PHARMACOLOGICAL ASSAYING.*

HISTORICAL AND DESCRIPTIVE.

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The first discovery of the value of medicinal substances and their later development was based very largely on pharmacologic observations. During more recent times this has been looked upon as almost the sole means for a rational selection of remedies and for the establishing of correct dosage.

It is only of comparatively recent years, however, that pharmacology has been recognized scientifically as a method of ascertaining the value of a medicinal preparation. For the most part if no chemical method existed for standardizing, entire dependence was placed on the standard methods for extraction and on certain physical tests. Later when it was recognized that a worthless sample of a medicinal drug would make an extract not differing in any apparent respect from one from an active sample, it was very evident that an assay process was a necessity.

Pharmacologic assaying cannot be applied to any drug which induces no typical reaction when administered to an animal or applied to living tissue, and it is unnecessary to apply it to those possessing an active constituent with well marked chemical characteristics. In general, the attitude on this subject is that whenever possible pharmacologic assaying is adapted for such drugs as are not amenable to a chemical assay.

On the other hand, there is the extremist, who voices the opinion of not a few when he says that every medicinal preparation amenable to a pharmacologic test should be so standardized. This, however, is scarcely a logical viewpoint.

The objection voiced against the biological standardization is not against the method as a general proposition but largely against the method in its particular application, that it is qualitative only. There is no question anywhere of the fact that only by animal or human experimentation can the properties of a drug be established. The question is whether the test can be made quantitative and the value of the substance be measured to establish the dosage. It is only of recent years that the therapeutic properties can even occasionally be assumed from the chemical composition. We are still to a certain extent dependent on the natives to suggest the importance of a drug, by the use they make of it—a use based on a more or less accidental observation of its effects on themselves or animals.

As illustration of these points it may be noted that hellebore was discovered to have medicinal properties by Melampe, a shepherd who traced the diarrhoea in his sheep to their having eaten of this plant.

Acetanilid was by accident found to have antipyretic properties by being given to one of Prof. Kussmanka's assistances, causing an alarming lowered temperature.

Many drugs, such as quinine and cocaine, were used by the natives medicinally with no record of their first discovery. In some cases human use followed the observation that animals apparently chose certain plants for relief from injury or disease. These records are, however, not very trustworthy.

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Confining this historical account to those drugs and medicinal substances now commonly standardized by use of animals eliminates much of interest in the history of the materia medica but leaves for consideration some of the most important medicinal substances, *Cannabis Sativa*, Ergot, the heart tonics of the Digitalis series, and the Suprarenal and Pituitary gland extracts.

The writer may be pardoned if he draws somewhat from his own experience, for 19 years of close acquaintance with the actual standardization of the drugs mentioned covers the greatest part of the period during which such standardization in its restricted sense has been practiced.

CANNABIS SATIVA.

Cannabis Sativa, or when grown in India, designated as *Cannabis Indica*, was known and used 1000 years B. C. It may have been the substance referred to under different names, as for example Nepenthe. Its effects are very wonderfully described in Dumas "Count of Monte Christo."

Cannabis Sativa was probably never standardized with any degree of accuracy by use of any other animal than the dog. Fraenkel[1] confirms the experience of most investigators in stating that rabbits are immune to its action. Guinea pigs are also practically without reaction to this drug in any reasonable quantity. Cats are susceptible but are unsatisfactory test animals in many respects.

My personal experience with its physiological assay on dogs began in 1899, but this was merely to continue a practice which had obtained since 1894–5. Houghton,[2] in 1897, read a paper on Physiological Standardization, in which he referred to its use in establishing the reliability of cannabis preparations, but at that time gave no details of the method applied. Twenty-seven samples were assayed, only thirteen of which proved to be active when administered to animals.

One of the first authors to mention the use of dogs and to describe specifically the effect of the drug is Ponthieu,[3] in 1901, who says: "To verify the action of Cannabis Indica the dog is used, and the drug is administered in the form of an extract; its physiological action manifests itself later in a vacillating gait, ataxia, depression of temperature, and finally complete insensibility."[4]

While no accurate description of the assay method originated by Houghton and regularly practiced since, appeared until 1908, a paper by Thomas Maben[5] was read before the Dundee meeting of the British Pharmaceutical Conference in 1902, on the physiological action of *Cannabis Indica*—a paper "based on observations communicated to him in the course of a discussion with H. C. Hamilton," quoted from Proc. A. Ph. A., 1903, page 804.

Famulener and Lyons[6] have recorded the first accurate description of the physiological assay of *Cannabis Indica* including doses of official preparations, characteristic effects, and the end-point to be observed in establishing the value of the drug.

Fraenkel,[1] in an article published the same year, described the action of the drug on dogs, but gave little data on dosage as the samples tested were cannabinol and its derivatives. The work there recorded was qualitative only.

The details of the method in practice at the time of my first acquaintance with the work were identical with those described by Famulener and Lyons with

the exception that in addition to recording the degree of incoördination, other symptoms, such as degree of preliminary excitement, of drowsiness, and of fall in temperature, were preserved as part of the record. At some time in 1900 record of these by-effects was discontinued as being non-essential and subject to greater individual variation than the degree of incoördination which is typical of cannabis intoxication. In reporting on the pharmacological identity of American and Indian cannabis Houghton and Hamilton[7] described the method as modified and regularly applied at that time.

While practically all the writers up to this time had selected the dog as the test animal, Goodall[8] writes, "At present my standard is that a dose of $^1/_4$ grain should kill or deeply narcotise frogs of 20 Gm."

Haskell[9] refers to Houghton's as the only assay method known.

This method is again described in concise form in the Report of the A. Ph. A. Committee on Physiological Testing.[10]

Pittenger,[11] in 1914, published the same assay method but with no dosage specified and no material changes.

Pearson[12] emphasizes the need of and difficulty in selecting susceptible dogs, also noting that continued dosing does not produce any immunity.

Eckler and Miller[13] seem to be the first to describe the use of a particular breed of dog but not in the sense of specifying the exclusive use of this breed. Hamilton, Lescohier, and Perkins[14] touched on a phase of cannabis standardization not apparently considered by other investigators. In order to corroborate for human therapy the fact established by animal experimentation that cannabis preparations are equally valuable from whatever source the crude drug is derived, and require only the ordinary phsyiological assay, these investigators carried out several series of tests on themselves, using both Indian and American grown drug. Their conclusion is that no difference in the effects of the two varieties could be detected.

Finally, we come to the U. S. P. Revision Committee's Report now embodied in U. S. P. IX[15] and made official for the assay of *Cannabis Sativa*. This report includes several steps not previously suggested as essential in assaying this drug, namely, 1st, Fox terriers for the test animal but not exclusively; 2nd, Doses of 0.03 mil for F. E. Cannabis, 0.3 mil for the tincture, 0.004 Gm. for the extract, 3rd, Preliminary fast of 24 hours for the dogs used.

Previous to this the only requirement in the test animal was susceptibility. The doses suggested by Houghton and Hamilton, Famulener and Lyons and Eckler and Miller were 0.01 Gm. of extract, 0.1 mil of fluidextract and 1.0 mil of tincture per kilo of dog weight. The period of fasting was suggested at several intervals up to 12 hours. The intention of the official method is to require these preparations to produce an observable reaction with the specified doses while previous authors made use of doses of such size that a weaker preparation would have a measurable reaction, evident but less intense than that required for a standard preparation. This feature shortens the test of a weak preparation in that a clue to its activity is likely to be obtained in the first test, while by the official method only a standard (or better) preparation would show an effect.

It is evident, therefore, that the official method differs only in some of its details from that previously followed but that these complicate the method by including non-essential details and by increasing the difficulties of the test.

ERGOT.

In very early times this drug was used in obstetrics by the Chinese and the Romans. Salerne,[1] in 1754, and Tessier,[2] in 1778, found that gangrene occurs in young pigs after administration of ergot.

Dietz noted that one to three ounces of ergot would cause gangrene of the combs of birds.

Wiggers[3] fed 9 grains of an extract he called "ergotin," obtained by alcoholic extraction, to a cock and caused convulsions and death. This probably occcurred too quickly for the typical bluing of the comb to appear since he noted only that the comb became cold.

Bonjean[4] obtained an aqueous extract purified by precipitation with alcohol to which he also gave the name "ergotine." This caused the typical bluing of comb and wattles and a narcotic condition which demonstrated to him that it contained the therapeutic agent.

Kobert,[5] in carrying out investigations of ergot bodies in 1884, used all the laboratory animals including cocks, frogs, pigs, rabbits, cats, and dogs. He used also the isolated uterus of the sheep and considered this the most suitable method of testing ergot, but as a second and final test it must produce abortion in pregnant animals with no other untoward effect.

Jacobi's work in 1897[6] was probably the most important to that date because he carefully checked up his chemical investigations by means of physiological tests. He noted its action on the uterus, on the cock's comb and on blood pressure, the three characteristic effects of this drug.

All the work recorded to this time has been on experimental bodies with no reference to standardization of commercial products. Houghton,[7] in 1898, proposed, in a paper before this Association, applying the Cock's Comb Method for the routine assay of commercial ergot preparations, the method of administration then followed, being that of feeding the crude drug, and by means of a catheter introducing fluid preparations into the rooster's crop.

About this time the work of Barger, Carr and Dale,[8] who wrote voluminously at this period, seemed to have cleared up much of the uncertainty regarding the identity and character of the active constituents of ergot. They showed that different constituents were responsible for the different physiological effects noted. Thus they demonstrated that the aqueous extract as well as an alcoholic can contain an active agent. Ergotoxine, an alkaloid, appears to be the agent causing the bluing of the cock's comb, *p*-oxyphenylethylamine or tyramine is the pressor agent although the alkaloid acts in this way too, while *B*-iminazoylethylamine or histamine is the principle which acts on the non-pregnant uterus and in most cases lowers the blood pressure of anesthetized dogs and rabbits. This work shows conclusively why no assay method based on the amount of any one active constituent present is adapted to the standardization of this drug.

Dohme and Crawford,[9] after considerable experimentation, evolved the method of injecting hypodermatically solutions of the fluidextract and of Keller's Cornutine using 5 Cc. of the former and equivalent amounts of the latter. They concluded that cornutine represents practically all of the therapeutically active substances of ergot and that an assay for Keller's cornutine is the correct means of standardizing this drug for its vaso-constrictor virtues.. Dohme[10] later con-

cluded that this was not a correct method because samples of ergot very low in cornutine were quite active when tested by physiological means.

Barger and Dale[11] suggested a method known as the vaso-motor reversal. The end-point of this reaction is the complete neutralization of the pressor effect of 0.1 mg. of adrenalin.

The later recognition of the various active agents of ergot easily explained why this method is very inaccurate.

Kehrer[12] found the isolated cat uterus suspended in Ringer's Fluid the most satisfactory method for assay and believed that the manufacturer has in that a means of assuring standardized products. From his work with this and with the cock's comb method he concluded that the same active agent is not concerned in the two effects since one disappeared more rapidly than the other.

Goodall[13] agreed with Kehrer in the use of cats, but employed as the end-point, the action on blood pressure—20 min. of liquid extract intravenously administered should raise the pressure 20 mm. of mercury in an animal of 1500 Gm. He, however, recognized the presence of both pressor and depressor substances and concludes that "In the present state of knowledge it is hardly possible to adjust the therapeutic dosage of ergot to physiological findings."

Cronyn and Henderson[14] pointed out that failure to obtain satisfactory blood pressure records is largely due to the anesthetic—a volatile anesthetic partially nullifying the pressor action. This has also been observed by others. They reached the same conclusion as that of Goodall that no thoroughly reliable method is known for establishing uniformity in ergot extracts. Their work with the cock's comb method is, however, open to criticism on account of using different breeds of roosters and not more carefully standardizing the technic.

Probably no more extensive experiments have been carried out to select a satisfactory assay method for ergot than were those of Edmunds and Hale,[15] who reviewed the literature critically, examined the various methods which had been suggested, and finally selected as least subject to inaccuracies the cock's comb method, the end-point recommended being that 1 mil of the fluidextract injected deeply into the breast muscles must blacken the comb in one hour. As a standard they suggest that 1 mil of fluidextract blacken the comb to the same extent as $2^1/_2$ mg. ergotoxin phosphate.

Pittenger and Vanderkleed,[16] in 1914, suggested as the most logical and accurate method for standardizing ergot, its action on the isolated uterus muscle. This method, in the light of the chemical and pharmacological investigations of Barger and Dale, is not applicable for their standardization. They considered ergotoxin to be the most valuable principle in ergot and also that it has little action on the uterus.

My personal experience with the standardization of ergot suggests the use of White Leghorn cocks not over 1 year old, sufficiently susceptible to the action of ergot so that 1 mil of the fluidextract shall blacken the comb in a typical manner and to a reasonable degree in one hour. The roosters for test purposes have an individual record of the average degree of blackening to be expected and are frequently retested with a standard fluidextract to verify their susceptibilities.

Knowing as we do that different constituents of ergot have different pharmaco-

logic effects it is difficult to select for test purposes a reaction which surely represents the desirable therapeutic action of the drug.

The chemist finds certain constituents to be present; the pharmacologist determines their typical effects but rarely has the physician had full opportunity to conclude as to which is the substance concerned in the therapeutic effects of the drug.

As an oxytocic agent one would naturally believe that only the constituent acting on the uterine muscle is of value, while as a hemostatic the pressor effect seems the logical measure.

But histamine, which has a selective action on the uterine muscle, has not proved to be a valuable oxytocic agent when used alone. Further, it lowers the blood pressure of most anesthetized animals and thus obscures the pressor effect and makes the blood pressure test of the drug an uncertain measure of its value as a hemostatic.

Tyramine, another of the constituents isolated from ergot, is said to act on the uterus and to raise the blood pressure both effects probably due to its action on unstriped muscular tissue.

Ergotoxin has much the same therapeutic effect, and pharmacologically has the typical action of bluing the cock's comb.

The logical conclusion, therefore, from this is that the cock's comb reaction is not obscured by counteracting substances as is the pressor test and with our present knowledge is the most satisfactory of the various tests proposed.

THE DIGITALIS SERIES OF HEART TONICS.

The earliest recorded attempts actually to standardize the members of this series are apparently those of Fagge and Stevenson[1] in 1866. They claimed that the physiological tests of these medicinal substances would be of great medico-legal importance.

They carried out tests on most of the members of the series but particularly on digitalin. The method used was that later called the Focke Method and consisted in exposing the heart of the weighed frog, which was then attached to a cork. The solution was injected subcutaneously into the thighs and the time noted when the heart stopped in systole. The time elapsing between injection and systolic stoppage was selected as the basis for the relative toxicities of the different samples. They considered frogs much more satisfactory than the higher animals because of ease in examining the different organs and the rapidity of their reaction to the drug.

Koppe[2] carried out similar experiments on the separated constituents of digitalis, using the same technic and end-point as the earlier writers. He used other animals also, dogs, cats and rabbits, to which the drug was administered mostly subcutaneously, noting in the dog and rabbit the changes in the rate and strength of the heart beat, in the cat and dog also the amount necessary to induce vomiting.

In 1881, Bennefield[3] undertook the examination of tinctures of digitalis from different parts of Germany. Chemical and physical methods failing, he applied physiological tests, using the rabbit. His technic was that later adopted by Hatcher for his cat method, namely, to inject the solution slowly into the jugular vein until the animal dies. The solution injected was prepared by evaporating

the tincture to constant weight and digesting the residue with water at a moderate temperature, using a filtered solution for injection.

Fraenkel,[4] in the same year, examined different preparations of digitalis on the dog. The animal was curarized and injected subcutaneously. He observed three typical effects of digitalis, the increased blood pressure, decreased rate and increased amplitude of the heart beat.

Gley,[5] in 1888, tested ouabain and strophanthin on the laid-bare frog's heart and observed that with equal doses systolic stoppage was accomplished in half the time with the former. He also determined their toxicity on guinea pigs, dogs, and rabbits.

Reusing[6] compared the action of strophanthus and digitalis using frogs. He not only studied the effect on the exposed heart, as in previous methods, but applied also a perfusion method.

Bardet[7] examined the various active constituents of digitalis employing frogs and rabbits in the method known as the M. L. D. method, the first recorded account of the use of frogs in this, the simplest application of the physiological test, the method adapted by Houghton for quantitative assay purposes.

Fouquet[8] also determined only the M. L. D., using frogs, dogs and rabbits, administering the material subcutaneously with as little alcohol as would dissolve the substance.

Prevost,[9] examining Swiss Pharmacopoeial digitalis extracts, used frogs and chose for the end-point the minimum systolic dose. He considered the frog by far the best adapted to the work.

Houghton,[10] in 1898, in October took the first step in advance of other pharmacologists of this time by adopting as a routine procedure the physiologic assay of the heart tonics on frogs, using the M. L. D. as the end-point. This method was further elaborated and presented before the Pharmaceutical Section of the 7th International Congress of Applied Chemistry on May 31, 1909, at which time he suggested a Heart Tonic Unit based on the M. L. D. and proposed this as an international standard for the assay of the digitalis series.

Jacquet,[11] recognizing the importance and the applicability of the physiological test in arriving at exact dosage of digitalis, described his method and recorded his results in 1897 but did not propose the routine application of the method until December, 1898. His method was the systolic stoppage of the frog's heart, but he used rabbits as well, adopting as an end-point the minimum lethal dose.

Fränkel,[12] taking up the subject again, used the systolic stoppage of the frogs heart as the end-point but made an important forward step in fixing the time at which stoppage in systole must take place at one hour, thus varying the dose instead of the time. This approaches still more closely the method of Focke and Gottlieb, and is almost identical with the present U. S. P. Method.

Famulener and Lyons[13] adopted the same general method probably about the same time in studying the relative values of different digitalis extracts and the active constituents of digitalis.

Ziegenbein,[14] using a method credited to Hans and Arthur Meyer but really originated by Fagge and Stevenson, examined a number of different species of digitalis and found them to differ greatly in toxicity. He selected a two-hour interval in which the drug should act to induce the systolic stoppage.

With all the imperfections which he had observed in the physiological methods he considered that only by the use of the frog could one obtain a degree of uniformity in the activity of digitalis preparations.

Moschkowitsch,[15] in 1903, on the basis of considerable experimentation, regretted to report that he failed to substantiate the results of Focke, Prevost, and others by use of frogs, but his failure seemed to carry no particular weight against physiological testing.

The reason for this is probably twofold, first, because some of his work is open to criticism and, second, because all who have experimented in pharmacologic assaying recognize the difficulties and discouragements involved, and, further, realize that chemical methods have even less evidence in their favor.

Focke[16] wrote voluminously on the physiological assay of digitalis and a method known by his name was given tangible form in 1902.

He applied the principal first used by Fagge and Stevenson, but modified for quantitative results. He obtains a value $V = p/dt$, in which p is weight of the frog, d the dose, and t the time of systolic standstill.

The objections to this, the second modification of the frog-heart method in its quantitative application, are first, that the short time selected for obtaining results, namely, 7 to 10 minutes, is too short for complete absorption of the drug and is thus inapplicable to extracts containing much inert material; second, the laying bare of the heart of an unanesthetized animal is contrary to the best pharmacologic procedure, and further, is certainly a factor in affecting the results adversely; third, the use of so few frogs does not allow for sufficient elimination of exceptional frogs—those much more or less resistant than the average. It has never found adherents in the U. S.

In addition to applying a quantitative method to digitalis assay, Focke experimented with leaves from different sources, of different ages, wild and cultivated, the effect of moisture on deterioration, and the seasonal and temperature effect on the frog. In all, while not much of his work was purely original, he added considerable to our knowledge of this drug and its standardization.

Santessen[17] used another modification of frog-heart method in that he observed the effect on the heart, but did not expose it until the heart had practically reached systolic standstill. He recognized the difficulties of physiological standardization and the importance of the factor of individual resistance as well as the general factors which affect results in such a method.

Hatcher[18] first adopted the systolic stoppage of the heart in one hour as the method and end-point for assay purposes, but later wrote on his experiments with the cat method, adapting the technic of Bennefield, that of slowly injecting the material into the vein over a period of 90 minutes, death to take place at that time. This is the first suggestion of the practical use of this method for quantitative assay and it may be said that while there seems little to recommend it, the method has appealed to some as of considerable importance. One of the arguments for it, or rather used against the M. L. D. frog-heart method, is that a product may possess toxic principles which are of no therapeutic value but which appear valuable by strictly M. L. D. methods. It should be noted, however, that the cat method is nothing more than an toxicity test with the disad-

vantage that the characteristic systolic stoppage cannot be observed to identify the cause of death.

Hatcher, himself, admitted that he found unaccountable variations of about 50 percent and his tables show even greater variation than he admitted.

Robinson and Wilson,[19] in some experiments to establish the character of the digitalis action on the cat's heart, observed a total variation of 100 percent in the lethal dose.

Reed and Vanderkleed,[20] objecting to the use of frogs because of the number of variable factors concerned, proposed the M. L. D. of guinea pigs as a quantitative method. They have, however, failed to prove that guinea pigs have a constant resistance and that death results from a direct action on the heart. It is another M. L. D. method with no technic to confirm the cause of death, such as is available in the frog-heart methods.

The work of Sollmann and others[21] in showing the influence of temperature on the toxicity of the digitalis series to frogs is well worth noting and is highly important. This variable factor, however, is offset in both the frog-heart methods by the use of the standard for comparison in every assay thus eliminating this as well as other uncontrollable factors, such as, climate and season and the species and weight of the frogs.

Heintz,[22] in 1912, recognizing the limitations of the various physiologic tests proposed, suggested applying not one but several tests, including the M. L. D. and M. S. D. on frogs, the M. L. D. on mice, and the pressor action on the circulatory system of rabbits and cats. The toxic dose on mice is by internal administration with food in pill form. His proposition has received little comment.

Krogh[23] used the isolated frog's heart and determined the lowest concentration of the drugs which would arrest the spontaneous rhythm. He considered the method accurate within 10 percent.

One other proposed method remains to be noted, namely, that of Pittenger,[24] who suggested the use of gold fish which are particularly susceptible to the influence to poisons in water. It is probably the simplest method heretofore proposed but it appears to have gained insufficient recognition for criticism. It is another M. L. D. method and allows of no means of identifying the poison that causes death.

Of the methods quoted only the M. L. D. and M. S. D. on frogs, and M. L. D. on guinea pigs and cats, are practiced in the U. S. The M. S. D. on frogs is the one suggested in U. S. P. IX as adapted to the standardization of this important series of drugs.

The cat method, as stated before, is purely a toxicity test and can be classed with that on guinea pigs as objectionable because death is almost invariable due to paralysis of the respiratory centers, and, therefore, not directly a measure of the heart tonic value.

As stated by Edmunds and Hale, there is little to choose between the M. L. D. and M. S. D. on frogs. To one who has been accustomed to the former, however, it has three advantages over the M. S. D. method, first, in the use of a larger number of frogs with less work and actual time involved; second, in the elimination of the factor of slow absorption, and third, in the fact that the end-point is not obscured by rough handling such as by the pithing and laying bare of the frog's heart. At the same time it has the only advantage claimed for the M. S. D.

method in that the frog's heart can always be examined to verify the identity of the toxic principle.

THE PITUITARY GLAND.

While the extracts of this gland have widely different effects, such, for example, as the pressor, diuretic, glactagogue, cathartic, and oxytocic actions, no undisputed chemical evidence has been brought forward to demonstrate the presence of more than one active constituent—a substance which acts on plain muscular tissue and is responsible for all the phenomena noted.

As assay methods, only three have been proposed and of these only two are generally used, one being official in the 9th Rev. of the U. S. P.

The first method proposed is that by Dale and Laidlaw,[1] who in 1912 described the method which with some modifications is now official. It consists, in brief, in using the isolated uterine muscle from a young guinea pig of not to exceed 350 Gm. weight. One horn is removed, suspended between a fixed and a movable attachment in artificial blood plasma (Locke's solution), heated to body temperature, and the solution to be tested is thoroughly mixed with the Locke solution to make a homogeneous mixture in contact with the uterine muscle. It is claimed that when all the conditions are rigidly followed the contractions of the uterus will vary according to the amount of active principle present in the solution.

There are many factors affecting the sensitiveness of the muscular tissue used in the test and, therefore, affecting the quantitative accuracy of the method such, for example, as the size, age and condition of the pig, the temperature changes of the solution in contact with the specimen, and the presence of foreign substances in the Locke's Solution or in the pituitary extract.

The method has been later described by a number of authors in each case with some slight variation in technic.

Führer[2] applied this method in attempting to prove the separation from the gland of four active constituents with different properties. One of these constituents was considered to be the active principle, but others had similar properties less strongly pronounced.

Heidelberg, Pittenger and Vanderkleed[3] consider the oxytocic to be the only method practicable for quantitative assaying, describing in minute detail the apparatus and technic used by them.

Guggenheim[4] used the rat uterus with approximately the same technic. There was no exceptional contraction observed, but after applying hypophysis extract to the uterus it maintained a steady contraction instead of the normal rhythmic contractions.

Roth[5] described the Dale method in minute detail with his modifications and concluded, on the basis of considerable experimentation, that it is the most satisfactory assay method, he having also applied tests by the intact uterus and the bood pressure. The intact uterus is so rarely used that it is not regarded as a distinct method.

For a standard in carrying out the uterine contraction assay Roth proposed and used a solution of histamine which acts similarly to pituitary on the uterine muscle but which more often lowers than raises blood pressure. The final dilution of histamine found best suited in most cases is 1 in 20,000,000, which, in comparison with two of the best known commercial preparations, diluted 1–15000

caused equal contractions. No tracings were submitted to substantiate these statements.

For the pressor test, histamine not being applicable, a commercial preparation was selected for comparison with the others. The blood pressure method was proposed by Hamilton[6] the same year that Dale's method was published.

Disregarding, because of expediency rather than for any other reason, the physiological effects of pituitary extracts other than that on the circulatory system, this author proposed a pressor test by which the activity of the extract could be measured with considerable accuracy.

The dog anesthetized with chloretone was used in the same general way as for standardizing suprarenal gland extracts, comparison being made between the sample and a standard prepared from the dried, defatted, powdered posterior lobe. The standard material was prepared in considerable quantity in order to represent an average product. The amount to be injected at one time was specified as 1 mil of the solution to be obtained by dissolving the soluble part in acidulated water, using 1 Gm. to 1000 Cc.

The method was described in greater detail by Hamilton and Rowe,[7] who critically analyzed the two methods pointing out the discrepancies in Roth's results and particularly calling attention to the failure of the oxytocic method as a measure of pressor action of pituitary extracts because of the use of histamine as a standard, since the latter is usually not a pressor agent. It also is questionable as a standard since its clinical action is not identical to that of a pituitary extract. The unqualified use of histamine as the standard was also criticised by Pittenger and Vanderkleed[8] and by Pittenger[9] who have not found different lots of histamine equally active while solutions have not been proved to be stable.

The U. S. P. 9th Revision Committee gave no details of the test but directed attention to the method as described by the Hygienic Laboratory. The strength of the official solution, however, has been specified as equal to that of histamine 1 to 1000, a value estimated by different authors as being from 10 percent to 40 percent as strong as a good commercial preparation. This has been noted by Pittenger,[9] Hamilton,[10] and Eckler,[11] and the result of the statement is that no official preparation has appeared on the market, since strict adherence to this standard would in some cases lower the activity of the preparations considerably below the present high standard.

The objections to the oxytocic method other than those concerning the character of the standard and the activity of the official product are principally to the fact that the uterine muscle is so sensitive to other stimuli than that specific for pituitary extracts, that uniform quantitatively accurate results are not obtainable, the reaction being rarely proportional to the amount of extract applied.

The principal objection advanced against the pressor test is that the dog, while least subject of all animals to this fault, does not remain sensitive to repeated injections but becomes progressively less responsive after the first two or three. This, however, has not proved a valid objection in the work of the author.[7,9]

The third method proposed is that of Spaeth,[12] who uses the melanophores of *F. heteroclitus*. "As a result of this work it appears that the melanophores of Fundulus and probably of all other teleost fishes must be considered functionally

modified smooth muscle cells." These melanophores or pigment cells of the fish are a part of the scales.

In the assay method these scales are placed in the pituitary solution and the resulting contraction is evidenced by the apparent bleaching out of the pigmented portion of the scales. This effect is also produced by potassium chloride in 0.1 *N* solution and it is proposed that a dilution of this solution shall be used as a standard for comparison.

The standard test solution is a mixture of 0.1 *N* KCl and it is proposed that a dilution of this solution shall be used as a standard for comparison. The standard test solution is a mixture of 1 part 0.1 *N* KCl and 2.5 parts 0.1 *N* NaCl. The solution of pituitary extract is to be mixed with an equal amount of 0.2 *N* NaCl—a dilution which he suggests as "a uniform standard for pituitary extract." As the author has had no experience with the method—this test animal not being available in inland towns—it will not be discussed except from its superficial aspects.

It is not an illogical test in that the same general effect of pituitary extracts as those acting in the other methods is made use of, namely, the constricting action on smooth muscle—an effect which should be fairly transferrable from any one kind of tissue to another. The use of KCl as a standard instead of a standard pituitary product is no more illogical than the use of histamine in the oxytocic test.

It is improbable that the use of a test object not easily obtainable in every locality would ever appeal to the average investigator unless the method were pre-eminently satisfactory. This has not been demonstrated either by the originator of the method or by any other investigator.†

The principal points to be considered in comparing the practicability of the first two methods (omitting the third since no data on it are available), are as follows: The pressor test measures the constricting effect of pituitary extract on smooth muscle and has been found free from many of the objectionable features of the oxytocic effect, such as its supersensitiveness and lack of uniformity in results. The dog contrary to Roth's statement (which he failed to prove), does not become progressively less reactive to pituitrin if the proper technic is followed.

The pressor test, therefore, seems equally applicable for assay of pituitary extracts and much more practical for routine analysis.

THE SUPRARENAL GLAND: ADRENALIN.

The physiological standardization of extracts of this gland depends entirely on its constrictor effect, and the assay method now used to the exclusion of all others is the constricting action on the arterioles causing an increased blood pressure. In fact, so completely has this effect taken the place of other reactions that the use of any other test is not considered.

Oliver and Schafer,[1] in 1894 and 1895, noted the marked blood-pressure-raising action of these extracts as did also a number of others about the same time.

Von Fürth, in 1898,[2] carrying out pharmacologic tests in conjunction with his chemical experiments, observed that when a substance isolated from the gland

† Unofficial reports from the Hygienic Laboratory are to the effect that this method was found to be inaccurate and impracticable.

was intravenously administered in a dose of 0.000025 Gm. to a rabbit the blood pressure rose 114 to 116 mm.

Among others who wrote of this reaction is Gottlieb, whose contribution[3] is noteworthy because of the tracings showing the action of suprarenal extract on the circulatory system of a dog, the blood pressure of which had been reduced to zero by paralyzing the heart with potassium nitrate.

This combined action on the heart and circulatory system is the effect which forms the basis of the method proposed by Houghton[4] in a paper read before this Association in 1901.

The method in brief consists in the use of an anesthetized dog, the injections being made intravenously and the results recorded from the carotid artery by means of a kymograph. The injections can most conveniently be made into one of the femoral veins and the record made and preserved on a sheet of blackened paper on the revolving drum of the kymograph. Tracings obtained in this way are shown in which the rise in blood pressure varies directly with the amount of active agent injected.

My personal experience with this method began in 1900 at which time the standard used for comparison was a carefully prepared and preserved extract of the gland so diluted before injection that the rise in blood pressure would not in general be greater than 20 to 30 mm.

In 1902, in a second paper by Houghton,[4] the method was amplified and the active constituent, adrenalin, was proposed as the standard with the test dose for the average dog 1 mil of solution of adrenalin chloride containing 0.00001 Gm. per mil. This is by no means a minimum active dose but was selected as the dose from which the reaction was most sensitive to minute changes. Thus it is easy to distinguish a difference of 5 percent more or less than this amount when injected into an average dog and, therefore, permits of standardization within those limits of error.

No material change from this technic was proposed by anyone until the Revision Committee of the 9th U. S. P. included the suprarenal gland among the drugs for which a physiological test was proposed. To the casual reader the two methods are identical but to the operator there are several points of difference which have received critical comment by Hamilton,[6] who pointed out sources of error in the described technic.

Cameron,[6] in 1906, after trying various other methods, chose the blood pressure method as the most convenient and reliable for the purpose of standardizing suprarenal products.

Hunt[7] and Sollman and Brown,[8] using the same general method, assayed and reported on several commercial samples.

Crawford[9] reviewed the literature and gives minute details used in applying this method, but suggested no additional methods.

Läwen,[10] using the same kind of a reaction, but applied by perfusion through the blood vessels of the frog, demonstrated the constricting effect but found the animals to vary considerably in their response.

He observed the effect of a mixture of cocaine and suprarenin, as Braun had first noted to be a localization of the former and thus to prevent its general action.

Ehrman[11] applied the reaction to the pupil of the enucleated frog's eye, the

constricting effect in this case being on the iris, thus causing a measurable dilatation of the pupil. He considered the method equally accurate with other applied methods but his results show that a dilution of adrenalin corresponding to the commonly used test solution, namely, Solution Adrenalin Chloride 1–1000 diluted 1 in 100, *i. e.*, 0.00001 Gm. per Cc. was inactive.

In this historical account of physiological standardization no attempt has been made to have the references exhaustive because many of the workers along these lines have made no attempt to apply the test quantitatively. On the other hand, a number of the authors quoted have not contributed directly to the development of drug standardization but their work has been very helpful in pointing the way and for that reason is too important to be omitted.

In collecting the bibliography I have been greatly assisted by consulting the records of a number of authors who have in some cases had access to more extensive libraries than were available to me.

It is evident from a study of the collected references abstracted that there has been no orderly development of the methods official or unofficial now in use to assay these important drugs. In two cases—Cannabis Sativa and Adrenalin solutions—there has been only one method for each adopted for general use and in both cases that method made use of one of the first typical effects observed. In the other three cases Ergot, the digitalis series and pituitary gland extracts—two or more methods are in common use and there seems no way to reconcile conflicting opinions.

The reason for this is that in no case is any opinion wholly right or wholly wrong and no one is inclined to give up a method which in his hands has given fairly satisfactory results for one which does not appeal to him as being either more logical or more accurate.

If it were possible to check up by clinical tests the results obtained by the different methods of pharmacologic assay—if for example, it were possible to determine which is the better of two samples of pituitary extract, alike by one method of assay and different by another—then differences of opinion as to the adaptability of any particular method would vanish. This, while apparently a simple means of eliminating discord has never been worked out in practice and we seem no nearer to a satisfactory solution of the problem than at any time in the past.

How serious these differences of opinion are as to which method of assay is correct the following quotation from a letter which passed between two state universities will serve to illustrate: "Permit me to call your attention to the fact that the studies of Roth and Edmunds and many other workers merely relates to the toxicity (of digitalis). Furthermore, in this connection the U. S. P. suggests a standard based on toxicity which, in the opinion of Hatcher and many other workers, is not the measure of the therapeutic value of the drug. A sample may be toxic and high in vaso-constrictor properties, and at the same time be absolutely contra-indicated in 90 percent of the cases where digitalis is called for."

The above quotation is evidence that the official recognition of a method is not sufficient to obtain its general recognition and shows how valuable some clinical evidence would become if it were practicable to obtain it.

The history of Physiological Standardization to date is a record of wonderful development in the face of many discouragements. The opposition has, however, largely broken down and thus the way is cleared for still greater achievement. With the active coöperation of the clinician much might be accomplished which under present conditions is next to impossible.

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O. HENRY, PHARMACIST.

C. Alphonso Smith, the author of the O. Henry Biography, has recently discovered the origin of O. Henry, the nom de plume of the late William Sydney Porter. He writes that he had long suspected the source of the name, but that his surmise was not confirmed until he received the following letter from Dr. Paul Barringer, president of the Virginia Polytechnic Institute. Doctor Barringer writes:

"At various times in my life I have run upon chemical analyses made by a Continental chemist who signed himself 'O. Henry.' While the substances under analysis were adapted to use in the Materia Medica, I had no idea until recently that the man was a pharmacist. In looking up the preparation of hydrocyanic acid in the United States Dispensatory, I found O. Henry twice referred to, in short search. Seemingly he was of Antwerp, as he wrote a good deal for the *Journal de Pharm. d'Anvers*, and also Paris pharmaceutical papers. In fact, I find his trail from 1833 to 1857, and he touched many of the lines a southern drug clerk would be interested in—quinine, cinchonine, etc. Can be it possible that this short, crisp, unusual name, that hits the eye from the page, ever caught the eye of the young drug clerk, Sydney Porter, and stuck?"

Mr. Smith says that on turning to the United States Dispensatory, which O. Henry used when he was a drug clerk in his uncle's store in Greensboro, N. C., he found frequent references to O. Henry. He comments: "When it is remembered that Will Porter had from early boyhood an unerring feeling for odd and narrative names, as well as faces, and that he was filling prescriptions when he first signed the name O. Henry to a short story, the evidence becomes, it seems to me, practically coercive that here and here alone the pen name took its origin."