a serious objection to the method. The same difficulty is seen in the cat unit method for digitalis, although here it is less serious because digitalis, unlike aconitine, exercises its chief effect on the heart and not the respiratory center.

I have carried out one more series of experiments in an attempt to overcome the anæsthetic difficulty. These were on cats and the technic was changed in that ether was given by insufflation through a tube carried down to the bronchi, with an artificial respiration maintained. Death of the animal should be due, therefore, to direct action on the heart, the respiratory failure factor being eliminated. Protocol 5 gives the result of these experiments. Three experiments showing wide variations are omitted because of gross experimental errors.

Protocol 5.—EXPERIMENTS ON CATS. ARTIFICIAL RESPIRATION AND ETHER BY INSUFFLATION. INTRAVENOUS INJECTION OF ACONITINE.

Weight in Kg.	Min. lethal dose Gm. per Gm. weight.
3.0	0.000,000,053
3.9	0.000,000,053
3.2	0.000,000,056
3.32	0.000,000,060

Although these experiments are few in number, the figures show fairly close uniformity and seem to warrant the conclusion that the intravenous method is reliable provided the effect of the anæsthetic on the respiratory center is either accurately controlled or eliminated. It may be noted that the doses in the last experiment are slightly under those for guinea-pigs, but this is probably due to differences in the rate at which the drug enters the blood stream.

DISCUSSION.

In the experiments described, it is shown that the guinea-pig method satisfactorily establishes a standard for aconitine potency. The procedure is simple and requires no unusual precautions. It is possible that seasonal variations in animals might have to be considered, but it would be a simple matter to re-fix the lethal dose at any time. The lethal dose for cats and dogs, as far as my limited experiments show, agrees fairly well with that for guinea-pigs, where the same method is employed.

When a fatal dose of aconitine is given to any of these animals, the symptoms are largely respiratory, and death is due in part to respiratory failure. It has been stated (21) that in biological assay the activity of any substance tested should be measured by its effects on the organ on which its therapeutic action is desired. My experiments show as do those of Roth that the onset and degree of cardiac inhibitory action from vagus stimulation bear no quantitative relation to the amount of aconitine injected. There is then a distinction to be drawn between therapeutic

and toxic effect, and however desirable the standardization of a strictly therapeutic dose may be, in practice the end-point of the standard reaction is some definitely measureable toxic effect. It is a matter of no great importance in the case of aconitine what may be the actual cause of death. The point is to establish a definite standard of potency, and the therapeutic dose can be established later by careful observations at the bedside.

In the intravenous injection methods, it has been shown that the results are too diverse and the control of anæsthetic dosage too difficult to allow of practical application. A study of the respiratory and blood-pressure record in the cat and dog experiments show that the respiratory action has a definite relationship to the fatal dose. Where the record shows an early onset of respiratory weakness, death is produced by a smaller dose of aconitine. When the respiration is fairly well maintained, the dose is larger. The blood pressure after an early fall, with a stage of vagus slowing, rises above its original level in either instance, and falls rather suddenly to zero when the fatal dose is reached. Gasping respiration continues a short time after the heart has stopped. Since an inefficient respiration brings about a weakening of the heart, any circumstance which in itself tends to depress the function of the respiratory center would make this more susceptible to a drug depressant action and in addition make the weakened heart also more susceptible. Such a circumstance arises where anæsthetics are used, since these are all respiratory depressants. Only when the anæsthetic dose can be as carefully measured as is the dose of the drug being tested, can the intravenous method, involving anæsthesia of the animal, give trustworthy results. It appears possible, according to my experiments, to bring this about, but the procedure involves a technic too complicated for practical application.

CONCLUSIONS.

1. A study has been made of methods for determining the minimum lethal dose of aconitine as a standard for biological assay of aconite preparations.
2. The pharmacopœial guinea-pig method, if carefully performed, gives uniform results and offers no technical difficulties.
3. The lethal subcutaneous dose for cats and dogs agrees fairly closely with that for guinea-pigs.
4. Intravenous injections on anæsthetized animals give unreliable results, if the anæsthetic is given in the usual manner.
5. If the anæsthetic is given in fixed amounts proportionable to the size of the animal or if respiratory failure is prevented by artificial respiration, the intravenous method appears to be reliable.
6. Vagus showing of the heart cannot be taken as a measure of aconitine toxicity.

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THE PERSISTENT RIDDLE OF BETTER DISTRIBUTION.

Dr. Julius Klein, Director of the Bureau of Foreign and Domestic Commerce of the U. S. Commerce Department, speaking to the American Association of Advertising Agencies, October 25th, pointed out how the science of distribution has failed to keep pace with improved manufacturing methods.

According to Dr. Klein we are at present trying all sorts of schemes, attempting to grope our way in this direction and that, without many clear principles to guide us.

Mail-order houses, which were supposed to derive much of their advantage from the fact that they had no salesmen or expensive retail establishments, are now finding it advantageous in many cases to establish retail display stores and many of them are employing the equivalent of traveling salesmen to place their catalogs in the hands of prospective customers.

Some chain stores which hitherto consistently adhered to the cash and carry system now find it advantageous to add a telephone and a delivery service. Department stores are taking on all kinds of service departments, including beauty parlors, golf schools, bus transportation, "baby checking" facilities and many other services.

We have retailers organizing coöperative wholesale buying associations, wholesalers organizing chains of retailers and manufacturers dealing directly with retailers or even undertaking house-to-house selling to consumers.

We have department stores organizing branches and retail stores consolidated under one management. And through it all we have hand-to-mouth buying, installment-selling and a host of similar movements.

Out of all this experience, we are, undoubtedly, developing information which will be of great service in the future but, in the meantime, we seem to be spending much money and exerting valuable energies in fruitless experiments. Experience may be a good teacher—at times—but the tuition fee is often prohibitive.

Perhaps one of the most fruitful fields for eliminating waste in distribution lies, ironically enough, in the curtailment of overlapping "efficiency research" efforts. To this end the Bureau publishes annually a catalog of "Market Research Agencies," which lists all important current market investigations and the organizations engaged in this field.

In shaping the strategy for the attack on all of these problems the trend of thousands of individual inquiries directed to the Bureau from all lines of business forms a valuable source of suggestion. Equally important also is the advice of numerous trade bodies and associations which collaborate constantly in these undertakings.