MANUAL

OF PRACTICAL

PHARMACEUTICAL ASSAYING

INCLUDING

DETAILS OF THE SIMPLEST AND BEST METHODS OF DETERMINING THE STRENGTH OF CRUDE DRUGS AND OF GALENICAL PREPARATIONS.

DESIGNED ESPECIALLY FOR THE USE OF THE STUDENT AND OF THE PRACTICAL PHARMACIST.

BY

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PREFACE.

The object of this treatise is eminently a practical one. It aims to present, as briefly and clearly as possible, an account of the assay processes which the pharmacist may advantageously use, and which he would use frequently were he not deterred by an exaggerated idea of the difficulty of the requisite chemical manipulations.

To ascertain exactly the proportion of each active constituent in a drug, or in a medicinal preparation, is often a task of great difficulty, and the professional chemist not unfrequently acknowledges himself baffled in the attempt. In fact, the chemist's idea of an assay process is so high that it is practically unattainable.

Pharmaceutical assaying, however, proposes for itself aims which are practical, rather than ideal. It seeks to ascertain approximately the relative therapeutic value of different specimens of a given drug or galenical preparation. The time is not far distant when physicians will insist on fixed standards of strength for the preparations of all active drugs. If such standards cannot be established for the galenical preparations of the drug, they will be driven to the exclusive use of the active principles, but they would generally prefer to employ standardized tinctures, extracts, etc., prepared directly from the drug, with whose action as a whole they are familiar.

Most of the published assay processes are needlessly complicated and laborious. It has been my aim to simplify and curtail the operations as much as is consistent with trustworthiness in the results. Only those methods of assay that have approved themselves in actual practice will be described in detail in this manual. To the labors of Prof. Dragendorff, especially, I find myself indebted for many of the processes that I shall offer, and I must acknowledge a like indebtedness to that indefatigable and enthusiastic American worker, Dr. Squibb, and to others whose contributions in this field have been less numerous. Many of the processes, however, which I shall describe have this measure of originality, that they have grown up under the exacting demands of the laboratory work in which I have for several years been engaged. From hints that have been gathered from various sources, these methods have developed into their present form, and every year's added experience will no doubt find still something in them to change and improve. They are offered for what they are worth, in the belief that to those at least who do not read German they will be of material service, and that from this small beginning there will arise before many years a literature in our own tongue of a subject of such growing importance.

AUGUST, 1886.

TABLE OF CONTENTS.

INTRODUCTORY.

1. For the simplified assay operations described in this manual very little apparatus is required. It is taken for granted that the use of the ordinary utensils employed in making simple chemical tests is familiar, and that the operator is provided with these, which are indeed in common use in every drug store. A few pieces of especial apparatus are, however, indispensable, and the more important of these will be here enumerated for convenience.

Fig. 2.

Fig. 3.

2. Graduated Pipettes. (a) *Measuring pipettes* to deliver respectively 5 and 10 cc. of fluid (Fig. 1). The form shown in the figure is to be preferred. The tube should not have an external diameter of more than 5 mm. ($\frac{3}{16}$ in.), and the portion below the bulb should be 12 or 15 cm. (5 or 6 in.) long. These measuring pipettes are filled by cautiously drawing up the liquid into them by suction with the mouth. The forefinger, which should be slightly moist, but not wet, is then dexterously applied to the opening at the top of the pipette, when the liquid will be prevented by atmospheric pressure from flowing

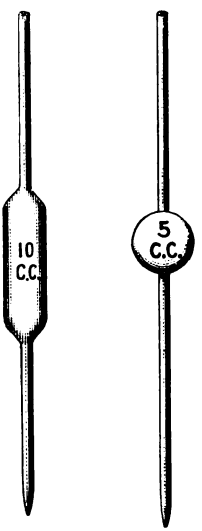

Fig. 1.
Measuring Pipettes.

out. The air is now to be allowed to enter by slightly relaxing the pressure of the finger, permitting the excess of fluid thus to escape. As soon as the level of the liquid reaches the mark, the finger is once more firmly pressed on the orifice, and the contents of the pipette may then be conveyed to the desired receiver.

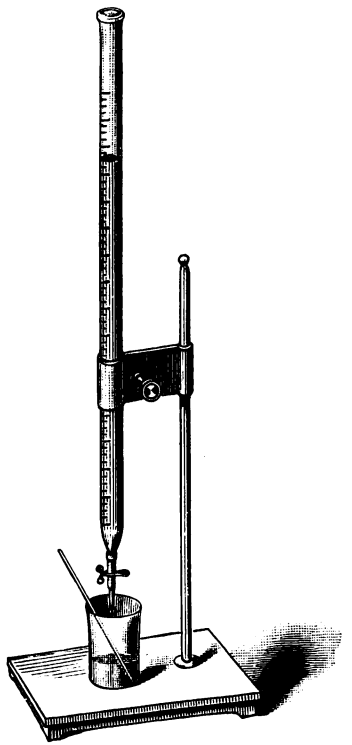

Fig. 5.
Mohr's Burette.

(b) *Graduated pipettes* (Fig. 2). Of these it is convenient to have one with a capacity of 5 cc. graduated to tenths or twentieths, together with one or two graduated minim "droppers" (Fig. 3.)

Fig. 4.

3. Graduated Flasks (Fig. 4). Those most useful are of a capacity of 25, 50, 100, 250 and 1,000 cc. Inexpensive substitutes for these flasks can be easily made from prescription vials. It is not necessary that the exact capacities mentioned above be insisted upon, but the measures must agree among themselves. Thus, instead of the 25 and 50 cc. flasks, we may use a 1 oz. and 2 oz. vial, so chosen that the capacity to mark of the latter shall be exactly double that of the former. By capacity in this connection is understood the quantity

of fluid that can be poured out of the vial or flask when filled to the mark, not the quantity it actually contains, for a little of the fluid will always adhere to the glass when emptied. The mark can be etched with hydrofluoric acid, or simply scratched with a file. The 1,000 cc. (1 litre) flask must be correctly graduated; it is to be used in preparing volumetric solutions.

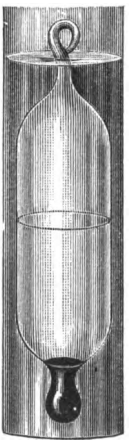

Fig. 6.

4. Graduated Burettes. The ordinary Mohr's burette (Fig. 5), provided with an Erdman's float (Fig. 6), for accuracy in reading, is the most convenient form for ordinary use. The burette may have a capacity of 50 cc., and should be graduated to tenths. A burette holder is also essential, although with a little ingenuity it will not be difficult to extemporize one, if economy is an object. The correctness of all graduated apparatus must, in every case, be tested before using it for quantitative work.

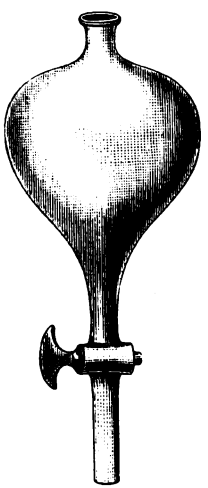

Fig. 7.

5. Separating Funnels. No piece of apparatus is more frequently called into requisition in the estimation of alkaloids than the separator. Two or three will be required; capacity 100 cc. (Fig. 7). The pattern designed by Dr. Squibb (Fig. 8) has some advantages over the ordinary form. In any case, the tube of the separator should be cut off so that it shall be not more than 3 to 5 cm. (one or two inches) long.

6. A few small **Glass Percolators** of cylindrical form, about 20 cm. (8 in.) long, and about 4 cm. (1½ in.) in diameter, will

be convenient. Smaller ones even than this are sometimes useful, and may be easily made from glass syringes, the top being cut off, if necessary. For the larger sizes, lamp chimneys, or narrow bottles from which the bottoms have been removed, often serve a good purpose.

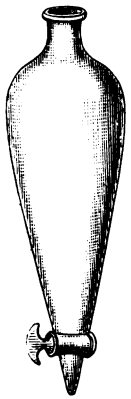

Fig. 8.

7. Apparatus for Hot Repercolation. For the rapid exhaustion by a volatile solvent of a vegetable powder there is no process to be compared with that of hot repercolation. Various forms of apparatus have been devised for this purpose. The most efficient is what is called Soxhlet's tube (Fig. 9.) The drug to be exhausted is placed in a test tube having an opening at the bottom, closed by a plug of asbestos or glass wool, and the whole is inserted into the Soxhlet's tube, or else the drug is simply wrapped in filter paper and placed in the Soxhlet's tube. This is then connected by aid of sound corks, with a good inverted condenser above [an upright Squibb's condenser answers well], and below with a flask containing the fluid to be employed in the extraction. When heat is applied to the flask, vapor rising from the fluid passes up through the small tube on the right in the cut, and so up into the condenser, whence it returns in form of a liquid, dropping into the extraction tube. When this tube is filled to the level of the bend of the syphon tube on the left, the liquid is drawn off by the automatic action of the syphon, being returned to the flask to be again volatilized and recondensed as long as heat is applied to the flask. The action goes on with perfect regularity, and exhaustion of the drug will be complete in from one to six hours.

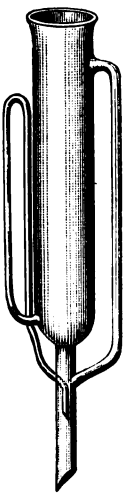

Fig. 9.

8. An apparatus for exhausting small quantities of drug by hot repercolation may be extemporized as follows: Select a pint flask having a rather long, straight neck. As a percolator, use a half ounce glass syringe of about 15 mm. (⅝ in.) in diameter, or a piece of glass tubing long enough to reach within 3 or 4 cm. (1¼–1½ in.) of the bottom of the flask, drawn almost to a point at the lower end. Fit this into the flask by means of a short section of rubber tubing, or by a perforated cork, making the joint vapor-tight. (The cork is to be preferred where a solvent like chloroform, which acts on rubber, is to be used.) By aid of a file, moistened with a solution of camphor in spirits of turpentine, make an opening in the upper part of this percolator, just below the joint, to allow the vapor to enter the condenser. Fit the percolator with a perforated cork, through which is to pass the point of the tube of an upright condenser, and the apparatus is complete (Fig. 10).

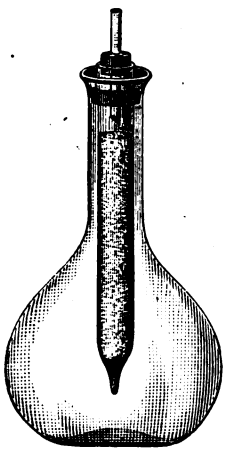

Fig. 10.
Extraction Apparatus.

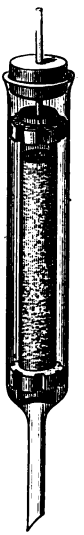

Fig. 11.

Still another form of extraction tube is shown in Fig. 11. The principle of its action is precisely the same as that of the one just described. In use it is to be connected above with an upright condenser, the tube of which is shown in the cut, below by means of a cork with a small flask containing the solvent.

REAGENTS.

9. Mayer's Solution of *potassio-mercuric iodide.* Used for the detection and the quantitative estimation of alkaloids. This solution, as made by Prof. Mayer, was of decinormal ($N\frac{1}{10}$) strength. A solution one half this strength ($N\frac{1}{20}$) is to be preferred for volumetric work, and will be understood in all cases where this reagent is mentioned in this manual.

FORMULA.

Mercuric chloride	6.775 grm.
Potassium iodide	25. grm.

Distilled water sufficient to make one litre.

10. Sonnenschein's Reagent. *Sodium phosphomolybdate.* Precipitate a solution of sodium phosphate with ammonium molybdate in excess, in presence of nitric acid. Wash the precipitate with water containing nitric acid, and dissolve in a hot solution of sodium carbonate. Evaporate to dryness, and ignite gently to expel ammonium salts. Moisten the residue with nitric acid and again ignite gently. Dissolve the residue in a mixture of one volume of nitric acid (sp. gr. 1.42) and 13 volumes of water.

A simpler mode of preparing this reagent is the following: Dissolve 2.16 grm. molybdic acid in a slight excess of solution of caustic soda; boil some time to expel traces of ammonia, and add 0.358 grm. crystallized sodium phosphate.

11. Scheibler's Reagent. *Sodium phosphotungstate.* Dissolve sodium tungstate 100 parts, and sodium phosphate 60 to 80 parts, in water 500 parts, and add nitric acid to acid reaction.

Both this and Sonnenschein's reagent may be employed like Mayer's reagent for the volumetric estimation of alkaloids.

12. Chinoidin Iodosulphate. Employed by Dr. De Vrij for the estimation of quinine.

Heat together on a water bath one part of chinoidin and two of benzol. Decant the clear solution, and agitate with an excess of dilute sulphuric acid. To the acid solution, contained in a capsule, add *slowly* with continuous stirring a solution of one part of iodine and two parts of potassium iodide in fifty parts of water. One part of iodine is required for every two parts of chinoidin contained in the acid solution. The precipitate of chinoidin iodosulphate subsides on warming, and is to be washed repeatedly with water by decantation. Dissolve one part of this precipitate in six parts of strong alcohol by aid of a water bath heat, and allow the solution to cool. Decant from the deposit, evaporate, and dissolve the residue in five times its weight of cold alcohol. This solution constitutes the reagent.

13. Prollius' Mixture.

FORMULA.

Concentrated ether	325 cc.
Alcohol	25 cc.
Concentrated solution of ammonia	10 cc.

Mix the alcohol and ammonia and add to the ether.

14. The **Prollius' Fluid Modified,** alluded to sometimes in subsequent pages, consists of:

Concentrated ether	250 cc.
Chloroform	80 to 100 cc.
Alcohol	25 cc.
Concentrated solution of ammonia	10 cc.

GENERAL METHODS FOR THE ASSAY OF CRUDE DRUGS.

15. Selection and Preparation of the Sample. It is necessary in the first instance, in every case, to secure a fair average sample of the drug. When the drug consists of seeds, leaves, etc., presumably of nearly uniform quality, it is only necessary to grind to a coarse powder a few ounces of the material, and then to take a sufficient quantity of this for the purposes of the assay, and by aid of mill, mortar and sieve reduce the *whole* of it to a powder of requisite fineness. In general, it may be said that the finer the powder the better. The coarsest powder admissible is a No. 30; for many assay processes a much finer powder—60 to 80—is required.

If the drug consists of large pieces, variable in quality, like rhubarb and most barks and tubers, it is best to select first a number of representative pieces; take from each a representative section or segment, and proceed with this as already described.

16. If the drug requires to be dried before grinding, the loss of weight during this operation must be noted, and the requisite correction made in the result.

Some chemists always report their results calculated on the thoroughly dried drug. It is better to report separately the amount of active principle and of moisture that are present. The air dried drug will in any case contain from 5 to 10 per cent. of moisture, or even a larger proportion, but this moisture is part of the drug in the condition in which it is bought, and in which it is weighed for making galenical preparations, and hence should not, in ordinary cases, be calculated out of the result of the assay. In ordinary work, indeed, an estimation of the moisture in the drug is unnecessary.

17. Methods of Exhausting the Drug. The choice will be between four processes, each of which has its advantages.

A. *Maceration.* The drug in fine powder is placed in a bottle or flask with a quantity of the chosen menstruum sufficient to ensure complete exhaustion—at least eight or ten times the weight of the drug—and allowed to macerate therein, with occasional shaking for from one to three days, according to the activity of the solvent. This is the simplest plan of all, and that which requires a minimum amount of the operator's time and attention. The result is not reached quite as soon, and in some cases this is a matter of importance.

B. *Percolation.* Details of this familiar pharmaceutical operation are hardly necessary here. The process has perhaps no advantage over maceration, except that it can be completed in a shorter time. The operation, however, consumes more of the operator's time, and requires more attention.

C. *Boiling*, with several successive portions of the chosen solvent

D. *Hot Repercolation.* This is altogether the most rapid and effectual mode of applying solvents. It requires an especial extraction apparatus, and it takes some time to mount this properly with condenser, but it materially shortens the time of the assay. When once set in operation, the action is automatic, and it is a favorite method with professional chemists. The apparatus required has been already described and illustrated (7) and (8).

18. The choice of solvent will depend, of course, upon the nature of the drug. In most cases the active principle of the drug is an alkaloid, which is present in the form of a salt, soluble in water or in alcohol. If we employ water as a solvent, we extract much inert matter, which is liable to embarrass subsequent operations. Alcohol is to be preferred—the stronger the better—as less likely to extract an excessive amount of inert matter, but it must be remembered that the resins taken up by alcohol are sometimes

more troublesome than the gummy matter extracted by water. Furthermore, alcohol does not penetrate the drug—provided it is dry—as well as water does, and it is hence often advantageous, even when we expect to employ alcohol, to first moisten and soak the drug with water. Messrs. Dunstan and Short have recently called attention to the usefulness of a mixture of chloroform and alcohol in treating many drugs containing alkaloids.

Acidulated water or acidulated alcohol was formerly very generally recommended as the solvent to be selected for the exhaustion of the drug. A freely soluble salt of the alkaloid is thus formed, and the drug speedily and very completely exhausted.

20. Another plan is to treat the drug first with an alkali, milk of lime, magnesia, or carbonate of sodium, dry and exhaust with strong alcohol, or with wood spirit. This plan succeeds with many drugs. It gives us a solution containing comparatively little inert extractive, and therefore better adapted to our purpose. The objections to it are: 1st, the liability to decomposition of sensitive alkaloids by the prolonged action of the alkali, especially where lime is used; 2d, the amount of time required to carry out the successive steps of the analysis. This method admits of various modifications, which may sometimes be advantageous. Instead of extracting the alkaloid with alcohol, we may employ some solvent, such as fusel oil, chloroform, ether, or petroleum benzin, which will take up very little besides the alkaloid We may conduct the extraction by the method of hot repercolation, and so shorten the time of the assay. For the assay of cinchona bark and some other drugs, which abound in alkaloid, this method, with or without modification, especially commends itself, and is generally practiced.

21. In general, however, for the exhaustion for assay of drugs which contain alkaloids, a solvent like that recommended by Prollius for the assay of cinchona bark is to be preferred to all others.

Prollius' Fluid consists of a mixture of ether, alcohol and solution of ammonia (13). This solvent has remarkable penetrating powers, the ammonia which it contains decomposing the salts of the alkaloids which exist in the drug, and the ether taking at once into solution the liberated alkaloid. It is to be preferred to ammoniated alcohol, because it takes up, besides alkaloid, only fatty matter, oleo-resins, chlorophyl and similar substances, present, generally, only in small quantity in the drug.

22. The easiest way to test a new drug for the presence of alkaloid is simply to macerate a little of it a few hours in Prollius' fluid, shake out with acidulated water, and test the watery solution with the general reagents for alkaloids. If such compounds are present, they will almost certainly be detected in this way.

GENERAL METHODS OF EXTRACTING ALKALOIDS FROM CRUDE DRUGS.

23. I. **Method Recommended by Dragendorff.*** Moisten the drug in fine powder, with twice its weight of water, containing one to three per cent. of sulphuric acid, macerate 24 hours in a well-corked bottle, add alcohol enough to make up a volume of ten cc. of solvent for each gram of drug. Macerate two or three days, shaking occasionally. From an aliquot portion of the solution thus prepared, the alkaloid may then be extracted by any of the well-known processes, but in most cases it is recommended to drive off the alcohol by evaporation, and estimate the alkaloid in the aqueous solution remaining by titration with Mayer's reagent. The method is practical, and consumes little time, but has only a limited range of application.

24. II. **Method of Lösch.**† Exhaust the drug by maceration with ten times its weight of alcohol containing hydrochloric acid. Reduce the tincture to one-third its volume by evaporation; filter when cold, and evaporate to the consistence of an extract. Exhaust this with water (two parts) containing sulphuric acid, filter, add saturated solution of alum (six parts), warm the mixture, add solution of ammonia in slight excess, and evaporate to dryness on the water bath. Pulverize the residue and exhaust with the appropriate solvent, amylic alcohol, chloroform or ether. The alkaloid is thus obtained in a crude form, requiring purification as a preliminary to weighing. Of course, the method is not adapted to the separation of the volatile alkaloids.

*Die Chemische Werthbestimmung einiger Starkwirkender Droguen, 1874.
†Pharm. Zeitung, 1879, No. 89; Am. Jour. Pharmacy, Jan., 1880.

25. III. **Method of Hager.*** Place in a tared flask 15 grams of the drug, in coarse powder, with 120 grams of water and 30 grams of dilute sulphuric acid (10 per cent). Having placed a small funnel in the neck of the flask, heat six or eight hours on the water bath, and then set aside two hours in a cool place; add water to make up the loss by evaporation, filter, and of the filtrate take for the assay an aliquot portion. It is assumed that air-dried seeds and barks will yield about twenty per cent. of extractive, roots and rhizomes, air dried, forty per cent.; flowers and leaves, air dried, fifty per cent. The amount of fluid, therefore, corresponding with 10 grams of drug will be, in the first case, 102 grams; in the second, 104 grams; in the third, 105.

26. More exact figures can, of course, be obtained by estimating the extractive actually present, a weighed portion of the fluid previously neutralized with ammonia being evaporated to dryness, and the calculated weight of the ammonium sulphate being deducted, but in ordinary practice this is an unnecessary refinement.

27. The portion of fluid weighed out for the assay is to be evaporated at a gentle heat to one-half its volume, 25 or 30 grams of finely pulverized litharge added, the heat being maintained; after half an hour 5 grams of lead carbonate is to be added, and the mixture brought to complete dryness by continued application of a gentle heat. Rub the residue to a powder, introduce into a flask, and exhaust by maceration with an appropriate solvent, absolute alcohol, ether, chloroform, or amylic alcohol. The filtered fluid will generally contain finely divided lead carbonate, which must be allowed to subside, and the clear fluid decanted or removed by a pipette.

28. If the drug contain a volatile alkaloid, the acid solution must be evaporated to a small volume, and a mixture of thoroughly dried litharge and terra alba added. After the lapse of an

*Handbuch der Pharmaceutischen Praxis, Supplement, p. 62.

hour, a further addition is to be made of well-dried lead carbonate in quantity sufficient to absorb all the sensible moisture, and the dry powder thus obtained is to be exhausted at once with absolute alcohol. The alkaloid may now be neutralized with oxalic or sulphuric acid, the alcohol evaporated, and the residue treated with caustic soda and ether.

29. IV. Methods practiced by the Author.*

General Assay Process No. 1.

Put into a bottle or flask 10 grains of the drug in moderately fine powder (No. 30 or finer). Pour in carefully 100 cc. of Prollius' fluid (13), cork securely and shake well. Set by for 12 to 24 hours, shaking at intervals. At the end of that time decant 50 cc. of the clear fluid into a beaker, and set this in a warm place until the ether has nearly all evaporated. Then add 5 cc. of water, containing ½ cc. of 5 per cent. sulphuric acid, and 10 cc. of ether. Stir to redissolve in the ether all the oily and resinous matter that may have separated, and to insure combination of all the alkaloid with the acid, evaporate the ether completely, together with any alcohol that may remain.

30. Filter the aqueous fluid through a very small filter into a 1-ounce vial, which must have a good lip. Wash the beaker with a little water (1 to 2 cc.), and pass this also through the filter. If you suspect that a part of the alkaloid has escaped solution in the acid, redissolve the oleo-resinous residue in ether, add 2 cc. of water containing a drop or two of the 5 per cent. sulphuric acid. Evaporate off the ether with constant stirring, and test a very small drop of the acid fluid for alkaloid by placing it on a mirror, and adding to it a drop of Mayer's reagent. If there is produced more than a faint cloud, the solution must be passed through the filter into the vial, and the filter finally washed with a few drops of water. Put into the vial 10 cc. of ether; cork and shake. The

*Druggists' Circular, August, 1884, p. 114.

ether will remove impurities, leaving the alkaloid in the aqueous solution.

31. When the ether has completely separated, pour it off carefully into a bottle kept for waste ether, and repeat the ether washing once or twice, or until nothing further is taken up by it from the acid solution.

32. Having decanted the ether as closely as possible, add to the solution ammonia enough to render it alkaline, and shake now immediately with 15 cc. of a mixture of ether (3 volumes) and chloroform (one volume), which will take up the liberated alkaloid. After 15 or 20 minutes, the fluid having separated into two distinct layers, decant the ethereal fluid into a tared evaporating dish, and evaporate off the solvent at a water bath heat. Meanwhile wash out the alkaline fluid once or twice again with 10 or 15 cc. of the same mixture of ether and chloroform, adding the solution, when perfectly clear, to the contents of the capsule. Test a drop of the residual solution for alkaloid by rendering it acid, and adding Mayer's solution. If the alkaloid is not wholly removed, wash once more, this time with chloroform, having first rendered the solution acid, and then made it alkaline again. Bring the chloroform to the surface by adding sufficient ether, separate, and add to the contents of the capsule.

33. When the alkaloid is completely dry, cool the dish in the desiccator and weigh. In absence of a desiccator, cool rapidly, and weigh at once. The weight of the alkaloid in decigrams, multiplied by two, will be the percentage of total alkaloid contained in the drug.

After weighing the crude alkaloid, dissolve it in a few drops of dilute acid. If there remain any residue, wash the acid solution with two successive portions of ether (10 and 5 cc.), separate, evaporate the ethereal solution, weigh, and deduct the weight from that of the crude alkaloid. For ordinary purposes, the weight of the crude alkaloid, uncorrected, serves sufficiently well as an indication of the value of the sample.

It is always safest to wash with water containing a little ammonia the alkaloidal solution which is to be evaporated for weighing. The same water (5 cc) may be used to wash the successive portions of solvent before they are transferred to the capsule.

GENERAL ASSAY PROCESS NO. 2.

34. In the case of many drugs the alkaloid may be washed out with acid water direct from the ethereal solution obtained by treating the drug with Prollius' fluid. Put the 50 cc. of fluid decanted from the drug residue (29) into a separator (Fig. 7). Add 2 cc. of 5 per cent. sulphuric acid and shake well. When the acid solution has subsided, draw it off into a 1 ounce bottle, following it with a few drops of distilled water. Add 2 cc. of distilled water to the fluid in the separator and shake. When this has subsided, draw it off also into the bottle, rinsing as before with a little water. Make sure that the whole of the alkaloid has been removed by this treatment, and proceed to wash the acid solution with ether (once will suffice) and to extract the alkaloid from it precisely as described above (33).

GENERAL ASSAY PROCESS NO. 3.

35. In place of Prollius' fluid we may advantageously use in certain cases some other solvent. Petroleum benzin 95 cc., absolute alcohol 4½ cc., and stronger water of ammonia ½ cc., may sometimes be used. The mixture of alcohol and ammonia must be added after the other articles have been placed in the bottle, and the mixture must then be at once vigorously shaken for some time. When the alkaloids are not freely soluble in ether, chloroform may be substituted for it, or, better, a mixture of chloroform, one volume, and ether, three volumes (14).

36. When petroleum benzin is the solvent used, we may be confident of removing all the alkaloid from it by mere agitation with acid water, as just described in process No. 2, above. When the solvent consists of chloroform we cannot be so sure of extracting the whole of the alkaloid in this way, but may do so with certainty by following the plan first described (29) *et seq.*

VOLUMETRIC ESTIMATION OF ALKALOIDS BY MAYER'S REAGENT.

37. Most alkaloids are very completely precipitated from acid aqueous solutions by the solution of potassis-mercuric iodide, known as Mayer's reagent. The precipitates are more or less soluble in alcohol, ether, acetic acid, iodides, and to a greater or less extent in an excess of the reagent. In alkaline solutions, ammonia also is precipitated. The fluid, therefore, to be tested with this reagent must be at least faintly acid in reaction, and must be nearly free from alcohol, and other fluids similar in their solvent action to alcohol. The presence in the solution of certain salts, notably of iodides, has a marked influence over the result.

38. It has been generally assumed that the precipitate consists of a double iodide of the base with mercury, containing either one, two or three molecules of the base to one of the mercuric salt. We are, therefore, told that 1 cc. of a reagent of semi-decinormal strength will precipitate a quantity of the alkaloid, which, expressed in milligrams, will be found by dividing its molecular weight by 60, 40 or 20, as the case may be. Thus it was stated by Prof. Mayer himself, and the text books generally have copied his figures, that for each cc. of reagent $N_{\frac{1}{10}}$ required for their complete precipitation, there will be indicated the following quantities of the respective alkaloids:

Aconitine.....	.0134	Coniine.....	.0021	Quinidine.....	.0060
Atropine	.00725	Morphine.....	.0100	Strychnine	.0083
Brucine	.01165	Quinine	.0054	Veratrine	.0135
Cinchonine	.0051				

39. Discrepancy of Fact with Theory. In attempting to verify these figures, and the assumption upon which

they are based, I have made numerous experiments, which have driven me to the following conclusions:

(1.) The precipitates produced by Mayer's reagent have not the composition that has been generally assigned to them, although that may be regarded as their normal composition. They contain almost uniformly a smaller proportion of mercury, often less than three fourths of the quantity that has been assumed to be present in them.

(2.) The precipitates are not constant in composition, although in this respect there is a great difference between different alkaloids. Even when we collect and weigh the precipitate, therefore, our results will lack exactness, although they may give close approximations to the truth in many instances, if we first ascertain by experiment what is the actual weight of the precipitate; we shall go far astray if we attempt to calculate it from the formulas generally accepted. In the case of certain alkaloids the precipitate will be found to vary excessively in weight.

(3.) If the reagent is added until precipitation ceases, there will be found in nearly all cases to be a large excess of reagent present. This excess will constitute a certain proportion of the fluid at the end of the titration, and hence *the more dilute the solution titrated, the larger the quantity of reagent required to complete the precipitation.* What this excess is in the case of each alkaloid we must ascertain experimentally, and we must then apply a correction to our result, subtracting therefrom, for each cc. of fluid present at the end of the experiment, a certain, often very considerable, amount. Thus, in estimating colchicine, we are obliged to deduct from the quantity of reagent used, for each cc. of fluid present not less than .08 cc. The instructions generally given direct that an additive correction be made instead of a subtractive, in case the solution titrated is unusually dilute, based on the solubility of the precipitate in water. This is a mistake—often an important one—which has arisen from relying too exclusively on theoretical considerations, the theory itself being erroneous.

(4.) The manner in which the reagent is added is not without influence on the result. The larger the initial addition, the greater the quantity, as a rule, that will be required.

40. The **practical deductions** to be drawn from the above observed facts are that to secure uniform results in titrations with Mayer's reagent, it is necessary that the solutions operated upon shall have as nearly as possible a uniform strength, and that the reagent shall always be added in exactly the same manner.

41. The manner in which the titration is best conducted is as follows: Put the solution to be titrated into a test tube, having noted its volume. Run into it from a burette the reagent ($N\frac{1}{20}$) as long as it produces a dense precipitate, or until about one-half the quantity necessary to complete the precipitation has been added. Filter into a second test tube, selecting a filter which will hold the whole of the fluid at the end of the titration, but not much more. As soon as a sufficient quantity of fluid has filtered through clear, set the funnel in test tube No. 1, and cautiously add a drop or two of reagent to the clear fluid; if a dense precipitate is produced, add 5 to 10 drops of the reagent, and return to the filter, using the fluid that has filtered meantime into the first test tube to rinse out the second. Proceed in this way until the precipitation begins to be scanty. When nearing the end of the experiment allow nearly the whole of the fluid to filter through before adding more reagent, and filter twice, if necessary, to secure a perfectly clear fluid. Except in the case of a few alkaloids there is no difficulty about obtaining a clear filtrate, and the filtration is very rapid.

42. The reaction is taken to be complete when the addition of a single drop, or at most two, of reagent does not produce at once a permanent turbidity in the fluid. It will often happen that one or two drops of the reagent will produce a faint cloud, which disappears as it mixes with the rest of the fluid. If a larger quantity of reagent is added, a permanent turbidity, or even a precipi-

tate, is produced, but this should be ignored. Uniformity of practice in fixing the end point of the reaction is, of course, indispensable. (For Hereth's method see 52.)

43. Titration of Tinctures, etc., with Mayer's reagent. When Mayer's reagent is employed in estimating the strength of complex fluids, such as tinctures, etc., the question will arise whether there is not likely to be something else present besides alkaloid, which will be precipitated with it, making the result of the titration too high. In addition to alkaloids, the reagent precipitates albuminoid substances, and these may not unfrequently be present. They may be in large measure excluded by the use in the preparation of the solution of such solvents as strong alcohol and ether. Unless we know that they are absent, we can place no confidence in our result.

44. Drugs containing several alkaloids. Many drugs contain several different alkaloids, requiring, it may be, very different quantities of Mayer's reagent for their precipitation. In such cases, the indications of a titration can be interpreted only on the assumption that the several alkaloids are present in average proportions. The total quantity of alkaloid having been, however, ascertained by some other method, a titration may enable us in such a case to estimate the proportion of each of two alkaloids, and this plan is in fact resorted to in the assay of nux vomica and ignatia.

45. Finally, it may be said that while titration with Mayer's reagent affords a ready means of estimating with tolerable precision small quantities of an alkaloid separated from a drug by some assay process, and known to be nearly pure, *its indications must be accepted only with reserve in the case of galenical preparations, and especially those in which there has been exposure to heat and to oxidation.* For it must be remembered that many alkaloids are easily altered by such exposure, retaining their general chemical character as alkaloids, but losing their identity, and having no longer their former medicinal activity.

46. Any method of assay in these cases is, however, open to similar objection, unless it places before us in definite crystallized form the active principle we seek to estimate, and that is generally quite out of the question. We must, therefore, wherever it is practicable, supplement the chemical by physiological quantitative tests, and upon the latter we may surely place implicit reliance.*

47. Precipitation equivalents of Mayer's reagent. In contrast with the figures given in most of the text-books for the precipitation equivalents of Mayer's reagent in the case of various alkaloids, I have brought together the results of a few out of many experiments which I have myself made, to endeavor to settle, for practical purposes, the values that should be adopted. The series of experiments, conducted at irregular intervals as opportunity has offered, is by no means complete, but it may serve to show that in the titration method of assay we must ignore theoretical considerations, except so far as theory and fact coincide.

48. Using solutions of known strength, I noted not only the quantity of Mayer's reagent required to precipitate completely 0.1 gram of alkaloid, but (a) the quantity required to produce an apparent excess of reagent in the solution, so that it would cause a precipitate in more of the alkaloidal solution ; (b) the excess of Mayer's reagent used, estimated by the quantity of mercury present in the solution. These results, with some other useful data, are given in the following table:

*Just as these sheets are going to press, I find that this same conclusion was reached more than twenty years ago by Thos. B. Groves, in a study of the subject, which has just come to my notice (Pharm. Journal, II, 6, 268).

49. Precipitation of various alkaloids by Mayer's reagent N1-20; 0.100 of alkaloid used in each experiment.

NAME OF ALKALOID.	Strength of Alkaloidal Solution.	Cc. of Reagent required to produce apparent excess.	Cc. of Reagent required for complete precipitation.	Excess of Reagent at end of experiment.	1 cc. of Reagent precipitates of alkaloid. (grams.)	Weight of precipitate collected immediately and dried at 100° C.
Aconitine	1:200	.....	7.1	2.	.0141	.180—.190
Atropine	1:200	7.	13.1	3.	.0077	.216—.220
Atropine	1:400	6.	14.	3.5	.0072	.....
Atropine	1:600	6.	15.	5.6	.0067	.....
Berberine	1:200	.....	3.8	.....	.0263	.192—.200
Berberine	1:400	.....	3.9	.....	.0257	.....
Berberine	1:600	.....	4.6	.....	.0218	.....
†Brucine	1:200	.....	8.	1.7	.0125	.200—.215
†Brucine	1:400	.....	8.8	.....	.0114	.....
‡Brucine	1:400	.....	9.8	.....	.0102	.....
†Brucine	1:600	.....	9.2	.....	.0109	.....
Cinchonidine	1:100	12.4	13.8	1.0	.0073	.....
Cinchonidine	1:200	12.4	13.5	0.7	.0074	.330—.375
Cinchonidine	1:200	.....	15.6	2.6	.0064	.....
Cinchonine	1:100	.....	12.8	0.8	.0078	.....
Cinchonine	1:100	.....	14.	1.2	.0072	.....
*Cinchonine	1:200	~~12.8~~ 7.9	10.8	.....	.0093	.333—.345
‡Cinchonine	1:200	8.	14.2	.....	.0071	.....
*Cinchonine	1:400	8.	12.4	2.4	.0082	.....
‡Cinchonine	1:400	9.6	14.—18.	.....	.007 to .0086	.....
Cocaine	1:200	.....	12.8	.....	.0078	.246
Cocaine	1:400	10.	14.4	4.6	.0069	.....
Cocaine	1:600	.....	16.	5.2	.0063	.....
Colchicine	1:200	3.2	9.2	.....	.0109	160±
Colchicine	1:400	4.2	11.4	.....	.0088	.....
Colchicine	1:600	5.	12.6	.....	.0080	.....
Colchicine	1:800	4.	14.6	.....	.0067	.....
Emetine	1:200	8.	9.4	0.4	.0106	.256±
Emetine	1:400	8.8	10.2	1.	.0098	.....
Emetine	1:600	.....	10.6	0.6	.0094	.....
Gelsemine	1:200	5.8	10.4	.....	.0096	.185—.200
Gelsemine	1:400	6.5	12.	.....	.0084	.....
Hydrastine	1:200	.....	7.4	.....	.0135	.200—.210
Hydrastine	1:400	.....	8.	.....	.0125	.....
Hydrastine	1:600	.....	8.4	.....	.0119	.....
Hyoscyamine	1:200	.....	8.5	.....	.0116	.220—.250
Morphine	1:200	~~7.~~ 7.91	~~7.~~ 7.91	.....	.0128	.190—.204
Morphine	1:400	.....	8.9	0.6	.0110	.....
Pilocarpine	1:200	4.8	16.8	.....	.0060	.240—.340
Pilocarpine	1:200	.....	20.	.....	.0050	.....
*Quinine	1:200	11.6	16.4	.....	.0061	.....
‡Quinine	1:200	12.4	18.	.....	.0056	.310—.335
Quinine	1:400	12.8	16.8	.....	.0060	.....
Quinine	1:600	12.2	20.	.....	.0050	.....
Strychnine	1:200	.....	11.	0.6	.0091	.260—.275
*Strychnine	1:400	11.6	12.	.....	.0084	.....
‡Strychnine	1:400	11.6	12.2	.....	.0082	.....
Strychnine	1:600	11.2	11.9	0.6	.0087	.....

*Neutral. †Nearly neutral. ‡Acid.

50. Conclusions from experimental data, tabulated above. A study of these figures will show that while an excessive quantity of reagent is almost in every case required to complete the precipitation, the weight of the precipitate is considerably lower than that theory would lead us to expect. There is in some cases a decided increase in the weight of the precipitate if it is allowed to stand some time in the fluid before collecting it. Thus, if the atropine precipitate be left a few hours to itself, it condenses, assuming in part a crystalline form, and adheres strongly to the containing beaker. The fluid can generally be decanted without disturbing it in the least, and the precipitate can then be washed with water, dried, and weighed in the beaker, and it will be found to fall little short of the theoretical weight of .245 from 0.1 of alkaloid.

51. No doubt results more closely coinciding with theory would be obtained by allowing some considerable time to elapse after each addition of the reagent, but this manner of conducting a titration robs the process of its single advantage, of rapidity of execution, and is not to be recommended, since, after all, there will be considerable, and often quite capricious, departures from theoretical figures. A mode of conducting the titration has been recently suggested by Frank S. Hereth,* which allows time for the completion of the reaction, without extending the time of the titration.

52. Hereth's method of titrating with Mayer's reagent. Hereth's method is as follows: Knowing approximately the alkaloidal strength of the solution to be examined, provide half a dozen or more of test tubes or vials, into each of which measure 10 cc. of the solution. To the first is added a quantity of Mayer's reagent, which is thought to be a little less than enough for the precipitation; to the second is added a quantity of reagent 5 per cent. greater; to the next a quantity 10 per cent. greater; and so

*Pharm. Record, July 1, 1886. p. 209

on. Let the test tubes stand at least eight hours; then test a portion of the clear supernatant fluid from each with two drops of the reagent. Among them there will be some which react strongly, others which do not respond at all. The first one which fails to react obviously has received of the reagent a little more than enough, and the amount of precipitate produced in the one preceding it in the series will enable us to fix quite accurately the point at which precipitation would have ceased had the titration been conducted in the usual manner.

53. If this method is to be adopted, it will be necessary, of course, to fix the value of the equivalent for each alkaloid empirically by experiments carried out in the same manner, and these would no doubt be found different from those given by previous observers who have followed a different mode of procedure.

54. According to Dragendorff (Plant analysis), these data being gathered from various sources, 1 cc. of Mayer's reagent $N\frac{1}{20}$ precipitates of the following alkaloids respectively:

Atropine	.00625	Nepalline	.0194
Berberine	.04125	Nicotine	.00203
Brucine	.00985	Physostigmine	.00688
Chelidonine	.0084	Sabadilline	.0187
Colchicine	.01585	Sabatrine	.0167
Coniine	.00625	Sanguinarine	.00743
Emetine	.00945	Strychnine	.00835
Hyoscyamine	.00698	Veratrine	.0148

GRAVIMETRIC ESTIMATION OF ALKALOIDS.

55. The following table*, giving the percentage of gold or platinum left on ignition of the double salts of various alkaloids, is useful in case exact estimations of the alkaloid are required:

	Gold.	Platinum.
Atropine	31.57	.....
Aconitine	22.06	.....
Amanatine	44.23	.....
Berberine	29.16	18.11
Brucine	.....	16.52
Cinchonine	.....	27.36
Cinchonidine	.....	27.87
Cocaine	.....	.....
Codeine	.....	19.11
Coniine	. ...	29.38
Curarine	.....	32.65
Delphinine	26.70	.....
Delphinoidine	29.00	15.8
Emetine	.....	29.7
Gelsemine	37.4 (36.9)	16.7 (16.4)
Hyoscyamine	34.6	.....
Morphine	.....	19.52
Muscarine	43.01	.....
Narcotine	15.7	15.9
Narceine	.....	14.52
Nicotine	....	34.25
Papaverine	.....	17.82
Pilocarpine	35.5	23.6 to 25.2
Piperine	.....	12.7
Quinine	40.	26.26
Strychnine	29.15	18.16
Thebaine	.....	18.71
Theine	37.02	24.58
Theobromine	.....	25.58
Veratrine	21.01	.....

*Dragendorff's Plant Analysis, § 173 (p. 181 of English translation).

ASSAY OF FLUID EXTRACTS, TINCTURES, WINES, &c.

56. Fluid Extracts. To ascertain the alkaloidal strength of a fluid extract we may generally adopt one of the following general methods:

a. Shake the fluid directly with chloroform (two or three portions) after rendering alkaline, and adding water. Wash the alkaloid from the chloroform into acid water, and finally extract the alkaloid with chloroform or other appropriate solvent from the aqueous solution after rendering again alkaline.

b. Dilute the fluid with strong alcohol, shake with lime, filter, acidulate with sulphuric acid, filter once more, and evaporate an aliquot portion, with addition of a little water, to procure an aqueous acid solution, from which, after filtration and ether washing, the alkaloid may be extracted by shaking with chloroform, or other suitable solvent, and ammonia.

c. Dilute the fluid with water largely, add excess of solution of lead sub-acetate and filter; remove lead with alkaline carbonate, sulphate or phosphate, filter, evaporate aliquot portion to a small volume, and extract alkaloid with chloroform.

d. Add a little water and a few drops of dilute acid, evaporate until all alcohol is driven off, make up to a suitable volume, and titrate with Mayer's reagent.

57. The general plan for treating **tinctures, wines, etc.,** is essentially the same, but the volume of the fluid may be generally reduced considerably by evaporation with advantage, as a preliminary step.

58. Syrups are to be rendered alkaline and shaken repeatedly with chloroform.

59. Solid Extracts may be dissolved in alcohol, dilute alcohol, or water, and the solution treated by one or other of the methods employed for a fluid extract.

SPECIAL METHODS OF SEPARATING ALKALOIDS IN A STATE OF PURITY.

60. I. **Scheibler's Process.** Precipitate the aqueous solution with phosphotungstie acid, collect the bulky precipitate, wash with water containing some phosphotungstie acid and ammonia. Rinse the washed precipitate into a flask, add caustic baryta or carbonate of potassium to alkaline reaction, and distill to obtain any volatile alkaloid. If the alkaloid is a fixed one, treat the precipitate with caustic baryta or lime, and dissolve out the alkaloid by chloroform.

61. II. **Wagner's Method.** Acidulate the fluid with sulphuric acid, precipitate with excess of a solution of iodine and potassium iodide, collect the precipitate and dissolve in a solution of sodium thiosulphate (hyposulphite), filter the solution, precipitate once more with iodine and potassium iodide, and dissolve the precipitate in sulphurous acid. On evaporation the alkaloid will remain in the form of a sulphate.

62. III. **Method Suggested by T. B. Groves.*** Precipitate the aqueous solution with Mayer's reagent. Collect the precipitate, wash slightly, suspend in water, add solution of silver nitrate (or of lead acetate) sufficient to decompose the precipitate. Excess of the reagent can easily be shown by testing a drop of the filtered fluid with solution of potassium chromate. The aqueous solution, after filtration, is to be rendered alkaline, and shaken with chloroform or other solvent to extract the alkaloid.

63. IV. **By Phosphomolybdate of Sodium.** Exhaust the drug with acidulated water, precipitate the solution

*Pharm. Jour. Trans. [II.], Vol. 6, 275.

by addition of a slight excess of subacetate of lead solution; precipitate excess of lead from filtered solution with sulphuric acid, cautiously added; concentrate by evaporation after partially neutralizing with soda; precipitate the alkaloid with sodium phosphomolybdate in slight excess; collect the precipitate, wash with a little water, press between blotting paper, mix with excess of carbonate of calcium or barium—or with calcium or barium hydrate, dry at a water-bath heat. The alkaloid will now exist in a free state in the mixture, and may be extracted from it by alcohol or other appropriate solvent.

64. V. **By Tannin.** An aqueous solution, prepared in the manner just described, is to be rendered exactly neutral by addition of soda. Solution of tannin is then to be added cautiously, avoiding excess, and preserving the neutrality of the solution by successive additions of soda solution. Collect the precipitate, wash slightly with water, press between blotting paper, mix with finely powdered litharge, or preferably with precipitated lead hydrate, or with zinc oxide, and a little water. Heat on the water bath, with frequent stirring, and renewal of evaporated water until the tannin is wholly removed, so that a portion of the mixture, shaken with alcohol and filtered, yields a filtrate which ferric chloride does not darken in color. Dry the mixture, powder, and extract the alkaloid with alcohol. The use of lime in place of lead or zinc oxide is not to be recommended.

SEPARATION OF ALKALOIDS FROM ONE ANOTHER BY SOLVENTS.

65. Dragendorff's Method. The alkaloids being in aqueous solution containing free **acid,** shake with successive portions of **petroleum benzin.** Besides volatile and fixed oils, camphors, etc., the solvent will remove (a) *crystalline,* capsicine, piperine (in part), and picric acid (in part); (b) *amorphous,* certain constituents of black hellebore, and products of the decomposition of aconitine; and (c) *volatile* carbolic acid.

66. Shake the still acid fluid with several successive portions of coal tar **benzene** (benzol). This will remove absinthin, cantharadin, cascarillin, caryophyllin, colchicine, cubebin, digitalin, elaterin, populin, santonin, theine, and traces of berberine, delphinine, physostigmine and veratrine; also remnants of piperine and picric acid.

67. Treat the fluid next with **chloroform** in the same manner. This solvent will remove cinchonine, convallamarine, digitalin, helleborin, jervine, narceine, papaverine, picrotoxin, saponin, senegin, smilacin, syringin, theobromine, and traces of brucine, delphinine, narcotine, physostigmine, veratrine, and remnants of some of the substances imperfectly removed by benzene.

68. Next shake the solution once more with petroleum benzin to remove traces of chloroform; reject this portion; add ammonia to **alkaline** reaction, and shake with fresh portions of **petroleum benzin,** which will now remove (a) *volatile alkaloids,* aniline, coniine, lobeliine, nicotine, sarracenine, sparreteine, trimethylamine; (b) *fixed alkaloids,* brucine, cocaine, emetine, quinine, sabadilline, strychnine, veratrine.

Gerichtlich-Chemische Ermittelung von Giften, 1876, p. 141.

69. The ammoniacal solution is shaken with **benzene,** which removes the remnant (generally considerable) of the fixed alkaloids partially taken up by the petroleum benzin, also aconitine, atropine, cinchonine, cinchonidine, codeine, hyoscyamine, napelline, napaline, thebaine, with remnants of brucine, delphinine, narcotine, physostigmine, veratrine.

70. From the solution, **chloroform** will still remove morphine (in part) with the remainder of the cinchonine, narceine and papaverine, and **amylic alcohol** in turn will remove morphine, salicin, solanine, remnants of convallamarine, narceine, saponin, senegin. The fluid, evaporated to dryness, with addition of powdered glass, will still yield to **chloroform,** curarine.

PROXIMATE ANALYSIS OF PLANTS.

71. An exhaustive treatment of the general subject of plant analysis is quite beyond the scope of this manual. Dragendorff's treatise on the subject is reasonably complete, and has been translated recently into English.* An outline, however, of the admirable general scheme of plant analysis devised by H. B. Parsons†—to whom, short as was his period of professional labor, pharmaceutical chemistry owes so much—may well find a place here.

72. Parson's Scheme of Analysis. Moisture is determined by drying a known weight of the finely divided substance at 105° C. (221° F.). The loss of weight represents water, and sometimes a little volatile oil.

73. Mineral matter is estimated in the usual manner by igniting at a low red heat in a platinum or porcelain crucible, and the constituents are determined by the familiar methods of mineral analysis.

74. Total nitrogen is determined by igniting the substance with soda lime and estimating the ammonia formed. The amount of nitrogen found may, if required, be calculated to its equivalent in albuminoids by multiplying by the factor 6.33, recognizing the fact, however, that the nitrogen present does not all necessarily exist in the form of albuminoids.

75. The systematic analysis of the substance depends upon the successive application in a definite order of solvents and reagents, as prescribed in the following tables:

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*Published by H. J. Vail & Co., 1884.

†Pharm. Journal Trans. [3], x. 793. Allen's Commercial Organic Analysis, 2d Ed., Vol. I, p. 356 *et seq.*

76. Table A.

Treat 5 grams of the finely divided substance with benzol, wholly distilling below 86° C., or, failing this, with chloroform, by hot repercolation, continued six hours.

Solution (a). May contain alkaloids, glucosides, free organic acids, chlorophyll, certain resins, fixed oils, fats and waxes, camphors, volatile oils, but no mineral matter. Examine by Table B.

Residue (a). Dry at 100° C., weigh and treat 12 hours by hot repercolation with alcohol, sp. gr. .848.

Solution (b). May contain mineral matters, tannin, organic acids, alkaloids, glucosides, certain extractive and coloring matters, resins and sugars. Examine by Table C.

Residue (b). Dry at 100° C., weigh and treat with a known measure of cold water. Macerate with frequent agitation eight or ten hours, then filter through fine washed linen, or paper if possible.

Solution (c). May contain soluble albuminoids, gums, and, in the analysis of fruits and fleshy roots, pectin bodies, salts of organic acids, dextrinoid bodies and coloring matters. Examine by Table D.

Residue (c). Wash with alcohol, dry at 100° C. and weigh, treat with 100 cc. of water and 5 cc. of concentrated sulphuric acid, and heat until a drop of the liquid gives no color with iodine.

Solution (d). May contain dextrin and maltose, from conversion of starch; also albuminoids, and occasionally organic acids, either as salts or free. Examine by Table E.

Residue (d). Wash thoroughly, dry at 110° C. and weigh. Boil two hours with 500 cc. of a 2 per cent. solution of caustic soda. Filter through washed linen.

Solution (e). May contain albuminous matters, pectous matters, cutose, humus, and products of decomposition. Examine by Table F.

Residue (e). Wash thoroughly in succession with hot water, alcohol and ether, dry at 110° C. and weigh. Treat with dilute bromine water and ammonia, until residue is completely bleached.

Solution (f). Contains lignin and coloring matter.

Residue (f). Wash, dry and weigh as cellulose.

77. Table B.

ANALYSIS OF SOLUTION IN BENZOL OR CHLOROFORM.

Evaporate carefully to dryness and weigh the residue. Treat with water, again evaporate to dryness at 100°C., heat to 110° C. and weigh again.

Volatilized. Volatile oils, camphors (partially), volatile alkaloids. The last may be detected by the alkaline reaction of the aqueous liquid, and their loss avoided by adding a drop of hydrochloric acid before evaporation.

Residue (a). Treat with a moderate quantity of warm water, and, when cold, filter through fine paper by aid of a filter pump.

Aqueous Solution. Divide into two portions, A and B. A. Evaporate to dryness and weigh *total extract;* ignite and weigh *ash.* B. Test portions for alkaloids and glucosides by special reagents, and for organic acids by solutions of barium, calcium, iron and lead.

Residue (b). Remove from the filter and vessels used by benzol or chloroform, and agitate the solution with warm, very dilute hydrochloric acid, and separate by means of a separating funnel.

Acid Solution. Test for alkaloids and glucosides.

Benzol Solution. Evaporate to dryness and treat residue several times with alcohol of sp. gr. .848. Filter through paper.

Alcoholic Solution may contain camphors, resins, chlorophyll, certain fixed oils.

Residue (c). Consists of fixed oils, fats, wax, and very rarely resin.

78. Table C.

ANALYSIS OF ALCOHOLIC EXTRACT.

Concentrate to a small bulk and remove, dry and weigh any crystals or powder which may separate from the cooled liquid. Dilute to 200 cc. with alcohol of sp. gr. .848, and divide into three portions; (a) 20 cc., (b) 20 cc., (c) 160 cc.

Portion (a). Evaporate to dryness, and weigh *total extract.* Ignite and weigh *ash.*

Portion (b). Evaporate nearly to dryness, add water, filter and evaporate, filtrate to dryness. Residue is *soluble extract.* Ignite to obtain *soluble ash.*

Portion (c). If much sugar or tannin be present, recognizable by the taste, follow "A"; if but little of either be present follow "B."

"A." Evaporate nearly to dryness, add water, filter, and make up filtrate to 160 cc.

Residue. May contain resins, coloring matters, albuminoids, especially from seeds, alkaloids and glucosides.

Solution. Divide into eight portions of 20 cc. each.

(1) Precipitate *tannin* with ammoniacal zinc acetate. Dry at 120° C. Weigh, ignite and weigh. Difference, *tannin.*

(2) Add neutral lead acetate. Loss of weight on ignition of dried precipitate represents tannic, gallic and other organic acids, coloring and extractive matters, and rarely albuminoids.

(3) and (4) Precipitate by basic lead acetate and treat as in (2). After separating lead, determine glucose in one-half of filtrate; invert the other half and determine glucose. Difference is glucose formed from sucrose and glucosides.

(5) and (6) Treat with basic lead acetate and filter, decompose both precipitate and filtrate with H_2S, and test first for organic acids, and second for alkaloids and glucosides.

(7) and (8) Use in case of accidents to other portions.

"B." Evaporate carefully to dryness, pulverize and treat with considerable portions of absolute alcohol. Filter.

Alcoholic Solution (a) Evaporate nearly to dryness and add water.

Aqueous Solution (b) Add basic lead acetate. Loss of weight on igniting dried precipitate=tannin, organic acids and some extractives. *Filtrate* may contain alkaloids, glucosides, extractive and coloring matters.

Residue (b). May contain (1) alkaloids, glucosides (rarely), and extractive, soluble in H Cl; (2) matters insoluble in dilute H Cl: (3) acid resins and colors soluble in dilute ammonia; (4) neutral resins, colors and nitrogenous matters insoluble in dilute ammonia.

Residue (a) from alcoholic solution. Treat with water.

Solution (c). Add basic lead acetate. Loss of weight on igniting dried precipitate represents colors, extractive, organic acids, albuminoids (rarely). From *filtrate* remove lead, divide into two portions; in one determine glucose direct; in the other, after inversion; difference will be glucose due to sucrose and glucosides.

Residue (c). Treat with dilute H Cl. (1) Dissolved, some alkaloids and glucosides; (2) insoluble, some resins and coloring and extractive matters. Dissolve in alcohol, evaporate to dryness and weigh.

79. Table D.

ANALYSIS OF SOLUTION IN COLD WATER.

Make up liquid to a definite volume, and divide into aliquot portions, a, b, c, d, e.

a Determine *total solid matter* by evaporating and drying residue at 110° C. Determine *ash* by ignition.

b Add solution of iodine. A blue color indicates soluble starch; a reddish-brown color, *erythro-dextrin*.

c Add ammonium oxalate. A white precipitate indicates calcium, probably as calcium *arabinate*.

d Evaporate, ignite residue with soda lime, and multiply nitrogen found by 6.33 to estimate albumin.

e Add dilute hydrochloric acid. A gelatinous precipitate consists of pectin or pectic acid; if the liquid be filtered and treated with four times its volume of alcohol, a further precipitate may consist of arabin or dextrin.

80. Table E.

ANALYSIS OF SOLUTION IN DILUTE ACID.

Boil with excess of barium carbonate, neutralize exactly with baryta water, filter, concentrate, and bring volume to exactly 50 cc., and take its specific gravity. Divide excess over 1,000 by 8, to obtain weight of starch. If density indicates but little starch, treat one-half the solution with 1 cc. conc. sulphuric acid three or four hours at 100° C.; neutralize and estimate glucose by copper solution. Multiply by 0.9 to obtain starch. Test the other half of solution with tannin for albuminoids.

81. Table F.

ANALYSIS OF SOLUTION IN DILUTE ALKALI.

Add slight excess of hydrochloric acid. A precipitate may contain pectic acid and other bodies, coloring matters, etc. Further precipitation usually occurs on adding alcohol.

ESTIMATION OF ALCOHOL IN FLUID EXTRACTS, TINCTURES, Etc.

82. Approximate Method of Carl Jungk.* Mix 5 cc. of the fluid extract with 20 cc. of stronger ether, and shake well together in a graduated tube. Allow the fluids to separate, and read off the volume of the lower stratum, from which, by aid of the accompanying tables, the proportion of alcohol can be deduced. In case the fluid is made with an alcohol of more than 70 per cent., use 50 cc. of ether and 5 cc. of water. Divide the result (measure in cc. of the lower stratum of fluid) by two, ascertain from the table the corresponding percentage of alcohol, and multiply this by two. The presence or absence of glycerine must be ascertained, and its quantity approximately estimated, as a preliminary step in the investigation.

TABLE I.—For fluids containing no glycerin.

Vol. of fluid used.	Vol. of ether used.	Vol. of aqueous stratum separated.	Vol. per cent. of com. alcohol. (sp. gr. .820.)
5 cc.	20 cc.	4.50	25.0
5 cc.	20 cc.	4.25	33.3
5 cc.	20 cc.	3.50	40.0
5 cc.	20 cc.	3.00	50.0
5 cc.	20 cc.	1.75	66.7
5 cc.	20 cc.	1.50	75.0
5 cc.	20 cc.	0.00	83.3

* Deutsch-Amer. Apotheker-Zeitung, May 1, 1886, p. 114.

TABLE II.—For fluids containing 25 per cent. (vol.) glycerin.

Vol. of fluid used.	Vol. of ether used.	Vol. of aqueous stratum separated.	Vol. per cent. of com. alcohol. (sp. gr. .820.)
5 cc.	20 cc.	4.75	18.75
5 cc.	20 cc.	4.50	25.00
5 cc.	20 cc.	4.00	30.00
5 cc.	20 cc.	3.50	37.50
5 cc.	20 cc.	3.00	50.00
5 cc.	20 cc.	2.75	56.25
5 cc.	20 cc.	2.00	62.50

TABLE III.—For fluids containing 50 per cent. (vol.) glycerin.

Vol. of fluid used.	Vol. of ether used.	Vol. of aqueous stratum separated.	Vol. per cent. of com. alcohol. (sp. gr. .820.)
5 cc.	20 cc.	5.5	12.5
5 cc.	20 cc.	4.5	25.0
5 cc.	20 cc.	4.0	37.5
5 cc.	20 cc.	3.0	50.0

This ready method of ascertaining approximately the proportion of alcohol in a tincture or fluid extract commends itself as eminently practical, although the nature of the extractive will often modify the results materially.

83. Exact determination of Alcohol. Where more exact results are required, it is best to resort to the usual expedient of distillation. Make a mark with a file on the neck of a two-ounce bottle. Fill the bottle to the mark with the tincture or fluid extract, having previously brought its temperature to 15.55° C. (60° F.). Transfer the fluid to a distilling flask, rinse the bottle with two portions of one fluid ounce each of water, adding this to the contents of the flask; connect this securely with a Liebig's condenser, and distil, using as a receiver the same bottle that was employed to measure the tincture. Continue the distillation until the bottle is filled exactly to the mark. Cork the bottle, mix its contents well by shaking, cool to standard temper-

ature, add distilled water to make up the measure, at this temperature, accurately to the volume of the original fluid, and take the specific gravity by the pycnometer. The proportion of alcohol will then be found by reference to the alcohol tables of the U. S. P.

84. In case the fluid has an acid reaction it should be neutralized with sodium or potassium carbonate, otherwise volatile acids might pass over and vitiate the result. If the proportion of spirit is greater than 50 per cent., it is best to add two volumes of water instead of one, and distil off two volumes; take the specific gravity as before, and find from alcohol tables the volume per cent. of spirit present in the distillate. Multiply this result by two to find the volume per cent. present in the tincture. It is convenient in these cases to use two bottles, one of exactly double the capacity of the other, when filled to the respective marks. Measure the fluid with the first, the distillate with the second.

ESTIMATION OF GLYCERINE IN FLUID EXTRACTS, ETC.

85. Approximate Method of Carl Jungk.* To 20 cc. of the fluid extract add two grams of washed magnesium carbonate, or calcium carbonate. Evaporate to dryness. Rub to a fine powder, and exhaust with a mixture of alcohol 10 volumes and chloroform 6 volumes, using 50 cc. of the mixture. Filter; avoiding evaporation. To 25 cc. of the filtrate in a graduated tube, add 80 cc. of concentrated ether. The glycerine will separate in pure form. Add to the measure, expressed in cc., 25 per cent. as a correction for loss and solubility, and move the decimal point one place to the right for volume percentage.

Thus, if 3.2 cc. of glycerine have separated, the fluid contained 32. + 8. = 40 per cent. vol. of this solvent. Of course, care must be taken in the evaporation to avoid a high temperature or long exposure to heat, otherwise there will be lost a variable proportion of glycerine.

86. Accurate Estimation of Glycerine, according to Fox and Wanklyn.† This method cannot be trusted without reserve in the examination of fluid extracts, since there may be other substances besides glycerine present, which are oxidized to oxalic acid. Alcohol, if present, must be driven off by evaporation, at a low temperature, remembering that glycerine itself is volatile to an appreciable extent at a water bath heat.

Take for the assay a quantity of the preparation containing not to exceed 0.25 grm. glycerine; add 5 grams of solid caustic potash, and powdered permanganate of potassium in excess. Keep the solution at the boiling point half an hour, then add sul-

*Deutsch-Amer. Apotheker Zeitung, May, 1886, p. 114.

†Chemical News, January 8, 1886.

phurous acid to decompose the excess of permanganate. Filter from the precipitated manganese oxide, acidify with acetic acid, and boil; add calcium chloride, collect the oxalate of calcium precipitated, and wash thoroughly with boiling water. Determine the oxalic acid in the precipitate in the usual way by titration with potassium permanganate. One equivalent of oxalic acid, $C_2 H_2 O_4$ (90 parts by weight), represents one equivalent of glycerine, $C_3 H_8 O_3$ (92 parts by weight).

ACONITE.

87. Active Constituents. According to Messrs. Wright & Duff, who have made a very careful study of the alkaloidal constituents of the several species of aconite, there are three or four distinct alkaloids met with, which are all exceedingly unstable bodies, decomposed by the action of mineral acids, and still more readily under the influence of alkalies, which act even in the cold.

The most important of these alkaloids are:

(1). **Aconitine,** $C_{33}\ H_{43}\ NO_{12}$, the principal alkaloid of Aconitum Napellus, but present in A. ferox, and in other species.

(2). **Pseudaconitine,** $C_{36}\ H_{49}\ NO_{11}$, like the first crystallizable and exceedingly active. The solution of both these alkaloids produce tingling and numbness of the tongue and lips.

(3). **Japaconitine,** from Japanese aconite roots, closely resembling aconitine, and almost identical with that alkaloid in composition, and by many chemists regarded as not in fact distinct from it.

(4). **Picraconitine,** an alkaloid of greatly inferior potency, producing no lip tingling, but forming very bitter salts.

(5). A **more crystallizable alkaloid** of little activity. These latter occur in the roots of A. Napellus.

88. Action of Alkalies on Aconitine. By boiling with alkalies, or with mineral acids, or even with water alone, aconitine is split into benzoic acid and a feeble base, aconine, which is physiologically inert. Under similar treatment pseudaconitine is split into dimethyl protocatachuic acid and pseudaconine.

89. The facts of prime importance as bearing upon assays

of aconite, are that the alkaloids of this drug are not all equally active, and that contact with alkaline solutions decomposes them more or less rapidly.

90. The alkaloids are all precipitated (aconine included) by Mayer's reagent, which therefore cannot serve as a reliable indicator of the medicinal strength of a preparation of aconite. A clear understanding of these facts is essential to a correct interpretation of the results which may be obtained in the various processes of assay that are to be described.

Assay of Aconite.

91. Dragendorff's Method.* Having reduced the drug (aconite root) to a fine powder, weigh out two grams of it. Place this in a flask, and add 20 cc. of water containing 0.1 gram sulphuric acid [tartaric acid is to be preferred]; cork the flask, shake until the powder is thoroughly moistened, and set aside for 24 hours. Then add 81.7 cc. of alcohol, cork, and allow to macerate two or three days, with occasional shaking. Filter, covering the funnel to prevent evaporation. Take for the assay 50 cc. of the filtrate, equivalent to 5 grams of drug; evaporate at a water bath heat, with addition of a little water, until all alcohol is driven off, not carrying the evaporation, however, to dryness. Add water enough to make up a volume of 8 cc., and titrate with Mayer's reagent, N$\frac{1}{20}$. Each cc. of reagent used indicates 15 milligrams† of alkaloid.

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*Werthbestimmung einiger Starkwirkender Droguen (St. Petersburg, 1884), p. 7 *et seq.*

†Dragendorff states that, adopting Duquesnel's formula for aconitine, each cc. of Mayer's reagent, N 1-20, should precipitate 13½ milligrams of alkaloid, while Planta's formula makes the equivalent a trifle less than this. Actual titrations with crystallized aconitine, as also with the alkaloid separated from aconite root, have given uniformly a higher equivalent, which may be stated approximately as 15 mg. Since the assay aims only at comparative valuations, and we are dealing with a drug containing several alkaloids, we may content ourselves with this arbitrary constant, but the results of the assay should always be stated in terms of Mayer's reagent consumed, rather than of alkaloid found.

Dragendorff applies the same method of assay also to aconite leaves, but uses double the quantity of drug.

92. Short Method of the Author. The following plan is by far the simplest and most rapid for this assay: [Compare (29).] Place in a flask or bottle 10 grams of the powdered drug (aconite root or leaves). Pour in 100 cc. of Prollius' fluid, and cork at once securely. Shake the flask occasionally during 12 hours. Then decant, or remove with a pipette, exactly 50 cc. of the clear fluid. Evaporate this spontaneously or by a gentle heat in a beaker, after adding 5 cc of water. When the ether is nearly all expelled, add 6 drops of 5 per cent. sulphuric acid and 10 cc. of fresh ether. Stir vigorously to make sure that the acid takes up the whole of the alkaloid. Continue the evaporation until all ether and alcohol are expelled, adding, if necessary, a little more water. Make up the volume of fluid finally to 8 cc. with water, and titrate with Mayer's reagent as above. Instead of estimating the alkaloid by titration, it will be found exceedingly easy to extract and weigh it. To do this, shake the 50 cc. of ethereal fluid with two or three successive portions (5, 3 and 3 cc.) of water containing hydrochloric acid (sufficient to give a sharp acid reaction after shaking with the alkaline fluid). Transfer the acid solutions containing the alkaloid to a 2 oz. prescription vial, with good lip and sharp shoulder, wash once with ether (15 to 20 cc.), render the fluid alkaline with ammonia, and shake immediately with ether. As soon as the ether has completely separated, pour it off into a tared evaporating capsule, and drive off the ether by a gentle heat. Wash the alkaline fluid once more with ether, evaporate the washings together, dry at water bath heat, and weigh. To obtain percentage, multiply the weight in decigrams by two.

93. The assay must be made rapidly. Since the alkaloids (aconitine and pseudaconitine) are decomposed even at common temperature by prolonged contact with ammonia, the assay must be carried through rapidly. If the drug is reduced to

a sufficiently fine powder, the 12 hours' maceration may be reduced one half, or even more, with advantage. The alkaloid decomposed is not wholly lost in the assay, whether the estimation be made by titration or by weighing, since it is still represented by the derivative alkaloids, aconine and pseudaconine. That the actual loss is not great is easily demonstrated by submitting the alkaloid obtained to the physiological test.

94. In place of Prollius' fluid, we may employ in this assay a combination of petroleum benzin with ammonia and alcohol, as in the assay of coca leaves, with no advantage, however, unless in the direction of economy. In the few trials I have made of this plan, I have obtained results a little lower than by the use of the Prollius' mixture.

95. For exact estimations of aconitine we may avail ourselves of the action of alkalies, weighing the benzoic acid produced by its decomposition. Exhaust the drug by percolation with alcohol, or by Dragendorff's method by maceration first with water and afterward with alcohol. Evaporate to a small bulk the alcoholic solution, which may advantageously contain tartaric, but not mineral, acids; add water and a few drops of sulphuric acid, 5 per cent., shake repeatedly with ether to remove all matters soluble in that fluid. Neutralize the residue and evaporate to a small volume, add alcoholic solution of potassa, and digest on the water bath until the alkaloid is completely "saponified." Evaporate off the spirit, acidify the aqueous residue and wash out the benzoic acid produced by decomposition of the alkaloid by shaking repeatedly with ether. One part of benzoic acid is equivalent to six parts of aconitine (103).

96. The details of this process, however, have not been fully elaborated, since we have a ready method of arriving at the medicinal activity of any preparation of aconite, the results of which are not dependent upon any skill in chemical manipulation, and do not even require isolation or identification of the active

principle of the drug. This method consists in testing by physiological experiment the actual power of the drug as evinced by the effects it produces, under prescribed conditions, on the nerves of the tongue and lips.

97. Physiological Test of Dr. Squibb.* For the reasons just explained, the physiological test should always be resorted to in corroboration of the results of a chemical assay. The test is applied to the root as follows: Exhaust 10 grams of the root in No. 30 powder, with alcohol, by percolation. It is convenient to continue the percolation until 100 cc. of fluid is obtained, and if the percolation is properly conducted this will surely exhaust the drug. Put 10 cc. of the percolate into water sufficient to make up 600 cc., unless the drug has been shown by the assay to be unusually strong or unusually weak. Having rinsed out the mouth well, take one fluidrachm of the dilute solution into the mouth and hold it in the anterior portion of the buccal cavity one minute by the watch. Eject the solution, and rinse the mouth once more. In a few minutes, if the root be of standard strength, the tingling sensation characteristic of aconite will be perceived in the tongue, and this will continue 30 to 45 minutes. Doubtless different persons will differ in susceptibility, so that each one should ascertain for himself just what effect he may expect from a drug of average strength, and this can only be done by repeated trials, but susceptibility does not vary as much as might be expected.

98. If the effect is greater or less than that expected, a second trial must be made, after an interval of several hours, and the relative strength of the specimen under investigation will finally be found with a considerable degree of precision.

The effect of aconite leaves is generally feeble at a dilution of 1:100. That of aconite root should be well marked at a dilution of 1:600.

* Ephemeris, p. 125 *et seq.*

99. The preparations of Aconite may be assayed best by an application of the same physiological test. Tinctures or fluid extracts are simply to be diluted so that 600 parts of the fluid represents one of aconite root. Solid extracts are to be dissolved in water, or in diluted alcohol, and the solution is then to be treated in the same manner. Oleate of aconitine is to be diluted with ether, and the alkaloid extracted from it by repeated washing with water containing hydrochloric or sulphuric acid.

100. We should expect to find that, judged by the physiological test, one grain of aconite root is the equivalent in activity of six to nine grains of aconite leaves, of 1½ grains ext. aconite leaves, of ½ grain abstract aconite, U. S. P., of ⅕ grain ext. aconite (root), U. S. P., and of $\frac{1}{100}$ grain crystallized aconitine.

101. Commercial aconitine we shall find exceedingly variable in activity. The physiological test should be commenced with a solution containing 1 part in 75,000, and if in a solution 1:25,000 we fail to obtain tingling comparable with that produced by a good sample of the root 1:600, we should condemn the article.

102. Chemical tests fallacious. The preparations of aconite may be also examined chemically, either with Mayer's reagent or by extracting the alkaloid and weighing, but very little dependence can be placed on the result of such an examination unless supported by the physiological test, which can never be omitted, and which seems to render all others superfluous.

103. Assay of Commercial Aconitine. Process of C. R. A. Wright and A. P. Luff.*

(a.) Estimate moisture in a sample by drying at 100° C. (212° F.), and noting loss of weight.

(b.) Dissolve 0.5 grm. in hydrochloric acid, and shake the solution repeatedly with ether. Evaporate the ethereal solution and weigh the residue as inert resinous matter, including probably some dimethyl protocatechuic acid.

* Pharm. Jour. Trans., September 1, 1877, p. 171.

(c) Heat 0.5 grm., with water, in a sealed tube for 24 hours, at 140° to 150° C. (284 to 302° F.), acidify with hydrochloric acid, and shake repeatedly with ether. Evaporate the ethereal solution and weigh the residue, which consists of a mixture of dimethyl protocatechuic and benzoic acids.

(d.) Distil the mixed acids obtained above with water, and extract benzoic acid from the distillate with ether, or estimate it otherwise.

One part of benzoic acid is equivalent to six parts of aconitine. One part of dimethyl protocatechuic acid is equivalent to four parts of pseudaconitine.

104. If the alkaloid consists mainly of aconitine, the residue from (c) may be considered to be benzoic acid simply; if it consist mainly of pseudaconitine, it may be taken to consist wholly of dimethyl protocatechuic acid. In the former case multiply by six, in the latter by four, to obtain the proportion of active alkaloid in the sample.

105. The authors, however, find that the inert picraconitine also yields benzoic acid when treated as above (c). The presence of this alkaloid is betrayed by the bitterness of the solution (highly dilute). It is proposed to separate this alkaloid from the aconitine by means of sodium carbonate solution, which precipitates aconitine, but not picraconitine.

106. These methods are tedious, circumstantial, and, at best, uncertain. The decomposition of the alkaloids can be effected with greater certainty and promptness by boiling a few hours with an alcoholic solution of potassa or soda. I should much prefer to trust to Dr. Squibb's physiological test, assuming that a solution containing one part of pure aconitine (or its therapeutic equivalent of pseudaconitine) in 60,000 parts of water will produce, under the conditions of Dr. Squibb's test, a numbness lasting one-half to three-quarters of an hour.

BELLADONNA AND STRAMONIUM.

107. Active Constituents. The important active principles of these two drugs are identical, being the alkaloids **atropine** and **hyoscyamine,** of which the former is more abundant in belladonna, the latter in stramonium. It is sufficient for the purposes of a pharmaceutical assay to ascertain the total quantity of these alkaloids in the drug. Of course we cannot bring the two drugs into comparison with one another in this way, but we may judge approximately the relative value of different samples, or of different preparations of the same drug.

108. Belladonna root, being nearly free from fatty, waxy and resinous substances, is very easily assayed. **The leaves** of belladonna and stramonium present greater difficulties, simply from the presence of a larger proportion of inert substances soluble in ether or chloroform. Stramonium seed abounds in fatty matter, which still further embarrasses the analysis. As a rule, however, these differences do not require modifications in the general plan, but merely in the details of the assay. There are several methods which give good results; individual manipulators will choose some one, some another, according to their habits of work.

Assay of the Crude Drug.

109. Dragendorff's Method. The drug is to be exhausted precisely as directed in the case of aconite root, by macerating first with acidulated water, then with alcohol added (91). The alkaloid is to be estimated with Mayer's reagent, either volumetrically or gravimetrically. In the former case we are directed to make up the aqueous solution for titration to such a volume that it shall contain between 0.2 and 0.3 per cent. of alka-

loid. In other words, the solution should measure for each gram of dry drug represented about 2 cc. in the case of belladonna root or leaf, or 1½ cc. in the case of stramonium seed or leaf. Under these circumstances we are to reckon for each cc. of Mayer's solution, $N\frac{1}{20}$, consumed 6¼ milligrams of alkaloid, adding as a correction for each cc. of fluid present at the end of the titration, $\frac{1}{20}$ milligram.

Thus, if the solution represent 5 grams of drug (belladonna), make up to a volume of 10 cc. Suppose there is required of the reagent ($N\frac{1}{20}$) 3.85 cc. There is then present of alkaloid $3.85 \times 6.25 + 13.85 \times .05$, or 24.82 milligrams. Multiply by two, and move the decimal point to the left two places to obtain the percentage, which is, therefore, 0.496.

110. The Titration Method. Although the mode of reaching the result is faulty, the result is itself a fairly close approximation to the truth, and is reached with very little expenditure of labor, and by a method which is as simple and direct as could be desired. The titration is, indeed, sometimes troublesome, from the fact that the fluid does not always filter clear—an evidence, generally, that the alcohol has not been completely expelled. The precipitate also is liable after a time to clog the filter, thus delaying the operation considerably. It becomes necessary, indeed, sometimes to change the filter, taking care, of course, to press out from the rejected filter as much as possible of the fluid it holds.

111. Gravimetrical Estimation by Mayer's Reagent. Instead of titrating the solution, we may precipitate it (after filtration) in a tared beaker, with an excess of Mayer's reagent, and after 24 hours, when the precipitate has completely subsided and adhered for the most part to the bottom and sides of the beaker, we may decant the fluid through a small filter, wash the precipitate in the beaker several times with distilled water, which is afterwards to be passed through the same filter, and finally dissolve the portion of the precipitate which remains upon

the filter in strong alcohol, run the solution into the beaker, evaporate at 100° C., and weigh. Multiply the weight of the precipitate by 0.449 to obtain the weight of the alkaloid it contains.

Results said to be fairly satisfactory, but it must be remembered that the precipitate is not of constant composition, and that its weight will therefore vary somewhat.

112. Method of Dunstan and Ransom.

(a) **Belladonna Root.*** Exhaust 20 grams of the dry and finely powdered root by hot percolation—preferably in an extraction apparatus (7) and (8)—with a mixture of chloroform and absolute alcohol, equal volumes; if an extraction apparatus is used, about 60 cc. of the mixture will be required. Agitate the percolate with two successive portions (25 cc.) of distilled water, which are separated in the usual way. Mix the aqueous solutions and shake once with a little pure chloroform to remove adherent coloring matter. Separate the chloroform, add ammonia to the aqueous solution, and wash out the alkaloid by shaking with two successive 25 cc. of chloroform. Wash the chloroform with a little water containing some ammonia, transfer to a tared capsule, and evaporate over a water bath until the weight is constant, which usually occupies a little less than an hour. The results of analyses made in this manner by the authors are much lower than the average results I have obtained by other methods of analysis. I should certainly add some hydrochloric or sulphuric acid before attempting to wash out the alkaloid with water from the mixture of chloroform and alcohol, although the authors state that this is unnecessary.

113. (b.) **Belladonna Leaves.†** Exhaust 20 grams of the dried and finely powdered leaves in an extraction apparatus with absolute alcohol, of which about 100 cc. will be required. Add to the percolate an equal volume of water with a little hydro-

* Phar. Jour. and Trans., Feb. 9, 1884, p. 623.

† Phar. Jour. and Trans., Sept. 12, 1885, p. 237.

chloric acid. From the slightly warmed liquid remove chlorophyl, fatty matter, etc., by repeated washings with chloroform, which must be continued as long as anything is removed by that solvent. The solution is then to be made alkaline with ammonia, and the alkaloid extracted precisely as in the assay of belladonna root, above.

114. Method of Dr. Squibb.* Take for the assay 50 grams of the powdered drug, moisten the powder uniformly with 39 cc. of alcohol, sp. gr. .820, to which has been previously added 0.1 grm. or about three small drops of strong sulphuric acid. Pack in a cylindrical glass percolator and exhaust by percolation (best by aid of a Sprengel pump) with strong alcohol, of which 300 cc. will suffice, with skillful manipulation. Evaporate at a low temperature in a shallow dish, stirring toward the end until the odor of alcohol is no longer perceived. Add to the liquid extract, while still warm, 25 cc. of water containing one or two drops of sulphuric acid, stir well, cool and transfer to separator, and rinse the dish with 1 or 2 cc. of water, to be added to the contents of the separator. Rinse the dish with two or three successive small portions of chloroform, which is also to be transferred to the separator. Add about three drops more of sulphuric acid, and agitate the contents of the separator for five minutes, not too violently lest emulsification take place. Separation of the liquids, if too slow, may be hastened by adding a little more acid and shaking again; and if still obstinate, by adding 10 cc. more of water and chloroform. When completely separated, which may be only after some hours, draw off the chloroform layer into a second separator, and repeat the chloroform washing with successive portions of 10 cc. each as long as the chloroform is colored. Wash the chloroform with 15 cc. of water containing one drop of sulphuric acid, and add the watery solution to the contents of the first separator. Add to this, now, 20 cc. of fresh chloroform, and

* Ephemeris, p. 849.

supersaturate with sodium carbonate—carefully, to avoid loss by frothing; about six grams of the salt will generally suffice. Now agitate the mixture thoroughly, and allow the chloroform containing most of the alkaloid to separate. Draw off into a tared beaker, wash a second time with 10 cc. of chloroform, and add this to the contents of the beaker. Finally dry the alkaloid thoroughly and weigh.

115. Physiological Test of Dr. Squibb. A simple, ready method of estimating the strength of a preparation of belladonna is afforded by the physiological effect which the alkaloid of the drug produces on the pupil of the eye. Dr. Squibb demonstrated by experiment that one drop of a solution containing $\frac{1}{200}$ part of a grain (.00032 grm.) of commercial atropine sulphate in a fluid ounce, when introduced into a healthy human eye, produced within 50 minutes a decided dilation of the pupil, lasting six hours. Individuals differ considerably in susceptibility; still by experimenting always under like conditions on the same subject, or better, by experimenting at the same time on a number of subjects—and animals, as cats, could be utilized for this purpose—it is possible to compare different specimens of belladonna and different preparations of the drug. A fluid extract of belladonna leaves, containing 0.342 per cent. of alkaloid, when diluted with water to 400 times its volume, produced a corresponding effect, while a fluid extract of belladonna root containing 0.52 per cent. of alkaloid, produced the characteristic effect only slightly in a dilution of 1:600. A standard drug, judged by this method, may be assumed to be one which will produce a distinct effect in a dilution of 1:500, but none, or one scarcely perceptible, in a dilution of 1:600, and a specimen that would yield to assay the full proportion of alkaloid, but would not answer to the physiological test, could not be accepted as really of standard strength.

116. Method of A. W. Gerrard. Exhaust the drug, in fine powder, with alcohol, evaporate or distil off the

spirit and dissolve the residue in water and ether. Add ammonia, and after shaking the mixture allow the ether to separate completely. Transfer the ether to a separator, shake with water containing acetic acid. Separate the aqueous acid solution, wash once with ether, add ammonia, and remove the alkaloid by shaking with ether.

In carrying out this assay the several washings should be repeated at least twice, and a mixture of ether and chloroform is certainly to be preferred to the simple ether.

117. The Author's Method. The simplest way to assay belladonna (root or leaves) or stramonium (seed or leaves) is that described (29) *et seq.* It is unnecessary to repeat here the details of the process, but I may call attention to its advantages over most of the methods that have been described: 1st. The result is reached speedily, and with very little labor. 2d. The alkaloid is extracted with comparatively little inert matter accompanying it, and the impurities are easily eliminated owing to their insolubility in acid water. 3d. Very little heat is applied in any part of the process, and there is practically no risk of decomposition of the alkaloid under the influence of the reagent employed. 4th. In practice the results compare favorably with those obtained by more lengthy and laborious processes. 5th. The process answers equally well for assays of different parts of the plant, and may be applied to many other drugs with equally satisfactory results.

118. In all manipulations with the mydriatic alkaloids, we must remember how easily they are decomposed and lost; even boiling with water destroys them.

119. While a gravimetric process of assay is always to be preferred, the expedient of titrating with Mayer's reagent affords a tempting short cut to results, which, although less exact, are, for practical purposes, equally good. Instead, therefore, of attempting to extract the alkaloid from the acid fluid obtained in the pro-

cess described, we may simply make it up to such a volume that it shall contain about one part in 400 of alkaloid, and titrate with Mayer's reagent ($N\frac{1}{20}$). Two portions of the solution may be titrated, the first result enabling us to bring the solution sufficiently near the required strength. Each cc. of Mayer's reagent ($N\frac{1}{20}$) will precipitate, when the solution contains 1:400 of alkaloid, about 6.25 milligrams, of the mixed alkaloids. If the strength of the solution is above or below the standard of 1:400, we may adopt the following rule for calculating from the result of the titration how much alkaloid is present: Subtract from the quantity of reagent used for each cc. of fluid present at the end of the titration .05 cc., and multiply the remainder by 7.6. The product will be the desired amount in milligrams, whence the percentage is easily computed.

120. Fluid Extract Belladonna, Root or Leaves. If the fluid extract is not too heavily loaded with inert extractive, the simplest mode of extracting the alkaloid in it is the following: Put into a separator 10 cc. of the fluid extract; add a few drops of solution of ammonia and 15 cc. of chloroform; agitate, add 10 cc. of water, and again agitate carefully. Allow the chloroform to separate completely, which it will do speedily, and draw it off into a second separator containing 5 cc. of water, to which has been added a few drops of dilute sulphuric acid, 1:5. Agitate, and when the chloroform has separated draw it off into a third separator containing 5 cc. of acidulated water. Agitate and separate the chloroform, which now contains no trace of alkaloid. To the contents of the first, add, meanwhile, 10 cc. of fresh chloroform, which is to be passed in succession through the several separators as before. A third portion of chloroform may be used if it be found that the contents of the first separator retain alkaloid. (Acidify a drop, and test on mirror with Mayer's reagent.) Now transfer the aqueous fluid in separator No. 3 to No. 2, render alkaline with ammonia, and extract the alkaloid by shaking with

three successive portions of chloroform, 15, 10 and 10 cc. respectively.

The above plan will be found more advantageous than that commonly pursued in similar cases, viz., adding acid to the fluid in the first instance, and washing repeatedly with chloroform to remove fatty and waxy matter, then rendering alkaline and washing out the alkaloid. It will be found if this is attempted that the impurities can be removed only by repeated washings with chloroform, and the alkaloid will after all require to be purified. A mixture of chloroform and ether cannot be used in this assay in place of the pure chloroform, except in the final washing out of the alkaloid.

121. Second Method. This is of general applicability, and, although it cannot be finished quite so quickly as that just described, its successive steps are easily carried out by the most inexperienced, and the beginner will perhaps prefer it on that account. Mix 10 cc. of the fluid extract with strong alcohol enough to make 50 cc. of the mixture. Pour this at once into a four-ounce vial containing about two grams of freshly slacked lime, and shake the mixture vigorously at intervals during half an hour. Filter, add to the filtrate a few drops of dilute sulphuric acid, 1:5, just sufficient to give it a distinctly acid reaction to litmus paper. Filter once more, and measure 25 cc. of the filtrate into a capsule; add 5 cc. of water and evaporate at a gentle heat. When reduced to a volume of 8 or 10 cc., add 5 cc. more of water, and continue the evaporation until the whole of the alcohol is removed.

122. The alkaloid in the solution may now be determined by titration with Mayer's reagent (119), or it may be extracted as follows: When cool, pass the fluid through a very small filter, which must then be washed with a little pure water. It is possible that the resinous matter that has separated may still hold alkaloid. Dissolve this, therefore, in the capsule in a little ether, stir with a little acidulated water until the ether evaporates.

Test a drop of the acid water with Mayer's reagent; if it shows presence of more than a trace of alkaloid, filter and add to the former filtrate, which has been received in a one-ounce prescription vial. Shake with 15 cc. of a mixture of ether and chloroform to remove traces of impurities. Reject the ether-chloroform, and repeat the washing once or twice if any color is imparted to the solvent. Finally add ammonia, and extract the alkaloid with ether-chloroform, using three portions of 15, 10 and 10 cc. respectively.

123. Tincture of Belladonna may be assayed in the same manner, using 50 cc. of the preparation, which should be evaporated by a gentle heat to a small volume as the first step in the operation.

All evaporations of aqueous solutions of unstable alkaloids, like atropine, cocaine, etc., must be conducted in a shallow, flat evaporating dish or basin, at a gentle heat, and yet as rapidly as possible. A strong current of air assists the evaporation greatly. The solution must always be as nearly neutral in reaction as possible.

124. Solid extract of belladonna or stramonium must be dissolved in a small quantity of spirit, and the solution treated precisely as described for a fluid extract. The best solvent to use is strong alcohol, but this will not generally dissolve the whole of the extract. Take 2 grams of the extract, dissolve it in 5 cc. of dilute alcohol, add 15 cc. of strong alcohol, decant the clear or turbid solution into a 50 cc. measuring flask, dissolve the precipitated gummy matter in the smallest practicable quantity of water, and again add strong alcohol. Repeat this operation until 50 cc. of solution are obtained, when the residuary gummy matter may be rejected. Treat the alcoholic solution with lime precisely as described above (121).

125. Powdered extracts of these drugs and **concentrations or abstracts** may be treated with Prollius' fluid

in the same manner as the crude drug, using 2 grams of the extract in place of 10 grams of the drug.

126. For the assay of solid extract of belladonna leaves, Messrs Dunstan and Ransom recommend the following plan, which is very direct but which necessitates the use of much chloroform to remove chlorophyl, etc : Warm one gram of the extract with dilute hydrochloric acid until as much as possible is dissolved. Filter the mixture, preferably through glass or cotton wool, and wash the residue thoroughly with hot dilute hydrochloric acid. Wash the acid liquid repeatedly with chloroform until nothing further is removed by that solvent. Then render alkaline with ammonia, and wash out the alkaloid with chloroform, as in the assay of belladonna root above (120).

CINCHONA BARK.

127. Estimation of total Alkaloids in the Bark. The method of **Prollius** is the most expeditious, and is to be recommended for general use. The process, as practiced by the writer, is as follows: Put into a flask or vial 5 grams of the bark in a powder at least as fine as No. 30, together with exactly 100 cc. of Prollius' fluid. Cork immediately, shake frequently during two hours, or at intervals during 24 hours. Decant into a separator 50 cc. of the ethereal fluid; add 10 cc. of dilute sulphuric acid (5 per cent), shake. Observe whether the fluid is sharply acid; if not, more acid is needed.

128. Draw off the acid fluid into a two-ounce vial, wash the ethereal fluid twice more with 10 and 5 cc. of acidulated water, which is to be added to the contents of the vial; add solution of ammonia to strongly alkaline reaction, and shake with 20 cc. of a mixture of chloroform one volume and ether three volumes. When separation is complete, decant the ethereal fluid into a tared capsule, wash the aqueous solution twice with fresh portions of the ether-chloroform, evaporate at 100° C. (212° F.) to constant weight, and weigh.

129. Method of Hager.* Mix 10 grams of the finely powdered bark with 100 cc. of water and 10 cc. of a solution of caustic potash, sp. gr. 1.35; heat and keep at the boiling point 15 minutes, add 15 grams of dilute sulphuric acid, sp. gr. 1.115, and boil 20 minutes, cool, transfer to a measuring cylinder, and dilute to a measure of 110 cc. (allowing thus 10 cc. for the bulk of the woody fibre, etc.); pass through a dry filter, and to 60 cc. of the filtrate, taken to represent 6 grams of bark, add 50 cc. of a cold

* Handbuch der Pharmaceutischen Praxis I, p. 828.

saturated solution of picric acid. After half an hour collect the precipitated picrates on a pair of mutually counterpoised filters, wash with a little cold water, dry at 100° C. (212° F.), and weigh. The product contains about 42.5 per cent. of its weight of alkaloids, which may be separated and weighed as such by suspending the precipitate in cold water, adding excess of caustic soda, and washing out with chloroform.

130. Assay by acid, according to **Dr. J. E. DeVrij.*** Mix 20 grams of the bark in fine powder with 20 cc. of water, to which has been previously added 3 cc of strong hydrochloric acid. Let stand a few hours. Add more water, stirring thoroughly, to form a liquid which can be poured. Let stand until foam has disappeared. Introduce into a cylindrical percolator, the orifice of which is closed with a loose plug of charpie. [Cheese cloth will answer equally well.] Continue the percolation until excess of caustic soda ceases to produce a precipitate in the passing percolate. About 180 to 200 cc. of percolate will be obtained if the process is carefully conducted. [The author lays stress on the avoidance of heat in the extraction of the bark with dilute acid.]

131. The total alkaloids may now be ascertained in either of the following methods: a. Precipitate with a large excess of caustic soda. Collect in a double filter. Wash until washings are nearly colorless. Drain the filter well on blotting paper until the precipitate ceases to adhere, then carefully detach it or transfer to a tared capsule. Dry on a water bath to constant weight. [To obtain anhydrous alkaloid a higher temperature should be used.] To the weight of alkaloid thus obtained add as a correction for solubility, for each cc. of filtrate and washings combined, 0.000585. Multiply by five to find percentage.

132. To estimate cinchotannic acid, the alkaline mother liquid may be exposed to the air in a shallow dish two or three days, the liquid heated, and hydrochloric acid added to slight acid

* Chemist and Druggist, Aug. 15. 1885.

reaction. After cooling, the precipitate of cinchona red is to be collected on a pair of mutually counterpoised filters, washed, dried and weighed. Multiply the weight of the precipitate by 1.2 for an approximation to the quantity of cinchotannic acid.

133. b. Put the acid solution in a bottle with one litre of commercial benzol, add caustic soda in excess, shake and let stand five minutes (not longer) to separate. Decant the clear benzol solution into a filter previously moistened with benzol, and transfer the remaining fluid to a separator. When separation is complete draw off the red alkaline liquid into the bottle previously used, and shake with 200 cc. of benzol. This last, together with the benzol remaining in the separator, is to be filtered and mixed with the first portion, and the alkaloids are then to be determined as follows:

134. Shake the solution with 70 cc. of decinormal sulphuric acid. Draw off the acid and add 30 cc. of water. Shake, draw off and mix with the acid fluid. Heat, add from a burette decinormal soda solution cautiously until the fluid is exactly neutral to litmus paper. Subtract the quantity of alkali used from 70, and multiply by .031 for the weight of alkaloid in 20 grams of bark, or by .155 for the percentage of alkaloid.

135. If preferred, the alkaloid may be separated from the benzol and weighed, by shaking the benzol solution with 30 cc. of very dilute nitric acid, separating, washing the benzol with 20 cc. of water. Add the water to the acid solution, heat to drive off traces of benzol, when cool transfer to a separator, and, having added excess of caustic soda, shake out with 200 cc. of ether. Separate and wash the alkaline fluid once more with 100 cc. of ether. Recover the ether by distillation, when the alkaloids will remain in a state of great purity.

136. Method of Dr. Otto Kaspar.* Put into a

* Schweiz. Wochenschrift für Pharmacie, Druggists' Circular, August, 1886, p. 175.

flask of 150 cc. capacity 50 cc. of alcohol of 90 per cent., 20 drops of diluted hydrochloric acid, and lastly 10 grams of the finely powdered bark. Heat for about 10 minutes and express; repeat the operation with 50 cc. of alcohol and 10 drops of acid, and a third time with 50 cc. of alcohol without acid. Filter the united liquids, distil off the alcohol over a water bath until the volume of fluid is reduced to about 30 cc., and evaporate with constant stirring to a weight of ten grams. Place this concentrated fluid in a glass cylinder about 30 centimetres (12 in.) long and 2 cm. (¾ in.) wide, add 15 grams of a 10 per cent. solution of caustic soda, and 15 cc. of ether. Let the mixture stand 12 hours, shaking it frequently, remove the ethereal layer with a pipette, and repeat the ether washing twice with 15 cc. of ether. By evaporating the filtered ethereal fluids we obtain ether-soluble alkaloids (crystallizable and amorphous quinine). The author lays stress on the time, twelve hours, as an important point in his assay process, cinchonidine and quinidine, which are at first taken up by the ether, after a time separating again in crystalline and comparatively insoluble form. The residue is to be treated with chloroform, 3 portions of 15 cc. each, to remove the alkaloids not soluble in ether, cinchonine, cinchonidine and quinidine. Fusel oil may be substituted for the chloroform, but is much less agreeable to handle.

Assay of the Alkaloids of Cinchona Bark.

137. Method of John Muter.* The alkaloids having been extracted from the bark by the use of lime and strong alcohol, as in the U. S. P. assay process, brought into the form of sulphates in a concentrated aqueous solution, and introduced into a separator, add solution of caustic soda, and wash out the alkaloids with chloroform, using four successive portions of 50, 25, 25 and 25 cc. (working with the product from 50 to 100 grams of bark). Evaporate the chloroform solution in a tared 5 ounce

* The Analyst, 1880, 223.

squat beaker, dry at 100° C. (212° F.), heat to 116° C. (240° F.), and weigh.

138. Dissolve in absolute alcohol, and divide into two equal portions, A and B. To portion A add from a burette volumetric sulphuric acid (11.6 grams of acid, sp. gr. 1.843, in one liter of water; each cc. = 0.1 gram crystallized quinine sulphate) until just faintly acid to delicate litmus paper, and note the quantity of acid used as a guide for future operations. Drive off the spirit and dissolve the residue in water at 85° C. (185° F.), using 5 cc. of water for each cc. of volumetric acid used. If necessary, add a little of the volumetric acid to effect complete solution. Keeping the temperature at about 85° C. (185° F.), add cautiously, drop by drop, decinormal solution of caustic soda until all but neutral. Cool rapidly to 157° C. (60° F.), keep at that temperature one hour, filter through a pair of counterbalanced filters, and wash the crystals with a little cold water (1.5 cc. for each cc. of volumetric acid used in the previous titration). Drain the crystals. Press, dry at 100° C. (212° F.), raising the temperature gradually at last to 116° C. (240° F.), and weigh as anhydrous quinine sulphate. Measure the filtrate and washings, and for each cc. of the fluid add to the weight actually obtained 0.000817 as correction for solubility of quinine sulphate in water. Multiply this sum by 0.8686 to obtain quinine alkaloid.

139. To portion B add dilute hydrochloric acid until it has a faint acid reaction, evaporate, and dissolve the residue in the least possible quantity of water at 38° C. (100° F.) Neutralize accurately with decinormal soda solution, add a saturated solution of Rochelle salt in excess, cool and keep for one hour at a temperature of 15 7° C. (60° F.), frequently stirring. Collect on a pair of mutually counterpoised filters, wash with 100 cc. of water at 15.7° C. (60° F.), dry at 104.4° C. (220° F.) and weigh. Add for each cc. of filtrate and washings 0.00083 to obtain weight of tartrates of quinine and cinchonidine. Deduct weight of quinine tartrate, found by multiplying weight of quinine sulphate previously found

by 0.915. The remainder multiplied by 0.804 will give cinchonidine alkaloid.

140. Concentrate the filtrate and washings from the tartrate to its original volume, cool, render faintly acid with acetic acid, and add excess of a saturated neutral solution of potassium iodide with constant stirring. After an hour or so, collect, wash, dry and weigh, precisely as in the case of the tartrate. Add 0.00077 for each cc. of filtrate and washings, and multiply by 0.7168 to obtain the quinidine alkaloid.

141. From the filtrate and washings precipitate the alkaloid by sodium hydrate, collect on a pair of filters, wash, dry and weigh, heat with spirit of 40 per cent. to dissolve out amorphous alkaloid. Dry the residue and weigh as cinchonine alkaloid. The difference between this weighing and the previous one gives the quantity of amorphous alkaloid, but must be corrected by deducting for each cc. of filtrate from the precipitated tartrates 0.00066, and for each cc. of filtrate from the quinidine hydriodide 0.00052.

142. Method of A. Petit.* Mix 40 grams of finely powdered bark with 800 cc. of Prollius' fluid. Shake every five minutes during one hour. Decant 600 cc. of the clear fluid, representing 30 grams of bark, into a separator. Add dilute sulphuric acid (containing 25 per cent. of H_2SO_4) just sufficient to supersaturate the bases present. About 20 cc. will generally be required. Shake, separate and draw off the aqueous fluid into a capsule. Shake the ethereal fluid with 5 cc. of the dilute acid and 15 cc. of water, separate, and add to contents of capsule.

143. Heat on water bath, to expel ether, add 80 cc. of water and precipitate the alkaloids by adding solution of caustic soda in excess. Cause the alkaloids to coalesce by stirring with a glass rod, wash, transfer to a tared capsule, dry at 100° C. (212° F.) and weigh. If the liquid is not perfectly clear, pass through a tared

* Chemist and Druggist, 1884.

filter, wash, dry, weigh, and add the gain in weight to weight of alkaloids in capsule.

144. Dissolve the alkaloids again in a slight excess of sulphuric acid, add 25 cc. of stronger ether and 5 cc. of solution of ammonia and shake. Decant the ether into a vial. Shake the alkaline solution again with 10 cc. of ether, which is to be added to the first portion. Let stand 15 minutes. Decant the clear solution into a separator, add 10 cc. of dilute sulphuric acid (1:20), shake, separate. Wash the ether a second time with 5 cc. of acid of the same strength, and then with water enough to make in all 25 cc. of fluid.

145. Heat the acid solution in a beaker to boiling, add cautiously dilute solution of ammonia (1:5) until the fluid is barely alkaline. Cool to crystallize quinine sulphate. When completely cold, collect on a tared filter, wash with saturated aqueous solution of quinine sulphate, dry at 100° C. (212° F.) to constant weight, and weigh as anhydrous quinine sulphate. To obtain quinine sulphate crystallized, multiply by 1.1689; to find quinine alkaloid, by 0.8686. The mother liquor may be treated with ammonia and ether to remove the remainder of ether-soluble alkaloid.

Estimation of Quinine in the Mixed Alkaloids from Cinchona Bark.

146. Method of De Vrij.* [If the bark under examination contains much cinchonidine, digest the crude alkaloid in powder first with ten times its weight of ether; after standing half an hour or more decant the ether, and wash the residue with a small additional portion of the solvent. Evaporate to dryness, and apply the test to the residue, which will contain all the quinine.]

147. Dissolve the mixed alkaloids in 40 times their weight

* Phar. Jour. and Trans., Dec. 11, 1875, p. 461. The Hague, July 5, 1880.

of alcohol of 92 per cent., containing 0.76 per cent. of sulphuric acid. [Or dissolve in normal sulphuric acid, using 6.2 cc. for each gram of alkaloid, evaporate to one-half, and add 46 cc. of strong alcohol for each gram of the alkaloids.]

148. To the solution, add solution of chinoidin iodosulphate (12) from a pipette, drop by drop, stirring constantly as long as a dark brownish red precipitate of herepathite is produced. As soon as all the quinine is precipitated and a slight excess of reagent added, the solution acquires an intense yellow color. The beaker is now to be covered and heated to boiling on the water bath. Cool, note the volume of fluid, filter, and wash the precipitate on the filter with a saturated solution of herepathite in alcohol of 92 per cent. Dry the residue at 100° C. (212° F.) and weigh. Add to this weight, for each cc. of fluid previous to filtration 0.0011 as a correction for solubility of herepathite in alcohol. Multiply the corrected result by 0.55055 to obtain anhydrous quinine, or 0.7409 to obtain crystallized sulphate of quinine.

149. Method recommended by Y. Shimoyama.* Dissolve the alkaloids (at least 0.5 gram.) in a beaker in 30 or 40 cc. of water by aid of the smallest possible quantity of dilute acetic acid. Filter into a tared beaker, wash the filter, neutralize exactly with dilute solution of soda. Add, for each gram of the alkaloids 10 cc. of a cold saturated solution of sodium oxalate. Concentrate on the water bath to 8 or 10 grm.; should a slimy mass separate during the concentration, it must be filtered off and well washed with boiling water. To the contents of the beaker add 10 to 15 cc. of water, and stir until a clear solution results. Set by three hours at a temperature of 18° C. (64.4° F.), stirring frequently. Collect the precipitate on a double filter, and wash thoroughly with a saturated solution of quinine oxalate. Dry at 110° C. (230° F.) Weigh, and add for each cc. of fluid previous to filtration 0.00064, to obtain the quantity of quinine as

* Arch. Pharm. [3], [23], 209-229.

oxalate. Multiply this by 0.878 to obtain quinine anhydrous, or by 1.1815 to obtain quinine sulphate, crystallized.

150. According to De Vrij, the separation from cinchonidine cannot be effected by this method, but it nevertheless, no doubt, serves sufficiently well the purposes of such an approximate assay as the pharmacist has often occasion to make. The method of De Vrij, given above, is open to the same objection, and has the added disadvantage of requiring the use of an especial reagent somewhat troublesome to prepare.

COCA LEAVES.

151. The leaves of Erythroxylon Coca contain probably several alkaloids, but only one of them, cocaine, has as yet been made the subject of exhaustive study. What is the precise relation of the other alkaloidal principles present to cocaine, either genetically, or as bearing on therapeutics, has not yet been ascertained. As in the case of such drugs as aconite, belladonna, etc., which contain several alkaloids, we must for the present base our judgment of the value of the drug on an estimation of the total alkaloid, or perhaps the total alkaloid extracted by some particular solvent, confirming that judgment by an appeal to organoleptic properties, *i. e.*, to physiological effects. For the manufacture of cocaine salts, it is of course necessary to ascertain the quality, as well as the quantity of alkaloid. The methods of assay given here are merely such as may serve the purposes of the pharmacist; with suitable modifications, and practiced on a sufficiently large scale, they may, however, be employed by the manufacturer of cocaine.

ASSAY OF COCA LEAVES.

152. Method of Dr. Squibb.* Moisten 50 grams of coca leaves, in fine powder, with 40 grams of strong alcohol, to which has been added .08 gram of sulphuric acid sp. gr. 1.843, or 0.16 grm. of hydrochloric acid sp. gr. 1.16. Pack in a percolator and let stand a few hours. Percolate with strong alcohol until exhausted, obtaining thus 450 to 500 cc. of product. Evaporate at a low temperature to expel alcohol. Dissolve the residue by the successive application of ether and water, using 25 to 30 cc. of

* Ephemeris, p. 784.

each, and transfer to a separator. Add 1 or 2 cc. of 10 per cent. acid and shake vigorously. If the mixture forms an emulsion which does not separate within an hour or two, a further addition of acid and a repeated agitation will cause a more prompt separation. Draw off the lower watery solution into a second separator, and wash the remaining ether with 10 cc. of water, which is to be added to the acid solution. Wash this now with successive portions of ether of 10 cc. each, as long as the ether takes up color, rejecting the ether washings. Add sodium carbonate crystals to supersaturate, avoiding loss by effervescence, and wash out the alkaloid by shaking with several successive portions of ether (10 cc.) Evaporate the ethereal solution in a tared beaker by a gentle heat, and weigh.

153. Other drugs containing alkaloids may be assayed by the same process. The acid may be omitted in the first step of the operation, or tartaric acid may be substituted. In general, the acid does not increase the yield of alkaloid, while in some cases it acts injuriously during the evaporation.

154. Method of the Author. Place in a bottle having a capacity of 120 cc. 10 grams of the drug in No. 30 powder. Pour over it 95 cc. of petroleum benzin, shake, add 5 cc. of a mixture of absolute alcohol 19 volumes, concentrated ammonia 1 volume, cork securely, and immediately shake vigorously for a minute or more. Set by twenty-four hours, shaking the bottle occasionally, say at intervals of half an hour during working hours. Decant rapidly 50 cc. of the clear fluid. If the decanted fluid is not perfectly clear, filter it, washing the filter with a little petroleum benzin. Transfer to a separator containing 5 cc. of water, to which has been added 6 or 8 drops of dilute sulphuric acid, 1:5. Shake vigorously. When the fluids have separated draw off the aqueous fluid into a 1 oz. vial having a good lip.

155. Add to the contents of the separator 2½ cc. of water with 1 drop of dilute sulphuric acid, shake, draw off into the vial.

A third portion (2½ cc.) of water may be shaken with the petroleum benzin, and then a drop of it tested on a mirror with Mayer's reagent to ascertain if all the alkaloid has been removed. If there is produced more than a faint turbidity, add another drop of acid, shake vigorously for some time, and again allow the fluids to separate, and draw off the water into the vial.

156. Add to the mixed aqueous fluids 15 cc. of petroleum benzin. Shake vigorously. When separation is complete pour off the benzin completely. Now put into the vial 15 cc. of stronger ether, U. S. P., and ammonia enough to render the mixture decidedly alkaline. Shake immediately. When separation is complete, decant carefully the ether into a tared capsule. Wash the residue in the vial with two or three successive portions of fresh ether, until the aqueous fluid is free from alkaloid. Evaporate the ether at a water bath heat. Finally dry the alkaloid to constant weight, weigh, and multiply the result, expressed in decigrams, by two to obtain percentage of crude cocaine.

157. In place of the petroleum benzin, ordinary ether may be used in this assay. The former solvent is much the cheaper, and although the yield of total alkaloid is not quite as large, it is probable that the results serve even better to indicate the comparative value of different samples of leaves.

158. Finally observe the physical properties of the alkaloid obtained. From leaves of the best quality it will be quite colorless or white, and will easily crystallize in the capsule. If it remain in the form of oily drops the proportion of crystallizable alkaloid is below the average. If the color is dark, the leaves have suffered, probably from heating or sweating when not thoroughly dry.

159. The leaves must always be tested "physiologically," by chewing a few and noting the effect produced on the tongue. A little practice will enable one to judge by this method quite accurately the value of a specimen of the drug, but it is impossible

to formulate rules. The alkaloid itself, obtained in the assay, may be submitted to physiological test. For this purpose, dissolve it, by aid of a minimum quantity of hydrochloric acid, in 124 times its weight of water, and apply one drop of the solution to the tongue. Compare the effect with that produced by a solution of pure crystallized cocaine muriate of corresponding strength (one part in 100).

160. Titration with Mayer's Reagent. Instead of extracting the alkaloid from the acid aqueous solution, obtained as above described, we may estimate it by titration, although this method is not to be recommended. If this plan is decided upon, the acid solution, representing 5 grams of drug, must be made up to a volume of 15 cc., and the reagent, $N\frac{1}{20}$, added as long as it continues to produce a precipitate in the clear filtrate. Under the conditions of the assay,

0.2	per cent.	of	alkaloid	will require	3.5 cc. of reagent.
0.3	"	"	"	4.6	"
0.4	"	"	"	5.7	"
0.5	"	"	"	6.8	"
0.6	"	"	"	7.9	"
0.7	"	"	"	9.	"

COLCHICUM.

161. The active principle of this drug is the alkaloid colchicine, which differs from most alkaloids in the following particulars: 1st. It is removed from acid solutions by shaking with chloroform; 2d. It is quite freely soluble in water; 2d. It is precipitated by Mayer's reagent only from strongly acid solutions. The alkaloid is furthermore very easily decomposed, its aqueous solutions rapidly losing strength even when quite neutral. Mineral acids, even quite dilute, decompose it on application of heat.

Assay of the Drug.

162. Method of Dragendorff.* Exhaust 5 or 10 grams of the drug with water or alcohol; the root, if dry, must be softened with water, before it is treated with alcohol. [From the proneness of aqueous solutions to change, it would appear to be best always to use alcohol, preferably of about 75 per cent., for the extraction.] Having expelled alcohol by evaporation, which should be conducted rapidly, but at a moderate heat, adjust the volume of fluid so that it shall contain about one part in 600 of alkaloid, and include in this a quantity of dilute sulphuric acid (1:5) equal to one-tenth of the whole volume. Titrate with Mayer's reagent, $N/_{10}$, and reckon for each cc. of reagent used 15.85 milligrams of colchicine.

163. It is necessary to use the large quantity of acid prescribed. If the proportion of acid is too small, precipitation is incomplete. In such case, addition of more acid to the clear solution in which Mayer's reagent no longer produces a precipitate will throw down further precipitate.

*Werthbestimmung einiger Starkwirkender Droguen, p. 73.

The method gives fairly good results, which, if not absolutely correct, serve to compare one specimen or preparation of the drug with another.

164. Personally I prefer to employ in the titration a more concentrated solution. I make the volume of the solution from each gram of drug 3 cc. in the case of colchicum seed, and 2 cc. in the case of the root. I then subtract from the result obtained in the titration (with Mayer's solution, $N\frac{1}{20}$) for each cc. of fluid present at the end of the titration 0.08 cc. Each cc. of the remainder will indicate 14.7 mg. of alkaloid. This arbitrary rule is based upon the observation that 1 cc. of Mayer's reagent, $N\frac{1}{20}$, precipitates:

In solutions	1:200	about	0.0106	alkaloid.
"	1:400	"	0.0085	"
"	1:600	"	0.0077	"

165. It will be observed that on the basis of these figures the result of a titration will be much less flattering than it would be were Dragendorff's equivalent, *i. e.*, that of Johannson, adopted. Repeated titrations not only of alkaloid separated from the drug and its preparations, but with Merck's colchicine, concur in indicating a very much lower equivalent than that ascribed to the alkaloid by Johannson. The titration, when made strictly in accordance with the directions given, but using the lower equivalent for the value of Mayer's reagent, indicates quite accurately the proportion of alkaloid, especially if this be first washed out with chloroform and redissolved in water.

166. The following table will aid in interpreting the results of a titration :

Quantity of Alkaloid.	Quantity of Mayer's Reagent N 1-20 required.			
	Vol. of fluid titrated, 5 cc.	Vol. of fluid titrated, 10 cc.	Vol. of fluid titrated. 15 cc.	Vol. of fluid titrated, 20 cc.
.010	1.2	....	....	....
.020	2.0	2.4	....	....
.030	....	3.2	3.6	....
.040	....	4.0	4.4	....
.050	....	....	5.2	5.6
.060	....	....	6.0	6.4
.070	....	....	....	7.2
.080	....	....	....	8.0
.090	....	....	.. ..	8.8

167. Method of the Author. Fairly accurate results are obtained by the general process described (29) *et seq.* Place 5 cc. of the drug (in No. 30 powder) in a flask or bottle. Add 50 cc. of Prollius' fluid modified (14), cork securely and macerate with occasional agitation 12 to 24 hours. Decant 25 cc. of the clear fluid, evaporate in a beaker by a very gentle heat. When the chloroform is all expelled, dissolve the residue in water containing 3 per cent. of sulphuric acid, using for colchicum seed 7.5 cc., for colchicum root 5 cc., and titrate with Mayer's reagent $N_{\frac{1}{2}\frac{1}{0}}$. This method is rapid and quite satisfactory.

168. Another plan is to exhaust the drug in a suitable apparatus, (7) and (8), by repercolation with a mixture of alcohol and chloroform, evaporate to dryness at a gentle heat, and determine the alkaloid in the residue by titration as above.

169. Still another method is to exhaust the drug by percolation with 60 per cent. alcohol, and treat by one of the methods to be presently described for the valuation of a tincture or fluid extract.

170. The galenical preparations of colchicum are

easily assayed by titration with Mayer's reagent. Extracts have only to be dissolved in water in the proportion of about 1:10 for a preliminary titration. A second experiment should be made in which the proportion of alkaloid in the solution prepared for titration shall approximate 1:250. The results of the titration will be the more conclusive if the alkaloid is first extracted from the solution in water or in dilute alcohol by repeated shaking with chloroform. The solvent is to be expelled by evaporation at a gentle heat, and the residue taken up with water containing 3 per cent. of sulphuric acid.

171. It is noticeable that the precipitate produced by Mayer's reagent in a solution from colchicum root is of a dull buff color, while that obtained from the extracted alkaloid is of a bright yellow, as is also that obtained from solutions prepared from colchicum seed. For this reason I hesitate to accept the result of a direct titration in the case of preparations of colchicum root, although, in the few test analyses I have made, the quantity of alkaloid extracted by the process described above has not fallen very far short of that indicated by direct titration.

172. Fluid Extracts or **Tinctures** may be evaporated to expel alcohol, the aqueous solution brought to such a volume that each cc. shall represent about 0.5 grm of drug, and titrated. In case acetic acid is present, it is advisable to wash out the alkaloid with chloroform—evaporate and take up with water containing 3 per cent. of sulphuric acid before attempting a titration.

173. From a Fluid Extract the alkaloid may generally be removed very easily by adding an equal volume of water, and shaking with chloroform, using several successive portions. To obtain it in a condition sufficiently pure for weighing, dilute 10 cc. of the fluid extract with 85 cc. of water, add solution of subacetate of lead in slight excess (*i. e.*, until the fluid has a distinctly sweetish taste), make up the volume to exactly 100 cc., filter; add to the filtrate, in powder, sulphate or phosphate of

sodium, shake, filter once more, and evaporate 50 cc. of the filtrate in a shallow capsule or basin until reduced to 15 or 20 cc. Transfer to a separator, and shake with three successive portions of chloroform of 15, 10 and 10 cc. respectively. Evaporate, dry to constant weight at 100° C. (212° F.) and weigh. A little of the alkaloid seems to be lost in the precipitation with lead sub-acetate, so that the results of the assay are likely to be low.

174. Colchicum is a drug peculiarly liable to variations in strength, and its galenical preparations ought always to be standardized by assay. In my experience, the seed has proved to be not only richer in alkaloid, but much more uniform in alkaloidal strength than the root.

CONIUM.

175. This drug depends for its medicinal activity upon a volatile alkaloid, coniine, which is present in all portions of the plant, but is most abundant in the immature fruit. It is accompanied by a small proportion of an allied alkaloid, conhydrine, but it is not necessary to attempt to discriminate between these in an ordinary assay. Many specimens of conium leaves, and not a few of conium seed, are almost inert, from loss of their volatile active constituent. Some method of ascertaining, even approximately, the value of the drug is an obvious desideratum. The strength of the odor developed by treating the drug with liquor potassæ, becomes, to one accustomed to the use of the test, a ready means of judging the relative, if not the absolute, value of given samples. Unfortunately a quantitative comparison of odors can be made, if at all, only by a preternaturally acute olfactory sense, so that the test may be considered as of very little practical value, except as showing the presence or absence of coniine.

176. Several plans of assay have been proposed, but as yet none that can be called wholly satisfactory. Mayer's reagent precipitates only concentrated solutions of coniine salts, and the precipitate remains suspended obstinately in the fluid, passing even through a double paper filter with the greatest facility. Dragendorff has recommended, nevertheless, titration with this reagent, in presence of chloride of potassium, stating that each cc. of the reagent, $N\frac{1}{10}$, precipitates .0125 of coniine. Time will be only wasted, however, in attempting to verify his conclusions. The only reagents which precipitate coniine in dilute solutions are sodium phosphomolybdate, tannin, sodium phosphotungstate and the solutions of bromine and iodine which are used as general rea-

gents for alkaloids. Picric acid produces precipitates in moderately concentrated solutions, containing *e. g.* more than 1:400, but being a crystalline precipitate, its formation is influenced by too many conditions to make it well adapted for analytical use.

177. Zinoffsky suggested the use of the phosphomolybdate of sodium for determining coniine by titration. He recommends to prepare the solution of such strength that 1 cc. of it shall precipitate 33.3 mg. of strychnine. Of coniine 1 cc. will then precipitate 50 mg. Since this reagent precipitates also ammonia, and ammonia is always present in conium, it is necessary to prepare the solution for titration in such a manner that coniine is retained and ammonia excluded. To accomplish this Dragendorff recommends to prepare the extract from the drug with strong alcohol containing sulphuric acid, allow the alcoholic solution to stand in a cold place for some time and filter, leaving ammonium sulphate undissolved. The solution is to be evaporated or distilled in a partial vacuum, to avoid loss of coniine. The operation is necessarily tedious and complicated.

178. Assay process of the Author. Coniine, when other alkaloids are absent, may be very easily estimated in the following manner: Prepare a volumetric acid solution—hydrochloric acid is to be preferred—of such strength that each cc. shall contain 2.2956 milligrams of HCl. This may be made from "normal" acid by mixing 62.9 cc. of this with distilled water sufficient to make one litre. Of this acid each cc. is equivalent to ten milligrams of coniine. Prepare a volumetric alkali of exactly corresponding strength.

179. Put into a two-ounce vial a quantity of the coniine solution containing approximately 50 to 80 milligrams of the alkaloid. If the strength of the solution is wholly unknown, a preliminary examination may be made in the following manner: Put into each of half a dozen clean vials exactly 15 minims of distilled water, measured with the minim dropper (Fig. 3). Add to No. 1

15 minims of the coniine solution. Mix thoroughly, and add 15 minims of the mixture to No. 2, and so on, producing solutions of $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$, etc., the strength of the original. Place on a mirror a drop of each of these dilutions, and add to each a drop of solution of sodium phosphotungstate, strongly acidulated with hydrochloric acid. Precipitates will be produced in the solutions which contain more than 1:3500 to 1:5000, none in those which are much more dilute. Hence if the fourth dilution is the last which shows a precipitate we may conclude that the original solution contained between 1:200 and 1:300 of coniine; if the fifth, between 1:100 and 1:150, and so on.

180. To return to the assay process. Put into the vial with the coniine solution 50 cc. of petroleum benzin, add a few decigrams of potassium carbonate, shake well, and allow the fluid to separate completely. Into a second vial introduce exactly 10 cc. of the volumetric acid. Decant into this vial the benzin solution containing the alkaloid, being scrupulously careful not to permit any minute drops of the alkaline aqueous fluid to go over with it. [It may be safest to decant the benzin into an intermediate bottle, where it may be washed with 2 or 3 cc. of distilled water, but with careful manipulation this is unnecessary.]

181. Shake the acid with the benzin, when it will quickly remove the alkaloid. Allow the fluids to separate completely, decant the benzin into the vial containing the alkaline fluid. Shake together, let stand, and return the benzin once more with the same precautions as before to the vial containing the acid. Shake together, and now after the fluids have completely separated, remove the benzin, add litmus as an indicator, and titrate the remaining acid with the standard alkali. Subtract the amount required to exactly neutralize from 10, and the remainder will give in centigrams the quantity of coniine present. Ammonia, if present, will remain behind in the alkaline solution.

182. The alkaloid may also be weighed as hydrochlorate if

care is taken to use only a small excess of acid in the final washing out of the alkaloid from the petroleum benzin. The solution must be evaporated at a very gentle heat, and exposed to this no longer than may be necessary to drive off the last traces of free hydrochloric acid. The drying may be completed in the desiccator, or, instead of weighing the salt, the chlorine in it may be estimated by means of volumetric solution of silver nitrate.

183. Dragendorff condemns this plan of estimating coniine on the ground that the salt is volatile even at ordinary temperatures. Test analyses, operating with pure coniine, and with crystallized hydrochlorate and hydrobromate, have proved, however, that the loss is inappreciable, where the operation is conducted in the manner described. It is altogether possible, however, that a portion of the alkaloid contained in conium may form excessively volatile salts, or salts easily decomposed, which may be lost consequently during evaporation. But such alkaloid certainly cannot be reckoned as coniine.

184. A more rapid method of approximately estimating coniine in absence of other alkaloids, and of ammonia, is by titration with a standard solution of sodium phosphotungstate —or as suggested by Zinoffsky (177), of sodium phosphomolybdate. I have not found either to give results at all exact, yet, in solutions of the same degree of dilution (about 1:200), and otherwise under similar conditions, the titration is as close as those made ordinarily with Mayer's reagent. The precipitate separates fairly well, although it is rather slow in forming, particularly towards the end of the titration. To standardize the reagent, use a solution 1:166 of coniine hydrochlorate, and make the reagent of such strength that each cc. will precipitate 10 milligrams of the alkaloid.

185. Method proposed for the assay of Conium. Extract the alkaloid from the drug—conium leaves or fruit—by the method adopted for the assay of coca leaves (155).

Follow the details of that process of assay up to the point where the alkaloid is to be finally washed out for weighing. Use instead of ether petroleum benzin for this final washing out, and for the ammonia substitute potassium carbonate. Instead of evaporating the benzin solution, wash out of it the alkaloid with water containing hydrochloric acid. From this point the assay is beset with difficulties that forbid us as yet to hope for anything but comparative results.

186. The alkaloids do not comport themselves toward reagents in the same way as coniine. They are precipitated by most reagents, notably by Mayer's reagent, by picric acid, and by iodine, in solutions considerably more dilute than are those of pure coniine; at least this is true of the alkaloids separated from galenical preparations of the drug. The alkalimetric method gives results lower than other methods, and does not seem to fairly represent the comparative alkaloidal strength of different samples.

187. I am inclined to think that the most useful information will be obtained by titrating the acid solution, which will be free from ammonia, with phosphotungstate or phosphomolybdate solution, as already explained. I am confident, at any rate, that the valuation of conium seed can be made satisfactorily, for all practical purposes, in this manner.

188. Assay of galenical preparations of Conium.

(*a.*) **Fluid Extracts.** To 5 cc. of the fluid add a little potassium carbonate (0.2 to 0.3 grams), and about 50 cc. of petroleum benzin. Transfer the benzin, after complete separation, to a vial containing 5 cc. of 1 per cent. hydrochloric acid. Shake, separate, return the benzin, as already explained, to ensure complete transference of the alkaloid to the acid solution. Bring this solution to a volume of 10 cc., and titrate with sodium phosphotungstate or phosphomolybdate.

A tincture must be reduced by evaporation, with addition, if necessary, of a little acid, and treated in the same manner as a fluid extract.

189. (*b.*) **Solid Extracts.** Dissolve in water or dilute alcohol, and proceed as in the case of a fluid extract. One gram of the extract is a convenient quantity to operate upon.

DIGITALIS.

190. Active Constituents. According to Schmiedeberg, the more important constituents of digitalis are the following.

1. Digitoxin ($C_{21}H_{32}O_7$?, Kuppe), crystals of a pearly luster, insoluble in water and in benzol, soluble in chloroform and in hot alcohol, converted into amorphous toxiresin by boiling in alcoholic solution with dilute acids; the principal constituent of Nativalle's digitalin.

2. Digitalin ($C_5H_8O_2$), crystalline, soluble with difficulty in cold water and in ether, easily in alcohol and in chloroform-alcohol; converted by heating with acids in alcoholic solution into digitaliresin and glucose; a constituent of the digitalin of Homolle and Quevenne.

3. Digitalein, as yet imperfectly known. The three foregoing constitute the active constituents of the drug.

4. Digitonin ($C_{21}H_{52}O_{17}$), a substance allied to saponin; white, amorphous, easily soluble in water, slightly soluble in alcohol, insoluble in ether, chloroform, benzol, soluble in ether-alcohol; precipitated from its aqueous solution by acetate of lead, tannin, subacetate of lead and ammonia.

191. The preparations offered as digitalin by different manufacturers differ widely in composition and in activity. The crystallized digitalin of Nativalle is conceded to be the most active product, and this is practically insoluble in water. The soluble German digitalin is, however, a very active preparation, having obviously quite a different composition. The different constituents which have been named above of digitalis, and others that have been described with equal minuteness, are apparently exceedingly unstable bodies, and it seems to be as yet impossible to decide

by a chemical examination alone how active a given sample of the drug is.

192. The method adopted by Dr. S. P. Duffield* in a comparative examination of samples of digitalis of American, English and German growth respectively, is, perhaps, as good as any that could be suggested.

Dr. Duffield simply followed the process of the U. S. Pharmacopœia of 1860 for the manufacture of digitalin, using in each experiment one pound of drug. The variability in activity of the different preparations that are sold as digitalin has led to the omission from the list of official preparations of this article, but the process formerly official is still given in the dispensatories, and need not be here repeated. The process of Nativalle is not so well known, and may be given here for the suggestions which it may afford in the direction of an assay process, but it must be remembered that Nativalle's digitalin is not the only active constituent of the drug.

193. Process of Nativalle (1874) for the manufacture of digitalin. Macerate 1,000 parts of powdered digitalis leaves for 24 hours in a solution of 250 parts of acetate of lead in 1,000 parts of water, then displace with 50 per cent. alcohol. Treat the percolate with 40 parts of sodium bicarbonate, distil and evaporate to 2,000 parts; when cool, mix with 2,000 parts water, let stand several days, decant. Collect the precipitate on a filter, drain, press out, mix with 1,000 parts of 80 per cent. alcohol, and heat to boiling. Add 10 parts of neutral lead acetate, continue the boiling a few minutes, cool and filter. Mix the filtrate with 50 parts of animal charcoal, dry at a low temperature, and exhaust the residue with chloroform, evaporate, dissolve the residue of impure digitalin in 100 parts of 90 per cent. alcohol. Add a concentrated solution of 1 part of lead acetate, and 10 parts of granulated animal charcoal, boil for a few minutes, cool, filter and evaporate.

* Proceedings Am. Pharm. Association, 1868, p. 413.

Dissolve the residue in 10 parts alcohol, add 13 parts of water and 5 parts of ether, and shake well together. Crystalline digitalin is deposited, which may be further purified, if necessary, by treatment with animal charcoal, and recrystallized from alcohol.

194. Assay process suggested by Nativalle.* In his earlier writing Nativalle proposed the following method of separating the active constituents of digitalis :

Macerate 100 parts of the coarsely powdered drug 12 hours in a solution of 25 parts crystallized lead acetate in 100 parts of water. Transfer the mixture to a percolator, and when the fluid has drained off, percolate slowly with water until 300 parts of fluid are obtained. This solution contains the digitalein. Add to it sodium phosphate (or sodium carbonate) in slight excess, to free it from lead, filter, treat the filtrate and washings with tannin. Warm the solution to cause the precipitate to separate. Wash the precipitate a few times with hot water, mix it with an equal weight of litharge in fine powder, kneading the mixture frequently during 48 hours. Dry the mixture, powder, and exhaust by percolation with strong alcohol. On evaporation there will remain crude digitalein.

195. The digitalin is contained in the residue left in the initial water-extraction. Percolate to exhaustion with 50 per cent. alcohol, add 4 parts of crystallized acetate of lead, filter, add sodium phosphate (2 parts) to precipitate excess of lead, distil off the alcohol, and reduce the fluid to ten parts. From the concentrated solution there will separate crystals of digitalin, together with crystals of an inert substance. Wash the crystals with a little 35 per cent. alcohol, purify by recrystallization from 80 per cent. alcohol. (In an *assay* this step would doubtless be omitted.) Digest the crystals 24 hours with twenty times their weight of chloroform, which will dissolve the digitalin, leaving behind the inert substance. Evaporate the chloroform solution and weigh.

* Die Pflanzenstoffe, Husemann-Hilger, p. 1231.

ELATERIUM.

196. The active principle of the drug is elaterin, $C_{20}H_{28}O_5$, a readily crystallizable substance, of a bitter, disagreeable taste. This is insoluble in water or glycerin, requires for solution 290 parts of ether and 125 parts cold alcohol, but is freely soluble in boiling alcohol. Its best solvent is chloroform. The drug is very variable in strength, and often contains a large proportion of mineral matter (calcium carbonate, alumina, terra alba, etc.)

197. Assay Process.

(1.) Ignite 0.25 grm. in a porcelain or platinum crucible until the ash is quite white, to determine mineral matter. The ash ought not to amount to more than .02 grm.

198. (2.) **Estimation of Elaterin.** Put into a small flask 2 grams of the drug in fine powder, with 15 cc. of chloroform, and macerate half an hour at a temperature of 55° C. (131° F.) Transfer to a small filter, and exhaust by percolation with chloroform. Evaporate the solution in a tared capsule, dry at 100° C. and weigh. Dissolve the residue in 15 cc. of ether, added at once, transfer immediately to a small beaker, cover, and set by to crystallize.

In my experience, the ether has always formed momentarily a perfect solution of the residue, from which crystals of elaterin have begun almost immediately to separate. If a residue remain, this may be dissolved in a few drops of chloroform, and added to the ethereal solution.

199. After a few hours decant the ether into another beaker, and allow the ether to evaporate slowly until reduced to a very small volume. Decant the remaining fluid carefully from the

crystals, and wash these with a little ether by decantation. Finally dry the crystals remaining in both beakers and weigh. The weight of the crystals is usually about one-half that of the chloroform extract. If it fall much below that, loss of elaterin in the crystallization may be suspected. The percentage of chloroform extract will generally serve as a reliable basis for judging of the value of the drug. Elaterium of good quality should yield at least 35 per cent. of chloroform extract, and 16 to 20 per cent. of crystallized elaterin.

GELSEMIUM.

200. Yellow Jessamine contains at least one alkaloid capable of yielding crystallizable salts. This is called gelsemine, or gelseminine. It is sparingly soluble in petroleum benzin, rather freely soluble in ether, and still more so in chloroform. It is accompanied in the root by an alkaloid which seems not to produce crystallizable salts, and which, like amorphous quinine, seems always to be more or less colored. The drug contains a fluorescent principle, having the characters of an acid—gelseminic acid—which is taken out of acid solutions by thorough washing with ether or chloroform, but a portion of which is liable to accom pany the alkaloid when extracted in the usual manner.

201. The assay of the drug may be conducted by the general process given in (29). Add, however, to the Prollius mixture one-fourth its volume of chloroform. It will be found that the alkaloid carries with it certain impurities which give it a dark color, and which seem to be not inconsiderable in amount. If the acid solution has been thoroughly washed with ether, and it be understood that the object of the assay is merely to obtain comparative results, we may be content to weigh the alkaloid in its crude form.

202. It would be well, however, to check this weighing by a subsequent titration with Mayer's reagent. Dissolve the crude alkaloid in two hundred times its weight of water containing 0.2 per cent. of sulphuric acid. Each cc. of Mayer's reagent $N\frac{1}{20}$ will then correspond with 10 mg. of alkaloid, nearly. If the solution contain a larger proportion of acid, or is more dilute, the quantity of reagent required for complete precipitation will be somewhat greater. Thus in a solution containing one part of alkaloid in

400, with 0.2 per cent. of acid, each cc. of the reagent indicates about 8.5 mg. of alkaloid, the end of the reaction not, however, being very clearly defined.

203. Instead of titrating, we may precipitate the filtered solution with a slight excess of Mayer's reagent. Collect on a pair of mutually counterpoised filters, wash slightly, dry at 100° C. and weigh, and multiply by 0.53 to obtain approximately the weight of the alkaloid.

204. Probably the alkaloid might be obtained in the first instance in a tolerably pure state if the original acid solution were somewhat diluted, and precipitated with lead subacetate, filtered, excess of lead removed by sulphuric acid; the solution partially neutralized, concentrated, rendered alkaline, and shaken repeatedly with ether.

205. To obtain the gelsemine in a state of comparative purity, substitute for ether-chloroform, petroleum benzin in extracting the alkaloid from the alkaline solution. A large quantity of the solvent, however, will be required, and it should be applied warm.

It is not certain after all that the portion of alkaloid not extracted by benzin is less active than the rest, and for the present it is best, perhaps, to be content with estimating simply total alkaloids.

206. A simple method of ascertaining approximately the value of a sample of the drug is the following: Exhaust 10 grams of the powder, which should pass through a No. 30 sieve, with a mixture of three volumes of alcohol with one of water, either by maceration (three days in 100 cc. of menstruum), or by percolation (to a volume of 100 cc.). Evaporate 50 cc. of the tincture, with addition of water and 2 drops of dilute sulphuric acid (5 per cent.) obtaining about 2 cc. of an alcohol-free solution. Titrate with Mayer's solution, $N\frac{1}{20}$, each cc. of which may be taken to indicate 10 mg. of gelsemine.

207. In precisely a similar manner we may estimate the alkaloidal strength of a fluid extract or tincture, using of the former 10 cc. of the latter 50 to 100 cc. for the experiment. Or the alkaloid may be extracted by shaking the strongly alcoholic solution first with lime, evaporating, after acidulating, an aliquot portion of the filtrate, washing the acid aqueous solution well with ether, and finally washing out the alkaloid after adding ammonia with ether chloroform or pure ether.

GUARANA AND OTHER DRUGS CONTAINING CAFFEINE.

208. Estimation of Caffeine. Dragendorff employs a modification of Mulder's process, as follows :*

Exhaust 5 grams of guarana with distilled water at 100° C. (212° F.) Evaporate the filtered solution with addition of two grams of calcined magnesia and five grams of powdered glass to complete dryness. Place the finely powdered residue in a flask with 60 cc. of ether, or better, a mixture of six volumes of ether with one of chloroform, and macerate 24 hours. Repeat the maceration three or four times with fresh portions of the solvent. Transfer the ethereal fluid to a tared flask, distil off the ether, and dry to constant weight at 100° C. (212° F.) The residue consists of pure crystallized caffeine. It is necessary to use plenty of ether. Doubtless the extraction would be better conducted by hot repercolation.

209. Method of Dr. F. V. Greene.† Boil 5 grams of the powdered guarana with 15 grams of finely powdered litharge in 150 cc. of water, until a colorless solution is obtained. About three hours' boiling will be required. According to J. H. Feemster (Am. Jour. Pharm., 1882, p. 523), this operation may be facilitated by adding toward the close a few drops of solution of subacetate of lead. Water must be supplied from time to time during the boiling. When cool filter the fluid, and wash the residue well with boiling water. Treat the filtrate and washings with sulphuretted hydrogen to remove the lead. [Addition of sodium phosphate will no doubt answer the purpose equally well, if

* Werthbestimmung einiger Starkwirkender Droguen, p. 56.

† Am. Journal of Pharmacy, July, 1877, p. 337, based on Prof. Wayne's method of extracting caffeine from tea and coffee.

indeed there be any need of removing the lead, when subacetate has not been added.] Evaporate to a small volume, and extract the alkaloid with chloroform, as in the other processes.

210. Method of Dr. Squibb.* Mix ten grams of powdered guarana and two grams of calcined magnesia with 100 cc. of water and boil five minutes. Add to the paste thus produced 30 cc. of strong alcohol. Stir thoroughly. Transfer to a filter, drain off the liquid and percolate the residue with 100 cc. of a mixture of water two volumes, and alcohol one volume. Boil the residue with 100 cc. of the same mixture of alcohol and water, drain and percolate until exhausted. Evaporate the fluid (300 to 350 cc) on a water bath to 20 cc. Transfer to a separator and wash out with three successive portions of chloroform.

210. (*a*) **Method of H. W. Snow.†** Place 5 grams of the finely powdered drug in a small flask, introduce 44 cc. of chloroform, cork, shake, then add 6 cc. of a mixture of alcohol 6 volumes, and stronger water of ammonia one volume, again shake vigorously and set aside with frequent shaking several hours, or allow to stand over night, Then filter rapidly through a small, dry, plaited filter paper 10 cm. (4 in.) in diameter. Measure 40 cc. of the filtrate, corresponding with 4 grams of drug, evaporate, and treat the residue with a little warm water, and filter through cotton, and afterwards through paper. Wash the residue and the filters until the washings cease to give a precipitate with phosphomolybdic acid. The operation of washing and filtering takes but little time ; if the cotton filter clogs, it is easy to stir up the insoluble waxy or fatty matter so as to permit the liquid to pass through. Transfer the aqueous solution, which will usually measure 40 to 50 cc. to a separator, and wash out the alkaloid with chloroform, using several successive portions, dry and weigh. The aqueous fluid should be tested with phosphomolybdic acid to make sure that the whole of the alkaloid has been withdrawn.

* Ephemeris, p. 614.

† Paper presented to Am. Pharm. Assn , Sept., 1886.

211. The Author's Method. Exhaust by percolation with a mixture of two volumes of alcohol and one of water, ten grams of guarana in No. 30 powder. The percolate may be made to measure 100 cc. Evaporate the percolate on the water bath to 20 cc. Add water to make up a measure, when cool, of 100 cc. Stir in a little magnesium carbonate and filter. Put into a separator 25 cc. of the filtrate, add 15 cc. of chloroform, shake, draw off the chloroform into a tared capsule. Evaporate at a gentle heat. Wash the fluid in the separator with two more portions of chloroform of 15 and 10 cc. and add to the contents of the capsule. When dry weigh. The product should be well crystallized caffeine, quite white, and wholly and easily soluble in warm water.

212. The advantage of this method over any described hitherto lies in the choice of solvent for exhausting the drug. Guarana contains much starchy matter which interferes with the use of water as a solvent. Alcohol of about 60 per cent., while it readily exhausts the drug, even in a coarse powder, leaves this troublesome matter behind.

213. It is possible that in the latter part of the assay trouble may sometimes be experienced, either from emulsionizing, or from withdrawal by the chloroform of other substances besides caffeine. The method has not been tested with a very large number of samples, but it is believed that it will prove satisfactory. A more circuitous method has been much used in my laboratory, and I am not yet sure that it does not give results as near the truth as that just described. The alkaloid extracted by either method is perfectly white, and apparently quite pure, but the quantity is appreciably less by the less direct method, which is as follows:

214. Alternative Method. Exhaust ten grams of the drug, as above, by percolation with a mixture of alcohol two volumes, and water one volume. Evaporate the percolate to about 40 cc. Pour into a 250 cc. measuring flask containing 190 cc. of water. Add solution of subacetate of lead in slight excess

[until the mixture has a sweetish taste]. Add water enough to make up 250 cc. Let stand a few minutes. Filter, and to the clear filtrate add a little potassium carbonate (dry) to remove the excess of lead. Filter once more. Evaporate 125 cc. of the filtrate, representing 5 grams of drug, in a shallow capsule or basin to a small volume. Transfer to a separator and wash out the caffeine with chloroform, using three successive portions of 15, 10 and 10 cc. respectively.

215. By this method of assay, guarana yields on the average 4.5 per cent. of caffeine, a quantity certainly not smaller than that obtained by the methods that have been heretofore employed, but the yield is always somewhat greater by the more direct process previously described, to which I therefore now give the preference.

216. The same method of assay is applicable to the fluid extract of guarana. Pour into 45 cc. of water 5 cc. of the fluid extract. Make up the volume exactly to 50 cc. Stir in a little magnesium carbonate, filter, and take for the assay 25 cc. of the filtrate, from which the alkaloid is to be washed out as above with chloroform.

217. Or, dilute 10 cc. of the fluid extract with an equal volume of 60 per cent. alcohol, and treat with lead subacetate, etc., precisely as described in (214). In any of these assays, double the quantity of drug or fluid may be taken, if the operator has not a sufficiently delicate balance to weigh the alkaloid accurately. Even with the quantity prescribed, however, the weight of the alkaloid ought to be as much as 100 milligrams.

218. The same general plan of assay is to be adopted for estimating the alkaloid in tea, coffee, maté, etc., and their preparations, but the details require more or less modification, in each case. Tea and roasted coffee are to be exhausted by macerating half an hour in boiling water. The infusion may be evaporated directly to a convenient volume and treated with chloroform, or

treated first with subacetate of lead and carbonate or phosphate of soda, and then evaporated. I am inclined to believe that there is always loss of alkaloid in evaporating the aqueous solutions, but am not prepared at present to suggest a better plan.

219. Raw coffee requires to be thoroughly dried and reduced to a fine powder, and boiled repeatedly with water, to extract the whole of the alkaloid. The proportion of alkaloid in tea varies from 1.5 to 3.5 per cent.; in coffee is about 1.2 per cent.

220. Assay of Tea, Dr. Squibb's process. Mix intimately in a mortar 10 grams of coarsely powdered tea and 2 grams of calcined magnesia. Stir the mixture into 100 cc. of boiling distilled water and boil five minutes. Filter and percolate the residue with 50 cc. of water. Stir it once more into 100 cc. of boiling water, boil, filter, and percolate the drugs to exhaustion. The liquid (about 350 cc.) is now to be evaporated on the water bath to 20 cc., evaporating the weaker portion first. Transfer to a separator, and proceed as in the assay of guarana.

221. The caffeine obtained in this way will be nearly pure. To separate any oily matter that may be present, dissolve it by gentle agitation in the capsule with several successive portions of distilled water, filter into another tared capsule, set in a warm place for spontaneous evaporation to dryness.

222. Assay of Coffee, Dr. Squibb's process. Follow the same process as for the assay of tea [employing, however, a finer powder], but after evaporating the aqueous solution to a volume of 20 cc , instead of attempting to wash out the alkaloid directly from this solution, precipitate the albuminous matter from it by pouring it into 100 cc. of strong alcohol. Filter, and wash the residue with strong alcohol. Evaporate the alcoholic solution and redissolve in a little water (several successive portions). Transfer to separator, and wash out the alkaloid with chloroform, as in the assay of guarana.

HYDRASTIS CANADENSIS.

223. Active Constituents. The drug contains two or more alkaloids. The most abundant is that to which it owes its bitterness and its deep yellow color, and is a constituent of many plants besides golden seal. The correct name of this alkaloid is berberine, although it is still confounded with the less abundant white alkaloid hydrastine, which is the distinctive active principle of the drug. Berberine forms salts which are generally only sparingly soluble in water, while the salts of hydrastine dissolve freely — the alkaloid itself being, however, almost completely insoluble.

224. The examination of powdered hydrastis has been generally conducted hitherto simply with a view to ascertain whether or not the powder was adulterated. For this purpose, an estimation of berberine will serve as well as a determination of the more important alkaloid. For estimating berberine several plans may be followed.

ESTIMATION OF BERBERINE.

225. Method of J. U. Lloyd.* Macerate 5 grams of the drug in fine powder with 11 grams of officinal alcohol four days, with frequent shaking. Decant the clear liquid, add alcohol to make up the former volume, macerate as before, decant, and repeat the operation a third time, mix the decanted solutions. After twelve hours filter, washing the filter with a little alcohol. Add to the filtrate one-third its volume of officinal sulphuric ether, and then 1.5 grams hydrochloric acid and 0.5 gram sulphuric acid. Mix thoroughly, and set aside in a cool place 48 hours. Then collect the precipitate of berberine hydrochlorate on

* Druggists' Circular, Feb., 1885, p. 22.

a pair of mutually counterpoised filters, wash with a mixture of equal parts of sulphuric ether and alcohol until free from acid, dry in a cool place and weigh. It is not stated whether the crystals found under these conditions contain water of crystallization or not. If anhydrous, they contain 90.18 per cent of alkaloid. The crystals obtained from aqueous solutions contain 82.21 per cent. of alkaloid.

226. Colorimetric Method of the Author. Exhaust 10 grams of the drug in moderately fine powder by percolation with 75 per cent. alcohol, of which 100 cc. may be used. [Comparative experiments have not been made to ascertain what menstruum best exhausts the drug; that mentioned certainly answers the purpose well.] Dilute with water to one litre a portion of the percolate representing one gram of the drug, and add a few drops of hydrochloric acid.

227. Prepare from pure berberine sulphate or muriate a standard solution containing one part in 25,000 of the alkaloid (0.04 gram in a litre). This standard may be made with a menstruum containing 25 per cent. of alcohol, and kept on hand, taking care that it is not exposed to a strong light.

228. Select two test tubes of equal diameter and thickness of glass. Into one of these pour exactly 10 cc. of the solution of golden seal. Into the other put enough of the standard solution to produce a depth of color, looking into the tube from above, sensibly equal to that of the fluid in the first tube. Before making the final adjustment of color, bring the volume of fluid in the second tube as nearly as possible to 10 cc. that the conditions for the comparison may be most favorable for a correct judgment. When the tints in the two tubes, held over white paper side by side, and viewed from above, are judged identical, note the quantity of the standard fluid that has been required. This quantity, expressed in cc., multiplied by 0.4, will give the percentage required.

229. In case more than 10 cc. of the standard solution were required, it would be best to make the comparison with 5 cc. only of the golden seal solution, adding 5 cc. of water. The percentage in this case will be found by multiplying the volume in cc. of standard solution used by 0 8 instead of 0.4.

230. This method is capable of giving, with a little practice, results of fair exactness, but obviously it is intended only to furnish a rapid off-hand mode of judging the quality of golden seal. It is equally applicable, however, to the examination of tinctures, fluid extracts, etc., of this drug, provided, of course, it can be shown that the preparation does not contain berberine to the exclusion of hydrastine.

231. To examine a fluid extract it is only necessary to dilute the preparation in the first instance with four or five times its volume of water, filter, then take a quantity supposed to represent 1 gram of drug, dilute with acidulated water to one litre, and make the comparison as already explained. If the preparation proves to be very deficient in strength, it may be necessary to take a quantity representing 2, 3, or even five grams of drug.

232. Should the preparation be a poor one, and made with weak spirit, the coloring matters other than berberine may interfere with this colorimetric process. In such a case, evaporate, if necessary, to a small volume, add strong alcohol, to precipitate the obnoxious coloring matter, decant the alcoholic solution, redissolve the precipitate in a little dilute alcohol and precipitate a second time, and continue this as long as the alcoholic solution is strongly yellow after precipitation of the gummy matter. Mix the alcoholic solutions, and bring to the required volume by addition of water.

Estimation of Hydrastine.

233. The value of golden seal, or of any galenical preparation of it, unquestionably depends chiefly on the hydrastine it contains. In the present state of our knowledge of the alkaloids of

golden seal, we cannot hope by assay of a small quantity of drug to ascertain exactly how much crystallizable hydrastine is present, but it is easy to estimate the crude alkaloid, after removing the most of the berberine, simply by precipitating it with ammonia.

234. Assay Process. The extract from 5 grams of the drug is to be evaporated to a small volume, hydrochloric acid added in excess (1 cc. of dilute acid 1:4), and the mixture allowed to stand in a cool place some time. Filter, washing the filter with water containing hydrochloric acid. To filtrate and washings add ammonia in excess, collect the precipitate on a filter, wash slightly with water, redissolve in hydrochloric acid. Add through the filter water enough to bring the volume to 10 cc., precipitate once more with ammonia, and collect the precipitate on a tared filter, dry and weigh, or else allow the precipitate to subside, decant as much as possible of the clear fluid, and dissolve out the precipitated alkaloid with chloroform, evaporate the solution to dryness and weigh. If chloroform is used at an earlier stage, it will take out much that is not alkaloid.

235. From a Fluid Extract the berberine may be separated as follows: Mix 5 cc. of the fluid extract with 15 cc. of strong alcohol. If no precipitate is formed, dilute still further with alcohol, and add to the solution ether, hydrochloric acid and sulphuric acid, in the proportions directed in (225). If a precipitate is formed, as will usually be the case, allow it to subside, and decant the clear fluid. Dissolve the precipitate in a little dilute alcohol and reprecipitate with strong alcohol. Repeat this operation until 50 cc. of alcoholic solution has been obtained, and to this add ether and acids precisely as directed in (225).

236. The residue, after filtering out the berberine hydrochlorate, may be neutralized, evaporated, and employed for estimation of hydrastine, but this estimation can be more rapidly made by using a separate portion of the fluid extract, proceeding according to the method of (234).

237. Mayers' reagent cannot be employed to any advantage in the examination of preparations of golden seal, except as a rough measure of their alkaloidal strength The several alkaloids of the drug behave very differently towards the reagent. It is noticeable that berberine is always thrown down first, although, of course, not without large admixture of other alkaloid or alkaloids. Each cc. of Mayer's reagent precipitates something like 50 mg. of berberine, about one-half that quantity of hydrastine, and a still smaller equivalent of the crude alkaloid which ammonia throws down. Variations in the dilution of the solution produce very great variations in the results of titration. For 10 cc. of a fluid which represents 2 grams of drug, we may expect to use between 6 and 8 cc. of the reagent $N\frac{1}{20}$, commonly used in these titrations.

HYOSCYAMUS.

238. Active Constituents. Henbane contains two principal alkaloids, hyoscyamine and hyoscine, closely related to one another chemically, and having similar physiological action. Hyoscyamine is a crystallizable solid, while hyoscine at ordinary temperatures is semi-fluid, although it forms crystalline salts. Recent studies of these two alkaloids have shown that it is the second to which the drug owes its distinctive character as a therapeutic agent. There is, however, no simple method as yet known of separating the alkaloids in assays, and we must be content to base our judgment of the drug upon its content of total alkaloid.

239. The assay of Henbane may be conducted in precisely the same manner as that of belladonna leaves, with certain modifications in detail. It must be remembered that the alkaloids of this drug are even more sensitive to the action of reagents—particularly of alkalies—than are those of belladonna, and that they are also more easily lost in evaporation of solutions. It is probable that the result in all cases where the attempt is made to estimate the alkaloid directly by extracting with chloroform, drying and weighing are low; nevertheless, if the evaporation is conducted at a very gentle heat, this method, as applied to the crude drug, is, on the whole, to be preferred to any other, and at least enables us to compare one sample with another.

240. Mayer's reagent may be advantageously employed for estimating the quantity of alkaloid, but its indications cannot be depended upon unless the solution titrated has been so prepared as to exclude substances which, although precipitated by the reagent, have not otherwise the reactions of alkaloids. The assay can be made most easily by the method described in (92), estimating the

alkaloids by Mayer's reagent $N/_{20}$, of which, according to Dragendorff, 1 cc. precipitates 0.00598 grm. "hyoscyamine." Following this method we may expect to find in henbane leaves about 0.18 per cent. of alkaloid. If we extract the alkaloid and weigh it directly, following the method of (29), we shall probably obtain scarcely more than 0.1 per cent.

241. Galenical Preparations of Henbane. It is when we come to the examination of the galenical preparations of the drug that we find our results unsatisfactory and contradictory. This is especially true of those preparations that have been subjected to the influence of heat, or have been exposed to the action of the air in evaporation. It appears that the alkaloids undergo in these operations changes which entirely alter their chemical behavior, and it is most probable that these changes are accompanied by loss of medicinal activity. Similar changes seem to take place merely with age in preparations of henbane, like the tincture or fluid extract, which have not been exposed to the action of heat.

242. We often find that these preparations when titrated directly, or after preliminary treatment with lime (56 b), show apparently, by Mayer's reagent, a large proportion of alkaloid, but that the usual treatment with chloroform and an alkali fails to remove the substance to which this reaction is due. We can only conjecture, since we do not observe the same thing in our examination of the drug itself, or of freshly prepared tinctures therefrom, that the alkaloids originally present in the drug have been split up, and we may therefore well hesitate to express an opinion with regard to the therapeutic activity of the preparation. We still may fall back on the physiological test, but the details of such a method of examination have yet to be worked out, and its value can only be ascertained by experiment.

IPECACUANHA.

243. The active principle of the drug is emetine, an alkaloid somewhat difficult to separate on account of its susceptibility to the action of alkalies. The proportion of alkaloid is large, amounting generally to more than 2 per cent. The estimation is most conveniently made by the titration with Mayer's reagent.

244. Method of Dragendorff, as modified by the author.* Place in a suitable bottle or flask 50 cc. of distilled water (without addition of acid), afterwards put in ten grams of ipecacuanha in fine powder; mix, cork the bottle or flask, and set by in a warm place, shaking occasionally. At the end of 24 hours add to the mixture 52 cc. of alcohol, making a total of 100 cc. of menstruum owing to condensation of volume; cork, and set aside again for three days, shaking well several times a day. Then measure out with a pipette for the assay 25 cc. of the clear fluid, which will represent as nearly as possible 2½ grams of drug. Put this in a capsule, add 5 drops of a highly dilute sulphuric acid (containing 6 per cent. H_2SO_4), evaporate at a gentle heat until all the alcohol is driven off, add water to bring the volume of fluid to 20 cc., digest a few minutes on the water bath, allow the mixture to cool, and proceed, without filtering, to titrate with Mayer's reagent, $N/_{10}$.

245. According to Dragendorff, 1 cc. of Mayer's reagent $N/_{10}$ precipitates 0.00945 grm. emetine, the degree of dilution not stated. This equivalent is obviously based on theoretical considerations, and experiment has proved its inaccuracy. The precipitation of emetine by Mayer's reagent is very complete, and the

* American Journal of Pharmacy, Nov., 1885, p. 531.

excess of reagent required is not large, but, as usual, a certain excess *is* required. The volume of fluid titrated, therefore, has an important influence on the result. A correction must be introduced, as usual, and we may adopt the following:

246. Rule. For each cc. of fluid, at the end of the titration, deduct from the result 0.0384 cc. [Mayer's reagent, $N\frac{1}{20}$, having been used], and multiply the remainder by 0.012075.

247. Or, reduced to a tabular statement:

Quantity of Mayer's reagent N 1-20 required to precipitate a given quantity of Emetine.

Quantity of Emetine.	Vol. of fluid titrated, 5 cc.	Vol. of fluid titrated, 10 cc.	Vol. of fluid titrated. 15 cc.	Vol. of fluid titrated, 20 cc.
0.010	1.06	....	....	....
0.020	1.90	2.14	.....	....
0.030	2.70	2.96	3.20	.....
0.040	....	3.80	4.00	4.3
0.050	....	4.62	4.80	5.1
0.060	.....	.....	5.60	5.9
0.070	.....	.....	6.40	6.8
0.080	.....	.....	.....	7.6
0.090	.....	.....	....	8.4
0.100	.....	.....	.. ..	9.2

248. If the solution contain between 1:200 and 1:500 emetine, the results of the titration, interpreted by the above rule or tabular statement, will be very near the truth.

249. If the solution is filtered previous to titration, the residue being thoroughly washed, the estimation of alkaloid may be made gravimetrically, by precipitating with a slight excess of Mayer's reagent, collecting on a pair of mutually counterpoised filters, washing with a little water, applied drop by drop, pressing between blotting paper, drying at 100° C. (212° F.), and weighing. Multiply the weight of the precipitate by 0.39 for alkaloid, 100 parts of emetine producing about 256 parts of precipitate (instead of 288, as theory would lead us to expect).

250. Other Methods of Assay. The general method of estimating alkaloids, given in (29), may be adopted also for ipecac, although the results are a little lower than those obtained by the foregoing process. The drug (5 grm.) must be in a fine powder, and the maceration must not be protracted beyond a few hours. The ether having been evaporated, take up the alkaloid with acid water (20 cc.), and titrate with Mayer's reagent, in preference to attempting to separate and weigh the emetine.

251. The following method is simple, rapid, and reasonably satisfactory: Place in a flask fitted with an inverted condenser 5 grams of finely powdered ipecac, add a mixture of strong ammonia 0.3 gram, alcohol 5 grams, chloroform 30 grams. Set in a warm place half an hour, then apply sufficient heat to keep the mixture boiling for one hour; then add 50 cc. petroleum benzin, boil half an hour, add benzin enough to make the mixture measure nearly 100 cc., filter, and add through the filter enough benzin to make 100 cc. Of this take for the assay 50 cc. Separate the alkaloid by shaking with several portions of acid water, bring the volume of the aqueous solution to 20 cc., and titrate.

252. Still another method gives good results, and consumes very little time. Exhaust 5 grams of the finely powdered drug by hot repercolation in a suitable extraction apparatus (7) with 40 cc. of a mixture of 3 volumes of chloroform with 1 of alcohol. Remove the alkaloid from the chloroform solution by shaking with acid water, and estimate it either by titration, or gravimetrically, separating the alkaloid by rendering alkaline, and shaking repeatedly with chloroform.

253. Flückiger has recently published a process of assay* which does not differ greatly from this last. He exhausts the drug with boiling chloroform, with addition of a very little ammonia. He reports a yield of only 1 per cent or less of emetine by this

* Pharm. Zeitung, Jan., 1886.

process. Either he operated on a poor drug, or the solvent does not extract all the alkaloid. I have seldom found a drug that yielded less than 2 per cent. of emetine, and the result in following the different methods of assay has not varied greatly.

254. The assay of galenical preparations of ipecac presents no difficulty; an aqueous solution is prepared, of which 20 cc. represents theoretically 2 to 4 grams of drug, acidulating with dilute sulphuric acid, and titrating with Mayer's reagent, as already explained. Of the solid extract 0.5 to 1.0 gram may be taken for the assay; of the fluid extract 2 to 4 cc.; of the wine or tincture 25 cc.; of the syrup 50 cc. (diluted with 100 cc. of water). In this last case, we must arbitrarily reckon, for each cc. of Mayer's reagent $N_{\frac{1}{10}}$ used, 0.0095 grm. emetine. Of course, the alcohol from fluid extracts, etc., must be expelled by evaporation previous to titration.

NUX VOMICA AND IGNATIA BEAN.

255. Active Constituents. These drugs contain the two alkaloids, strychnine and brucine, with possibly a third—although igasurine, with its numerous varieties, is probably to be classed among the myths of science. Strychnine, in nux vomica, constitutes 40 to 50 per cent. usually of the total alkaloid. In ignatia the proportion is larger. It is believed that the medicinal action of the two alkaloids is similar in character, but that of strychnine is much more powerful.

256. Contrary to the common opinion, brucine is the more bitter of the alkaloids. Solutions containing 1 part of brucine in 500,000 are decidedly bitter—as much so to my individual sense as those of strychnine three or four times as strong. The proportion of brucine in a mixture of the two alkaloids may, indeed, be approximately ascertained by simply preparing a sufficiently dilute solution and comparing its bitterness with that of standard solutions of brucine and of strychnine, but I find that to some there is not the difference in bitterness between the two alkaloids that, to my own sense of taste, is so obvious.

257. Dragendorff's Method of Assay.* Reduce the drug by rasping or otherwise to as fine a powder as possible. Exhaust 15 grams of the powder by boiling with three successive portions (150 cc.) of distilled water, containing sulphuric acid. After each boiling strain and press the residue. The marc should finally be nearly free from bitterness. Evaporate the mixed solutions to the consistence of honey, after having nearly neutralized with magnesia. To the syrupy fluid add 2½ times its volume of

* Chemische Werthbestimmung, etc., p. 61.

alcohol, of 90° Tralles, heat to boiling, filter while hot, and wash the residue on the filter thoroughly with hot alcohol of 65° Tralles. Distil off the spirit, add some sulphuric acid, filter. Wash repeatedly with benzol to remove fatty and waxy matter; finally add ammonia, and wash out the alkaloids by shaking with several successive portions (25 cc.) of chloroform. Evaporate the chloroformic solution to constant weight in a tared capsule, and weigh the residue as "total alkaloids."

258. Method of Messrs. Dunstan and Short.* Mix 5 grams of powdered nux vomica with 10 grams of crystallized sodium carbonate, and form into a paste with water. Dry over a water bath and reduce to powder. Exhaust this in a suitable extraction apparatus (7 *et seq.*) with a mixture of 30 cc. of chloroform and 10 cc. of absolute alcohol. This is accomplished in from one to two hours. Transfer the solution to a separating funnel, add 25 cc. of dilute sulphuric acid (10 per cent.), shake well, and allow the fluids to separate. (The separation is much aided by gently warming the mixture on the water bath.) Draw off the chloroform into another separator containing 15 cc. of the dilute acid, shake well together, separate, and, having rejected the chloroform, add the acid liquid to the contents of the first separator. Add ammonia and 25 cc. of chloroform, shake, separate. Wash the chloroform with a little water containing ammonia. Evaporate in a tared capsule over the water bath and weigh. The last of the alkaloid may be obtained by a second and even a third washing with chloroform. The percentage of alkaloids is found by multiplying the weight of the residue expressed in decigrams by two.

259. Method of the Author. Place in a flask or bottle 5 grams of the drug in No. 30 powder, add 50 cc. (accurately measured) of the modified Prollius fluid (14) Cork the flask at once, shake well, and set by 12 to 24 hours, shaking occa-

* Phar. Jour. and Trans., Feb. 17, 1883, p. 65.

sionally. Transfer of the clear fluid exactly 25 cc. to a separator. Wash out the alkaloids with dilute sulphuric acid, 5 per cent., using two successive portions of 10 and 5 cc. respectively. Draw off the acid solutions into a 2 oz. vial, add ammonia, and wash out the alkaloid by shaking with three successive portions (20, 15 and 10 cc.) of a mixture of chloroform 1 volume, ether 3 volumes. Evaporate at a gentle heat, dry to constant weight at 100° C. (212° F.), and weigh.

260. It is not claimed for this method that it completely exhausts the drug, but it gives a sufficiently close approximation to the correct result to answer all practical purposes, and it requires very little time and no especial apparatus to carry it out.

261. Assay of galenical preparations of nux vomica and ignatia.

(a) **Fluid Extracts,** Tinctures, etc. Measure 10 cc. of a fluid extract, or 25 cc. of a tincture into a capsule. Add 1 cc. of dilute sulphuric acid, 5 per cent., and 10 to 20 cc. of water. Evaporate to drive off alcohol, adding, if necessary, more water. Pour the watery solution, measuring about 10 cc., into a 2 oz. vial with a good lip, rinse the dish with ether and a few drops of water, and add the rinsings to the contents of the vial. Wash the acid fluid with several successive portions of ether, using for the final washing a mixture of chloroform 1 volume, and ether 3 volumes. Having decanted this, add ammonia to strongly alkaline reaction, and wash out the alkaloid with three successive portions (15, 10 and 10 cc.) of the same mixture of ether and chloroform. Dry and weigh as usual.

262. (b) **Solid Extract.** Dissolve 1 gram of the extract in 10 cc. of water containing 1 cc. of dilute sulphuric acid (5 per cent.) in presence of some ether. Pour the solution into a 2 oz. vial, and proceed exactly as in the last paragraph.

263. Use of Mayer's reagent for valuation of nux vomica. Although the method cannot be recommended for exact-

ness, and has little advantage over the gravimetric in point of rapidity or simplicity, titration with Mayer's reagent is capable of yielding results in the examination of galenical preparations of nux vomica of practical utility. Strychnine is precipitated even in quite strongly acid solutions very perfectly by this reagent, and the excess required is small, so that the quantity of alkaloid precipitated by 1 cc. of reagent under different conditions is nearly the same, and is nearly what theory would lead us to expect, viz., 8 milligrams. Not so, however, with brucine. The quantity of reagent required is, in the first place, less than authorities state it, and the influence of any excess of acid, the presence of alcohol or of iodide of potassium, etc., even in small proportions, produces a sensible variation in the result of a titration.

264. Excluding alcohol, and operating with a solution containing the slightest excess of acid, and having a dilution of 1:200, we may expect that 1 cc. of the reagent, $N\frac{1}{20}$, will precipitate about $12\frac{1}{2}$ mg. of brucine. In a solution containing 1:500 this will be reduced to 11 mg. or less; presence of acid will diminish it materially. The conditions, however, can be easily adjusted so as to insure tolerable uniformity in the results, and we may assume that of the mixed alkaloids 1 cc. of Mayer's reagent, under the conditions of the titration, will precipitate of the mixed alkaloids about 10 mg.

265. If a fluid extract is to be examined, put 5 cc. into a capsule, add 5 minims of dilute sulphuric acid (5 per cent.) and 5 cc. of water, and evaporate on the water bath to expel alcohol. Bring the aqueous fluid to a volume of 20 cc., and titrate with Mayer's solution, $N\frac{1}{20}$, each cc. of which will correspond with 10 mg. (approximately) of alkaloids. Of a tincture of nux vomica, take for the assay 10 cc. and treat as above, making up the aqueous solution to 10 cc. for titration. Of the solid extract take 0.5 grm., bring into solution in water by aid of 5 minims of dilute sulphuric acid, 5 per cent., and a sufficient quantity of ether,

which must be expelled by evaporation, and make up the solution to 20 cc.

266. If the precipitates produced by a slight excess of Mayer's reagent in the above solutions (previously filtered) be collected on double filters, dried at 100° C. and weighed, we may again obtain a close approximation to the quantity of alkaloid present by multiplying the weight of the precipitate by 0.42.

267. Separation of Strychnine from Brucine. The method of Dunstan and Short,* although criticised by Dr. Schweissinger,† is the best that has yet been proposed: Dissolve the alkaloids (0.2 grm. or more) in 10 cc. of dilute sulphuric acid, 5 per cent. (volume), make up to 175 cc. with water, and add 25 cc. of a 5 per cent. solution of potassium ferrocyanide. Stir occasionally during six hours, when the strychnine will have been completely precipitated in the form of a crytallized ferrocyanide. Collect the crystals on a filter, wash with a little acidulated water, treat on the filter with strong solution of ammonia and chloroform, separate the chloroformic solution, evaporate, dry cautiously at 100° C. (212° F.). and weigh as strychnine.

268. The proportion of strychnine found in the total alkaloids from nux vomica is generally about 40 per cent., but it varies greatly. The simplest method of estimating the strychnine is that suggested by Dr. Schweissinger,† based on the difference in saturating power of the two alkaloids. One cc. of a decinormal solution of hydrochloric acid will neutralize 0.00394 grm. brucine, but only 0.00334 strychnine. Having weighed the mixed alkaloids, therefore, dissolve them in a slight excess of decinormal acid, noting exactly the quantity used. Titrate back to neutrality with decinormal alkali, using litmus as an indicator. Subtract alkali used from acid taken to find the quantity of acid required to neutralize the alkaloids. Divide weight of alkaloids in milligrams by

* Year Book of Pharmacy, 1883, pp. 469-475.

† Archiv. der Pharm.; Am. Druggist, 1885, p. 230.

this difference, expressed in cubic centimetres. Subtract the quotient from 3.94. Divide the remainder by 6, and move the decimal point three places to the right, to obtain percentage of strychnine.

269. Example: Weight of alkaloids taken, 75 mg. Deci normal acid used to dissolve, 35 cc. Decinormal alkali required, 14 75 cc. 35−14.75=20 25 75÷20.25=3.704 3.94−3.704=0.236 0.236÷6=0.0393, giving 39.3 per cent. of strychnine=29.5 mg.

270. Solutions of brucine being much more bitter than those of strychnine, it may be possible to estimate the proportion of each present in a mixture by comparing the bitterness of an exceedingly dilute solution with that of solutions of the same strength of brucine and of strychnine respectively.

271. The differences in weight of the precipitates produced by Mayer's reagent in strychnine and brucine solutions may also be made the basis of a means of roughly estimating the relative proportion of each.* This is to be preferred to Dragendorff's plan of basing the calculation on titration merely with the same reagent, especially if we adopt the theoretical equivalents, in which he seems to put complete confidence.

272. For the ordinary purposes of the pharmacist, an estimation of total alkaloids in nux vomica or its preparations may be considered sufficient.

* Druggists' Circular, June, 1886, p. 137.

OPIUM.

273. U. S. Process of Assay. The details of this process need not be repeated here, but attention is called to the fact that the results of the assay are always low. This is attributed by Herbert Lloyd (Am. Druggist, Dec., 1885, p. 221) to solubility of morphine in the excess of ammonia present, and it is proposed to add to the amount of morphine obtained 5 per cent. of its weight, together with a constant correction of 0.050, which will approximately compensate the loss.

274. Method of J. Howard Wainwright,* of the U. S. Laboratory attached to the Custom House. Put into a 4 oz. wide mouth vial 10 grams of the opium with 100 cc. of boiling water. Cork securely, and allow to stand, with frequent hard shakings 12 to 24 hours. Decant the clear liquid upon a filter of convenient size, and collect the filtrate in a beaker. To the residue in the bottle add 30 to 50 cc. of boiling water. Agitate well, and transfer the mass to the filter, with the aid of a little hot water. Drain well on the filter, wash with a very little hot water, applied drop by drop around the edges of the filter.

275. Return the residue to the bottle, and shake up with 50 cc. of hot water. Pour again upon the filter, and collect the filtrate in an evaporating dish. Percolate the residue with hot water until completely exhausted. Evaporate the filtrate, and when reduced to a small volume add to it the first filtrate, a little at a time, until the whole is reduced to a volume of 20 to 25 cc. Transfer, with aid of the least possible quantity of water, to a tared Erlenmeyer flask, and, when cool, add 10 cc. of strong alcohol, and a volume of ether equal to that of the mixture, cork and

* Journal American Chemical Society, VII., 48.

shake well. Immediately add 4 cc. of a 10 per cent. solution of ammonia, cork again, and shake until crystals of morphine begin to separate. Set aside 12 hours in a cool place, or else shake continuously for half an hour or more. [The best plan is to shake vigorously at intervals during 48 hours. There is often a perceptible increase in the amount of morphine after the first 12 hours.]

276. When the morphine is completely precipitated, decant the ether upon a pair of mutually counterpoised filters. Add to the contents of the flask 20 cc. more of ether, and decant this also through the filter, which is to be washed with a little ether, applied drop by drop to its edge. Remove the last traces of ether from the surface of the heavier liquid by the aid of a strip of filter paper. When the filter is dry, pour upon it this dark colored fluid, containing the crystals of morphine. Wash the crystals from the flask by aid of a portion of the filtered fluid.

277. When all the fluid has passed through the filter, rinse the flask with a little distilled water, and wash the crystals and filter first with this, then with pure distilled water, until this comes through colorless. Drain the filter thoroughly, remove carefully from the funnel, fold together and press between dry filters as long as moisture is given up. Finally dry at 100° C. (212° F.) and weigh. If any crystals have remained in the flask this must also be dried and weighed.

278. This assay process differs only slightly in some details from that of Dr. Squibb, and is to be preferred decidedly to the lime process of the Pharmacopœia. It is simple, and requires no especial manipulative skill.

279. Method of J. Perger.* Boil 10 grams of the opium a short time with 150 cc. of water and 15 grams of caustic baryta. Filter and boil the residue repeatedly with small quantities of water until it fails to give a reaction with sulpho-molybdic acid. Excessive boiling is to be avoided, and the filtrate should

* Journ. pr. Chemie [2], 29, 97, 110. Jour. Chem. Society, Nov., 1884, 1217.

not measure more than 400 cc. Pass through the solution a current of carbonic acid gas to precipitate excess of baryta. Evaporate rapidly on the water bath to dryness. Moisten the residue with absolute alcohol, transfer to an Erlenmeyer's flask, and exhaust by repeatedly boiling with absolute alcohol, of which 200 to 300 cc. will be required. Distil off the alcohol, add 15 cc. of water containing some ammonia, and allow to stand some time. Stir well with a glass rod, and collect the morphine on a tared filter, dry at 40° C. (104° F.), and treat repeatedly with chloroform. The product is crude morphine, but contains only an inconsiderable amount of impurity.

280. Method of Flückiger.* Place 8 grams of powdered opium upon a filter of 80 mm. (3¼ in) diameter, and wash with 25 cc. of ether, keeping the funnel well covered. Force out the last drop of ether by tapping the funnel, dry the opium on the water bath, transfer to a flask containing 80 grm. of water at 25° C. (77° F.) [the authority we quote says 25° C. (59° F.); one or the other of the figures must be wrong]. Cork and shake well repeatedly. After 12 hours pour the mixture on the previously used filter, and collect 42.5 grams of the filtrate in a small flask, to which add 14 cc. of strong alcohol, 13.5 cc. of ether and 1 cc. of solution of ammonia. Cork securely, shake, and set aside at a temperature of 12° to 15° C. (53° to 59° F.), shaking occasionally.

281. After 24 hours, moisten a new tared filter of 80 mm. (3⅛ in.) diameter with ether, pour upon it the ethereal layer in the flask, add 14 cc. more of ether to the contents of the flask, shake, and when separated transfer the ethereal layer to the filter. When this has passed, and the filter is dry, pour the whole contents of the flask on the filter, and wash the crystals of morphine twice with a mixture of 2 grams of dilute alcohol, 2 grams of water and 2 grams of ether. Dry at a gentle heat, finally at 100° C. (212° F.)

* Arch. der Pharmacie, Am. Druggist, Aug., 1885, p. 149.

and weigh, adding the weight of the morphine which has remained adhering to the flask. The result expressed in decigrams multiplied by two will be percentage of morphine in the opium.

282. Solid Extract of opium (6 grams) is to be dissolved in water, the solution diluted somewhat (as long as water continues to produce a precipitate), and, if necessary, filtered. The filtered solution is to be concentrated to 25 cc., and 10 cc. of alcohol and 30 cc. of ether added, the assay being completed as above.

283. Tincture of opium (120 cc.) is to be evaporated to a small volume, and water added to make 25 cc. If a precipitate is produced, add water as long as it produces a precipitate, filter, and evaporate filtrate and washings to 25 cc., and treat as above.

PHYSOSTIGMA.

284. Calabar Bean contains two alkaloids, physostigmine, also called eserine, and calabarine. It is to the former of these that the useful therapeutic properties of the drug are due. When alkaline solutions containing both alkaloids are shaken with ether, the physostigmine alone is taken up.

285. Assay process. Exhaust 10 grams of the drug in fine powder by boiling with three or four successive portions (50 to 75 cc.) of alcohol, to the first of which 0.25 grm. of sulphuric or tartaric acid may advantageously be added. Distil off or evaporate the alcohol on the water bath. Dissolve the residue in water (10 cc.) and ether (25 cc.), adding also a few drops of dilute sulphuric acid. Transfer to a 2 oz. vial, shake the fluids together and let stand. When completely separated, decant the ether carefully into the waste ether bottle, and wash the aqueous solution with two or three successive portions (15 cc.) of fresh ether, and decant as before. Add now 20 cc. of fresh ether, and solution of ammonia in slight excess, shake, decant the ether when completely separated into a tared capsule, and evaporate at a gentle heat, and protected from a strong light. Treat the alkaline fluid with fresh ether until no more alkaloid is taken up. Evaporate the mixed ethereal solutions to constant weight at 100° C. (212° F.) and weigh as physostigmine.

286. Solid extract of calabar bean must be dissolved in water and ether precisely as above, 1 gram sufficing for the estimation. Fluid extracts or tinctures are to be evaporated to expel alcohol, and treated in the same manner.

287. Even apart from the circumstance that calabar bean contains two alkaloids, only one of which can be regarded as its

active principle, titration with Mayer's reagent gives no satisfactory results, owing to excessive variations in the quantity of reagent required for the precipitation under different conditions. The gravimetric estimation, however, is easy, provided the operator has at his command a sufficiently sensitive balance.

PILOCARPUS.

288. Poehl* gives the following method for assaying jaborandi leaves: Extract 10 grams of the leaves with 100 cc. of water containing 1 per cent. of hydrochloric acid. Precipitate the infusion with subacetate of lead, remove excess of lead with hydrochloric acid. Filter, precipitate the filtrate with phosphomolybdate of sodium, collect the precipitate, wash with water containing hydrochloric acid, dry at 100° C. and weigh. Multiply by .4566 to obtain the weight of the alkaloid.

289. Titration of solutions of pilocarpine with Mayer's reagent gives results varying very widely even when the conditions seem similar. The precipitate seems to have at first the consistence of a soft resin, but eventually assumes a crystalline form. The precipitate, however, in solutions obtained from fluid extracts, tinctures, etc., of the drug subsides very slowly, and passes easily through a filter, so that titration is quite impracticable.

* Year Book of Pharmacy, 1881, 28, 141.

PODOPHYLLUM.

290. Active Constituents. The researches of Podwyssotsky have demonstrated that the active constituent of mandrake root is podophyllotoxin, a neutral resinous principle constituting about 50 per cent. of the officinal resina podophylli, or podophyllin. This compound is soluble in alcohol, chloroform, ether (less readily), but insoluble in petroleum ether, and very sparingly soluble in water. The other constituents of podophyllin are, 1st, an inert, oily substance, soluble in petroleum ether, and having the peculiar disagreeable odor which characterizes the drug; 2d, a substance (podophyllinic acid) soluble in chloroform but not in ether or petroleum ether; 3d, a substance (podophyllo-quercitrin) present in considerable quantity, soluble in ether, but insoluble in chloroform.

291. To assay the drug one of two methods may be adopted. We may either exhaust the drug with chloroform, reduce the chloroformic solution to a small volume, and pour in a fine stream into ten times its volume of petroleum ether, whereby the podophyllotoxin will be precipitated, or we may first exhaust the drug with petroleum ether, and subsequently with chloroform, evaporate the chloroformic solution to dryness, and weigh as podophyllotoxin. Podophyllinic acid may be excluded in either assay process by adding to the concentrated chloroformic solution six to ten times its volume of ether, free from alcohol, which will precipitate it. The quantity taken up of this compound, however, by cold chloroform is so small that the impurity may be neglected in an assay process whose object is to ascertain the relative value of different samples of drug.

292. The details of the assay are as follows: Place in a small percolator 10 grams of mandrake root, in moderately fine powder. Exhaust with petroleum ether, of which 100 cc. may be used. Remove the residue from the percolator, and when the petroleum ether it contains has been dissipated by exposure to a gentle heat, transfer to a bottle in which has previously been placed 50 cc. of chloroform. Cork securely, and allow to macerate 24 hours, shaking occasionally. Evaporate 25 cc. of the chloroformic solution to a volume of 1 or 2 cc., add 10 or 15 cc. of ether free from alcohol. If a precipitate is produced, continue addition of ether as long as it causes further precipitation. Allow the precipitate to subside and adhere to the sides and bottom of the containing vessel, decant the clear solution into a tared capsule, evaporate at a water bath heat to constant weight and weigh. The yield from an average sample of drug should be 100 mg. or 2 per cent. of the root.

293. Assay of Podophyllin. The podophyllin of commerce varies greatly in color. There are two principal varieties, one of a bright yellow color, said to be due to the use of alum in precipitating the resin, the other of a fawn color, which we may assume has been made by the official process. The German pharmacopœia requires that podophyllin shall dissolve completely in 10 times its weight of alcohol, or in 100 times its weight of solution of ammonia, sp. gr. 0.960. These requirements are not strictly met by the podophyllin of American manufacturers, and are too exacting. Except by eliminating the oily constituent, it seems to be impossible to produce an article which will dissolve completely in alcohol. The requirements of the German pharmacopœia have no relation whatever to the proportion of active constituent present in the resin.

294. To make a complete analysis of a sample of podophyllin: (1) Determine mineral matter in 0.5 grm. by igniting in the usual manner. (2) Determine the oily constituent in 2.5 grm. of the sample, by exhausting with petroleum ether, evaporating and

weighing. (3) Exhaust the residue from the preceding operation with chloroform by maceration and percolation. Dissolve the residue in alcohol, evaporate in a tared capsule to constant weight [the residue is apt to hold chloroform persistently], and weigh as total constituents insoluble in chloroform. (4) Evaporate the chloroformic solution in a tared capsule to a thin syrup, and add ether free from alcohol as long as it produces further precipitation. Decant the clear fluid from the agglutinated precipitate, wash this with a little ether, dry and weigh as podophyllinic acid. (5) Evaporate the ethereal solution in a tared capsule to constant weight, and weigh as podophyllotoxin. It is this last estimation alone that is really important. The proportion of podophyllotoxin, estimated in this manner, is generally between 40 and 45 per cent. of the whole.

295. The assay of **tinctures** or **fluid extracts** of mandrake may be made by reducing to a small volume and pouring into water containing a little hydrochloric acid. The precipitated resin is to be collected carefully, dried and weighed, to give an approximate indication of the value of the preparation. The portion of this resin soluble in chloroform serves, however, as a better criterion of its quality. The solubility of the resin and of its active constituent in water must of course be taken into account in making this estimation, but the exact value of the constant for correcting the results obtained remains yet to be experimentally determined.

QUEBRACHO BARK.

296. Until the several alkaloids of quebracho shall have been more carefully studied, it must suffice to estimate the total alkaloids in the bark as indicating its value. This may be done by either of the following methods:

297. Process A. Exhaust 10 grams of the finely powdered bark with a mixture of 2 volumes of alcohol with 1 of water, by percolation. Evaporate to a small bulk, add a few drops of dilute sulphuric acid and a little dilute alcohol to dissolve the most of the deposited matter. Pour the clear solution into a measuring flask containing about 40 cc. of strong alcohol, and rinse the dish with alcohol, using sufficient to make up the volume to 50 cc. Add a gram or two of freshly slacked lime, shake well together, filter, and take for the assay 25 cc. of the filtrate, equivalent to 5 grams of drug.

298. Add sulphuric acid to faint acid reaction, evaporate to a small volume, transfer to a 1 oz. vial, using a little ether and water, applied alternately, to rinse the capsule. Wash the acid solution with several successive portions of ether, as long as this solvent removes anything. Render alkaline with ammonia, and wash out the alkaloid by shaking with several successive portions of a mixture of chloroform 1 volume, and ether 3 volumes. It is not easy by this method to extract completely the alkaloid, which is removed somewhat more readily by pure chloroform. An approximate result may be obtained by making up the volume of the aqueous acid solution to 40 cc., and titrating with Mayer's reagent, $N\frac{1}{20}$. One cc. of the reagent precipitates about 10.5 milligrams of alkaloid under these conditions.

299. Process B. Assay by acid. Take for the assay 10 grams of quebracho bark in fine powder. Mix 1.3 grams of strong hydrochloric acid with 13 cc. of water, and moisten the powder with the mixture. Let stand 24 hours, adding a little more water if necessary. Transfer to a small percolator, and exhaust with water, of which 150 to 200 cc. will be required. Partially neutralize with potassa and evaporate to a small volume. Add strong alcohol to make up a volume of 50 cc. Shake the solution with 1 gram of freshly slacked lime, filter, take of the filtrate 25 cc., representing 5 grams of drug, and proceed as described (298).

RHUBARB.

300. Active Constituents. The drug contains 1. Cathartic acid; 2. chrysophan; 3. emodin; 4. often chrysophanic acid, derived from decomposition of the chrysophan; 5. tannic acid of a peculiar variety. According to Dragendorff, cathartic acid is the purgative principle in rhubarb, as it is in senna. The tonic action of the drug, this writer ascribes to tannin, but he finds in the powerful antiseptic action of chrysophan and allied substances (emodin, erythroretin, phœoretin etc.) the explanation of the remarkable influence rhubarb exerts in many cases of intestinal catarrh. It is evident that with our imperfect knowledge of the action of this drug, we cannot as yet form any very exact estimate by assays of the relative value of different specimens.

301. Dragendorff's Process of Assay.* Macerate 5 grams of rhubarb in No. 30 powder, with water sufficient to make a volume of 100 cc., with frequent shaking, two days. Allow the powder to subside a few hours, and filter the supernatant fluid. By evaporating 10 cc. of this solution the amount of aqueous extract may be determined, but this is of no importance.

302. Cathartic Acid. Mix 20 cc. of the filtrate with 60 cc. of strong alcohol, let stand 48 hours, collect the precipitated gum on a filter and wash with alcohol. Distil off rapidly, in a partial vacuum, the alcohol from the filtrate and washings, reducing the volume of fluid to 5 cc. Mix this with 40 cc. of absolute alcohol, which will precipitate the cathartic acid, together with some inorganic salts. Collect the precipitate on a pair of mutually counterpoised filters, wash with absolute alcohol, dry

* Pharm. Zeitung, Feb., 1878. Phar. Jour. and Trans., Apr. 20, 1878, p. 826.

and weigh. The ash may be determined and deducted for exact estimations, but the quantity is only one or two milligrams.

303. Tannin and Chrysophan. To another 20 cc. of the aqueous infusion, a slight excess of copper acetate is to be added, and the precipitate collected with as little delay as possible on a tared filter, washed with a little water, dried and weighed. It is now to be ignited, with aid of ammonium nitrate, in a porcelain crucible, and the ash (oxide of copper) to be deducted from the weight of the precipitate, to give the combined tannin and chrysophan. (Instead of making this ignition, multiply the weight of the precipitate by 0.732 for an approximate result.)

304. Resinous substances, including chrysophanic acid, emodin, erythroretin, phœoretin, etc. Digest the dried marc left after extracting completely with water, in absolute alcohol six days, filter, and wash the residue with alcohol. Evaporate the alcoholic solution to obtain total resinous constituents of the drug.

TOBACCO.

305. The estimation of nicotine in tobacco may be conveniently made by the following method, after Dr. J. Skalweit.* The tobacco is dried at 50° C. (122° F.), finely powdered, and the moisture estimated in a weighed sample. Put into a flask 20.25 grams of the powder, with 200 cc. of 98 per cent. alcohol and 10 cc. of normal sulphuric acid, connect with a reversed condenser, and boil two hours. Transfer the liquid when cool to a measuring flask of 250 cc. capacity, rinse the flask with several successive small portions of absolute alcohol to obtain the required measure of 250 cc.; 100 cc. of the clear liquid are placed in a flask provided with a funnel tube, terminating near the bottom in a fine point, and with a bent tube for carrying off the alcoholic vapors; the greater portion of the alcohol is distilled off, 40 cc. of solution of potassa, sp. gr. 1.149, are added, and the distillation is continued until the liquid dropping from the condenser shows no reaction on litmus paper. The distillate is titrated with $\frac{1}{10}$ normal sulphuric acid, and by dividing the cubic centimeters found by five, the percentage of nicotine in the tobacco examined is ascertained. The absence of ammonium sulphate is proved by evaporating to dryness and dissolving in 98 per cent. alcohol. To obtain accurate results it is recommended that a slow current of hydrogen gas be passed through the distilling flask during the progress of the distillation.

306. Method of R. Kissling.† The tobacco is stripped, cut, dried for one or two hours at 50° to 60° C. (122° to

* Am. Journal of Pharmacy, Feb., 1882, p. 60, from Archiv. d. Pharm., July, 1881, pp. 36-41.

† Zeitschrift für Anal. Chemie. XXI, No. 1.

140° F.), and reduced to a coarse but uniform powder. Twenty grams of this powder are carefully moistened with 10 cc. of a dilute alcoholic solution of sodium hydrate (6 grams sodium hydrate, 40 cc. of water, and 60 cc. of alcohol); the moist powder is enveloped in filter paper, introduced into an extraction tube, and extracted with 100 cc. of ether for two or three hours. The ether is carefully and not quite completely distilled off, the residue mixed with 55 cc. of dilute aqueous solution of sodium hydrate, 4:1000, and subjected to distillation in a current of steam, the distillation being carried on as energetically as possible until 400 cc. of distillate are obtained in four portions of 100 cc. each, which are titrated with sulphuric acid, rosolic acid being used as indicator.

The writer has had no experience in the use of either of the above methods of assay, nor indeed with any other. He would be strongly inclined to try Prollius' fluid for the extraction of the alkaloid, but the details of the assay could only be worked out experimentally.

VERATRUM VIRIDE.

307. Active principles of the drug. The several species of veratrum contain a number of alkaloids, which are as yet only imperfectly known. The more important of these, according to Messrs. Wright and Luff, are **cevadine,** identical with Merck's veratrine; **veratrine,** identical with the base so name by Courbe, but not the veratrine of commerce, which is a mixture of several alkaloids, nor yet the pure veratrine of Merck; **cevadilline,** peculiar to cevadilla seed; **jervine** and **veratralbine,** the last being the most abundant alkaloid in white hellebore. According to these authors, cevadine and jervine are the important alkaloids of American hellebore. Charles Bullock inclines to the view that jervine is the only alkaloid present.

308. In absence of any exact informatian with regard to the relative therapeutic activity of these several alkaloids, and of any detailed method of assay by which the alkaloids may be separately estimated, we must be content at present to form our judgment of the relative value of samples of the drug, or of its preparations, by roughly estimating total alkaloids. The alkaloids may be extracted from the powdered drug by the modified Prollius fluid (14), but the writer has not worked out the details of the assay process, although these will be essentially those of the general assay processes described (29 *et seq.*). It must be remembered that jervine resembles berberine in the circumstance that it is precipitated by mineral acids, and care must be taken not to lose the alka'oid on this account.

ASSAY OF GALENICAL PREPARATIONS OF VERATRUM VIRIDE.

309. Method of H. W. Snow.* The comparative value of different samples of fluid extract green hellebore may be approximately judged by titration with Mayer's reagent. Place in a small flask 15 cc. of the fluid extract, add 5 grams of powdered pumice stone and 60 cc. of water containing ½ to 1 per cent. of acetic acid. Shake together and filter through a dry filter paper. Take of the filtrate 50 cc., equivalent to 10 cc. of the fluid extract, render nearly neutral with lime or magnesia, and concentrate to a volume of 10 cc. Transfer the concentrated solution to a separator, dissolve any substance that may have deposited during the evaporation by aid of a little ammonia, acidify with acetic acid, and add to the contents of the separator. Render the solution alkaline with ammonia, and wash out alkaloid with chloroform, using several successive portions. Evaporate the chloroformic solution on the water bath, dissolve the residue by aid of a little acetic acid in water, making up the volume to 10 cc., and titrate with Mayer's reagent ($N\frac{1}{20}$).

310. The average titration equivalent of the mixture of alkaloids obtained from this drug has not yet been experimentally determined. We may assume it to be in the vicinity of 15 milligrams. The average result obtained by Mr. Snow in his examination of commercial fluid extracts was 6.26 cc. of reagent ($N\frac{1}{20}$) for precipitation of the alkaloids of 10 cc. of the fluid. According to my own observation the quantity of reagent required ranges from 5 to 15 cc. in different samples of the drug, the usual range being, however, from 5 to 10 cc.

311. Alternative process. Mix 15 cc. of the fluid extract with 60 cc. of alcohol containing about 6 cc. of a saturated solution of lead acetate, shake, and allow to stand a few minutes. Filter through a dry filter, take 50 cc. of the filtrate, equal to 10 cc. of the fluid extract, remove excess of lead by H_2S, evaporate

* Paper read before Mich. Pharm. Assn., 1886, not heretofore published.

to expel alcohol, add water, with a little acetic acid, to make up a volume of 10 cc , and titrate as above.

312. The drug itself may be assayed obviously by the same general method, by exhausting it first with strong alcohol, by percolation, and concentrating to about the strength of an ordinary fluid extract, and proceeding exactly as described above. No doubt the drug may be advantageously exhausted by hot repercolation in an extraction apparatus with an appropriate solvent, perhaps the mixture of chloroform and alcohol, which proves so successful in the exhaustion of belladonna root, but no experiments have as yet been made in this direction.

313. Estimation of Jervine. Since jervine is probably the most important alkaloid present, the drug may be provisionally valued by an estimation of this base. Advantage is taken, to make this estimation, of the almost complete insolubility of jervine nitrate in a solution of potassium nitrate. The process is conducted by Mr. Snow in the following manner: Dissolve the crude alkaloid obtained as above described (209) in dilute acetic acid, filter the solution and add an equal volume of a saturated solution of potassium nitrate. Set by for 12 hours, then collect the jervine nitrate on a pair of mutually counterpoised filters, wash with a solution of potassium nitrate, and finally with a little water, press between blotting paper, dry at 75° to 80° C. (167° to 176° F.) and weigh. Assuming that the nitrate is an anhydrous salt of normal composition, we shall not be far out of the way if we assume that 89 per cent. of the weight of the precipitate is alkaloid. Simons' formula would make the factor 0.8844. Tobiens' would make it 0.8932. Mr. Snow obtained an average of 0.345 per cent. of jervine from eight samples of fluid extract, the range being between 0.233 and 0.485.

AMYL NITRITE.

314. Method of the Author,* based on Allen's method of estimating ethyl nitrite in spirit of nitrous ether. Either the nitrometer, or a burette, preferable one with a glass stop cock, may be used for the assay, or a 4 oz. bottle may be employed. If the burette is used, provide for the experiment (1) a saturated solution of common salt, (2) a 10 per cent. solution of potassium iodide, (3) some dilute sulphuric acid, (4) a hydrometer jar, or tall bottle, can or other container.

315. Pour into the hydrometer jar 150 cc. of the salt solution. Invert the burette in the jar, and draw up by suction enough of the brine to fill it completely to the stop cock. Fit to the point of the burette by the aid of a short piece of rubber tube a small funnel. Pour into this 5 cc. of a mixture of 1 volume of the amyl nitrite with 19 volumes of alcohol. By cautiously opening the stop cock allow this to enter the burette, being careful that no air enters with it. Rinse the funnel with 2 or 3 cc. of alcohol, and allow this also to enter the burette.

316. Introduce in the same manner in succession 5 cc. of the iodide of potassium solution and 5 cc. of the dilute sulphuric acid, taking care in each case to admit no air. This in practice will be found very easy. The moment the acid is admitted a vigorous reaction takes place, accompanied with effervescence, and separation of free iodine. Shake the upper part of the burette, taking care not to permit any air to enter from below. Let stand a few minutes. Pour carefully into the hydrometer jar in such a manner that it may run quietly down the side water enough nearly

* Pharmaceutische Rundschau, June, 1886, p. 135.

to fill it; raise the burette, allowing the brine to flow out, its place being taken by water. Now bring the level of the fluid within the burette into coincidence with that of the water outside, by raising or lowering the burette, and read off the volume of gas resulting from the reaction.

317. Since burettes are graduated from the top instead of the bottom, it will be necessary to take as the volume of the gas collected the difference between the contents of the instrument as graduated and the "reading" of the burette, *plus* the capacity of the ungraduated portion of the burette below (above) the scale, which must of course be ascertained by preliminary experiment. For example: Suppose the burette to be graduated to 100 cc., and to contain below the 100 mark 10.25 cc., and that the gas fills it in the inverted position to the mark 65.8, then 100.−65.8=34.2; add 10.25 and we have 44.45 as the measure of the gas.

318. Each cc. of the gas, measured at ordinary temperature and pressure, indicates a trifle less than 5 milligrams of amyl nitrite. To obtain percentage, multiply the volume of gas in cc. by 2.25, or, more exactly (after making corrections for temperature and pressure), multiply by 1.983 and divide by the specific gravity of the sample. In case the nitrites of ethyl, propyl or butyl are present, the results will of course be high, but these probably are not frequent impurities in this product.

319. In absence of a burette, the experiment may be made as follows: Fit a 4 oz. bottle with a cork having two perforations. Through one of these insert a short piece of glass tubing, fitted above by a short piece of rubber tubing with a small funnel. The tubing is to be provided with a pinch-cock. The second opening of the cork is to be fitted with a glass tube reaching to the bottom of the bottle inside, and bent, outside, at a right angle. To this a rubber tube is to be attached, long enough to reach the table on which the apparatus is placed. This we will call the overflow tube.

Fill bottle and tubes completely with brine. Place the extremity of the overflow tube in an empty graduated jar, and proceed to introduce into the bottle through the funnel the amyl nitrite and reagents, one after another, exactly as when the burette is used, keeping an exact record of the volume of fluid which is thus introduced. As the gas is evolved, a quantity of brine of corresponding volume is expelled through the overflow tube. At the end of the experiment, before reading off the volume of this fluid, lift the graduate, or depress it until the level of the fluid in it coincides with that of the mixture in the bottle, then pinch the overflow tube, and lift it out and read off the correct volume of fluid collected in the graduate. From this deduct the volume of the fluid, reagents, etc., that have been introduced into the bottle, and the remainder is the volume of gas evolved. The result is calculated as before. If a metric graduate is not at hand, divide the measure in minims by 16.2312 to obtain result in cc.

SPIRIT OF NITROUS ETHER.

320. The method of assay prescribed by the Pharmacopœia is troublesome, and not trustworthy. Its object is to ascertain the proportion of nitrous ether present in the preparation. It is not certain that the therapeutic value of "sweet spirit of nitre," as made by the old approved processes, depended wholly, or even mainly, on the proportion of this ether present, but in the U. S. P. preparation this is taken for granted, and the assay must be based on the same assumption.

321. Process of A. H. Allen.* To ascertain the proportion of nitrous ether present in a sample of the spirit, it is decomposed by potassium iodide in presence of sulphuric acid, nitric oxide (N O) being given off, as shown by the following equation:

$$(C_2 H_5) NO_2 + K1 + H_2 SO_4 = (C_2 H_5) OH + KHSO_4 + I + NO$$

Each cc. of the gas measured at ordinary temperature and pressure, corresponds with 0.0031756 grm. ethyl nitrite.

322. If we allow 1.5 cc. for solubility of the gas evolved, we must expect from 5 cc. of a spirit containing 4 per cent. of nitrous ether, 50.5 cc. of gas. We may say, therefore, that where 5 cc. of the spirit is used for the test, as is commonly recommended, the percentage strength of the preparation, as compared with standard, is obtained by simply multiplying the volume of gas obtained in cc. by 2. Thus, if we obtain 25 cc. of gas, we say that the specimen contains 50 per cent. of the standard quantity of acid. The actual per cent. by weight of nitrous ether will be found approximately by multiplying the volume of gas in cc.

* Phar. Jour. and Trans., Feb. 21, 1885, p. 673. The process is a modification of that of Prof. J. F. Eykman.

by 8, and moving the decimal point two places to the left. (Theoretically, multiply cc. of gas by 0.0635 and divide by specific gravity of the spirit.)

323. The assay may be made in an instrument constructed on purpose, and called a nitrometer, but it may be carried out equally well in the same manner as already explained in the account of the assay of amyl nitrite (314 *et seq.*), using 5 cc. of the undiluted spirit for the experiment. The assay operation requires scarcely five minutes' time, and the results are conclusive. The only fault that can be found with it is that it will oftener than not condemn the article on which it is made to give judgment, but this is only because its judgment is inflexibly just.

PEPTONE AND PEPSIN.

324. To estimate Peptone in an extract of meat or any similar preparation, prepare a standard solution of pure peptone 1:1000. Dissolve 1 gram of the extract of meat in cold water, and make up the solution to 100 cc. Filter. Put 10 cc. of the filtrate into a narrow test tube, and into a similar tube put 10 cc. of the standard solution of peptone. Add to each a few drops of a solution of caustic soda or potassa (5 per cent.), and then, drop by drop from a graduated minim pipette a solution of sulphate of copper, 1:500, shaking after each addition, and observing the effect on the color of the fluid.

325. It will be found that the solution of pure peptone assumes as the copper solution is added a delicate pink color, deepening up to a certain point, when it becomes suddenly violet, and, on continued addition of the reagent, will pass into a violet blue. The addition of copper solution must be stopped as soon as the color becomes at all violet, and the quantity added noted. This will be found to be about 32 minims.

326. The solution prepared from the extract may be already of a rather deep color, so that it is not so easy to tell exactly when the violet color first appears. If 30 minims of reagent produce it the quantity of peptone is small—not exceeding 10 per cent, and if the initial color of the solution is dark it may not be possible to make a satisfactory exact comparison. We will suppose, however, that the preparation is rich in peptone, and that it is found necessary to add 100 or 125 minims of the reagent to obtain a violet or blue shade of color.

327. Dilute a portion of the solution to exactly four times its volume, and test 10 cc. in the same manner as before. It now

requires 32 minims of the reagent to change the color perceptibly to violet. Compare the depth of tint with that of the peptone solution. If it is paler, repeat the experiment, using 12 cc. (or sufficient) of the solution. If the color is deeper, remove from the test tube a portion of the mixture until the tint, in looking down the two tubes, is as nearly as possible the same. In this way ascertain just how much of the solution is required to produce the same depth of color as in the standard solution.

328. Since our solution of the extract now contains 1:400, divide 400 by the quantity just found, expressed in cc., and the quotient is percentage of peptone in the original preparation. Thus if we had found 9.5 cc. of the solution sufficient, the percentage will be $400 \div 9.5 = 42.13$. If 11 cc. had been required, the percentage indicated would be $400 \div 11 = 36.36$.

329. An exact comparison of tints is generally not possible, from the circumstance that the solution has more or less color in the outset. This does not prevent us from drawing conclusions from the experiment that are sufficiently near the truth for our purpose. The quantity of copper solution used affords us a useful check on the correctness of our results, and in case the color of the solution is so dark as to render accurate comparison of tints impossible, we may base our conclusion wholly on this datum. This is especially true in dealing with specimens poor in peptone. This method gives, of course, only a rough approximation to the truth, and is liable to fallacy, especially if we have to deal with complex fluids.

330. The colorimetric method, indeed, is not of universal applicability, and it completely fails in certain cases. Extract of malt, for example, prevents the development of the characteristic color. No careful study has, however, been made as yet of the cause of this failure, or the method of overcoming the difficulty. In using the test, its limitations must always be borne in mind.

331. Defresne* estimates Peptone in solutions

* Repertoire de Pharmacie, Vol. 14, p. 262.

free from gelatin, dextrin, etc., by pouring the concentrated solution (1 part) into a mixture of absolute alcohol 10 parts, and concentrated ether 5 parts. The precipitated peptone is allowed to subside completely, the clear fluid decanted, and the peptone dried at 100° C. (212° F.) and weighed. A correction, equal to $\frac{1}{10}$ the weight of the solution, should be added for solubility of peptone in ether alcohol. This correction, I believe, is excessive.

332. In case **gelatin** is present, this must be separated from a second portion of the solution, by adding, at a boiling temperature, magnesium sulphate as long as it is dissolved. The gelatin separates as an elastic viscous mass, which can be easily separated. It cannot, however, be weighed directly. The nitrogen in it must be estimated and deducted from the total nitrogen separately estimated, and the difference multiplied by 6.05 to obtain the quantity of peptone. In case dextrin or other similar substances are present, the estimation of peptone must be made in a similar indirect manner.

333. Estimation of peptone by the colorimetric method promises to find a useful application in the valuation of **pepsins.** It has been customary to estimate the activity of pepsin by causing it to act on coagulated albumen, in presence of hydrochloric acid, under prescribed conditions of temperature, etc. [as in the U. S. P. test], and observing how much of the albumen is dissolved. The test is fallacious, in that mere solution of the albumen is not evidence of peptonization, and, in fact, it will be found that on neutralizing the acid solution at the close of the experiment a large proportion of the albumen, which has been converted simply into acid albumin, will be reprecipitated. The true test of the activity of a pepsin is its power of converting albuminoids into peptone.

334. Although I have not worked out the process in detail, I believe it will be entirely practicable to ascertain the activity of a specimen of pepsin by causing it to act, under rigorously prescribed conditions, on an excess of coagulated albumen, or of fibrin, and estimating the quantity of peptone produced. In case

the pepsin itself contains a large proportion of peptone, it may be necessary to estimate this and deduct it from the total amount present at the close of the experiment.

335. Assay of Pepsin, according to French Codex. In this connection the French pepsin test, which depends on peptonization of fibrin, may be given. It is as follows:

Put into a wide-mouthed flask 0.5 grm. of pepsin, with 60 cc. of distilled water, 0.6 grm. of hydrochloric acid (sp. gr. 1.171) and 10 grm. fibrin of pork in the fresh condition, well washed and freed from superfluous moisture. Digest six hours at a temperature of exactly 50° C. (122° F.), shaking the flask frequently until the fibrin is all dissolved, and after that at intervals of one hour. At the end of six hours add to 10 cc. of the filtered solution 20 to 30 drops of nitric acid (sp. gr. 1.39), which should not produce any precipitate, indicating complete peptonization of the fibrin. It will be seen that the test is a limitive one merely, and does not show the actual strength of the pepsin unless supplemented by other experiments, in which a smaller or greater quantity of pepsin is used.

336. The Official Pepsin Test. The U. S. Pharmacopœia requires that saccharated pepsin shall be of such strength that 1 part of it dissolved in 500 parts of water acidulated with 7.5 parts of hydrochloric acid shall be capable of dissolving at least 50 parts of hard boiled egg albumen in 5 or 6 hours, at a temperature of 38° to 40° C. (100° to 104° F.) The directions are not sufficiently explicit to give the test any value whatever. The same pepsin may be made to show a digestive activity of 100 or of 500, without deviating from the conditions laid down. The test is a defective one in the first place, because it depends merely on the dissolving of the albumen, without reference to whether it is changed to acid albumen, to peptone or to parapeptone. It is customary in making pepsin tests to use the white of an egg that has been boiled for a specified length of time, generally 15 minutes, and rubbed through a sieve having 30 meshes to the linear inch,

to dissolve the pepsin in acidulated water previous to adding the egg albumen, and to take care that the fluids at the beginning of the experiment have the standard temperature, and unless these conditions are definitely stated the results of the test are absolutely of no value.

337. The quantity of acid prescribed in the official test is too large to obtain a maximum solvent action from the pepsin, and many advocate the use of one-half the quantity. If this change is made, the requirement of the test as to the quantity of albumen to be dissolved should be raised to maintain the present standard. Many operators prefer to carry on the digestion at a temperature of 120° to 130°, the time being reduced to one-half hour, and this is no doubt much to be preferred, although it is not certain that the relative value of different samples of pepsin under such conditions would be the same as under those of the official test. In any case it is of the utmost importance, in comparative tests, that the temperature be maintained exactly at the standard selected. A range of 4° F., such as the U. S. P. test permits, is much too great.

338. Finally, the official process does not provide for any mode of estimating how much white of egg has been actually dissolved in the experiment. In practice it is necessary to use an excess of the albumen—but not too large an excess. At the close of the experiment the residue is to be collected on a filter, or preferably on a piece of muslin, drained, transferred to a tared capsule, dried at 100° C. (212° F.), and weighed. The weight multiplied by 7.5 will give approximately the weight of the undissolved albumen in its moist condition, and this is to be deducted from the quantity of albumen used in the experiment. Frequent agitation of the mixture during the process of digestion is an important detail that must not be forgotten.

The results obtained are, of course, only of comparative value. At present there is no standard of comparison that can be maintained as authoritative.

APPENDIX.

The following tables embody some of the more interesting details from records of analyses that have been made by myself and my associates during the past few months. In selecting the examples, I have aimed to present a fair average of what may be expected in examining the several drugs as they are offered in the market, but I have also included some exceptional extremes, to show the range of variation that may be met with:

ACONITE ROOT.

No.	Moisture.	Extractive yielded to alcohol.	Physiological Test.	10 grams require Mayer's reagent N 1-20.
1	8.9	10.5	1:500	5.6 cc.
2	9.3	10.6	1:700	6.4
3	8.5	13.2	.....	5.8
4	11.2	9.3	1:550	5.2
5	8.7	16.1	1:600	5.8
6	8 5	19.8	1:700	6.2
7	8.2	16.8	.....	10.8
8	9.4	12.5	.....	7.2
9	9.6	13.1	1:600	6.0
10	8.8	13 2	1:400	3.7
11	8.5	14.0	1:800	8.0
12	9 8	12.3	1:600	6.1
13	10.2	12.2	1:500	4 6
14	10.5	12 5	1:600	5 0
15	9.8	13.2	1:650	6.6
16	10.1	13.8	1:600	6.2
17	8.8	16.0	1:650	6.1

GUARANA.

No.	Alkaloid.	No.	Alkaloid.
1	4.5	5	6.5
2	4.1	6	3.9
3	4.6	7	5.0
4	5.1	8	5.5

BELLADONNA LEAVES.

No.	Moisture.	Per cent. extractive yielded to 66 per cent. alcohol.	10 grams require Mayer's reagent N 1-20.	Alkaloid by titration.	Alkaloid weighed.
1	8.2	10.2	15.7 cc.	0.64	0.43
2	....	7.0	17.6	0.44	.....
3	.....	12.1	19.5	0.75	.....
4	.....	7.6	17.0	0.47	.....
5	8.5	6.7	20.5	0.41	.....
6	.....	6.8	19.2	0.44	.....
7	12.2	6.6	30.7	0.42	0.40
8	....	7.6	25.5	.....	0.42
9	....	.....	22.8	....	0.41
10	....	.....	21.2	....	0.69
11	9.4	.....	23.8	.....	0.52
12	10.6	....	17.5	.....	0.86

BELLADONNA ROOT.

No.	Moisture.	Extractive yielded to 66 per cent. alcohol.	10 grams require Mayer's reagent N 1-20.	Alkaloid by titration.	Alkaloid extracted and weighed.
1	9.2	30.1	14.4 cc.	0.90	.....
2	7.8	23.4	8.6	0.84	.....
3	8.5	29.3	24.4	1.52	.....
4	5.6	22.5	10.8	0.67	.....
5	8.4	24.7	7.6	0.47	.....
6	9.3	23.1	10.8	0.67	.....
7	8.9	26.2	13 8	0.86	.....
8	7.5	26.7	21.6	1.35	.....
9	9.2	31.5	12.6	0.79	.....
10	8.8	24.8	9.4	0.59	.....
11	6.8	23.5	8.6	0.54	0.55
12	8.5	31.2	10.2	0 64	.....
13	9.0	27.2	.....	.....	0.52
14	8.4	25.7	9.6	0.60	.....
15	8.2	25.9	.....	.....	0.61

COCA LEAVES.

No.	Alkaloid.	No.	Alkaloid.
1	0.46	5	0.92
2	0.65	6	0.64
3	0.57	7	0.79
4	0.76	8	0.72

CALABAR BEAN.

No.	Extractive yielded to strong alcohol.	Total alkaloid extracted by ether.	No.	Extractive yielded to strong alcohol.	Total alkaloid extracted by ether.
1	3.9	0.23	8	2.0	0.19
2	2.7	0.20	9	1.8	0.15
3	2.3	0.21	10	.....	0.18
4	2.5	0.25	11	.....	0.25
5	2.3	0.23	12	.....	0.15
6	1.9	0.20	13	.....	0.18
7	2.3	0.21	14	2.7	0.19

CINCHONA BARK.

No.		Total alkaloids.	Quinine.
1	"Calisaya"	4.5	2.2
2	"	3.0	1.7
3	"	5.1	3.3
4	"	2.2	0.2
5	"Pale"	2.8	.....
6	"	3.7	.....
7	"	1.8	.....
8	"	4.3	.....
9	"Red"	5.4	2.8
10	"	6.5	3.5
11	"	4.2	1.2
12	"	8.8	2.5

VERATRUM VIRIDE.

No.	Per cent. extractive yielded to strong alcohol.	10 grams require Mayer's reagent N 1-20.	Per cent. jervine.
1	10.8	7.3 cc.	.....
2	13.3	16.0	.....
3	11.5	10.2	.....
4	10.3	9.4	.....
5*	.....	4.5	.246
6*	.....	11.1	.283
7*	.....	7.3	.469
8*	.....	10.0	.485

* Fluid extracts, examined by H. W. Snow.

COLCHICUM ROOT.

No.	Extractive yielded to 66 per cent. alcohol.	10 grams require Mayer's reagent N 1-20.	Alkaloid by titration.
1	17.8	7.2 cc.	0.72
2	17.3	6.2	0.62
3	16.4	8.4	0.84
4	16.2	5.0	0.50

COLCHICUM SEED.

No.	Extractive yielded to 66 per cent. alcohol.	10 grams require Mayer's reagent N 1-20.	Alkaloid by titration.
1	15.2	8.4 cc.	0.84
2	20.3	7.8	0.78
3	19.5	6.2	0.62
4	20.8	10.6	1.06
5	15.5	8.4	0.84
6	14.3	9.0	0.90

STRAMONIUM LEAVES.

No.	Per cent. extractive yielded to 66 per cent. alcohol.	10 grams require Mayer's reagent N 1-20.	Per cent. alkaloid by titration.
1	20.2	7.2 cc.	0.45
2	25.3	6.8	0.43
3	24.5	7.6	0.48
4	21.0	6.5	0.40
5	19.5	6.9	0.43
6	23.8	8.4	0.52
7	21.5	7.2	0.45
8	22.1	7.5	0.47

STRAMONIUM SEED.

No.	Per cent. extractive yielded to strong alcohol.	10 grams require Mayer's reagent N 1-20.	Per cent. alkaloid by titration.
1	6.2	8.5 cc.	0.53
2	7.5	8.2	0.51
3	3.3	5.3	0.33
4	6.5	7.2	0.45
5	6.3	8.8	0.55

GELSEMIUM.

No.	Per cent. extractive yielded to 66 per cent. alcohol.	10 grams require Mayer's reagent N 1-20.	Per cent. crude alkaloid by weight.
1	13.2	8.5 cc.	0.27
2	15.4	5.7	....
3	16.1	9.7	....
4	13.5	6.4	.....
5	10.0	5.2	. .....
6	15.3	8.1	0.25

HENBANE.

No.	Per cent. extractive yielded to 66 per cent. alcohol.	10 grams require Mayer's reagent N 1-20.	Per cent. alkaloid deduced from titration.
1	17.5	4.2 cc.	0.26
2	19.5	4.3	0.27
3	15.5	2.2	0.14
4	22.5	3.8	0.24
5	25.4	4.4	0.28
6	13.6	3.7	0.23
7	18.5	2.5	0.16
8	21.0	2.9	0.18

NUX VOMICA.

No.	Alkaloid.	No.	Alkaloid.
1	2.68	5	2.15
2	3.11	6	3.17
3	2.72	7	2.75
4	3.13	8	4.86

QUEBRACHO.

No.	10 cc. require Mayer's reagent N 1-20.	No.	Alkaloid.
1	11.3 cc.	5	1.22
2	9.6	6	1.81
3	13.3	7	1.65
4	11.5	8	1.43