PROCEEDINGS OF THE LOCAL BRANCHES

"All papers presented to the Association and its branches shall become the property of the Association, with the understanding that they are not to be published in any other publication than those of the Association, except by consent of the Committee on Publication."—By-Laws, Chapter X, Art. III.

Reports of the meetings of the Local Branches should be mailed to the Editor on the day following the meeting, if possible. Minutes should be typewritten, with wide spaces between the lines. Care should be taken to give proper names correctly, and manuscript should be signed by the reporter.

DENVER.

The Denver Branch, A. Ph. A., held its regular monthly meeting Tuesday evening, April 20, 1920, at the Metropole Hotel. An excellent dinner was served at 6.30. President Geo. Gregory presided and seventy-five members were present.

Before the meeting, Mr. Greshen, one of the Committee on the Presbyterian Hospital Association, made a talk and a plea for subscriptions to this very worthy cause. In response to Mr. Greshen's plea $575.00 was subscribed, which amount added to that already subscribed by various druggists throughout the city made a very commendable showing for the Retail Drug Trade in general. Following Mr. Greshen's talk the Minutes of the March Meeting were read.

Mr. Samuel T. Hensel made a motion that an amendment be made to the Minutes of the March Meeting wherein the action taken by the Executive Committee on H. R. Bill No. 8078 be shown, also a copy of the letter sent to each of the Senators and Representatives be added. This action was seconded and carried and the Secretary instructed to make the proper amendment.

AMENDMENT TO MINUTES OF MEETING HELD MARCH 16, 1920.

It was moved at the April Meeting of the Denver Branch, A. Ph. A., by Mr. Samuel T. Hensel, that the Minutes of the March Meeting be amended as follows:

To include action taken by the Executive Committee of the Denver Branch regarding H. R. Bill, No. 8078, introduced in the sixty-sixth (66th) Congress, to regulate the importation, manufacture, etc., of Coal-Tar Products.

The Executive Committee approved this, or some similar Bill, and the Secretary was requested to write the two Colorado Senators and four Representatives relative to the action taken in said Bill.

A letter was written and a copy of same is attached.

Following the reading of the Minutes, thirty-five new applications were read, voted upon and all elected to membership of the Denver Branch.

Charles J. Clayton next made a few remarks regarding the report of alcohol. This report must give the quantity used and the purpose for which it is used during each month, and report must be turned in to the Government on or before the 5th of each succeeding month.

The President took up the question of prices on drugs, drug merchandise, etc., and Mr. Chedister, of the Price Committee, was called upon for a report. Mr. Chedister gave a very clear explanation of the method of prices and explained the book which would be gotten out containing the various price lists. Much discussion was entered into regarding prices, and results which were satisfactory to all concerned were eventually reached. The prices of soda fountain beverages were afterward discussed by E. J. Hellwig and others.

Carl Shirley was responsible for the entertainment of the evening, which was warmly applauded by all present.

R. A. WHITE, *Secretary.*

COMMITTEE REPORTS

REPORT OF THE COMMITTEE ON PHYSIOLOGIC ASSAYING OF THE AMERICAN PHARMACEUTICAL ASSOCIATION, 1920.

This Committee reported last year that it was unanimous in its opinion that the "Biologic Assay Methods" of the U. S. P. IX are unsatisfactory, due to the fact that in many cases

they lack the details which workers in the practical laboratory have found essential in order to obtain accurate results. In other words, the 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 and therefore do not show as well as they might, the degree of efficiency to which biologic assays have been developed.

Due to this fact very little attention has been paid to the methods as set forth in the U. S. P. IX, as all evidence tends to prove that they are less accurate and reliable than the methods in common use.

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."

As the Tenth Decennial United States Pharmacopoeial Convention will meet in this city next week, we are of the opinion that this Committee can render the most service at this time by reporting what we consider to be "points of weakness" which exist in the present U. S. P. methods.

We therefore submit the following detailed constructive criticisms of the present U. S. P. "Biologic Assay 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 the dog swallow a capsule by the above method. Pulling the 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."[1]

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

Until a standard extract is furnished by some central authority to be used in adjusting the strength of our standard preparation nothing is gained by comparing the effects produced by the unknown with those produced by a standard preparation. According to the U. S. P., a manufacturer should prepare a standard by adjusting a preparation until it is of such strength that 0.03 mil per kilo of the fluidextract will produce incoördination.

Why not adopt 0.02 mil 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 mil per kilo instead of the amount of the standard necessary to produce the same effect? If the standard is of proper strength it will require 0.03 mil per kilo. The only object 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

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

and not to standard dogs. The operator is just as liable to have dogs which are over or under normal susceptibility when adjusting the standard as when assaying the unknown, thus making the standard slightly over or under strength. 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 a standard we only occasionally have a 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 is essentially 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 gone only a step farther and, as suggested by Pearson,[1] 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.

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

> "When assayed biologically Fluidextract of Cannabis produced 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

> "When assayed biologically not more than 0.03 mil per kilogramme of body weight, of Fluidextract of Cannabis should be required to produce incoördination when administered to dogs."

ACONITE.

The proposed "time limit" of 12 hours is very objectionable, as this means 12 hours after the guinea-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. We would suggest a 24-hour "time limit."

Pittenger by recording the results obtained by using 2, 3 and 24 hours as a "time limit," proved that the most concordant results are obtained by using 24 hours as the "time limit."

We do not doubt but that a 12-hour method is just as accurate as the 24-hour method, but it is very objectionable for the reason stated.

Your Committee cannot too strongly recommend that the biologic assay instead of the chemical assay for Aconite be made compulsory.

The chemical assay is a very accurate determination of the alkaloidal content of Aconite, but it is not nearly as good an index to the therapeutic value of the drug, or its preparations, as the pharmacodynamic test. It has been definitely proven by many workers that the results of the present chemical assay do not parallel the therapeutic activity of the drug. In other words, it often happens that a preparation of Aconite will run high in the chemical test and low in the physiologic test, due to the fact that a chemic assay does not express the true activity of the drug because it also estimates other alkaloids of lower activity than Aconitine.

This discrepancy between the two tests is easily understood when we consider the nature of Aconitine and realize how the acetyl and benzol groups are split off, thus reducing the physiologic activity of the product without destroying its alkaloidal nature, allowing it still to be estimated as an alkaloid in the chemical assay process.

The present pharmacopoeia makes the chemical test compulsory and at the same time recommends that the drug be assayed by the pharmacodynamic method. For the reason stated above it is impossible to standardize the product by both methods when the results obtained by one do not parallel those obtained by the other.

It is to be recommended, therefore, that the pharmacodynamic test be made compulsory in place of the chemical test.

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[1] Pearson, A. PH. A., Nov. 1916.

The principal criticism of the method as given in the Pharmacopoeia 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 quantity 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 hundredths 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 know only the amount of solution placed in the conical vessel and not the amount actually injected.

The U. S. P. method for standardizing the "digitalis series" has the following features which commend it strongly as a satisfactory method for commercial or scientific use:

(*a*) It is reasonably economic.
(*b*) It is quickly applied.
(*c*) It is fairly accurate.
(*d*) It makes use of one of the most typical effects of the drug.

On the other hand, the advocates of the "M. L. D." frog heart method claim that the U. S. P. method possesses the following features which are serious defects in establishing the degree of activity of the digitalis series as therapeutic agents:

(*a*) On account of the relatively short time between the dosing of the animal and reading the end-point. Slow or delayed absorption may cause a low valuation to be given to a drug having high therapeutic value.

(*b*) The inflexibility of the time of reading the end-point does not allow for variability in the frogs and the small number of frogs used may not correct this error. It is difficult at times to conclude whether the heart should be classed as beating or stopped at 60 minutes, nor what to conclude if it resumes a normal beating or comes to rest in systole soon after the hour.

(*c*) The rough handling in pithing and laying bare the heart cannot but effect the results unfavorably. While every frog is subjected to the same treatment this does not entirely remove the objection.

This Committee would, therefore, recommend that before re-adopting the "One-hour" frog method as the official method, the Revision Committee carry out sufficient comparative laboratory experiments with the "One-hour" and "Twelve-hour" methods to decide definitely which is the better of these two methods.

The standard, ouabain, adopted by the Committee for comparison in measuring the activity of the digitalis series of heart tonics has been criticised because different lots are not uniform in comparison.

"While it is true that the standard, in physiological assaying, is merely to measure the resistance of the frogs, this resistance is of such a complex character that it should be measured by a standard, not in any respect open to criticism. The standard, if not identical with the sample, should be one whose composition and identity have been established.

The description of ouabain indicates that it is derived from a non-official strophanthus seed, that its composition is indefinite in that it crystallizes with varying quantities of water and that it does not yield a crystalline strophanthin and cannot, therefore, be assayed chemically to establish uniformity. That it is not uniform is shown by Rowe,[1] a conclusion which may be deduced from the fact that the M. S. D. of ouabain accepted by the U. S. P. Committee is 0.0000005, while the average of the three samples tested by Rowe is 0.00000086 or 76 percent more—a difference not due to temperature, because in all cases the tests were carried out at 20° C. Further, it is an expensive substance and obtainable only by importation." (Hamilton[3]).

Strophanthin from Kombe seed can be made of uniform composition and activity according to Brauns and Closson[2] and is, therefore, preferable, to ouabain for every reason, but its use seems especially logical because of being derived from the official strophanthus seed.

This Committee would, therefore, recommend that before readopting *Ouabain* as the "standard substance" for standardizing animals which are subject to seasonal variation, a thorough study of Strophanthin Kombe should be made, as this substance is to be preferred to ouabain for the reasons stated.

SUPRARENAL GLAND.

As stated by Hamilton,[3] "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 be much more easily measured by use of a pipette than by injecting with a syringe, through a rubber connection and cannula, 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."

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 bulldog clamp. The preparation thus injected is entirely carried into the circulation by means of the main current of blood in the femoral vein.

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.

The majority of this Committee would recommend that the "Isolated Uterus" method be *retained* as the official method for testing Liquor Hypophysis.

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[1] Rowe, J. A. PH. A., Nov. 1916.

[2] Brauns and Closson, J. A. PH. A., May 1913.

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

We are of the opinion, however, that more concordant results can be obtained by employing the *whole* one horn of the uterus of a 350 to 425-Gm. pig as suggested by Pittenger,[1] instead of only a *segment* of the one horn of the uterus of a 250-Gm. guinea-pig.

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.

Under "Liquor Hypophysis," however, we find that the product must be tested "as directed by the United States Hygienic Laboratory."

We would recommend that the complete details of this test be included in the U. S. P.

"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.

It seems unwise as well as unnecessary to choose as the standard substance one which has only one of the typical physiological effects of hypophysis, and which alone has no therapeutic application equivalent to that of extracts of the pituitary gland." (Hamilton).[2]

Before re-adopting a complex substance like beta-iminazolylethylamine hydrochloride as a standard for adjusting the strength of commerical preparations, a thorough study of the following points should be made:

1. Degree of uniformity in the physiologic action of different available samples of the proposed standard substance.
2. Rate of deterioration, both in solution and powder.
3. Effect of repeated doses on the isolated uterus.

A similar study should be made of each of the following substances:

1. Dried, defatted, powdered Posterior Lobe as suggested by Hamilton.[3]
2. Water-soluble powder prepared by Aldrich.
3. Potassium Chloride, suggested by Spaeth.[4]

After a thorough study and comparison of the above substances, the one best suited for the purpose should be adopted.

The standard adopted by the U. S. P. IX is very low because it has been shown by Pittenger[5] 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 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.

It is to be hoped, therefore, that the next edition of the Pharmacopoeia will contain definite requirements as to the purity and uniformity of activity of the standard test substance and that an accurate coördination of the required U. S. P. strength of Liquor Hypophysis and that of the common pharmaceutical practice may be secured.

We would also recommend that Ergot be included among the drugs to be biologically assayed and that the Committee during the next year carry out the necessary experiments to develop the method.

In conclusion, your Committee would recommend that a copy of this report be sent to the Chairman of the U. S. P. Revision Committee for consideration in compiling the next edition of the U. S. P.

Respectfully submitted,

(Signed) H. C. COLSON,
H. C. HAMILTON,
W. A. PEARSON,
P. S. PITTENGER,
Chairman.

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

[2] Hamilton, *Amer. Jour. Pharm.*, Feb. 1917.

[3] Hamilton, A. PH. A., Oct. 1912.

[4] Spaeth, *J. Pharm. and Exper. Ther.*, Apr. 1920.

[5] Pittenger, Hamilton[4] and Eckler[6], J. A. PH. A., Feb. 1917.