hand. The microburette having been thoroughly cleansed in the usual way, is next thoroughly cleansed with alcohol, ether, alcohol and water to remove any grease—special attention being paid in this respect to the portion DEI. The two parts are now joined and are held together by a small rubber band which engages the lugs, B, C, D and E, and maintains a moderate tension upon the two parts. No burette stand or clamp is employed. The microburette is held in the hand. HI is used as the stirring rod while titering. A finger, by pressure against lug D or lug E, rotates DEI and in this manner starts or stops the flow of standard solution. When F is not in apposition with KL there is no flow. The flow is established when F and KL are in apposition, which is known by looking at F and at the register mark N. The flow is from M, past KL, out of F and down the outside of the stirrer HI. This flow is satisfactory only when the stirrer has been properly cleansed as described above. In filling the apparatus prior to a titration, use a minute funnel at A. Fill nearly to A and then establish the flow until the level sinks to zero. The stirrer is now coated with a very thin film of standard solution and the titration should be begun at once.

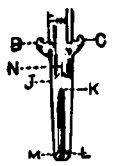

Fig. 2.

While the microburette will commend itself for its convenience and elegance in use, its main advantage arises from the fact that its flow is partly controlled by capillary action on the outer surface of the portion FI. This feature enables one to deliver small fractions of a drop from the microburette with as much ease as whole drops can be delivered from the ordinary burette. The readings are to 1/200 cc.

In calibrating the microburette and in running test titrations, the assay balance should be employed.

THE ASSAY PROCESSES OF THE U. S. P.

A. R. L. DOHME AND H. ENGELHARDT.

On various occasions we have pointed out that several assay processes of the present U. S. P. are very much in need of being thoroughly revised, both because the methods are rather cumbersome, and the results are far from giving the true percentage of the active principle. Since the methods are to be thoroughly discussed at this meeting we thought it necessary to again give our views in regard to the processes, although several points given here may have been discussed by us on previous occasions.

We still believe that the aliquot part method, when worked with precaution, gives more accurate results than the percolation method. The drug is more thoroughly exhausted by shaking with the menstruum than by percolating. A percolator perhaps is our most unscientific piece of apparatus. A channel might be formed in the packed drug, the parts adjoining this channel may be exhausted, while other parts of the drug come in contact with the menstruum only superficially. The method, besides, is very tedious, especially when such a fine powder

(No. 60) as prescribed by the U. S. P. is employed, and also requires a larger amount of menstrumm for the exhaustion.

We, therefore, strongly recommend the adoption of the aliquot part method, having proven by numerous experiments that results by this method compare favorably with those obtained by exhausting the drug completely by percolation.

For the final shaking out of the alkaloids, we recommend to use whenever possible, simple menstrua, viz., ether or chloroform and not mixtures of both in various proportions. As a rule, simple menstrua are less liable to produce emulsions than mixtures, and the mentsrua are more easily recovered for future use than mixtures, which always require tiresome adjusting.

For the extraction of the drugs, however, a mixture of ether-chloroform is to be preferred. Such a mixture seems to penetrate the cell-walls better than a simple menstruum, and consequently to extract the alkaloids more thoroughly. It is also recommended to allow the drug to stand with the menstruum for at least one-quarter hour before adding the ammonia, as the results obtained by doing so are somewhat higher, in our opinion, than those obtained by adding ether-chloroform and ammonia to the drug at once.

Whenever possible the alkaloids should be estimated by titration; in some cases when hydrolysis is liable to take place as in aconite, coca leaves, etc., a check by gravimetric estimation might be of advantage.

Of all the indicators for alkaloids, we have found cochineal to be the best, since only in titrating the alkaloids of ipecac is any difficulty experienced with this indicator. Iodeosin. at present used in the U .S. P. is rather unreliable, since the aqueous liquid is not always colored red when the end point is reached, but at times a red scum is formed at the contact of the two layers, the color of this scum increasing in intensity with the addition of the alkali. It is difficult to judge, in case this happens, when the end point is reached.

In regard to the various drugs and the galenical preparations thereof, we beg to offer the following suggestions:

Aconite Root.—To avoid hydrolysis as much as possible, ammonia might be replaced by sodium carbonate or bicarbonate solution. The present process is very tiresome, only in the case of a larger dilution can a somewhat rapid filtration be effected. Keller's aliquot part process, using ether-chloroform and sodium bicarbonate for extracting the drug, and ether alone for the final extraction of the alkaloid, after having made the acid solution alkaline with sodium bicarbonate, gives very good results. The wording, "not less than 0.5 per cent of aconitine" should be replaced by "not less than 0.5 per cent of ether soluble alkaloids," since the residue, although it consists for the greatest part of true aconitine, is always contaminated with other basic substances. The Squibb's test has been found to be too much dependent on individuality.

Extract of Aconite.—No matter how carefully this extract is prepared, a deterioration of the alkaloids is liable to take place, and the physiological strength consequently is largely reduced. Extract aconite should never be prepared. In assaying extract of aconite, the following simple process gives fairly accurate results: Dissolve the extract (2 grams) in 10 cc. of dilute alcohol, transfer the solution to a separator, make alkaline with sodium bicarbonate solution and shake

out with several portions of ether. From the ethereal solution the alkaloids are extracted by shaking with several portions of acidulated water, and from the latter, after making alkaline with sodium bicarbonate, the alkaloids are removed by shaking with several portions of ether. From ethereal solutions, after filtering to remove any suspended bicarbonate, the ether is distilled off, etc.

Fluidextract of Aconite.—Ten cc. are transferred to a separator, made alkaline with sodium bicarbonate, and then assayed as just given.

Tincture of Aconite.—One hundred cc. of the tincture are evaporated at a temperature not exceeding 60° C., the residue taken up in 10 cc. of dilute alcohol, and this solution assayed as given under extract.

Solution of Hydrogen Dioxide.—The method for determining the acidity should be revised. By evaporating 25 cc. of hydrogen peroxide solution to 10 cc. in the presence of 5 cc. of N/10 potassium hydroxide solution, not all the hydrogen peroxide is destroyed. This can be effected only by evaporating the solution in a platinum dish or by adding a suitable catalyzer, such as platinum black, etc.

Asafetida.—Owing to the scarcity of this article, it would be advisable to decrease the percentage of alcohol soluble matter, and to increase the allowable percentage of ash.

Aspidium.—The activity of this drug depends almost entirely on those substances present in what is generally termed "crude filicin". A reliable method has been worked out for determining crude filicin. The microscopic requirements given in the present U. S. P. will be met by a physiologically inactive drug also.

Belladonna Root and Leaves.—The assay process adopted for the new U. S. P., viz., the aliquot part method, has a decided advantage over the present process, and gives very satisfactory results.

Fluidextract and Extract of Belladonna.—The assay processes for these preparations are satisfactory. It is, however, advisable to increase the amounts of both the immiscible solvents, and the acidulated water.

Cantharis and Its Preparations.—These should be assayed; several reliable methods have recently been published. A suitable menstruum for preparing the mixture should also be looked for, as by the present menstruum only about 50 per cent of the cantharidin is extrated from the drug when used in the proportion 1:1.

Capsicum.—We have met with several specimen of inferior capsicum. Why not give and estimate the percentage of oleoresin?

Cinchona.—For the U. S. P. IX, unfortunately, an assay process has been proposed, which is similar to the one now official, differing from it only by the larger amount of menstruum taken for extracting the alkaloids from the drug. Although this is a step in the right direction, we doubt very much whether the increased quantity of menstruum will hold in solution the alkaloids from high-grade drugs. The Fromme process depending on the breaking up of the cells by the use of hydrochloric acid has always given us satisfactory results. It is a short one, and a determination can easily be carried out in two hours. That such a process is of great importance to chemists who have to make a dozen or more cinchona assays at the same time (as in our laboratory, when numerous samples for purchasing the drug are submitted) is obvious. We wish to mention again

that the alkaloidal residue should be dried at a temperature not exceeding 60° to 70° C., as otherwise it is strongly discolored. Any traces of chloroform should be driven off by treating the residue twice or three times with ether. In our laboratory, we invariably control the gravimetric results by titration, because the alkaloidal residue very frequently includes waxy and other substances which naturally increase the weight. The titration when carried out strictly according to Panchaud's direction, is not at all difficult.

Coca.—Here also the percolation process should be abandoned in the assay method. Keller's method, using plain ether, gives very satisfactory results. In case emulsions occur, which frequently takes place on account of the large amount of mucilaginous matter in the drug, tragacanth should be used for breaking up the emulsions.

Cochineal.—It is advisable to include in the U. S. P. a determination of the color strength of the drug, also an estimation of the moisture.

Colchicum Seed and Corm.—We have pointed out on various occasions that the results obtained by the present assay methods are absolutely wrong, that the residue calculated as colchicine contains only about 50 per cent of the alkaloid. The assay processes should be thoroughly revised. Dr. Lyons has given valuable information in what way these processes could be improved. For the estimation of pure colchicine in the alkaloidal residue, several methods are available also. We do not care to go into details about these improvements since we have given a compilation of them some time ago.

Conium Seed.—The assay method for this drug also should be revised. It is very cumbersome and could easily be replaced by a more expeditious process.

Conium Leaves.—This drug, although not official, should never be used. All the samples submitted to this laboratory for examination were almost void of Coniine.

Cubebs.—An estimation of and requirements for the percentage of oleoresin should be given. Cubebs vary considerably in the amount of oleoresin.

Belladonna Plaster.—A few slight modifications of this assay process, which work very well, have recently been recommended.

Ergot.—On various occasions we have mentioned a simple process to estimate the approximate amount of cornutine present in the drug. If it can be proven beyond doubt that the percentage of cornutine is in proportion to the physiological activity, this test should be adopted for the U. S. P.

Ferrum Reductum.—The assay process could be improved on.

Gelsemium and Its Preparations.—Assay processes for these substances have been recommended on various occasions. We believe, however, that such a process is only of relative value as long as the proportion of the active substance to the inactive is not known in the residue determined as total alkaloids. Quite recently a good deal of light has been thrown on the constituents of gelsemium and possibly in the near future an assay process based on the estimation of the active principle alone will be worked out.

Glandulae Suprarenales et Thyroidae.—Colorimetric estimation of the active principles is desirable.

Granatum.—The total alkaloids in pomegranate bark can easily be estimated.

Guarana and Its Preparations.—The assay processes are good.

Hydrastis and Its Preparations.—The amount of golden seal taken for the assay is entirely too large, considering the high percentage of hydrastine in the drug. There is no reason why the assay of the fluidextract should not be based on the same principle as the assay of the drug.

Hyoscyamus and Its Preparations.—All that is said about Belladonna applies to these products also.

Ipecacuanha and Its Preparations.—The amount of drug prescribed for the assay process should be reduced considerably, say to about 6 grams. The assay process otherwise is satisfactory. We have pointed out above that the titration of the alkaloidal residue is somewhat difficult, and it would be desirable to try other indicators which might prove to be more satisfactory.

Jalap.—A shorter process depending on the exhaustion of the root with hot alcohol and taking, after cooling and readjusting the weight, an aliquot part has been recommended by us on a former occasion. In connection with this drug it may be said that the quality of the various samples and shipments during the last twelve months was superior to that in previous years. Would it not be advisable to control the galenical preparations of jalap by simple assay processes?

Kola and Its Preparations.—These should be assayed by a process similar to that given for guarana. To estimate the amount of theobromine, acid instead of water has to be used for extracting the alkaloids from the chloroformic solution.

Malt and Extract of Malt.—It is advisable to give assay processes for the determination of maltose and diastatic power. We have met with numerous samples of malt which were deficient in both respects.

Nux Vomica and Its Preparations.—Keller's aliquot part method, using ether and chloroform, gives fairly good results; it must, however, be admitted that the results obtained by using the U. S. P. menstruum are slightly higher. The amount of the powdered drug can be reduced on account of the high percentage of alkaloids in the drug. It is to be regretted that the U. S. P. IX again shall adopt a method for determining the strychnine. The present official method and the numerous modifications thereof give fairly accurate results only in the hand of experienced workers. We doubt very much that the variation of the proportion of strychnine and brucine in the drug is greater than the variation obtained by assaying the same drug by various chemists. Only such methods should be adopted in the U. S. P. which are simple and give fairly accurate results, and not such ones which require much ability and experience. The U. S. P. is not written for experienced chemists, such as are generally found in the laboratories of the large wholesale houses, but for the retail pharmacist also who very seldom has and will have a thorough experience in assaying drugs. We have mentioned on other occasions that of all the pharmacopœias only the English directs the strychnine to be estimated, and this is done by a method which is still inferior to the old Gerock method and its modifications. We are afraid that by adopting the strychnine determination much trouble and numerous litigations will be caused. If it is important to determine the strychnine alone, why has not a process for

doing so been adopted by the Swiss, German, etc., pharmacopœias, which without doubt are up-to-date works? Is brucine therapeutically absolutely inert, and can it be entirely neglected? In our opinion, the determination of the total alkaloids (which by no means is such a very simple one, on account of the ammonia bases and the soap which are liable to be formed during the assay process) is a better criterion for the quality of the drug than an unreliable and incorrect estimation of the strychnine alone.

Extract of Nux Vomica.—The easiest way of assaying this extract is to convert the extract into a fluidextract by dissolving in diluted alcohol, rendering the solution alkaline with ammonia water, shaking out with several portions of chloroform, etc.

Fluidextract and Tincture of Nux Vomica.—Evaporate the quantity prescribed for the assay to dryness, take up residue in dilute alcohol and proceed as just given.

Opium.—In regard to this drug, we wish to refer to an article submitted to the A. Ph. A. (Proc. A. Ph. A., 1910, page 829) a year ago. There is no doubt that by the present official process almost the entire morphine contained in the drug is obtained, although Delbourdeaux, Journ. de pharm. et chem. VII, IV, 68, claims that by further exhaustion with water still more morphine can be extracted. He also claims that if the crude morphine, as obtained by the U. S. P. process, is not washed thoroughly, lime-water soluble substances are determined as morphine, rendering the percentage of the latter too high. We have obtained very good results with the present method, we think, however, that a shortening of the process would be desirable.

Extract and Tincture of Opium.—The assay methods work satisfactorily.

Pancreatin.—For the assay process the use of potato starch should be recommended. The milk test is unreliable and should be deleted.

Pepsin.—We have at times experienced considerable trouble with the assay process, which apparently was due to the age of the eggs. Recently we have only used eggs five to ten days old, and have obtained with such material rather concordant results. At the Indianapolis meeting of the Am. Chem Soc., a paper will be read dealing with the use of dry egg albumin in the assay process of Pepsin. If the results obtained by using dry albumin are encouraging, this modification should certainly be tried by the Revision Committee. Dry albumin can more easily be obtained in a uniform quality than fresh albumin, which contains a varying amount of water, according to the age of the eggs.

Physostigma and Its Preparations.—Slight modifications as to the quantities of immiscible solvent and acidulated water should be made.

Extract of Physostigma.—The use of sand and evaporation to dryness is to be avoided. We prefer to use powdered glass and to evaporate the liquid until the alcohol is expelled. Such a moist mass can be transferred to a bottle much easier than the hard mass obtained by the official process. Results just as accurate can be obtained by converting the solid extract into a fluidextract by dissolving it in dilute alcohol, rendering alkaline with sodium bicarbonate shaking out with ether, etc.

Fluidextract and Tincture of Physostigama.—The modifications just mentioned apply to the assay of these preparations also.

Pilocarpus and Its Preparations—.Replace the percolation process in the assay method by the aliquot part method. In case emulsions should be formed, use tragacanth for breaking up.

Fluidextract and Extract of Pilocarpus.—The modifications suggested under physostigma apply to the assay processes of these preparations also. Fluidextract of pilocarpus can be assayed by shaking out directly with chloroform after making alkaline with ammonia. Emulsions which are liable to be formed can be avoided by using a large amount of chloroform.

Piper.—The percentage of oleoresin should be determined.

Podophyllum.—Podophyllum with less than 4 or 4.5 per cent of resin is frequently met with on the market. An assay process for this drug therefore seems necessary.

Fluidextract of Podophyllum.—The percentage of resin should be determined.

Sanguinaria.—An estimation of the total alkaloids of blood-root might be valuable, although such a determination possibly does not indicate the therapeutic value of the drug.

Scopola and Its Preparations.—All that is said in regard to belladonna applies to this drug also.

Sinapis.—An estimation of allylthiocarbamide can be recommended.

Stramonium and Its Preparations.—See modifications recommended under belladonna.

Strophanthus.—There is no reason why this potent drug should not be assayed. A reliable process has been worked out.

Veratrum.—An estimation of the total alkaloids has been recommended on various occasions.

In conclusion we wish to say again that we hope that in the U. S. P. IX such assay methods will be adopted as are easily carried out, with the simplest apparatus, and in as short a time as possible, which, however, give at the same time reliable results, not theoretically accurate but practically accurate.

DISCUSSION.

Prof H. M. Gordin thought that since both the percolation method and the method of aliquot parts had merits and demerits, it was advisable to combine them so as to produce a method containing the merits of both.

Prof. L. D. Havenhill was doubtful as to the possibility of combining both processes. He agreed that percolation was the most uncertain process and would like to see it banished from pharmacopœial assays. In the hands of the average pharmacist he believed the aliquot part method would yield the better results.

Mr. F. R. Eldred agreed that the percolation method required the most careful manipulation, and for that reason would expect that novices would obtain better results by the aliquot method than by percolation; on the other hand, he thought that when the assayers were properly skilled better results could be obtained by percolation. He did not believe that the analytical objection of channeling would apply to this form of percolation as it did to the percolation of drugs on a larger scale. In his experience he had never had any trouble in exhausting the drug, though it is necessary to be sufficiently skillful to know when extraction is complete.

Mr. L. E. Warren agreed with the statement of Mr. Eldred. He had never found any difficulty in percolation of a drug or in determining when a drug had been exhausted.

Mr. C. E. Vanderkleed said that in his extended experience the aliquot part method had proved to be satisfactory, and he could not see any profit in trying to improve upon something that was giving entire satisfaction. The aliquot method had the great advantage of consuming less time than the percolation method, though with certain menstruua there is a possibility of error, as for example, in the extraction of cinchona bark, where in 5 volumes of water and 5 volumes of alcohol in 100 cc. one-half of the extracting liquid would not necessarily be represented by 50 cc., but probably by less than 50.

Dr. George F. Payne was strongly in favor of the aliquot part method, because the saving of time and convenience of handling the work was considerable, and he had used it almost exclusively in making a large number of assays in state work. It had the additional advantage in providing the analyst with a reserve portion of liquid in case of the loss of the first portion taken because of a broken flask or beaker, etc.

Dr. J. M. Francis and Prof. C. H. LaWall also expressed their preference for the method of aliquot parts.

Prof. Charles E. Caspari said that he was also in favor of the aliquot method, but not for the reason expressed by Dr. Payne. He did not believe it wise to infer that a remaining portion of the liquid represented exactly the first portion taken, since in the majority of cases after the operator has used the first portion he fails to take proper care of that remaining. As a concrete example he cited the assay of Nux Vomica, where it was difficult to pour off the first portion without stirring up the solid matter in the bottom of the flask. If the first determination is lost it would be necessary, or at least advisable, to percolate or filter the remainder and to use a still smaller aliquot portion, and he did not believe that in such cases the remaining portion could be relied upon to give a fair basis for comparison.

Prof. A. B. Stevens said that in drug assay work we must not expect such exact results as in the assay of inorganic salts, like those of silver. In the use of aliquot parts there is a little loss by evaporation while measuring the aliquot part, but the error is slight and not so important as that the method should give uniform results. The principal object in proximate assay work is to exclude drugs of inferior quality. In most cases the Pharmacopœia requires that drugs and preparations shall not be below a certain specified strength, but do not exclude those of higher alkaloidal content.

In reply to a question by Dr. Francis, he stated that if he were allowed but one indicator for all purposes he certainly would prefer cochineal.

Prof. E. V. Howell mentioned a plant which in his section was known as "Blue Bottle," "Baby's Breath" and "Cow's Breath," the tincture of which was very sensitive to acids and alkalies. One drop of a centinormal solution of alkali would change it from red to green; or one drop of a centinormal solution of acid, from green to red. He used the flowers but the stems were also available. He mentioned it with the hope that some one doing a good deal of analytical work would try it out as an indicator.

Prof. A. B. Stevens said that the principal objections made to the U. S. P. method for the assay of Aconite were the difficulty of filtration and length of time required for evaporation, both points of which are very easily overcome. He had assayed aconite obtained from many sources and had no trouble whatever in filtration where he followed the method published by him some years ago of adding about 10 gm. of pumice stone. In evaporation all that is necessary is to drive off the alcohol, which materially reduces the time required. In a number of experiments he had not found that a somewhat higher temperature had an injurious effect upon the active constituent and believed that a long, slow evaporation was more injurious than rapid evaporation at a higher temperature.

When ammonia was added to aconite, ether or chloroform would not exhaust the drug using the complete extraction method by percolation. He had tried various mixtures of ether, chloroform and alcohol; alcohol and ammonia, and alcohol 70 parts to water 30 parts. He had formerly thought that alcohol was a better solvent than a mixture of alcohol and water, but after experimenting had found that a mixture of 70 parts alcohol and 30 parts water was really better.

In a number of experiments tried on the same sample of aconite, with chloroform and ether, and ether alone, and the pharmacopœial mixture without ammonia and with ammonia, he had found that invariably the present solvent gives the best results. For illustration, the residue

from the pharmacopœial assay was physiologically active when dissolved in water in the proportion of 1 part aconite to 1400 of solution, while the residue from the ether-ammonia assay was not active in solutions greater than 1 to 1200.

Prof. Charles E. Caspari referred to a recent paper on Pepsin Assay, and stated that two different investigators had apparently proved that pepsin exerted its greatest action on egg albumen when the egg was about eight days old, after which the activity decreased until about the twentieth or twenty-first day.

Prof. Virgil Coblents referred to the employment of dried albumin in testing pepsin, and said that physiologists had generally adopted dried blood fibrin for this purpose. It possesses the advantage of uniformity, can be reduced to powder of any degree of fineness, and dried to any degree desired. After testing with pepsin the undigested excess can be removed, washed and dried, thus placing the testing of pepsin on a more nearly quantitative basis.

STANDARIZATION OF SOLUTIONS FOR ALKALOIDAL ASSAY.

A. B. STEVENS AND A. F. SCHLIHCTING.

It occurred to one of us that some of the variations in results obtained by chemists when assaying the same sample of drug, might be due to different standards used in preparing their standard solutions, possibly also to the indicator used. This suggested that it might be interesting and instructive to determine to what extent these factors affect the results, when conducting experiments under exactly the same conditions as to temperature, apparatus, etc.

A quantity of solution of potassium hydroxide was prepared, as nearly N/50 as convenient. The exact factor was then determined by means of the various standards used by chemists in drug assay. In testing these standards phenolphthalein was used as an indicator. The results are given in factors for the potassium hydroxide.

Standard	Potassium Bitartrate	Oxalic Acid	Succinic Acid	Sulphuric Acid	Hydrochloric Acid
KOH Factors	1.0561	1.0648	1.0638	1.0035	1.124
	1.0613	1.0627	1.0683	1.0057	1.112
	1.0629	1.0625	1.0706	1.0083	1.113
	1.0584	1.0611	1.0661	1.0972	
	1.0571	1.0618	1.0661	1.1006	
Average	1.0592	1.0625	1.0669	1.0962	1.118

Bear in mind that these results simply show the relation of one standard to another, or the variation between standards used.

Oxalic acid is preferred by some but has fallen into disrepute because it contains water of crystallization, a portion of which might be lost during drying, to free it from adhering moisture. Succinic acid is free from water of crystallization, hence can be dried without loss. The principal objection that can be raised to any of these for alkaloidal assays, is the fact that one indicator must be used in the standarization of the alkali, and another in the actual assay. It is proposed to overcome this objection by the use of pure anhydrous morphine as a standard. Morphine does not readily give up its water of crystallization. It requires a tem-