a year ago I think that there were three cases of post action of tetanus in New Jersey which I had occasion to investigate. I found that the pharmacist and physician both knew very little on the subject. They read from the text books published long ago information that no longer is considered reliable, and based their arguments on those points that they found in the text books; but if they had known that the Bureau of Hygiene at Washington has investigated the subject and found that they cannot infect a person—that is they cannot infect healthy tissues—by mixing tetanus germs with vaccine and injecting it into the healthy tissue, unless there is also pus infection, they would have had a very different view of the entire subject and been saved a great deal of trouble. As I said before, there are so many points from which this subject can be discussed that we might talk a long time and still be taking up points of exceeding value and interest to the Association.

JACOB DINER: More than five or six years ago, I presented a paper on vaccine before this section, wherein I endeavored to point out to the pharmacist that he must interest himself in the newer methods of treating disease. It is the pharmacist's duty to be conversant with the remedial agents, their origin, composition, and mode of acquisition. While he, in a manner, does familiarize himself with the chemical and medical drugs which he handles, there has been a woeful lack of interest in the matter of allied remedial agents, particularly vaccines, and antitoxins. There are many pharmacists who do not know the difference between vaccine and antitoxin; and I might say in all fairness that there are a number of physicians who do not know the difference. Pharmacists shou'd have more knowledge relative to biological products, not only that they may intelligently handle them but to discuss the subject with physicians. There is no excuse for the prevailing lack of information, as the opportunities for acquiring it is not only afforded by text-books but also through related articles in the journals.

W. M. BOWMAN: I want to call attention to one thing in biologicals. That is the point of scientific honesty. I think that when it comes to the handling of biologicals—the sale of biologicals to physicians—you will find that point something which can very well be borne in mind. In the sale of biologicals to physicians you are coming in contact, seventy-five percent of the time, not with the ultra scientific man, but with the man who doesn't know very much about biological products. The main thing in handling biologicals from the pharmacist's point of view is to thoroughly know the products; and one cannot know these only through a thorough study of the whole subject. And above all, whatever information is supplied should be backed by a knowledge of facts; when these are communicated it may occasionally result in loss of business, if the product is not adapted for the intended or contemplated use, but this is the correct proceeding, that is what I call scientific honesty.

BIOLOGICAL ASSAY METHOD OF THE U. S. P. IX.*

BY PAUL S. PITTENGER.

The history of standardization may well be divided into five important steps.

The *first step* was made by Dr. Lyman Spalding, who, in 1817, submitted to the Medical Society of the County of New York City the project for the formation of a National Pharmacopoeia, the adoption of which resulted in the publication of the first National Pharmacopoeia in 1820.

The *second important step* was the organization of the American Pharmaceutical Association in 1852 to improve and regulate the drug market.

The *third important step* consisted in the adoption of the Purity Rubric and of assay processes for galenical preparations by the Pharmacopoeial Convention of 1890.

The *fourth important step* consisted in the securing of legislation known as the

* Read before Scientific Section, A. Ph. A., Indianapolis meeting, 1917.

Pure Food and Drugs Act of June 30, 1906, by which the standards of the Pharmacopoeia were made law for Interstate Commerce in drugs and medicines.

The *fifth important step* is the inclusion of a Chapter on "Biologic Assays" in the U. S. P. IX. for certain drugs and their preparations which are not amenable to chemical standardization.

The incorporation of this chapter on "Biologic Assays" is an epoch in the history of standardization and, as stated in my former paper,[1] it is to be hoped that with this start a much wider publicity and experience will be gained so that the next Committee of Revision will readily be able to select from the proposed method and make official the methods which prove to be the most satisfactory and convenient for each drug.

In the second paragraph of the chapter on "Biological Assays" in the U. S. Pharmacopoeia, the following statement appears:

"Brief descriptions of the more commonly accepted methods are given here in order, first, to direct attention of manufacturers to them; second, to ascertain the points of weakness which may exist in them; and finally, to outline methods and establish standards which those interested may adopt, should they desire to assay their products and have them conform to the standards proposed."

In regard to the first intention of the Committee, I would only draw attention to the fact that the larger pharmaceutical manufacturers have biologically standardized their preparations for the past eight to ten years and were in many cases the *originators* of the tests in use at the present time. These tests were improved and developed by them to a practical working basis.

Due to the fact that the methods of the Pharmacopoeia in many cases *lack the details* which workers in the practical laboratory have found essential in order to obtain accurate results, I feel that the U. S. P. methods are in many instances *not as accurate and up-to-date* as the methods in common use at the present time in the commercial laboratories. In other words, the methods do not, according to my mind, show as well as they might the degree of efficiency to which biologic assays have been developed.

Most of my remarks will therefore, be limited to what I consider "points of weakness" which exist in the present U. S. P. methods.

CANNABIS.

Page 605. "Before administration the animal should not be fed for twenty-four hours in order to hasten absorption."

It is not necessary to withhold food for more than ten to twelve hours before making a test as the stomach will be completely emptied in this time and it will not be so hard on the animal.

"The head of the animal being held, its mouth is opened and the capsule or pill is placed upon the back of the tongue. Usually the drug is easily swallowed when given in this way, but this may be facilitated by giving the animal a small amount of water to drink."

This method works sometimes, but as a general rule the dog does not feel inclined to take capsules so easily. In practical work it will be found that it is almost impossible to make a dog swallow a capsule by the above method. Pulling the

[1] "An Improved Apparatus for Testing the Activity of Drugs on the Isolated Uterus."

tongue well forward, placing the capsule far on the back of it and then releasing the tongue, is an improvement, but the best method is the following:

"Open the animal's mouth by forcing the thumb and index finger of the left hand between the jaws, back of the teeth. The capsule is then placed on the back of the tongue with the right hand and the mouth quickly closed; while still holding the mouth shut, the animal can be made to swallow the capsule immediately by slapping it on the throat."[2]

By this method the most obstinate dog can be made to swallow the capsule on *first* attempt.

In lieu of a standard extract furnished by some central authority such as the U. S. Hygienic Laboratory, what is the use of running an assay each time on a standard preparation when the strength of the standard is obtained by adjusting a preparation until it is of such strength that 0.03 Cc. per kilo of the fluid extract will produce incoördination? Why not adopt 0.03 Cc. per kilo as a standard and calculate the strength of the unknown by comparing the dose of it necessary to produce incoördination with the above 0.03 Cc. per kilo instead of the amount of the standard necessary to produce the same effects? If the standard is of proper strength will it not require exactly 0.03 Cc. per kilo? The only object so far as I can see for assaying the standard preparation each time would be to avoid errors due to the variation in the susceptibility of dogs. The use of a standard preparation, unless supplied by some central authority, will not avoid this error because the standard preparation is adjusted to the above *standard dose* and not to *standard dogs*. Are you not just as liable to have dogs which are over or under normal susceptibility when you adjust the standard as when assaying an unknown, thus making the standard slightly over or under strength? If so, by adopting the longer process of assaying both standard and unknown each time, the error due to variation in susceptibility is only increased because you adopt as a standard preparation one which may be slightly over or under strength and then adjust all subsequent preparations to this, thus making the same error in all, whereas by the shorter method of adopting a definite dose as standard we only have an occasional preparation a little off strength, due to an over or under susceptibility of the dogs used on that particular assay.

Due to the variation in susceptibility of different dogs, the method must essentially be comparative and not absolute. This necessitates the adoption of an arbitrary standard with which the activity of the unknown can be compared. The U. S. P. method would, therefore, be very satisfactory had the committee only gone a step farther and, as suggested by Pearson,[3] made arrangements for supplying manufacturers with a suitable standard with which to compare the activity of their preparations. Until such a standard is supplied, however, it is only a waste of time to run an assay on a standard preparation, *which the manufacturer has prepared himself;* each time an unknown sample is tested.

Some workers have objected to the standards adopted by the Pharmacopoeia for Cannabis, claiming that they are too high. Personally I have found no difficulty in meeting the U. S. P. requirements for preparations of Cannabis. In going over the physiologic reports on fluid extracts of Cannabis I find only four or

[2] Pittenger, "Biochemic Drug Assay Methods," page 101.

[3] Pearson, Jour. A. PH. A., Nov. 1916.

five samples out of the last thirty submitted to the laboratory which have failed to come up to the U. S. P. standard. There have also been very few samples of Cannabis drug submitted to the laboratory which have not produced marked incoördination in the standard U. S. P doses.

In order to observe marked incoördination in the animals with the U. S. P. dose, it is, however, necessary to take all the precautions mentioned in the text, such as keeping the animals in a perfectly quiet room free from disturbance and separated so they cannot see each other.

I find that I obtain much more accurate results by using as an end-point a reaction which can just be distinguished when all of the above precautions are taken as by this method a sharp line can be drawn between the dose which just produces incoördination under the above conditions and the next smaller dose with which it is impossible to detect any symptoms of incoördination. If a very marked effect is used as an end-point for instance, an effect sufficiently marked that the animal will show incoördination even when its attention is attracted by movements of the operator, other dogs, etc., I find that not nearly as accurate results can be obtained because very little difference in the degree of incoördination can be noted between dogs receiving doses sufficiently large to produce marked incoördination under these conditions and those which have received 20 or 40 percent larger doses.

I have found, therefore, that by adhering strictly to the U. S. P. method, no difficulty in noting marked incoördination in animals receiving the U. S. P. standard dose of Cannabis.

The method of stating the standard, however, is open to criticism. The U. S. P. states:

"When assayed biologically Fluidextract of Cannabis produces incoördination when administered to dogs in a dose of not more than 0.03 mil per kilogramme of body weight."

According to the above statement a dose larger than 0.03 mil per kilo would not produce incoördination. The words "not more than" should either be omitted or changed to "in a minimum dose of 0.03 mil per kilo."

Some workers have objected to the action of the Committee in making the test for Cannabis compulsory because it is one of the least satisfactory tests we have, and would, therefore, be a hardship on the retail druggist in that he would be held accountable for the activity of his Cannabis preparations when only an expert could satisfactorily carry out the test.

This criticism would be justified had the Committee adopted a standard reading "the minimum dose of fluid extract of Cannabis necessary to produce incoördination should be *not less than* (—) mils per kilo, *nor more than* (—) mils per kilo."

The standard adopted, however, only specifies a *minimum* activity in order to guard against fraudulent, inert or badly deteriorated drugs and does not specify "limits" as in the chemical assays for alkaloidal drugs.

No hardships are imposed upon the inexperienced operator, therefore, because it is only necessary that Cannabis preparations possess a certain minimum activity and it is not compulsory that they actually be standardized.

Unlike most chemical assays the assay for Cannabis is such that a preparation which passes the inspection of an inexperienced operator is more active than

one passed by the expert because the expert can notice marked signs of incoördination in dogs before the first signs are appreciable to the inexperienced.

Of course, *the expert is better qualified to actually standardize these preparations*, but, as before stated, a person need not be an expert in order to determine whether or not a particular preparation of Cannabis conforms to the requirements of the U. S. Pharmacopoeia.

ACONITE.

The proposed "time limit" of 12 hours is very objectionable as this means 12 hours after the pigs are injected. When you add to this the time of weighing animals, preparing solutions for injections, making injections, etc., the test consumes 13 hours, which cannot be included in the ordinary working day and makes a rather long week for men employed in laboratories which run these assays almost daily. I would suggest a 24-hour "time limit." Three years ago we made a record of the results obtained on several thousand pigs at the end of 2, 3 and 24 hours and found that we obtained the most concordant results by using 24 hours as the "time limit." We immediately changed from the old 2-hour to a 24-hour "time limit" and have employed this "time limit" ever since with very satisfactory results.

I do not doubt but that a 12-hour method would be just as accurate as the 24-hour method but according to my mind it would be very objectionable for the reason stated.

DIGITALIS—STROPHANTHUS—SQUILL.

The method recommended for the above drugs is the so-called "one-hour frog" method. Personally I prefer the guinea-pig method to the frog method. My principal criticism of the method given in the Pharmacopoeia, however, is in regard to the technique recommended for injecting the doses into the frogs. The U. S. P. states:

"After the frogs have been weighed as described, the doses to be given are calculated according to their weights and are *measured into small conical glasses by means of a finely graduated pipette.* The doses of the preparation which are to be injected should be as uniform in quality as possible and *should not exceed 0.015 mil for each gramme of body weight of frog.*" * * * * * * * "When the doses are ready, they may be injected into the anterior lymph sac of the animal. This is done by means of a *glass pipette* which is drawn out to a fine point. The frog is held on its back in one hand and the pipette with the contained drug in the other, the mouth of the frog is opened with the point of the pipette and, carefully avoiding the tongue, the floor of the mouth is punctured and the point of the pipette is then seen to enter the anterior lymph sac of the frog. The contents of the pipette are now forced into the sac, either by gravity or by gently blowing, if necessary. In the latter case, care should be taken not to introduce air into the sac."

It is *absolutely impossible* to obtain accurate results if this technique is followed. It will be noted that the average frog should weigh 20 Gm. and that the dose injected should not exceed 0.015 mil for each gramme or 0.3 mil for a 20 Gm. frog. You are directed to measure this 0.3 mil by means of a *finely graduated pipette* into a conical glass. This *very small dose* (0.3 mil) is then sucked up into another *sharp-pointed pipette* and forced into the lymph sac by blowing.

The error due to the amount of solution left in the conical vessel and the second pipette is indeed great when compared with the very small dose given.

The use of the second pipette and the conical glass vessel is no doubt recommended because it is impossible to force the preparation into the lymph sac by blowing and at the same time accurately measure the dose to the hundredth of a mil.

The two pipettes and the conical glass vessels should be replaced by an all-glass or "Record Tuberculin Syringe," which is graduated in hundredth of a mil. By the use of one of these syringes the *actual amount of the preparation injected* can be measured to the hundredth of a mil, whereas by the U. S. P. method we only know the amount of solution placed in the conical vessel and not the amount actually injected.

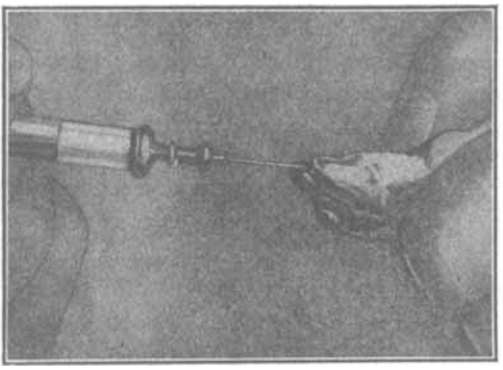

FIGURE 1.—METHOD OF INJECTING FROGS.
From Pittenger's "Biochemic Drug Assay Methods."

On page 608, first line, the directions state:

"The dose thus found is then compared, etc."

The text fails to state which dose is *the dose* to be compared. It is not stated anywhere that the *smallest or minimum* dose necessary to bring about the end reaction is the one to be used in computing the strength of the preparation. In other words, the directions give no definite outline for carrying out the tests, but take it for granted that the operator understands the technic of giving the doses in series, progressively increasing or decreasing until the M. L. D. or M. S. D. is found, etc.

SUPRARENAL GLAND.

As stated by Hamilton,[4] "the biologic assay of products of the suprarenal gland is open to criticism in only two particulars, *i. e.*, in the method of measuring and administering the doses and in attempting to check the results as described."

"Using both femoral veins for injecting sample and standard is to obviate the possible mixing of the two solutions if both are injected into the same vein. But it introduces a very much greater source of error. The amount injected can much more easily be measured by use of a pipette than in a syringe, and the dose after being injected can be easily and completely washed into the blood stream by a follow-up injection of 2 mils physiologic salt solution. When this procedure is followed, no mixing of two injections is possible."

[4] Hamilton, "Biological Standardization," Amer. Jour. Pharm., Feb. 1917.

Another very good method is to *expose the saphenous vein at its junction with the femoral.* When giving injections the needle of an all-glass syringe is inserted far enough through the saphenous vein to allow the point to project directly into the blood stream in the femoral vein. After injecting the preparation, the needle can be withdrawn and the saphenous vein clamped with a bulldog clamp. The preparation thus injected is entirely carried into the circulation by means of the main current of blood in the femoral vein.

My views also coincide with Hamilton's in that the "checking of an assay by making injections of sample and of standard into opposite sides from the first used is no check except in so far as it checks conditions on the two sides of the dog. This feature can better be eliminated by using only one side. Further, by the official method, if it is impossible to complete the test and the check on a dog, no option is left, but to repeat both test and check on another dog. It is occasionally necessary to check an assay on a second dog when conditions during the first test were unfavorable for accuracy but no advantage results from a retest on the same dog."

PITUITARY EXTRACTS.

It is gratifying to note that the Committee has adopted the isolated uterus method for Testing Liquor Hypophysis, for, as stated in another paper contributed to this Section,[5] "This method is the best so far proposed, as differences of activity which are only just appreciable by the blood-pressure method, under the best conditions, are at once obvious in the test on the uterus without any special care in controlling the regularity of the response."

I am of the opinion, however, as stated in the paper mentioned above, that more concordant results can be obtained by employing the *whole* one horn of the uterus of a 350 to 425 Gm. pig instead of only a *segment* of the one horn of the uterus of a 250 Gm. guinea pig; also by controlling the contractions of the uterus by means of an escapement wheel and bucket for holding shot instead of the small heart lever recommended. When the whole horn is used the heart lever is not heavy enough to allow sufficient weight to be added to control the contractions of the muscle.

The assay for Liquor Hypophysis requires more experience on the part of the operator than any other biologic test in the Pharmacopoeia, and, although compulsory for a U. S. P. product, it is not included in the chapter on Biologic Assays.

The principal criticism of the U. S. P. method for testing Liquor Hypophysis, however, is not with the method itself but with the standard adopted. The author's views upon this subject were set forth in a paper read before this Section last summer[6] in which the following statements were made:

"Before adopting a complex substance like the above (beta-iminazolylethylamine hydrochloride) as a standard for adjusting the strengths of commercial preparations it would have been better, perhaps, to make a thorough study of a number of problems such as the following:

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[5] Pittenger, "An Improved Apparatus for Testing Drugs upon the Isolated Uterus."

[6] Pittenger and Vanderkleed, "Preliminary Note on the Value of Beta-Iminazolylethylamine Hydrochloride as a Standard for Testing Pituitary Extracts," JOUR. A. PH. A., Feb. 1917.

1. Degree of uniformity in the physiologic action of different available samples of the proposed standard substance.

2. Rate of deterioration of solutions of this substance.

3. Effect of sterilization on solutions of this substance.

4. Rate of deterioration of the substance itself.

5. Effect of repeated doses on uterus.

6. The toxicity of the substance as compared with Pituitary Extract.

7. The relative toxicity of a Pituitary Extract of the strength proposed by the U. S. P. IX and that of the commercial extracts as supplied by the leading Pharmaceutical Manufacturing Houses.

The results of experiments are then given which tend to prove that the standard substance deteriorates quite rapidly and that "the standard adopted by the U. S. P. IX is very low because by comparison we find that the commercial extracts prepared by the leading pharmaceutical houses, which have been on the market for several years and to which the physicians have become accustomed as to dosage, etc., are from three to five times as active as an extract of the new U. S. Pharmacopoeia standard strength. This is unfortunate, as there is no reason why a weaker preparation than the one to which physicians have become accustomed should be placed on the market." The findings of the author as reported in the above paper have since been corroborated by Eckler[7] and Hamilton.[8]

It is to be hoped, therefore, that before it becomes necessary to revise the Pharmacopoeia again definite requirements can be drawn up for the test substance itself and that an accurate coördination of the required U. S. P. strength and of the common pharmaceutical practice may be secured.

PHARMACODYNAMIC LABORATORY,
H. K. MULFORD COMPANY,
August 15, 1917.

ON THE DETERIORATION OF CRUDE INDIAN CANNABIS.*

BY C. R. ECKLER AND F. A. MILLER.

It has long been known that crude Indian Cannabis loses its activity quite rapidly, and Marshall[1] and others have shown that the deterioration is due to oxidation of the active principles, but the rate of deterioration during commercial storage has not been determined, and this was of particular interest to us. For the purpose of learning something on this point, two sets of experiments were carried out, our intention being to imitate the different conditions under which the crude drug might be kept.

One lot of drug was stored in a cool basement in three portions, one portion sealed in alcohol, one portion sealed dry, and one portion unsealed dry. Another lot was stored in a warm attic in four portions, one portion, granulated, sealed;

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[7] Eckler, *Amer. Journ. of Pharmacy*, May 1917, p. 195.

[8] Hamilton, *Amer. Journ. of Pharmacy*, Feb. 1917.

* Read before Scientific Section, A. Ph. A., Indianapolis meeting, 1917.

[1] Marshall, "Experiments on the Cause of the Loss of Activity of Indian Hemp," *Pharm. Jour.*, Vol. 82, p. 418 (1909).