Section on Scientific Papers

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PHYSIOLOGICAL DRUG TESTING AND THE PHARMACOPOEIA.

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The chemical assay of aconite, cannabis indica, ergot, and the "heart tonics" is not practicable. In view of the great importance of some of these drugs, the attempt has been made to standardize them by tests upon the lower animals. That methods of physiological drug testing are of undoubted value is generally admitted, but that by these methods sufficient accuracy is secured to warrant their adoption as official methods of assay is not so universally believed. It has seemed to me that a discussion of physiological assaying as related to the Pharmacopoeia is particularly appropriate at this time.

Roughly, the methods of physiological testing of drugs may be divided into two classes. In the first of these, a determination is made of the amount of drug necessary to cause the death of an animal; in other words, we endeavor to ascertain the killing power of the particular drug. In the second class, the attempt is made to measure the effect of the drug upon the function of living tissue.

It would seem, on theoretical grounds, that the methods based upon the determination of the lethal dose are less likely to give reliable results than are those methods where the attempt is made to measure some peculiar physiological action of a drug; some action which has been shown clinically to be of therapeutic value. If, however, it is proven that the lethal action is always due to the therapeutically active principles and that the action is always upon the same vital centers, then this theoretical objection is removed. Until then, however, as has already been pointed out, simply because one specimen of drug is twice as poisonous as a second gives us no reason for assuming that the former is twice as active therapeutically as the latter.

The prime requisite for any method of assay is, of course, accuracy. The degree of accuracy being the same, methods showing cheapness, simplicity and rapidity are to be preferred and it will be of value for us to keep these points constantly in mind.

For the assay of aconite, two methods have been proposed. The first of these belongs to class two of the arbitrary division, and depends upon the fact that aconite has the property of so stimulating sensory nerve terminals as to cause a tingling sensation. Dr. Squibb endeavored to learn at how great dilution an aconite preparation was still capable of causing this sensory reaction when the solution was held is the mouth.

The other method is simply a lethal dose method; guinea pigs, frogs or other animals being used in the experiments.

Dr. Squibb's method is unsatisfactory because the personal equation is apt to influence the results. A very weak solution and a strong imagination will enable one observer to experience the same tingling that a second would experience with a stronger solution and a less active imagination.

The lethal dose method has also not been carefully enough studied to prove its reliability, so an intelligent discussion is not practicable.

So far as I am aware, the only assay method for cannabis indica is that proposed by Dr. Houghton, or some modification of Dr. Houghton's method. In sufficiently large dose, cannabis indica is capable of causing intoxication in a dog, first evidenced by a swaying from side to side when the animal stands. By determining the amount of the drug necessary to cause the first evidence of intoxication, it is claimed that fairly accurate results can be secured.

The personal equation of the observer is again a factor to be reckoned with. What constitutes the first symptoms of intoxication? One may select the slightest swaying, in which event a normal dog may often deceive him; another observer may demand very marked evidences, for which a much larger dose is required. Moreover, the reaction of the dogs is apt to vary, from the fact that minimal exciting doses are given, these small doses being more apt to bring out individual pecularities than are large ones. It is also true that accurate standardization of cannabis indica is not as essential as it is in the case with some other drugs, owing to the low toxicity of cannabis and the number of efficient substitutes that we have.

In ergot we have a drug of great value and one of such complex character that the chemical assay will probably never be satisfactory. Keller's method was formerly believed accurate, but the consensus of opinion seems that the results secured by the determination of the "cornutin" are not to be relied upon. The method proposed recently by Dr. Wood has also been found wanting, and we are forced to turn to physiological tests.

All of the methods now commonly used for the physiological assay of ergot are based upon attempts to measure directly the therapeutic action of the drug as shown by its effect upon the function of the lower animals or upon their excised organs. The cock's-comb method, which owes its prominence to Dr. Houghton, depends upon the power of most fresh ergot preparations to cause, when administered to a chicken, changes in the comb which may be so pronounced as to result in gangrene. It was formerly believed that this effect upon the comb was due to the vaso-constrictor action of ergot, but this view has recently been questioned. If, as Ellinger claims, identical changes can be produced by cantharidin, it is evident that the action can not be considered characteristic of ergot, and the method can be thought reliable only when it has been shown by careful clinical tests that this gangrene-producing power of ergot as measured on the chicken runs parallel with the therapeutic activity of the drug. The work of Edmunds and Hale, showing the agreement between the cock's comb test and the test upon the uterus of one of the lower animals is very suggestive.

The cock's comb test has been adversely criticized, but it would seem that in

some instances the poor results were due not so much to weakness of the method as to faulty technic. In our laboratory we aim to use fowls of the same breed and of as near the same age and weight as is practicable. The drug, in the form of a fluidextract, is injected into the pectoral muscles or into one of the wing veins. If the fowls are picked up at random and not kept under precisely similar conditions, accuracy can not be hoped for. It has seemed to us, also, that the oral administration of ergot to chickens is objectionable, because certain of the active constituents are not supposed to be absorbed from the gastro-intestinal canal.

It is certainly true that the reaction not being automatically recorded is a serious disadvantage. Here, as in some of the other tests I have mentioned, the individual bias of the observer is apt to interfere with accuracy, and the results secured by different men may differ widely owing to the different degrees of reaction aimed at.

Some years ago, Dixon proposed that the vaso-constrictor action of ergot, as evidenced by the rise in the blood pressure of a mammal, be made use of in the attempt to standardize ergot preparation. This method has been extensively used in England, but seems to have at least two serious disadvantages. In the first place, it has been shown (Goodall, Dale, Edmunds and Hale) that the effect upon the blood pressure and upon the uterus *are not always parallel.* So far as I can learn, there is no experimental evidence for supposing that the two sets of effects (upon the blood pressure and upon the uterus) are part of a widespread general action of the drug, and the assumption that vaso-constrictor action is an index of the potency of uterine action is not justified. Dale and Laidlaw have pointed out that B-iminazolylethylamine, one of the constituents of ergot, causes tetanic uterine contraction with a coincident fall in blood pressure.

Even were this method to represent a measure of the therapeutic value of ergot, it does not seem practicable to secure an accurate measure by means of it. Unlike adrenalin, ergot, in an amount large enough to produce an appreciable rise of blood pressure, has a very lasting effect and it is not practicable to make more than one injection into the same animal for purpose of comparison. If the same preparation be given to a number of different animals, it is apparent that these different animals will show great differences in the blood-pressure effects.

Finally, there are the two methods in which the effect of ergot upon the uterus itself is observed. In Kehrer's procedure, the organ is excised and the ergot, in solution brought into contact with it. In the method advocated by Dr. Edmunds, the movements of the uterus of a cat are observed by means of opening the abdomen of an anaesthetized animal in salt solution, the drug being injected intravenously.

There are several points which seem to render these methods undesirable. In the first place, both methods are technically rather difficult. Again, it is claimed that the condition of the uterus as regards parturition influences the reaction to ergot. Certain of the active constituents isolated by Barger stimulate the

parturient, but inhibit the virgin uterus. Then spontaneous movements often set up and cease from some unknown cause.

The most important drug on our list is digitalis. The fact that digitalis is so widely used and that variations in strength on either side of a mean is liable to have disastrous consequences renders it extremely desirable to secure some means of standardizing the medicinal preparations of this drug. A discussion of digitalis would also include apocynum, convallaria, strophanthus, and squill, since there is at present no satisfactory chemical assay for any of these.

The lethal dose method for testing digitalis was the first method to be employed commercially in attempting to standardize drugs by pharmacological experiments. To Dr. Houghton belongs the credit for this important step, which he took when he devised his 12-hour frog method for the assay of the heart tonics. This method is based upon the determination of the lethal dose of digitalis for frogs.

Dr. Houghton had also used guinea pigs in standardizing the heart tonics, but found them unsatisfactory. Dr. Reed, however, believed more accurate results could be secured by using guinea pigs rather than frogs.

Dr. Hatcher recently announced his cat method, by which he claimed great accuracy could be obtained, while, at the same time he considers the method to be simple and cheap.

I have already mentioned briefly a possible source of error is testing a drug by lethal dose methods. Does the therapeutic value of digitalis run parallel with its toxicity for lower animals? Is, as has been claimed, this lethal action of digitalis upon a guinea pig or a cat simply an exaggeration of the therapeutic action of the drug, the poisoning of the heart being the cause of death? Dr. Reed, Dr. Githens, Dr. Hatcher, all claim this to be the case. Cushny, on the other hand, states that even the glucosides of therapeutic value act largely upon the central nervous system; while Edmunds and Hale believe that the death of mammals after digitalis poisoning sometimes results from failure of the heart; sometimes from failure of the respiration. Nestor has come to the conclusion that death of rabbits from the lethal action of the glucosides is always due to respiratory failure, and, in some instances, he was able to save animals from the "dose always fatal" by the maintainance of artificial respiration. From a few experiments upon guinea pigs, I have always found the heart beating strongly after complete cessation of respiration and apparent death of the animal. In a series of experiments carried out in our laboratory by Mr. Eckler, using Dr. Hatcher's method, the respiratory movements of the cats were continued after apparent cessation of the heart beat, but the respiration was often seriously embarrassed before any appearances of cardiac failure. When "pure principles" are used, it is probable that the death of frogs results from cardiac poisoning, and, consequently, this method would represent a measure of the therapeutic action of such pure principles.

If we could assume that it was the bodies of therapeutic value alone that caused the death of the animal, it would not be so important how death was caused, provided there was shown to be a constancy in the dosage required. But in the Galenical preparations of digitalis we have very complex mixtures.

Suppose, as Dr. Hale pointed out, there should be a relative excess of digitonin present in a tincture of digitalis. Owing to the lethal action of this glucoside, very misleading results would be obtained by using a lethal dose method, and a preparation not only therapeutically weak, but capable of causing serious harm if used clinically might be considered of good strength.

On theoretical grounds, Dr. Cushny's frog heart method seems to avoid these objectionable features. This is a qualitative test of undoubted value, for I do not know of any substances present in digitalis leaves capable of producing the typical "digitalis heart" except the glucosides of the heart tonic series which are of value therapeutically. Even could we measure accurately the toxicity of digitalis for lower animals, it can not be claimed that we are always sure of measuring the therapeutic activity of the drug, for not only may a relative excess of the undesirable digitonin be present, but it is possible that injuriously acting decomposition products may arise with the aging or manipulation of a preparation, and it is certainly conceivable that as a tincture ages and deteriorates, it may increase in toxicity for mammals and at the same time not only lose in therapeutic efficiency but actually acquire an increasing power to do harm if used clinically. By observing the action on the frog's heart, we gain positive information concerning the desired glucosides, for they alone, so far as I can learn, are capable of bringing about the characteristic changes in the heart. Focek's method also possesses this advantage, but the barbarity it necessitates will prevent its adoption. The perfusion of the isolated heart involves a complicated technic and can not be considered very accurate.

It has been urged that the frogs vary markedly in their reaction to digitalis, but from my limited experience, I can agree with Hale and Focke that the unsatisfactory results secured are rather due to lack of care on the part of the operator than to unfitness of frogs. When we see the concordant results of Famulener and Lyons and Hale we can not but feel that the method of Cushny is accurate. It may be of interest to state that with ouabain, a pure substance, the same results were secured in Dr. Houghton's laboratory and ours, these results being obtained in each place without knowledge of the work in the other laboratory. To eliminate the possibility of variations due to season or locality, a definite chemical compound, such as strophanthin, suggested by Dr. Houghton, or, preferably, ouabain may be used, and the frogs themselves "standardized."

On the other hand, some authorities would have us think that mammals do not show appreciable individual variation. In the hands of Dr. Hatcher, remarkably uniform results were secured in the early work with his cat method, but he has recently reported an error of 50%+.

I have been unable to find the report of any very satisfactory evidence showing that guinea pigs react uniformly to digitalis regardless of age, weight, sex, season and diet. It would seem advisable to publish such evidence in view of the marked variation sometimes shown by guinea pigs in their resistance to bacterial poisons and the interesting experiments by Dr. Hunt, showing the effect of different diets on the resistance of guinea pigs to poisoning by acetonitrile.

Dr. Houghton has, I believe, found his method involves an error less than

10%. Using "pure principles" Hale has found the one hour method fully as accurate as this. In our own laboratory we have made our work, carried out independently, check within 10%.

There can be no question as to the economy of the different methods. Frogs for an assay cost us about 50 cents. Guinea pigs would cost us about $4.00. Cats could not be secured in Indianapolis in sufficient numbers for our use.

As regards simplicity, there is little to choose between Houghton's and Cushny's method. The guinea pig can not be handled by one man; while Hatcher's method is quite complicated.

The one hour frog method enables us to complete an assay in, at most, three hours. Houghton's method requires at least 24 hours, as does the guinea pig method. The actual time needed to run one cat, according to Hatcher's method, is 90 minutes. If, as seems necessary, three animals are used, the whole day is taken up, the preparation of the animals requiring some time.

Accuracy, cheapness, simplicity, speed. It would seem that in none of these points is Cushny's method excelled. Houghton's method is more time consuming, and it is conceivable that it may give erroneous results when other poisons besides the active glucosides are present in large amount.

It seems that the frog heart method is the only one that has been controlled clinically. Pratt, in this country, has shown how the therapeutic efficiency of digitalis leaves varied as did their strength as determined by this method. Focke, also, mentions similar comparisons. The worth of digipuratum, which is standardized by a modification of Cushny's method, has been shown by many clinical tests.

In conclusion, it may be said that in the one hour frog heart method is offered a means of standardizing digitalis which compares favorably with chemical assay methods when the test is carried out with due precautions by trained men.

It would probably be unwise to adopt as official any of the methods now used for the pharmacological assay of aconite, cannabis indica, or ergot. Further study is needed before it can be determined which are most suitable, but in the meantime it is very desirable that manufacturers use these methods, thereby insuring more nearly uniform preparations and also acquiring valuable data upon the methods used.

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VARIATION IN THE SUSCEPTIBILITY OF THE GUINEA PIG TO THE HEART TONIC GROUP.

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Pharmacologists are divided in their opinion as to the best method for determining the strength of preparations of the digitalis series by biologic means. Many papers have appeared during the last few years advocating the use of this or of that method, but a careful review of the literature shows that, in the opinion of