Herman L. Emmick 1/99

HAND BOOK

OF PRACTICAL

ASSAYING OF DRUGS

AND

GALENICALS

A Manual for the Pharmaceutical Student, and a Guide to the Pratical Pharmacist who has occasion either to standardize his own preparations or to test the Drugs and Galenicals he dispenses.

—BY—

A. B. LYONS, F. C. S.

DETROIT.
NELSON, BAKER & CO., Publishers.
1899.

ERRATA.

P. 2, l. 1 for Marme's read Marmé's

P. 30. l. 1, 2. for measured volume read weighed quantity.

P. 30. l. 5. for volume read weight.

P. 55. in table for 233 read 333 as the normal equivalent of strychnine.

P. 71. l. 16. for hydiodic read hydriodic.

P. 168. l. 2 from bottom. for (312) read (311).

PREFACE.

Twelve years have passed since the Author published his Manual of Practical Pharmaceutical Assaying. During the greater part of the intervening time he has been occupied in a different line of work. Others, however, have been engaged diligently in bringing under more complete cultivation the field in which his work was so largely of a pioneer character, and the old Manual can no longer be said to be abreast with the times. Decidedly it is time for a new book.

The new book, like its predecessor, is addressed to the needs of the student and of the practical pharmacist. It aims to furnish simple and rapid assay processes by which the therapeutic efficiency of samples of a crude drug or of galenical preparations may be judged at least approximately. To ascertain exactly the proportion of each active constituent of a drug is often a task that baffles the skill of the professional chemist. Still, these drugs are constantly used by the physician, and supplied for such use by the pharmacist, and, while waiting for fuller knowledge, the latter at least is under obligation to give the full benefit of his confessedly imperfect knowledge towards the securing of the best results possible from medication.

It is gratifying to note that American pharmacists have done their full share in improving assay processes for alkaloidal drugs. The name of one foreigner, C. C. Keller, has

come into prominence since the former publication was issued and his improved processes have been very generally adopted, sometimes with modifications in the minor details. On this side of the water, Schwickerath has developed the perforator method, Lloyd has taught us the use of ferric hydrate in extracting alkaloids from complex organic mixtures Thompson having already given us the most useful sawdust wrinkle, and finally, not to extend this list unduly, Prescott and his collaborators have worked out, from scientific data, the periodide method for several important drugs.

Prominence is given throughout the book to the simple, procedures which may be carried out without elaborate apparatus, and which do not require much time. I have endeavored, at the same time, to describe in sufficient detail the methods adopted in the more fully equipped laboratories. A number of assay processes such as those for conium, (268) to (270), for nux vomica, (390) and (364), for sanguinaria (421) et seq. are published for the first time. The forms of apparatus figured on p. 18 are new, but these form a very small contribution to the art, still in its infancy, which is the subject of this little volume. Twelve years have wrought great and radical changes in the detail of its methods, still I shall be gratified indeed if, at the end of another twelve years, there shall survive of the assay processes of to day as much as remains of those approved in 1886.

Laboratory Nelson, Baker & Co.,

December, 1898.

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APPARATUS.

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 special apparatus are, however, indispensable, and the more important of these will be here enumerated for convenience.

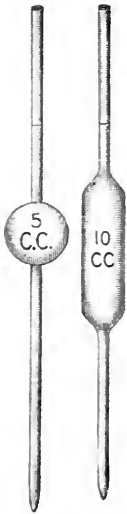

FIG. 1.

2. Graduated Pipettes. (a) *Measuring Pipettes* to deliver a given volume of fluid (fig. 1). Those most frequently used are the 5 cc. and the 10 cc. but the operator should be provided also with pipettes delivering respectively one, two and twenty cc. The tube of the smaller pipette should have an external diameter of not more than 5 mm. (3-16 in.), the portion below the bulb should be 12 to 15 mm. (5 or 6 in.) long; the mark should be at least 8 cm. (3 in.) from the upper end of the pipette and this should

be cut off square as in the illustration, not flared as they are often made. These pipettes are filled by cautiously drawing up the liquid into them by suction with the mouth until it rises a little above the mark. The fore-finger, which should be slightly moist, but not wet, is then dextrously applied to the opening at the top of the pipette, when the liquid will be prevented by atmospheric pressure from flowing out. The air is now to be allowed to enter by slightly relaxing the pressure of the finger, thus permitting the excess of fluid to escape. As soon as the level of the fluid comes exactly to the mark, the finger is once more firmly pressed on the orifice, and the contents of the pipette may then be conveyed safely to the desired receiver.

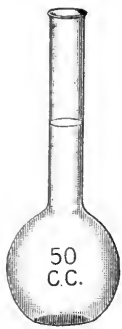

FIG. 2.

(b) *Graduated Pipettes.* Of these it is convenient to have one with a capacity of 10 cc. graduated to twentieths, and one or two graduated minim droppers, capacity about 2 cc.

3. **Graduated Measuring Flasks** (fig. 2) Those most useful are of a capacity resp. of 20, 25, 50, 100, 250 and 1000 cc. Dealers now supply at a moderate price ungraduated flasks precisely similar to these (having slender necks), which the operator can graduate for himself, marking them on the neck either simply with a file or by etching with hydrofluoric acid. It is convenient to have one or two measuring flasks graduated on the neck from 50 to 55 cc. and from 95 to 105 cc. The liter flask (1000 cc.) must be quite accurately calibrated; it is to be used for preparing volumetric solutions.

4. N.B.—All graduated apparatus, from whatever source

it is procured, should be tested before it is used. One liter of distilled water that has been recently boiled weighs at 15° C. (59° F.) 998.111 grammes. One cc. will therefore weigh practically 0.998 grm. To test a pipette or flask then, measure with it into a tared beaker or flask distilled water having a temperature of 15° C. weigh and see how nearly the actual coincides with the calculated weight. Make at least three tests of each piece of apparatus and take the mean of the weights, which should not differ from the theoretical by more than 20 mg. Of course we cannot expect the accuracy of the balance in measurements made with apparatus of this kind.

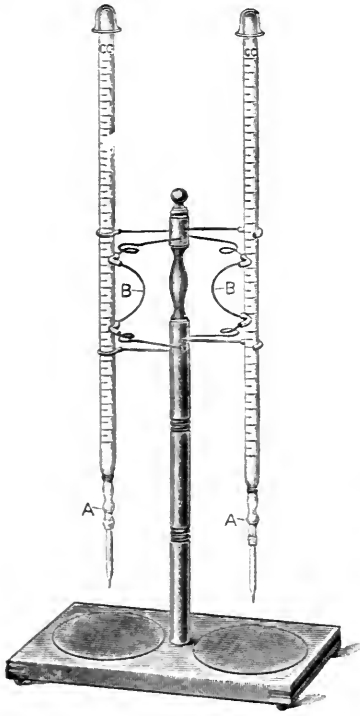

FIG. 3.

5. Graduated Burettes. For volumetric work at least two good burettes (fig. 3) will be required; those of 50 cc.

capacity graduated to tenths are most serviceable. Preference should be given to those provided with a blue or black enameled strip on white ground, running the length of the instrument at the back. The effect of refraction at the surface of the liquid in the burette is to produce an apparent break in this band as shown in the illustration (fig. 4), thus rendering possible very exact readings without the use of the float. The most convenient burette holder is that shown in the figure, the instrument being held quite firmly by the wires (shown at B in the figure, covered where they grasp the burette with rubber tubing) yet readily raised, lowered or removed. The bead valves shown at A in the figure are convenient, although they are a little liable to leak. A ground glass stop-cock is best of all, but is liable to be broken. The burette should be provided with a glass cap as shown in the figure, or else should be corked except while in actual use. Of course reagent should not be allowed to remain an indefinite time in the burette. Unless assays are daily made it is best to empty the burette after each operation and refill when occasion requires. The plates of white porcelain set into the foot board of the stand are a convenience when color indicators are used.

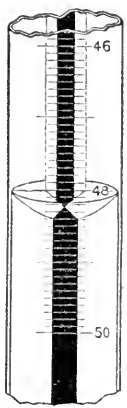

FIG. 4.

6. Burettes to be used for accurate volumetric work must be tested throughout the scale. Run out 2 cc. of distilled water at 15° C. into a tared flask and weigh, add 2 cc. more and weigh again and so on throughout. Repeat this

tedious operation three times and record the averages obtained. For refined work select a portion of the scale and test for every half cc. or even for every 2-10 cc. and construct for this portion of the scale a table giving the value of each fraction so determined. My own recommendation is to ascertain simply whether the instrument is or is not uniformly graduated and reasonably correct. If it is not, reject it, as the labor of correcting all readings of the instrument is very burdensome. There is a much easier way to secure accuracy. Instead of measuring the fluid used in a titration *simply weigh it*. Determine the weight of 1 cc. of the reagent at ordinary room temperature, and then, instead of running it out of a burette, put it in a dropping flask, weigh this at the beginning and at the end of the titration, and divide the difference in weight by the known weight of 1 cc. of the reagent. If the weighing plan is generally adopted, it is well to standardize the solution to a weight of 1000 grammes, instead of a volume of 1000 cc. It is not necessary to use the analytical balance in these weighings. It is impracticable to make additions of less than 20 or 30 mg. at once. Hence a weighing within 5 mg. is as accurate as the method can possibly demand and such a weighing can be made on a good prescription balance.

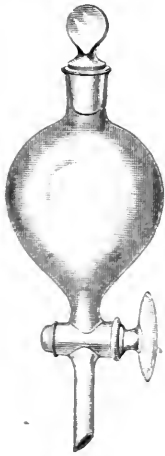

FIG. 5.

7. Separating Funnels. No piece of apparatus is more frequently called into requisition in the estimation of alkaloids

than the separator (fig. 5). The operator should have at least three. Those most serviceable are of a form approximating the globular, capacity 75 to 100 cc. The delivery tube should be quite short, not too narrow and cut off obliquely at the end. It is well to be provided with some larger ones, 150 to 250 cc. capacity. The pattern designed by Dr. Squibb (fig. 6.) has some advantages and is certainly to be preferred to that in which the bulb tapers into a long narrow neck above the stop-cock.

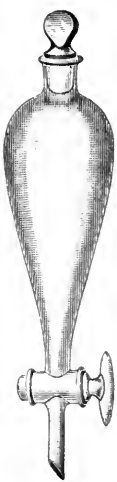

FIG. 6.

8. 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 for use in exhausting crude drugs by simple percolation. Smaller ones even than this are sometimes useful, and may be easily made from the barrels of glass syringes, the tops being cut off, if necessary.

9. Apparatus for Hot Repercolation. For the rapid exhaustion of a vegetable powder by a volatile solvent, there is no process to be compared with hot repercolation. Various forms of apparatus have been devised for this purpose and are illustrated in the catalogues of dealers. The most efficient is the Soxhlet apparatus (fig. 7). In use, the tube shown in the figure is connected below with a flask and above with an upright condenser. The drug to be exhausted is packed in a test tube having an opening in the bottom, closed by a plug of asbestos or glass wool, a d the whole is inserted into the barrel of the Soxhlet tube, or else

the drug in fragments is simply wrapped in muslin and placed in the 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 used 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 the form of a liquid, dropping into the extraction tube. When this tube is filled to the level of the bend in 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. 7.

10. An apparatus for exhausting small quantities of drug by hot repercolation may be extemporized as follows: Select a pint flask having a rather long wide neck. As a percolator use the barrel of a half-ounce glass syringe, about 15 mm. (5-8 in.) in diameter, or a piece of glass tubing long enough to reach within 3 or 4 cm. (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 soft 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 oil of turpentine. make an opening in the upper part of the percolator, just below the joint, to allow the vapor to pass up into the condenser. Fit the percolator with a perforated cork through which to pass the point of the tube of an upright condenser and the apparatus is complete (fig. 8.)

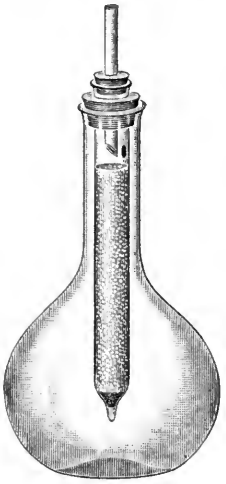

FIG. 8.

11. Still another form of extraction tube is shown in (fig. 9). 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 perforated cork, with a small flask containing the solvent.

FIG. 9

12. The principle of **Repercolation applied to Liquids.** For the extraction from aqueous solutions of small quantities of alkaloids by a minimum amount of immiscible solvent, the apparatus of Hulsebosch (fig. 10.) sometimes called a "perforator" is very useful. The solvent is placed in the flask to which heat is applied by a water bath. The vapor passes up into a condenser, not shown in the

cut from which it is returned in liquid form, dropping into a funnel tube open at the bottom, from which it escapes to pass upward in small drops through the denser fluid from which it is to extract the alkaloid. This fluid should fill the bend in the extraction tube about one third full. The lower end of the funnel tube should be very slightly notched with a file so as to allow the solvent to escape in several different directions. If notched on one side only, it may happen that precipitated alkaloid on the opposite side may remain undissolved. The solvent flows back into the flask to be again vaporized and repeat the same circuit again and again as long as heat is applied. The alkaloid can be thus extracted very completely in an hour or two with a minimum of solvent and by a process that is practically automatic.

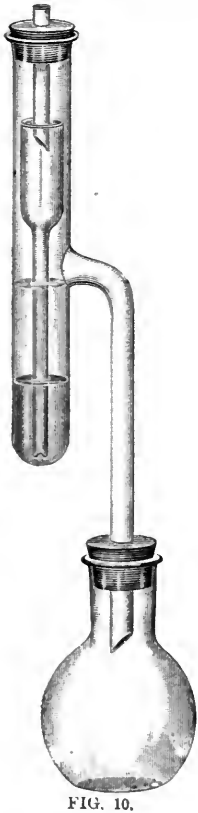

FIG. 10.

13. If the solvent is a fluid like chloroform heavier than water, the same apparatus may be used, substituting for the funnel tube a test tube having an opening in the bottom, or else let the apparatus take the modified form shown in (fig. 11). In either case, the bend of the extraction tube must be first filled half full with

the solvent. The aqueous solution is then introduced into the inner tube, and should be so adjusted in volume as to fill this to the constriction at B when the solvent is at the overflow level, and should not come nearer than 1 cm. to the opening A. At the constriction a disc of wire gauze should be placed. The vapor from the flask will pass up through the opening E into the condenser, from which the condensed liquid will drop on the wire gauze disc and so enter the aqueous solution in small drops which will sink through the lighter liquid, abstracting the alkaloid on their way, to pass through the opening A and return ultimately to the flask.

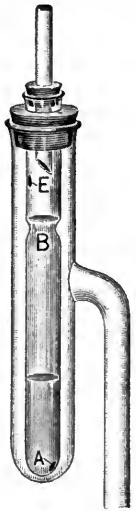

FIG. 11.

14. Another modification of the inner tube for chloroform extraction is shown in fig. 12. It is shaped like a test tube, but has a small tube extending from near the bottom to an outlet near the top. This must be filled to a depth of about 1 cm. with the solvent, and then the aqueous fluid introduced. When it is in use, the solvent of course passes up through the little tube, and so finds its way back eventually into the flask.


FIG. 12.

15. Another very useful piece of apparatus for use in the assay of crude drugs particularly, is a **Mechanical Shaker,** which may be operated by an electric motor, a small water motor, or any other source of power. A rocking and oscillatory motion may be easily communicated to a small platform or

a swinging shelf on which the bottles or flasks to be shaken are set. Ordinary ingenuity will devise the particular means of accomplishing the desired result in any given case.

REAGENTS.

16. Mayer's Solution of Potassium Mercuric Iodide. Used for the detection and volumetric determination of alkaloids. The solution as made by Prof. Mayer was of decinormal (N 1-10) strength. A solution one half this strength (N 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 (N 1-20).

Mercuric chloride, - - -	6.775 Grm.
Potassium Iodide, - -	25.000 Grm.
Distilled water, sufficient to make	One Litre.

17. Wagner's Reagent, Iodine in Potassium Iodide Solution. An exceedingly delicate qualitative test for alkaloids, which Prof. Prescott and his collaborators have recently employed with excellent results in volumetric determinations of atropine and of morphine.

FORMULA (N 1-10).

Iodine. - - - - - -	12.66 Grm.
Potassium Iodide, - -	32.00 Grm.
Distilled water, sufficient to make	One Litre.

18. Marmés Reagent, Potassium Cadmium Iodide, a delicate qualitative test for alkaloids, is prepared by boiling one part of cadmium iodide with two parts of a saturated solution of potassium iodide, and adding two parts more of the same saturated solution.

19 Thresh's Reagent, quite as delicate a qualitative test for alkaloids as the preceding, is also available for their volumetric determination.

FORMULA.

Bismuth Oxide, - - -	4.68 Grm.
Potassium Iodide, - - -	20.00 Grm.
Hydrochloric Acid (B. P.) - -	80 cc.
Distilled water, sufficient to make	One Litre.

Dissolve the bismuth oxide in the acid, diluted to 300 cc., dissolve the potassium iodide in water q. s. to make 700 cc., and mix the solutions.

20. 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 ten times its weight of a mixture of nitric acid (sp. gr. 1.42) one volume and water thirteen volumes.

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.

21. Scheibler's Reagent, Sodium Phosphotungs

tate. 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 determination of alkaloids.

22. Other General Reagents used for the detection of alkaloids are the following: Mercurio-potassium bromide (Mercuric bromide, 36.0 Grm., Potassium bromide, 26.6 Grm., water to make one litre); **Valser's Reagent,** mercurio potassium iodide, (mercuric iodide 45.4 Grm., Potassium iodide 33.2 Grm., water to make one litre); Mercuric chloride, 5% solution; Picric acid, saturated aqueous solution; Tannin, 5% solution; **Froehde's Reagent,** prepared only when needed, by dissolving with aid of heat one milligram of molybdic acid or of ammonium molybdate in 1 cc. of concentrated sulphuric acid, to be used immediately, when cold.

23. Chinoidine Iodosulphate, employed by Dr. De Vrij for the estimation of Quinine. Heat together on a water bath one part of chinoidine 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. The precipitate of chinoidine iodosulphate subsides on warming, and is to 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.

24. Prollius' Mixture, employed to extract alkaloids from crude drugs.

FORMULA.

Concentrated Ether, - - -	325 cc.
Alcohol, - - - - - - -	25 cc.
Stronger water of Ammonia, - -	10 cc.

Mix the alcohol and ammonia and add to the ether. A "weaker" Prollius' mixture is often preferable, containing only one half or one fourth as much ammonia.

25. Prollius' Fluid Modified, used for the extraction of alkaloids sparingly soluble in ether, consists of:

Concentrated Ether, - - - -	250 cc.
Chloroform, - - - - -	80 cc.
Alcohol, - - - - - -	25 cc.
Stronger water of Ammonia, - -	10 cc.

26. For Alkalimetric Estimation of Alkaloids prepare solutions of Sulphuric Acid, Normal; Hydrochloric Acid, Decinormal (N 1-10), and N 1-25, and corresponding solutions of Potassium Hydrate. The alkaline solutions should be frequently renewed or else kept in containers from which carbon dioxide is rigorously excluded. The simplest way of preserving them for a length of time is to put in the bottle a little Kerosene oil, making sure that this is perfectly free from acidity. The solution can be easily withdrawn for use with a pipette. Standardizing of the solution is best done by referring all others to the normal acid, which is to be prepared in the following manner.

NORMAL SULPHURIC ACID.

27. Put into a suitable flask 1000 cc. of distilled water, add carefully 30 cc. of chemically pure concentrated sulphuric

acid and mix thoroughly. Cool to about 25° C., the ordinary temperature of the laboratory, measure accurately two portions of 10 cc. each into tared beakers, add to each pure water of ammonia in excess and evaporate to dryness at a water bath heat, dry 20 minutes at 110° C., cool in a desiccator and weigh. If the weighings do not agree closely, dry once more at 110° C. cool and weigh as before. Take the mean of the weights found in milligrams, divide by 659.2. The quotient will be 1, followed by a decimal fraction. Drop the 1., point off three decimal places, read the result as cc. and add that quantity of distilled water to one litre of the acid solution to bring it to standard. Test the solution by evaporating 10.35 grams to dryness after adding excess of pure water of ammonia. The residue when completely dry must weigh 0.659 Grm. As a control, a separate standard may be prepared by dissolving in 90 cc. of distilled water 6.285 Gm. of pure crystallized oxalic acid and adding distilled water to make exactly 100 cc.

It is hardly necessary to give instructions for preparing the normal alkali, or of the more dilute standard solutions.

28. Indicators. Of the indicators for which the U. S. Pharmacopœia gives formulas, those most useful in alkalimetrical determination of alkaloids are the solutions of Brazil wood, cochineal and methyl orange. A solution of haematoxylin in alcohol (1:100) is employed by some; I prefer a more dilute solution (1:500) of which not more than two or three drops should be used. Iodeosin is another indicator which is regarded by some operators as quite indispensable.

GENERAL METHODS OF ASSAY OF CRUDE DRUGS.

29. Selection and Preparation of the Sample. It is necessary in the first instance to secure a fair average sample of the drug. A handful of the drug should be taken from as many different parts of the package as is necessary to insure such an average. 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 of these a representative section or segment, reduce all to a coarse powder, of this take a sufficient quantity—generally thirty to fifty grams—and by aid of mill, mortar and sieve reduce *the whole* of this portion to a powder of the requisite fineness. In general it may be said that the finer the powder the better. The coarsest powder admissible is a No. 30. In the case of drugs of compact texture, barks, seeds, etc. a much finer powder—No. 60 to No. 80—is required.

30. When the drug consists of seeds, leaves, etc., nearly uniform in quality, it suffices to reduce to a coarse powder in the first instance 200 to 300 grams, taken from different parts of the package as already suggested, and then to reduce to a uniform powder of the requisite degree of fineness 50 grams of this.

31. 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. Such a correction would be of course only approximative. Vegetable crude drugs generally are hygroscopic, gaining weight in a damp atmosphere and losing it in dry weather. The object of an assay is to ascertain the proportion of active principle present in the drug *in the condition in which it is purchased or used.* Here at the outset is introduced into the assay an element of uncertainty which is intolerable to the professional chemist. If the weight of the material assayed is subject to variations amounting possibly to five per cent. it is clear that the result of the work will be an approximate, not an exact figure. The ideal of the chemist requires for a basis the thoroughly dried drug as a positive invariable quantity. In practice we deal with the variable, hygroscopic drug, and must be content with practical, not ideal results, exactly as the practical mechanician uses for the fine measurements by which he adjusts part to part in a piece of delicate machinery, instruments "of precision" indeed, but only of practical and approximate, not of scientific and ideal, precision.

Of course in the case of a drug not to be used at once, particularly if it is to go into market with a guaranteed assay, the moisture in the drug should be determined and reported as an essential part of the assay.

32. Methods of exhausting the Drug. The choice will be between four processes, each of which may have, in a particular instance, its advantages.

A. *Maceration.* The drug in fine powder is placed in a bottle or flask, securely corked, with a measured or weighed quantity of the chosen menstruum sufficient to insure complete exhaustion—at least eight or ten times the weight of the drug

—and allowed to macerate therein with frequent or continuous agitation several hours at least; in many cases from one to three days. The mechanical shaker reduces materially the time required for this operation, which commends itself by its simplicity, and consumes a minimum amount of the operator's time and attention. In some cases, obviously, the method is inadmissable when a result must be reached within twenty four hours.

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

C. *Boiling,* with several successive portions of the chosen solvent. This is efficient and rapid, but generally more troublesome than the following method, and heat sometimes destroys a part of the alkaloid.

D. *Hot Repercolation.* This is altogether the neatest and most effectual mode of applying solvents. It requires a special extraction apparatus, and it takes some time to mount this properly with condenser, but it enables us in a minimum of time and with a minimum of solvent, to exhaust the drug When once set in operation, the action is automatic, so that the method is after all economical of time, particularly where assays require to be made frequently.

33. The choice of solvent will depend, of course, upon the nature of the drug. In most cases the active principle 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 sure 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 may be as troublesome as 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.

34. A mixture of chloroform and alcohol generally extracts alkaloids from crude drugs very perfectly, and leaves behind most of the inert matter. Acidulated water or acidulated alcohol was formerly much used in exhausting alkaloidal drugs. A freely soluble salt of the alkaloid is formed, and the drug is speedily and very completely exhausted, but much inert matter is removed at the same time, and the plan is now seldom used.

35. Another plan is to treat the drug first with an alkali, milk of lime, magnesia, or sodium carbonate, dry, and exhaust with alcohol or with wood spirit. The plan succeeds well with some drugs, particularly those rich in alkaloids. It gives us a solution containing little inert extractive, from which it is therefore comparatively easy to separate the alkaloid in a condition of purity. The objections to it are: 1st, the liability to decomposition of sensitive alkaloids by the prolonged action of the alkali, particularly when lime is used; 2nd, the amount of time required to carry out the successive steps of the analysis. The method admits of various modifications which may sometimes be advantageous. Instead of

extracting the alkaloid with alcohol, we may employ some solvent such as amylic alcohol, chloroform, ether or petroleum ether which will take up very little besides the alkaloid. We may conduct the extraction by the method of hot repercolation, particularly when the last named solvents are used, and so shorten the time of the assay.

36. Alkaline Solvents. At present the solvent used for the exhaustion of alkaloidal drugs is almost always some modification of the mixture recommended some years ago by **Prollius** for the assay of cinchona bark. The original Prollius' mixture consisted of ether, alcohol and solution of ammonia (24). The ammonia sets free the alkaloids which are immediately taken into solution by the ether. In case the alkaloid is one not readily soluble in ether, some more appropriate solvent may be substituted, that which has proved most efficient being a mixture of chloroform one volume with ether three to six volumes (25). A mixture of chloroform and petroleum ether has been recommended, and is a good general solvent for alkaloids. Petroleum ether alone answers in a few cases very well, used of course in connection with ammonia. It is understood that these various solvents are to be employed for exhausting the drug by maceration. It is surprising how quickly and how completely they accomplish this object, particularly when aided by the mechanical shaker. In the ordinary routine twelve hours may be allowed for the exhaustion, but it is frequently complete in one third of that time, and in some cases the alkaloid which is taken up in the first hour or two, will begin to crystallize out of the solvent (particularly petroleum ether) within half a day, so that it will not do to leave it on the drug more than four hours.

37. **Keller** has modified the procedure by using a measured volume of the solvent (ether or ether-chloroform) and then adding a certain amount of water of ammonia, which is supposed to be absorbed by the drug without affecting the volume of the solvent. After the maceration, water is added to the mixture to be absorbed by the drug, causing it to collect in balls, and allowing the solvent to be poured off clear. Of course when the solvent is ether, there will be a partial solution of each of the fluids in the other, so that the volume of the ethereal solution will no longer be what it was at the beginning, and it may be expected that the result of the assay will be high, if an aliquot portion of the ethereal solution is used as the author directs. A similar criticism may be made of the use of any of the compound solvents mentioned. No doubt the drug will absorb from them water and perhaps alcohol, reducing the volume of the solvent, but the proportion of the water and alcohol are insignificant, and it is probable that enough alkaloid remains undissolved to fully compensate the error arising from this cause. However, where scientifically exact results are desired, it is always possible, as suggested recently by W. A. Puckner, to exhaust the residue of the drug by percolation with the appropriate solvent, and so obtain from the portion of drug taken for the assay the total alkaloid.

38. 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' mixture, shake out with acidulated water, making sure that acid is really in excess, and test the acid solution with the general reagents for alkaloids. If such compounds are present in notable amount, they will almost certainly be detected in this manner.

GENERAL METHODS OF DETERMINING ALKALOIDS IN CRUDE DRUGS.

39. Method Recommended by Dragendorff.* Moisten the finely powdered drug 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 10 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 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 is now of little more than historic interest, although for a few drugs, notably ipecac, it may be advantageously employed where immediate results are not demanded.

40. Method of Lösch.† Exhaust the drug (one part) by maceration with ten parts 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

*Die Chemische Werthbestimmung Einiger Starckwerkender Droguen, 1874.

†Pharm. Zeitung, 1879, No. 89; Am. Journ. Pharmacy, Jan. 1880.

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. The method is needlessly cumbersome, but is suggestive. An iron alum, or simply ferric chloride may be advantageously substituted for the alum, or at least for a portion of it, and sodium bi-carbonate may be employed in place of the ammonia. In any case the method is not adapted to the separation of the volatile alkaloids.

41. Method of H. 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, add water to make up the loss by evaporation and then set aside two hours in a cool place; filter, and of the filtrate take for the assay an aliquot portion. It is assumed that air dried seeds and barks will yield about 20 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, coresponding with 10 grams of drug will be, in the first case, 102; in the second, 104; in the third 105 grams.

42. The portion of fluid weighed out for the assay is to be evaporated at a gentle heat to one half its volume, 25, or if much coloring matter is present 30, grams of finely pulverized litharge added; the heat being maintained, after half an

*Handbuch der Pharmaceutischen Praxis, vol. III p. 62.

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, 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. On evaporation of this solution, the alkaloid is frequently obtained pure enough for weighing.

43. If the drug contains a **volatile alkaloid,** the acid solution (above) must be evaporated to a small volume and a mixture of thoroughly dried litharge and terra alba added. After the lapse of an hour, a further addition is to be made of well dried lead carbonate, in quantity to absorb all sensible moisture, and the dry powder thus obtained is to be exhausted at once with absolute alcohol. If the extract is colorless, it may be neutralized with hydrochloric or oxalic acid and dried, if colored it is to be neutralized with sulphuric acid, some water added, the spirit evaporated off and the alkaloid shaken out with ether after adding caustic soda in slight excess.

44. The foregoing are among the best of the methods that were practiced previous to the year 1881, when Prollius* proposed an improved method for the assay of cinchona bark, from which have been gradually evolved the methods now most approved.

45. The Method of Dieterich which is still practiced in Germany, and is similar in principle to that of **Cazeneuve,** consists in mixing the drug with calcium hydrate and a little water, drying, powdering and extracting by hot repercolation

*Arch. Pharm. 1881, 85; Am. Journ. Pharm. 1882, 59.

with ether. The residue of impure alkaloid, remaining on evaporating the ether, is dissolved in alcohol and titrated with centinormal hydrochloric acid.

46. Method of O. Schweissinger* Ten grams of the finely cut dry herb (aconite, belladonna, etc.) are put into a flask with 200 cc. of water containing 1 cc. of diluted sulphuric acid, corked securely, and digested in a warm place over night, the mixture being occasionally agitated. The mixture is then allowed to cool, strained, the residue pressed. Of the strained liquid, 160 cc. corresponding with 8 grams of drug, are evaporated on the water bath under constant stirring, at a temperature of about 65° C., until reduced to about 20 grams. This solution is then poured in a thin stream into 80 cc. of strong alcohol, the volume made up to exactly 100 cc., the mixture well shaken and allowed to rest until the sediment is well deposited. Of the filtered alcoholic solution 50 cc., equal to 4 grams of drug are measured out, evaporated to 10 cc., transferred to a separatory funnel, 1 cc. of ammonia water and 40 cc. of a mixture of chloroform 3 volumes, ether 5 volumes, added, the whole shaken together gently (to avoid emulsionizing) for some time and allowed to separate. Finally 20 cc. of the ether-chloroform are measured out, evaporated in a capsule and titrated with centinormal acid, using cochineal as an indicator.

47. Method of Grandval and Lajoux† I. Make a mixture of ether 8 cc., alcohol, 3 cc. and water of ammonia, 2 cc. With this moisten 10 grams of the drug and pack in a miniature percolator, which may consist of the barrel of a small glass syringe. A little absorbent cotton is placed

*Pharm. Centralh., 31, 583, 771. Am. Druggist 1891.p. 351. when the method is said to have been published "some years ago."

†J. Pharm. Chim., 1893, pp. 99, 152.

beneath the drug in the percolator and another portion above it. The drug is then exhausted by slow percolation with ether, about 100 cc. being required. Make sure that the drug is exhausted by evaporating a few drops of the percolate on a watch glass and adding a drop of normal sulphuric acid and a drop of Mayers' reagent.

48. Introduce the ether into a separator, add 5 cc. of normal sulphuric acid, shake together, let separate and draw off the acid liquid into a suitable vial. Wash the ether several times with water, 4 cc., adding this to the acid solution. Finally render the solution alkaline, perferably with caustic soda, and shake out the alkaloid with ether.

49. II. 100 grams of the finely powdered drug are well triturated with 100 grams of basic lead acetate, then exhausted by percolation with water, which must be continued as long as the percolate gives any precipitate with Mayer's reagent. Most of the organic acids present remain as insoluble lead compounds, together with much of the coloring and extractive matter and a large proportion of the proteids. Dilute sulphuric acid is added to the solution in slight excess and the lead sulphate filtered out: the alkaloids are then precipitated in the filtrate by Mayer's reagent, variable amounts of albumin and coloring and extractive matters also coming down. After standing some time the precipitate is collected and treated by one of the two following processes:—(1) Potassium cyanide, a little caustic soda, and ether (or other appropriate solvent) are well shaken with the precipitate. The ethereal solution is caused to separate from the emulsion produced, by the addition of a little olive oil and is then treated with sulphuric acid, the acid solution separated and washed repeatedly with ether to remove

olive oil, and finally made alkaline with caustic soda and shaken out with ether. (2) The precipitate by Mayer's reagent is digested in a flask with a slight excess of sodium sulphide after which the alkaloid is extracted precisely is in method No. 1. The second process is that to which the authors give preference. Liquid alkaloids, except sparteine, they state, cannot well be separated by this method, which is unsatisfactory also as applied to belladonna and other drugs containing atropine.

50. Method **of C. C. Keller*** Dry the drug completely at a low temperature, perferably in a desiccator over sulphuric acid. Reduce to a fine powder, at least No. 80. Use for the assay from 10 to 20 grams of the dry drug, according to the quantity of alkaloid it contains. In most cases 20 grams may be used. Introduce into a suitable container, and add a mixture of ether 100 grams and chloroform 25 grams. Shake the mixture well and macerate five to ten minutes. Shake once more, add 10 grams of water of ammonia, 10%, and shake immediately and vigorously so that the drug powder shall be moistened uniformly. Shake occasionally during half an hour, then add a sufficient quantity of water (20 to 40 cc.) to cause the drug powder to agglutinate. Pour off of the clear ethereal fluid 100 grams, representing 16 grams of drug. It is not always possible to obtain the full quantity thus prescribed. In such case weigh the fluid obtained, care being taken to avoid loss by evaporation and calculate the quantity of drug it represents. [The relative proportion of ether and chloroform may be varied to suit the drug, but the proportion of chloroform should not exceed 25 or 30 per cent. by weight of the mixture.]

*Schweiz. Wochenschrift f. Chem. u. Pharm. 1892, 501, 509.

51. In case the fluid drawn off is turbid, it should be left at rest in a well stoppered flask until it becomes quite clear, after which the requisite quantity is to be poured off. Place the ethereal fluid in a separator, and wash out the alkaloid it contains with hydrochloric acid, 0.5%., using three portions of 25, 15 and 10 cc. respectively. From the mixed acid solutions, the alkaloid is removed by shaking out with ether or a mixture of ether and chloroform, after addition of ammonia. The solvent is used in three successive portions, the first being added before rendering alkaline with ammonia. In all about 100 cc. of the solvent should be used. The fluid is transferred to a tared Erlenmeyer flask of 150 to 200 cc. capacity, the solvent recovered by distillation, the vapor of ether removed from the flask by a current of air from a bellows, the flask dried on the water bath for periods of 15 minutes each, and weighed in the intervals, until a constant weight is reached. The alkaloid is generally quite pure, but for assurance of this it may be titrated with standard acid. Dissolve in 5 to 10 cc. of neutral absolute alcohol, add water to the beginning of turbidity, then a few drops of haematoxylin indicator, and finally from a burette hydrochloric acid N. 1-10 or N. 1-20 until the color changes. The quantity of alkaloid present in the drug can be then readily calculated. (88)

52. In case of drugs like ergot and nux vomica which contain fixed oil in considerable quantity, it is advisable to treat the drug with ether by percolation, before attempting to extract the alkaloid. The ether may itself remove notable quantities of alkaloid, hence it should always be washed with acidulated water, which is to be then tested for alkaloid.

If this is found to be present, it must be removed by the usual method, and added to the alkaloid separated in the subsequent assay process.

53. Method of K. Schwickerath.* Digest 20 grams of the powdered drug with 150 cc. of a weak Prollius' fluid, in which a mixture of petroleum ether, three or four volumes, and chloroform, one volume, may often advantageously replace the ether. Agitate frequently, or preferably continuously by aid of a suitable mechanical device, during twelve hours. The drug may be moistened with water to advantage at the beginning of the operation, or water is added after the maceration.

54. Filter off through cotton 75 cc. of the ethereal fluid, corresponding with 10 grams of drug, place in a shallow evaporating dish, add 10 cc. of a 1 per cent. solution of sulphuric acid, set aside in a moderately warm place to evaporate the solvent. The fatty and waxy matter is deposited on the sides of the capsule while the alkaloid goes into solution in the acid water. The acid solution is filtered through a very small filter into a separatory funnel, or preferably into a "perforator", (fig. 10, p. 17.) The waxy residue in the capsule is treated with 1 cc. of acidulated water and a little ether, which is allowed to evaporate and the aqueous solution passed through the filter and added to the first acid solution. This may be repeated once more if necessary. The alkaloid is then recovered from the solution by treatment with an alkali and the appropriate solvent, the perforator method being preferred.

55. Use of the Bosman-Schwickerath Perforator.

*Pharm. Rundschau, 1893, 282; 1894, 36.

Introduce into the perforator (fig. 10, p. 17) the acid solution from which the alkaloid is to be extracted, this should not fill the extracter more than half way up to the overflow tube. Put in also a bit of litmus paper. It is well to introduce just below the level of the overflow tube and above the aqueous liquid, a ring of absorbent cotton, to prevent the carrying over of droplets of the aqueous liquid. Place in the flask first 30 cc. of petroleum ether, connect the apparatus and apply a water bath heat so regulated that the fluid will be returned from the condenser in a constant small stream. Allow the process to continue one hour, to wash out thoroughly from the acid solution fatty and waxy matter.

56. Now disconnect the apparatus, pour out the petroleum ether, both from the flask and from the perforator. Place in the flask 30 cc. of ether, or such solvent as may be chosen, add to the acid fluid sufficient ammonia or sodium carbonate to render it distinctly alkaline, reconnect the apparatus and apply a regulated heat as before for two hours. If there is a very large quantity of alkaloid present, there may remain even then some portion of it undissolved, which will generally be plainly seen. In such cases it is best to disconnect the apparatus, add drop by drop hydrochloric acid to strong acid reaction, thus dissolving the remaining alkaloid, again render the solution alkaline, and continue the extraction an hour or until the whole of the alkaloid is dissolved. Make sure of this by testing a drop of the solution, after acidulating, with Mayer's reagent (which may, however, possibly produce a turbidity due to something besides alkaloid.) Finally transfer the ether in the flask to a tared beaker, rinse the flask with the ether which had remained in the

extraction apparatus and which is readily poured off by inclining the apparatus, add this to the ether in the beaker, evaporate to dryness and weigh. The result can be verified by dissolving the alkaloid and titrating with standard acid. (See Alkalimetry p. 51 et seq.)

57. If the solvent be one heavier than water, the modified form of apparatus (fig. 11, p. 18) must be used, but the process is not otherwise different. The great advantage of the perforator method is that a number of assays can be progressing at once under the supervision of a single operator, and that extraction of the alkaloid is accomplished with a mininum amount of costly solvent.

58. Method of Lyman F. Kebler,* a modification of that of Keller. Place 10 grams of the powdered drug in a 250 cc. flask, add 25 grams of chloroform and 75 grams of ether, cork the flask securely, agitate well for several minutes, add 10 grams of 10 per cent. ammonia water and agitate frequently during one hour. On adding five grams more of water of ammonia, the suspended powder agglutinates into a lump, the liquid becomes clear after standing a few minutes and can be poured off almost completely. 50 grams of the clear fluid are taken for the assay and treated by one of the following processes.

59. I. Place the ethereal fluid in a beaker, evaporate on a water bath, add 10 cc. of ether and evaporate once more. Dissolve the residue in 15 cc. of alcohol, add water to slight permanent turbidity, haematoxylin, Brazilwood or cochineal indicator, and a measured excess of volumetric acid; titrate back with centinormal alkali.

* Am. Journ. of Pharm. 1895, 499, 509.

60. II. Transfer the ethereal fluid to a separator, treat at once with 20 cc. of acidulated water; after thorough agitation and complete separation, remove the aqueous solution into a second separator. Repeat the above operation twice with 15 cc. of slightly acidulated water. Wash out the alkaloid from the aqueous liquid, after adding ammonia in excess, with three portions, (20, 15 and 15 cc.) of a mixture of chloroform three volumes, ether one volume. The ether-chloroform is evaporated off in a tared beaker, the residue twice redissolved in ether (8 cc.) to carry off the last traces of chloroform, dried on the water bath to constant weight and weighed. It may then still be dissolved in alcohol and titrated with standard acid and alkali. The first procedure yields naturally a somewhat higher figure.

61. The methods of Schwickerath, Keller and Kebler are modifications of, and in some respects improvements upon those originally worked out by the author,* following the general plan of Prollius' short method for the assay of cinchona bark. The author's original process is reproduced here, almost without modification, as short Assay Process No. 1, in the belief that it is still worthy of a place among the best methods to recommend to the pharmacist.

62. It may not be out of place to restate here the advantages which were claimed by the author for this general assay process, since these remain today the criteria by which the merits of new methods are judged. "The advantages of the author's method are:† 1st. The result is reached speed-

*Druggists Circular Aug. 1884, p. 114: Pharmaceutical assaying, 1886 p. 20 et. seq.

†Manual of Pharmaceutical Assaying (1886) p. 59.

ily, and with very little labor. 2nd. The alkaloid is extracted with comparatively little inert matter accompanying it, and the impurities are easily eliminated owing to their insolubility in acid water. 3rd. 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 reagents 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 drugs with only minor modification of detail."

METHODS PRACTICED BY THE AUTHOR.

SHORT ASSAY PROCESS, NO. 1.

63. Put into a 4 oz. prescription vial 10 grams of the drug in moderately fine powder (29). Pour in carefully exactly 100 cc. of Prollius' fluid (24), generally the "weaker", cork securely and shake vigorously several times at intervals of a minute or two. Place in the mechanical shaker four hours, or else shake at frequent intervals during that time.† At the end of that time, decant into a measuring flask exactly 50 cc. of the clear fluid, transfer to a shallow capsule‡ and set in a warm place (or expose to a current of air) until the ether has nearly all evaporated. Then add 5 cc. of highly dilute sulphuric acid (1%) and 10 cc. of fresh ether. Stir to redissolve in the ether all the oily and waxy matter that may have separated, and to insure combination of all alkaloid with acid, evaporate the ether completely together with any alcohol that may remain.

*If from lack of proper facilities for powdering the drug, it has been necessary to use a powder coarser than No. 60, the time should be extended accordingly, for a No. 30 powder to 10 or 12 hours.

†Druggist Circular, Aug, 1884, p. 114; Pharmaceutical assaying, 1886, p. 20 et seq.

‡Or else follow [73].

64. Filter the aqueous fluid through a very small filter into a one ounce prescription vial, which should have a square shoulder and a good lip, [or else filter directly into a perforator, completing the analysis as in the method of Schwickerath.] Treat the residue in the capsule with 3 cc. of water slightly acidulated and 5 to 10 cc. of ether, stir well together, evaporate off the ether and pass the aqueous liquid through the same filter into the vial [or perforator]. Test a small drop of this fluid with Mayer's reagent on a mirror. If it contains more than a trace of alkaloid, repeat the washing of the residue once more with 2 cc. of water and 5 cc. ether. This should not often be necessary, but the filter may be washed finally with a few drops of water.

65. We have now an aqueous solution containing all the alkaloids from five grams of the drug. In this for rough approximate work we may estimate the alkaloid by titration with Mayer's reagent, when this is applicable, or by other volumetric processes, as by Wagner's reagent, but in exact work the alkaloid must be extracted and weighed or titrated with standard acid, and this consumes hardly more time than a direct titration of the acid fluid.

66. The procedure for extracting the alkaloid is as follows: Put into the vial containing the acid solution 15 cc. of ether, shake well, let separate, pour off into a second similar vial containing 3 cc. of slightly acidulated water. Shake together, let separate completely and pour off the ether into a container for waste ether. [Rarely a second washing of the ether with water may recover traces of alkaloid, and it is always best to take this precaution.] Repeat the double washing of the contents of No. 1 and No. 2 with one or two suc-

cessive portions of fresh ether (15 cc.) until all traces of chlorophyll and fatty or waxy matter are removed; the ether is to be poured off each time as closely as possible without loss.

67. Now add to No. 2 two or three drops of water of ammonia (10%) and 20 cc. of ether, shake immediately fifteen seconds and let separate. [Make sure that ammonia is in excess by testing the vapor in the vial with red litmus paper, which must not be allowed to touch the neck of the vial. If the paper is not made blue, add more ammonia and repeat the shaking.] Pour off the ether into No. 1, add water of ammonia in excess, perhaps 10 drops, and immediately shake vigorously 30 seconds. Let separate, return the ether to No. 2, but shake this only once and then let stand several minutes, so that the ether will separate completely from the watery fluid. Add to No. 1 fifteen cc. of fresh ether, shake well together and let separate. The ether is now to be decanted carefully from No. 2 into a tared beaker, to be replaced by that from No. 1.

68. A third portion of ether, passed in succession through No. 1 and No. 2 will generally extract all the alkaloid if it is one freely soluble in ether. [If not, it is better to use a mixture of ether 15 cc. and chloroform 5 cc. for the first washing, to be followed generally with pure ether for the subsequent washings.] In any case test the residual aqueous solution in No. 1, which should be generally quite clear, by placing a drop of it on a mirror, adding acid in excess and a drop of Mayer's reagent. There should not be produced more than a faint cloud, although traces of alkaloid will often be still found after five or six washings.

69. Evaporate the ethereal solution on the water bath to constant weight. If chloroform has been used in the extraction, redissolve the residue once or twice in alcohol and evaporate to expel persistently adherent traces of chloroform. Weigh the residue with a delicate balance. The weight of the alkaloid in decigrams multiplied by two, or the weight in centigrams divided by five will be the percentage of total alkaloid contained in the drug.

70. After weighing the crude alkaloid, dissolve it in 5 cc. of alcohol, add 5 cc. of N 1-25 hydrochloric acid and 25 cc. of distilled water. Be sure that the alcohol and the water contain no traces of acid or of alkali, add two drops of Brazil wood indicator [or haematoxylin or cochineal] and titrate back with N. 1-25 alkali. The amount of alkali consumed must be subtracted from 5 cc. to give the acid equivalent of the alkali present. In case of alkaloids having only feeble alkalinity, the alkalimetric test is of course superfluous.

71. For rapid work, in the case of alkaloids that admit of determination by alkalimetry, the assay may be abbreviated by evaporating the original ethereal extract (50 cc.) to dryness, taking up with acid-free ether, 10 cc., evaporating once more, adding 10 cc. of acid free ether, together with 5 cc. of N 1-25 acid, evaporating off the ether and titrating back with N 1-25 alkali as described in the last paragraph. The alkaloid can be afterwards recovered from the solution in the usual way if this is desired.

SHORT ASSAY PROCESS, NO. 2.

72. Put into a 4 oz. prescription vial twelve grams* of

*This quantity is suitable in the case of drugs containing less than 0.8 per cent. of alkaloid. If there is as much as one per cent., 10 grams of drug is sufficient, if five per cent. not more than four grams should be used.

the drug in fine powder. Pour in exactly 100 cc. of a mixture of ether eight volumes and chloroform one volume, cork securely and shake well. After ten minutes, shake once more, add 5 cc. of water of ammonia (10%), cork and immediately shake vigorously, repeating this several times at intervals of a minute or two. Place in the mechanical shaker four hours, or else shake at frequent intervals (once in five or ten minutes) during that period, then, if the drug does not settle promptly leaving a clear supernatant fluid, add water sufficient to cause it to aggregate into lumps on shaking (from 15 to 25 cc. generally suffice.) In any case decant exactly 50 cc. of the clear ethereal fluid into a measuring flask, and thereafter follow the instructions given under assay process No. 1. (63 et seq.)

73. Instead of evaporating the ethereal fluid, it is practicable to wash out from it the alkaloid, in the following manner: transfer the fluid to a separator, add about 2 1-2 cc. of dilute hydrochloric acid (10%) and shake together. Make sure that the acid is in excess, best by adding a drop of some indicator, add more acid if necessary and water enough to make up 5 cc. in all of aqueous fluid. Shake together 30 to 60 seconds and let separate. Draw off the acid fluid into a one ounce prescription vial, treat the ethereal fluid with four or five successive portions (3 cc.) of slightly acidulated water until all the alkaloid is removed. Test a small drop each time after the second washing on a mirror with Mayer's reagent. Add the first portion to the contents of the vial (No. 1,) put the next two or three washings into a second vial (No. 2) and the last into a third vial.

74. To recover the alkaloid, add first to No. 3 a drop of

water of ammonia and 5 cc. of chloroform mixed with 15 cc. of ether; shake, let separate, decant the ethereal fluid into No. 2; add 2 or 3 drops (excess) of water of ammonia, shake, let separate and decant into No. 1; add excess of water of ammonia (perhaps ten drops, but make sure that the ammonia is in excess by testing the vapor in the bottle with blue litmus paper), shake together well for 30 seconds or more, let separate and decant into No. 3, which contains little but water; shake together once, let stand at least two minutes for complete separation and decant into a tared beaker. Put into No. 2, 20 cc. of pure ether, which is to be passed in succession though No. 1 and No. 3, in the same manner as before, and follow this with one or two additional portions of ether, 15 cc. each, until assured that the whole of the alkaloid is removed.

75. Finally evaporate the solvent at a water bath heat and dry to constant weight as in Process No. 1 (69) and (70). I have given the details of this assay process quite minutely for the benefit of beginners. The object of the final washing in vial No. 3 is to avoid the possibility of carrying over with the alkaloid any of the aqueous mixture of No. 1, which is heavily charged with a salt of ammonia.

SHORT ASSAY PROCESS NO. 3.

76. This differs from process No. 2 only in using for the extracting of the drug light petroleum (petroleum benzin) instead of the mixture of ether and chloroform. A benzin having a boiling point of 80° C. is generally suitable, and even to be perferred to that having a lower boiling point. In particular assays, it will be found advantageous no doubt to use even a less volatile hydrocarbon oil,

but I have not yet experimented in this line. It will be found best, generally, where petroleum benzin is used to follow the procedure of (73) unless emulsification occurs, and this in my experience is not common.

SHORT ASSAY PROCESS NO. 4.

77. Proceed exactly as in process No. 2, but use for extraction of the drug a mixture of chloroform one volume, ether four volumes.

78. Some modifications of the above processes suggest themselves as likely to be of service, although not yet tested fully in practice. These involve the use in the first stage of the process of some agent to fix organic acids and other compounds which may go into solution in ether or chloroform. Ferric chloride or lead acetate suggest themselves; possibly litharge might answer the purpose. The first may be used in the following way. Treat the drug in the beginning with an ethereal mixture, 100 cc., containing 0.2 to 0.5 gram of ferric chloride, agitate frequently or continuously during one hour, then add excess of water of ammonia and continue the process in the usual manner. Or the drug may be moistened in the beginning with an aqueous solution of lead acetate, or of ferric chloride, and after an hour treated with ether (ether-chloroform or other solvent) and ammonia in excess in the usual way.

79. All assays should be made in duplicate. The careful operator will always provide for verification of his result by some form of duplicate assay. In most cases it is not necessary to make two wholly independent assays, although that is the most satisfactory procedure. In the assay of a crude drug we more often make a single weighing of the

drug, which is exhausted by an appropriate solvent, and the solution made up to a definite volume. Of this we then take for assay two aliquot parts, following preferably different modes of treatment. A method of assay which in itself gives only approximative results, as e. g., a titration with Mayer's reagent, furnishes valuable confirmation of results reached by more scientific and exact methods, and such a duplicate may be all that the operator has the time to carry out.

ESTIMATION OF ALKALOIDS BY ALKALIMETRY.

80. The alkaloidal residues obtained by extraction with immiscible solvents, and commonly weighed as "total alkaloid" are never quite free from foreign substances. With reasonable care in manipulation they are generally so nearly pure that we shall make no serious error in accepting their weight as practically that of the alkaloid itself, and when the amount of alkaloid is large, as in cinchona bark, the gravimetric determination is close enough to the truth to satisfy even the professional chemist. Where the quantity of alkaloid, however, is very small, the error is very considerable; impurities may constitute from ten to fifty per cent. of the alkaloidal residue. We must therefore have some check upon the results obtained by weighing, and this in a majority of cases, is supplied in the alkaline reaction of the uncombined alkaloids. The most of them can be determined with great exactness by titrating with standard acid.

81. Unfortunately in drug assays, the residue generally contains two or more alkaloids, having different equivalent values, so that even alkalimetry does not give us the definite, exact result we desire. It enables us, however, to check our gravimetric result, and in most cases the limit of error is not greater than five per cent. of the entire amount, as against a possible thirty or even fifty per cent. in the gravimetric result. I must not be understood to say that errors as large

as this are common where gravimetric methods are followed; it is sufficient that even with reasonably careful work they are possible, and hence the operator should always control his work, when practicable, by alkalimetry. The beginner must remember, however, that in inexperienced hands, the alkalimetric process admits large possibilities of error. See (86.)

82. Direct titration with standard acid. Dissolve the alkaloid in ether, chloroform or other immiscible solvent (10 cc.) put the solution into a stoppered vial of clear glass, add water (5 cc.) colored with methyl orange (A. H. Allen) or other suitable indicator. Add the standard acid (hydrochloric, N 1-25) little by little, at last a drop at a time, shaking after each addition. The end reaction is sharply marked by change of color in the aqueous fluid.

83. Residual titration with Iodeosin as Indicator. Dissolve the alkaloidal residue in a little acid free ether containing iodeosin (2 milligrams to the litre) add an accurately measured quantity of standard acid N 1-25 in excess of what is required to neutralize, transfer to a stoppered vial or flask of clear glass, and titrate with alkali N 1-25, shaking the container well after each addition. As soon as the alkali is in excess, the iodeosin is taken up by the aqueous solution imparting to it a red color. The indicator is not suitable for titration of quinine, but answers well with most of the common alkaloids.

84. The ordinary procedure. (1) Dissolve the alkaloidal residue in 10 cc. of acid free ether, add water 10 cc. and a drop of indicator, preferably Brazil wood, cochineal or haematoxylin; add from a burette hydrochloric acid N 1-25

until after stirring well it is in decided excess. Allow the ether to evaporate. It should leave scarcely any residue. If there is any appreciable residue pour the acid solution into a clean beaker, treat the residue with 5 cc. of fresh ether and 1 cc. of acid N 1-25, evaporate off the ether and add the acid solution to the contents of the second beaker. Now titrate back with alkali N 1-25 until the color of the solution changes permanently, i. e. so that the original color does not return within 20 or 30 seconds. Subtract the quantity of alkali used from the total acid taken, and calculate the quantity of alkaloid by the table of equivalents below (87). Note that the relative strength of acid and alkali must be fixed *under conditions similar to those of the actual titration, and with use of the same indicator.*

85. (2) *Alternative Method.* Dissolve the alkaloidal residue completely in 5 cc. of acid free alcohol, add hydrochloric acid N 1-25, 10 cc. or such larger quantity as shall be more than sufficient to neutralize all the alkaloid. (The weight of the alkaloidal residue, already ascertained, will indicate the quantity required.) Add water 40 cc., two drops of indicator, and titrate with alkali N 1-25. It is important that the solution contain not more than one fifth its volume of alcohol. A larger proportion will interfere with the sensitiveness of the indicator, particularly haematoxylin. One plan is to add excess of standard sulphuric acid and evaporate off most of the alcohol before titrating. There is possibility of loss of some delicate alkaloids, however, from the action of an excess of sulphuric acid during the evaporation.

86. Make sure in all cases, when an alkaloidal solution is evaporated to dryness previous to titration, that there is no

possibility that it be reached by acid vapors, also that the heat to which it is subjected be insufficient to cause chemical changes in the alkaloid. It is advisable, indeed, in the case of the more unstable alkaloids to avoid any application of heat exceeding say 45° C. (113° F.) It is possible that in driving off chloroform the solvent itself may become decomposed, furnishing acid to vitiate the result. In any case if the titration gives results much lower than the weighing, we should suspect some source of error affecting the former rather than the latter, and a duplicate experiment should be made, avoiding as far as possible the above mentioned dangers.

87. **Determination of alkaloids in salts** by alkalimetry. While to most indicators alkaloids are alkaline in reaction they are nearly all neutral towards phenolphthalein Consequently, when a solution containing an alkaloidal salt is titrated with standard alkali, in presence of this indicator, the whole of the acid appears by the titration as free acid. If a solution containing an alkaloidal salt together with excess of acid is divided into two equal portions, and these are titrated with standard alkali, using in one case haematoxylin, cochineal or Brazil wood and in the other phenol phthalein, we shall get different results, the difference representing the quantity of alkaloid present in one half the solution. We may thus determine the amount of alkaloid even though it has been partially neutralized through accident or carelessness. The method is not applicable in the case of atropine, narcotine, coniine, colchicine, caffeine and some other alkaloids. It is important that the standard alkali used for the titration be free from carbonate.

88. Normal equivalents of the more Important Alkaloids. The following table is useful in interpreting the results of titrations.

	1 cc. Normal acid neutralizes of alkaloid milligrams.	1 cc. Acid N 1-25 neutralizes of alkaloid milligrams.	Per cent. alkaloid indicated by 1 cc. acid N 1-25 from 2 grams drug.	Per cent. alkaloid indicated by 1 cc. acid N 1-25 from 5 grams drug.	Per cent. alkaloid indicated by 1 cc. acid N 1-25 from 6 grams drug.	Per cent. alkaloid indicated by 1 cc. acid N 1-25 from 10 grams drug.
Aconitine [a]	647	25.9	1.294	0.518	0.431	0.259
Atropine [b]	289	11.6	0.578	0.231	0.193	0.116
Berberine	335	13.4	0.670	0.268	0.223	0.134
Brucine	393	15.7	0.786	0.314	0.262	0.157
Cinchonine	293	11.7	0.586	0.234	0.195	0.117
Cinchona Alkaloids	314*	12.6	0.628	0.251	0.209	0.126
Cocaine	303	12.1	0.606	0.242	0.202	0.121
Coniine	127	5.1	0.254	0.102	0.085	0.051
Emetine [c]	254	10.2	0.508	0.203	0.169	0.102
Gelsemine [d]	408	16.3	0.816	0.326	0.272	0.163
Hydrastine‡	397	15.9	0.794	0.318	0.265	0.159
Morphine	284	11.4	0.568	0.228	0.190	0.114
Nux Nomica Alkaloids	363*	14.5	0.726	0.290	0.242	0.145
Physostigmine	275	11.0	0.550	0.220	0.183	0.110
Pilocarpine [e]	208	8.3	0.416	0.166	0.139	0.083
Quinine	323	12.9	0.646	0.258	0.215	0.129
Strychnine	333	13.3	0.666	0.266	0.222	0.133
Veratrum Alkaloids	687*	27.5	1.374	0.550	0.458	0.275

a. Also aconite root and leaves, but results are without value.

b. Also Belladonna leaves and root; Hyoscyamus, and Stramonium leaves and seed.

c. Also Ipecac.

d. Also Yellow Jesamine.

e. Also Jaborandi.

*Approximately.

‡Only feebly alkaline; with ordinary indicators, titration not satisfactory

89. Kjeldahl's Iodometric Method for alkaloids. Another plan for the exact determination of alkaloids, based upon their power of neutralizing acids, has been proposed by Christensen.* It is a modification of Kjeldahl's iodometric method of estimating free ammonia. The alkaloid or alkaloidal residue is dissolved in an overplus of sulphuric acid, N 1-25. An overplus of neutral solution of potassium iodide (1-15) and of one of potassium iodate (1:25) is then added, whereupon iodine is liberated in quantity exactly proportioned to the free acid remaining. If the solution is then titrated with a solution of sodium thio-sulphate N 1-25 and the quantity of this solution required deducted from the quantity of standard acid taken, the remainder will be the quantity of acid N 1-25 consumed in neutralizing the alkali. It is a pretty method, but seems to have no advantage over the simple alkalimetric process.

90. Volumetric Estimation as Chloride. Another procedure may be adopted for determination of alkaloids which form strong combinations with acids, and which are not too easily decomposed by excess of acid. The alkaloid in ethereal solution may be super-saturated with an ethereal solution of hydrochloric acid, evaporated by a gentle heat, the residue taken up with alcohol once or twice and dried again to drive off completely excess of acid, and the residue dissolved in distilled water and titrated with silver nitrate standard solution N 1-100 to determine the chlorine and thus indirectly the quantity of alkaloid present.

*Chem. Ztg., Oct. 4th, 1890; Chem. and Drugg. 1890 p. 602.

GRAVIMETRIC ESTIMATION OF INDIVIDUAL ALKALOIDS.

91. For exact gravimetric determination of individual alkaloids, the precipitates produced by gold and platinum chlorides are well adapted. When these precipitates are ignited, the residue consists of metallic gold or platinum as the case may be. In general the procedure is like that for determining potassa as a platinum compound. The alkaloid is evaporated with a slight excess of hydrochloric acid and an excess of the reagent nearly to dryness, the crystalline precipitate is washed with a little water or with dilute spirit, care being taken to remove all excess of reagent and all mineral salts, the residue ignited and weighed, best in a Gooch crucible.

92. The following table gives (a) the weight of the respective residue of gold or platinum corresponding with one hundred parts of the pure precipitate* (b) the factor by which to deduce from the weight of the residue the alkaloid it represents. [For example a residue of 27 milligrams of gold in an atropine estimation would correspond with a precipitate of the double chloride of gold and atropine weighing 27×100

*The figures in the table, except those for gelsemine and cocaine are from Dragendorffs' Plant Analysis, § 173.

÷31.37 milligrams or with pure atropine weighing 27×1.46 =45.4 milligrams]

TABLE OF GOLD AND PLATINUM EQUIVALENTS.

	Per cent of Gold in precipitate.	Factor to give alkaloid.	Per cent. of Platinum in precipitate.	Factor to give alkaloid.
Atropine	31.37	1.46	.....	.....
Berberine	29.16	1.70	18.11	3.43
Brucine	.....	.....	16.52	4.03
Caffeine	37.02	0.98	24.58	1.99
Cinchonine	.....	.....	27.36	1.50
Cocaine	30.68	1.54	.....	.....
Codeine	.....	.....	19.11	3.06
Coniine	.....	.....	29.38	1.30
Emetine	.....	.....	29.70	1.30
Gelsemine (Gerrard)	36.90	1.04	16.70	2.09
Gelsemine (Thompson)	29.80	1.63	18.58	3.30
Hyoscyamine	34.60	.....	.....	.....
Morphine	.....	.....	19.51	2.92
Nicotine	.....	.....	34.25	0.62
Pilocarpine	35.50	1.06	23.6‡	2.13
Piperine	.....	.....	12.70	5.85
Quinine	40.00	0.82	26.26	1.66
Strychnine	29.15	1.69	18.16	3.42
Theobromine	.....	.....	25.55	1.85
Veratrine	21.01	3.01	.....	.....

VOLUMETRIC ESTIMATION OF ALKALOIDS BY MAYER'S REAGENT.

93. Most Alkaloids are very completely precipitated from acid aqueous solutions by the solution of potassio-mercuric iodide known as Mayer's reagent. The precipitates are more or less soluble in alcohol, ether, acetic acid, solutions of iodides and so 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.

94. If Mayer's reagent is added little by little to an acid solution of an alkaloid, the first portions will produce a dense precipitate but after a time a point will be reached where only a faint cloud is produced by a drop of the reagent. It may be still necessary to add a considerable quantity, ten or twenty drops, of the reagent before the point is reached when a further addition produces no effect, and it will be then found, in the case of most alkaloids, that a large excess of the reagent has been added, so that addition of a fresh

alkaloidal solution causes a heavy precipitate. The excess will constitute a certain proportion of the fluid at the end of the titration, hence, *the more dilute the solution titrated, the larger the quantity of reagent required to complete the precipitation.*

95. We cannot therefore fix for each alkaloid a definite invariable titration equivalent. We find moreover that the degree of acidity of the alkaloidal solution, the manner of adding the reagent, any variation, in short, in the mode of procedure is liable to influence the result materially, so that quantitative determinations of an alkaloid by titration with Mayer's reagent are of value only when carried out under certain prescribed conditions. We find, further, that at best there is a provoking variability in the composition of the precipitates, so that even when we collect and weigh these, we obtain only approximate results.

96. In spite of these drawbacks, a good many alkaloids *can* be determined with reasonable exactness by titrating with Mayer's reagent, and no doubt in the hands of an inexperienced person the method will be undertaken with more confidence and will actually give better results than alkalimetry. For certain alkaloids, it is true, it is worthless, for some others too troublesome to be recommended; the same may be said of the akalimetric method; in either case we are to prove all methods and hold fast all that is good. In my own practice, I still find a use for Mayer's reagent in rough valuations of ipecac and of colchicum, and in determinations of strychnine and of berberine. Besides this, it is useful in determining rapidly whether or not a given tincture or fluid extract is deficient in alkaloid.

97. Method of tritration with Mayer's Reagent. Put the solution (10 to 20 cc.) to be titrated, which should contain as nearly as possible 0.5 per cent. of alkaloid, into a small cylindrical measuring glass, or a roughly graduated test tube, and note its volume. Run into it from a burette, Mayer's reagent, N 1-20, 15 drops, or, if you have an idea of the quantity of reagent you are likely to require, one-half of that quantity. 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.

98. When the fluid has nearly all run through, set the funnel in test tube No. 1, and add to the filtrate two drops of Mayer's reagent from the burette; if this produces a dense precipitate as it should, add 10 or even 15 drops of the reagent, return to the filter, using the fluid that has filtered meantime into No. 1 to rinse No. 2, returning all to the filter. Proceed in this way until the precipitation begins to be scanty, when the quantity of reagent added at once must be reduced accordingly, until finally it becomes a single drop. As the end of the precipitation is neared, allow nearly the whole of the fluid to pass through the filter before adding more reagent, and filter twice if necessary to secure a perfectly clear fluid.

99. 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 will produce a faint cloud, which disappears as it mixes with the rest of the fluid. If a larger quantity of reagent be added, a permanent turbidity, or even a precipitate, is produced, but this should be

ignored. Uniformity of practice in fixing the end point of the reaction is, of course, indispensable. The operator should practice with solutions containing a known quantity of alkaloid, and so determine its equivalent as modified by his personal equation. If at the close of a titration it appear that the original solution was stronger or weaker than that recommended above (0.2 to 0.5%) a new solution should be made of a strength approximately of 0.5 per cent. and the titration repeated with this.

100. Hereth's Method* of titrating with Mayer's reagent consumes rather less time than that given above, and is carried out as follows: Knowing approximately the alkaloidal strength of the solution to be examined, provide half a dozen or more test tubes or vials, into each of which measure 10 cc. of the solution. To the first add a quantity of Mayer's reagent which is believed to be a little less than enough for the precipitation [make sure that such is the case, before proceeding further, by filtering and testing the filtrate with more of the reagent.] To the second portion add a quantity of reagent 5 per cent. greater, to the third a quantity 10% greater and so on. Let the tubes stand eight hours, then test a portion of the clear supernatant fluid from each with a drop of the reagent. Among them there will be some that 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 additional precipitate 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 carried on in the usual manner.

*Pharm. Record, 1886 p. 209.

101. 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, which would differ more or less from those based on the ordinary procedure.

102. Gravimetric Determinations by Mayer's Reagent. Results somewhat more exact and uniform may be obtained in the use of Mayer's reagent if we collect and weigh the precipitate instead of depending upon the quantity of reagent consumed. The error would rarely be more than five per cent. of the total weight—not an ideal exactitude of result, it is true, and yet close enough to give useful information. The reagent must be added in slight excess, and time given for complete reaction and separation of the precipitate; in the ordinary routine say two hours. The precipitate is to be then collected on a pair of mutually counterpoised filters, washed with the smallest practicable quantity of water, applied so as to wash the *filters* especially. When the filters have drained, press first between folds of filter paper, to remove most of the water, then dry at 100° C. to a constant weight and weigh. Or, after the precipitate is washed and drained it may be dissolved in strong alcohol, evaporated in a tared beaker and weighed.

103. Titration of Tinctures, etc., with Mayer's Reagent. It is sometimes recommended to titrate directly with Mayer's reagent solutions prepared from tinctures or fluid extracts by acidulating, evaporating off the spirit and diluting if necessary with water. Such a procedure may enable us to detect any notable deficiency of active principle, but is liable to be very misleading since other substances be-

sides alkaloids are precipitated by the reagent. Solutions prepared with strong alcohol or with ethereal solvents are less likely to contain such substances (albuminoids) but in general *titration with Mayer's reagent should be practiced only on solutions so prepared that they can contain little besides alkaloidal salts.*

104. It must be admitted, however, that, in the writer's somewhat extensive experience, direct titration applied to galenical preparations does correctly indicate in most cases their relative medicinal strength and a standardization based upon such titration is much better than no standardization at all.

105. Where several alkaloids are present in a drug as is so often the case, the titration method—whether with Mayer's reagent or with standard acid—can be only of limited application unless preceded by a separation of the alkaloids. It may, however, sometimes serve, when the total alkaloid has been determined, to indicate the relative proportion of the constituents, if there are but two as in nux vomica, and after all a standard based upon "total alkaloid" may be as rationally fixed by titration as by weighing.

106. Precipitation equivalents of Mayer's reagent. Only by the experimental method can the correct precipitation equivalents of the several alkaloids be determined for practical purposes. If solutions are always made so as to contain very nearly 0.5 per cent of the alkaloid, and the titration is made in solutions always containing the same proportion of free acid, there should be with most alkaloids but little variation in the titration equivalent, and it is with these conditions in view that I have made out in the accompanying table the column of "Practical equivalents" which should be used when the titration is conducted in the manner described above (97) et seq. I give for comparison Dragendorff's figures,

which, like my own, are empirical, but based upon titrations conducted in a somewhat different manner.

107. When the solution contains much less than 0.5% of alkaloid, closer figures will be obtained by using the "corrected equivalents" of the table after having deducted from the quantity of Mayer's reagent used a correction, which consists of a certain per cent. of the whole volume of fluid at the end of the titration. Thus in a titration of cocaine, suppose the volume of the solution before titrating were 12 cc., and that 5.25 cc. of Mayer's reagent N 1-20 have been required for complete precipitation. Then at the end of the titration the volume of fluid was 12+5.25=17.25 cc. The correction by the table is 8% of this, viz. 1.38 cc. Subtract this from 5.25, we have 3.87 cc. as our corrected figure for Mayer's solution used. This, according to the corrected equivalent of the table (.0098), indicates the presence in the solution of .038 Grms. of cocaine, instead of .0405, the figure we should have deduced by the use of the column of "Practical equivalents."

108. A second titration should be made, if time and material permit, with a solution concentrated so that 8 cc. contain as much alkaloid as the 12 cc. used in the first titration. In this case, if it were a question of concentration by evaporation of the aqueous solution, I should prefer to abide by the figure already obtained, rather than expose the alkaloid to risk of destruction—a principle to be borne in mind in all operations in which we deal with chemical compounds so unstable as many of the alkaloids.

109. The last column in the table, based upon experimental data, is to facilitate calculations where the alkaloid is to be determined by the weight of the precipitate produced by Mayer's reagent. Multiply this weight by the mean value of

the factor to find approximately the weight of the alkaloid contained in the precipitate. Thus a precipitate from a brucine solution weighs 93 milligrams, the factor is stated to be 0.465 to 0.500. The mean of these is 0.4825. The quantity of alkaloid is therefore about 45 mg., or somewhere between 43.2 and 46.8 mg

110. The reason why titration with Mayer's reagent is unsatisfactory is, in most cases, that the end of the reaction is not sharply defined, precipitation continuing long after the reagent is in excess. Better results could no doubt be obtained were there any simple method of determining the excess of reagent after precipitation ceases. One plan I have used with some satisfaction consists in adding to the solution at first an excess of Mayer's reagent, filtering and adding to the clear filtrate, after the precipitate has completely drained on the filter, 5 cc. of a solution of strychnine containing nine milligrams to the cc., titrating the excess of strychnine and so ascertaining the excess of Mayer's solution present. (It may be necessary to use more than 5 cc. of the strychnine solution.) Of course the titration equivalent to be adopted will be higher than that given in the table as the practical equivalent; it will approach more nearly to the "corrected" equivalent, but must be determined in each case experimentally.

111. Another way of determining the excess is by diluting the filtrate, adding to it ammonium sulphide, and comparing colorimetrically with Mayer's reagent diluted in a similar way. Make the latter dilution 1:1000; make up the filtrate to a litre, and from this make dilutions $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, etc., for the comparisons. I have not worked out the titration equivalents where this method is followed; they will be not very different from the corrected equivalents of the table.

112. TITRATION EQUIVALENTS OF MAYER'S REAGENT N 1-20.

Name of Alkaloid.	Results.	Titration Equivalent Dragendorff.	Practical titration Equivalent Lyons.	Correction.	Corrected titration Equivalent (S2)	Gravimetric Factor.
Aconitine	good	0.01350	0.0141	.....	.....	0.526-0.555
Atropine.....	unsatisfactory	0.00625	0.0077	5%	0.0088	0.448-0.461
Berberine.....	good	0.02063	0.0263	.....	.....	0.500-0.520
Brucine (a).....	fair	0.00985	0.0125	3%	0.0139	0.465-0.500
Chelidonine.....	.....	0.00840	.....	.....	.....	....
Cinchonidine.....	unsatisfactory	.....	0.0070*	.....	.....	0.266-0.303
Cinchonine.....	fair	.....	0.0071	.....	.....	0.290-0.300
Cinchonine(b)....	unsatisfactory	.....	0.0090*	.....	.....	.....
Cocaine	fair	.....	0.0078	8%	0.0098	0.406*
Colchicine(c).....	fair	0.01580	0.0109	8½%	0.0149	0.625*
Coniine.....	unsatisfactory	0.00625	.....	.....	.....	.....
Emetine.....	good	0.00945	0.0106	3%	0.0116	0.390*
Gelsemine.....	fair	.....	0.0096	7%	0.0120	0.500-0.540
Hydrastine.....	fair	.....	.....	.....	.....	0.476-0.500
Hyoscyamine....	unsatisfactory	0.00938	0.0116	.....	.....	0.400-0.454
Morphine.....	unsatisfactory	.....	0.0128	.....	.....	0.478-0.526
Nepalline.....	.....	0.01940	.....	.....	.....	.....
Nicotine.....	.....	0.00203	.....	.....	.....	.....
Pilocarpine.....	unsatisfactory	.....	0.0055*	.....	.....	.....
Physostigmine...	.....	0.00688	.....	.....	.....	.....
Quinine.....	unsatisfactory	.....	0.0056*	.....	.....	0.299-0.323
Sabadilline.....	.....	0.01870	.....	.....	.....	.....
Sabatrine.....	.....	0.01670	.....	.....	.....	.....
Sanguinarine.....	fair	0.00743	.....	.....	.....	.....
Strychnine.....	good	0.00835	0.0091	2½%	0.0102	0.363-0.385
Veratrine.....	fair	0.01480	.....	.....	.....	.....

(a) Nearly neutral. (b) Neutral. (c) Solution must contain 3% sulphuric acid. *Approximately.

VOLUMETRIC DETERMINATION OF ALKALOIDS BY WAGNER'S REAGENT.

113. Although Wagner's reagent precipitates alkaloids very completely from their aqueous solutions, chemists generally until quite recently have had the impression that the precipitates are of too variable composition to make them available in quantitative work. The researches of Dr. Prescott and his collaborators during the past two or three years have proved that this impression was not well grounded; that in the case of certain alkaloids if not of all, conditions of precipitation can be easily secured which will give us compounds of perfectly definite and constant composition, and that it is easy to determine the iodine in these by simple volumetric tests.

114. Volumetric Estimation with Wagner's Reagent. Prescott and Gordin* give the following general method of determining alkaloids by residual titration after precipitating with Wagner's reagent in excess: To 10 cc. of decinormal iodine solution (U. S. P.) diluted with a little water, add one cc. of the acidulated alkaloidal solution and shake the mixture well a few minutes. Should the precipitate separate out very quickly, leaving the fluid light yellow or greenish, the

*Jn. Am. Chem. Soc. Sept. 1898, p. 722.

quantity of alkaloid is too large and the experiment must be repeated using a more dilute alkaloidal solution, so that the solution after separation of the precipitate shall remain of a dark red color.

115. Having thus ascertained the approximate amount of alkaloidal solution to use in the assay, repeat the experiment, using 25 cc. of the decinormal iodine solution, and shaking, after addition of the accurately measured dilute alkaloidal solution, until the supernatant liquid is perfectly transparent and of a very dark red iodine color. [The iodine solution must be throughout the experiment largely in excess, hence it will not do to add more of the reagent if it be found that the quantity used was insufficient. The process must be repeated from the beginning.] An aliquot part of the liquid is filtered off and the excess of iodine in it is determined by titration with decinormal sodium thiosulphate (U. S. P.) Subtract the number of cc. used from 25 to find the quantity of decinormal iodine solution required to precipitate the alkaloid. The following factors have been established experimentally. Each cc. of the decinormal iodine consumed corresponds with atropine 3.6048 mg., strychnine, 5.5555 mg., brucine 6.5530 mg., morphine, 9.4794 mg., caffeine, 4.85.†

116. Other volumetric processes that may be sometimes serviceable depend upon the use of standard solutions of some of the other precipitants of alkaloids. Thus coniine which is very imperfectly precipitated by Mayer's reagent may be titrated with Sonnenschein's reagent (sodium phosphomolybdate) and this indeed admits of quite extended

†Gomberg, Jn. Am. Chem. Soc. 18, p. 339.

use. With our improved methods, however, of extracting the alkaloids directly from the drug, and obtaining them thus in a pure form these indirect methods of procedure have mostly fallen into disuse.

117. Better results than those given by Mayer's reagent are claimed, and no doubt justly, by Mr. Thresh* from the use of a solution of potassium and bismuth iodide (19) The solution is to be used exactly as Mayer's reagent is, being added to the acidulated ("H Cl or other acid") aqueous solution until it ceases to give a distinct precipitate. The precipitates separate promptly and the end of the titration is more sharply defined than with Mayer's reagent. The following are the quantities of the several alkaloids named which correspond with 1 cc. of the reagent:

Aconitine	.00280
Apomorphine	.00425
Atropine	.00388 to .00400
Bebeerine	.00206
Brucine	.00457 to .00463
Caffeine	.00358
Cinchonidine	.00258
Cinchonine	.00272
Codeine	.00404 to .00428
Emetine	.00258
Morphine	.00404
Narceine	.00416 to .00437
Quinidine	.00290
Quinine	.00273
Strychnine	.00496
Theine	.00433 to .00448

*Pharm. Jour. Trans. 1880. p. 809.

SPECIAL METHODS OF SEPARATING ALKALOIDS IN A STATE OF PURITY.

118. Scheibler's Process. Precipitate the aqueous solution with phosphotungstic acid, collect the bulky precipitate, wash with water containing some phosphotungstic acid and ammonia. Rinse the washed precipitate into a flask, add caustic baryta or potassium carbonate 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 with chloroform.

119. Wagner's Method.* Acidulate the aqueous solution with sulphuric acid, precipitate with excess of Wagner's reagent (17). Collect the precipitate, dissolve it in a solution of sodium thiosulphate (hyposulphite), filter, precipitate once more with excess of Wagner's reagent, dissolve the precipitate in excess of aqueous sulphurous acid, evaporate to dryness on the water-bath, keeping the sulphurous acid in excess until the hydiodic acid is all expelled. The alkaloid is left finally in the form of a sulphate.

120. Kippenberger's Method.† Precipitate the slightly acidulated aqueous solution with an iodine solution containing 12.7 grams of iodine and 60 grams of potassium

*Dingler's Polyt. J. 161.40; Ztschr. Anal. Chem. I. 102.

†Ztschr. Anal. Chem. 34.294, 407; Apoth Zeit. 12.459, 467.

iodide to the litre. Collect the precipitate on an asbestos filter, wash thoroughly with cold water, dissolve in a small quantity of purified acetone, dilute with water and shake with light petroleum (boiling point 30° to 50° C.) first with addition of caustic alkali, then after rendering acid with hydrochloric acid. Draw off the aqueous solution, evaporate off acetone at a gentle heat, add a few drops of solution of sodium thiosulphate to remove free iodine, finally render the solution alkaline with sodium carbonate or with ammonia and shake out in the usual way with chloroform or other appropriate solvent.

121. By Mayer's Reagent. T. B. Groves* directs to 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 out with an appropriate immiscible solvent. Prescott† directs to triturate the washed iodomercurate precipitate with stannous chloride solution and caustic potash added to strong alkaline reaction and then extract with an appropiate solvent. (If potassium carbonate be substituted for the caustic alkali, strong alcohol may be used as the solvent.)

122. Another plan suggested by Prescott, is to dissolve the iodomercurate precipitate in alcohol, adding acid if necessary and precipitating the mercury with hydrogen sulphide gas. The filtrate can be freed from iodine, if this be desired

*Pharm. Journ. Trans. [II.] vol. 6. p. 275.

†Organic Analysis, 1887. p. 46.

after expelling the excess of hydrogen sulphide, by adding excess of silver nitrate, filtering, adding hydrochloric acid to remove the excess of silver and filtering again, the alkaloid being now in the form of a chloride.

123. By Phosphomolybdate of Sodium. Exhaust the drug with acidulated water, make nearly neutral and precipitate the solution with solution of lead subacetate in slight excess; precipitate excess of lead from the filtered solution with sulphuric acid cautiously added, partially neutralize with soda, filter, concentrate by evaporation; precipitate with sodium phosphomolybdate in slight excess, collect the precipitate, wash with a little water containing phosphomolybdic and nitric acid; mix the moist precipitate with excess of carbonate of calcium or barium, or with calcium or barium hydrate, dry at a water bath heat and extract the alkaloid, which is now free, with alcohol or other appropriate solvent.

124. By Tannin. Prepare an aqueous solution in the manner just described; neutralize accurately with solution of soda, add solution of tannin cautiously, avoiding excess, and preserving the neutrality of the solution by successive additions of soda solution. [The following alkaloids require for complete precipitation presence of an excess of *acid:* aconitine, physostigmine, veratrine; and some others may be precipitated without loss from an acid solution, but this is the exception.] Collect the precipitate, wash slightly with water, 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.

SEPARATION OF ALKALOIDS FROM ONE ANOTHER BY SOLVENTS.

125. The comprehensive scheme of **Prof. Dragendorff*** which I transcribe here will be of assistance in case it is desired to separate one alkaloid from another. 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 decomposition of aconitine, and (c) *volatile*, phenol.

126. Shake the still acid fluid with several successive portions of **benzol.** This will remove absinthin, cantharidin, cascarillin, caryophyllin, cubebin, digitalin, elaterin, populin, santonin and the *alkaloids*, caffeine, colchicine with traces also of berberine, delphinine, physostigmine and veratrine; also remnants of piperine and of picric acid.

127. Treat the acid fluid next with **Chloroform** in the same manner. This solvent will remove convallamarin (in part) digitalin, helleborin, picrotoxin, saponin, (in part) senegin, (in part) smilacin and syringin with the *alkaloids*,

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

cinchonine, jervine, narceine, (in part) papaverine, theobromine and traces of brucine, delphinine, narcotine, physostigmine and veratrine, also remnants of some of the substances imperfectly removed by benzol.

128. 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, lobeline, nicotine, sarracenine, sparteine, trimethylamine; (b) *fixed alkaloids*, brucine (partly) cocaine, emetine, quinine, sabadilline, strychnine and veratrine.

129. Shake the solution next with **benzol,** which removes the remnant (generally considerable) of the fixed alkaloids partially taken up by the benzin, also aconitine, atropine, cinchonine, cinchonidine, codeine, hyoscyamine, napelline, nepaline, thebaine, with remnants of brucine, delphinine, narcotine, physostigmine and veratrine.

130. From the solution, **Chloroform** will remove morphine (in part) with the remainder of the cinchonine, narceine and papaverine, and **Amylic Alcohol** in turn will remove salicin, solanine and remnants of convallamarin, saponin, senegin, morphine and narceine. The fluid, evaporated to dryness, with addition of powdered glass, will still yield to **Chloroform** curarine.

ASSAY OF GALENICAL PREPARATIONS.

131. Fluid Extracts. To ascertain the alkaloidal strength of a fluid extract we may generally adopt one of the following general methods, of which the author would recommend for usual routine either e or a; some, like b or i, are of only limited utility.

a. Add ammonia and **shake out directly** with an immiscible solvent; wash the alkaloid from this into acid water, separate, add ammonia and shake out once more with the appropriate solvent. See (132)-(139).

b. Dilute the fluid largely with water, add excess of **lead sub-acetate** and filter; add sulphuric acid sufficient to remove excess of lead, filter once more, concentrate if necessary (after rendering nearly neutral) add ammonia and shake out with chloroform.

c.* Add to the fluid extract **ferric chloride** in excess, then sodium bicarbonate sufficient to produce a stiff magma. Triturate this with successive portions of chloroform until the whole of the alkaloid is removed. This may be sufficiently pure for weighing, but requires generally to be washed out with acid water, and extracted from this once more by adding alkali and shaking out with the appropriate solvent. See (140.)

*J. U. Lloyd, Proc. A. Ph. A. 1891, p. 128.

d.* Add to the fluid extract ten to twenty times its volume of weak **Prollius' mixture,** shake together for some time. Pour off an aliquot part of the ethereal fluid and treat as in the assay of a drug by Prollius' mixture. See (142.)

e.† Mix the fluid extract with oak **sawdust,** dry at a low temperature, treat the residue exactly like a crude drug with Prollius' mixture, etc. See (141.)

f. Evaporate off alcohol at a low temperature, add **plaster of Paris** mixed with a little sodium bi-carbonate, allow the mixture to set and harden; when sufficiently dry pulverize and extract the alkaloid by hot repercolation, or else by simple maceration, with ether, petroleum ether and chloroform (mixed) or other appropriate solvent. The alkaloid must be purified as in process c.

g.‡ (**The latest** original method published). Extract the alkaloid directly from the fluid extract by adding caustic soda and shaking out repeatedly with ether, remove traces of aqueous fluid containing alkali by addition of plaster of Paris; the ethereal solution is then titrated directly with centinormal acid using iodeosin as an indicator. The method is not applicable if chlorophyll, fixed oils or salts of ammonia are present. See (147) and (149).

h. 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.** See (103.)

i. Pour the fluid extract into nine times its volume of

*J Steiglitz, Pharm. Rundschau N. Y., 1891, 287.

†F. A. Thompson, Proc. A. Ph. A. 1892, p. 446, from Proc. Mich. Pharm. Assoc. 1891.

‡J. Katz. Arch.d. Pharm. 1898. 1. Am. Druggist, 1898, 281.

water containing acid, filter, **precipitate** the alkaloid from an aliquot part of the filtrate by adding **ammonia,** collect on a tared filter, wash, dry and weigh, or else after drying dissolve in an appropriate solvent, evaporate and weigh.

k. Put into a two-ounce vial one gram of freshly slaked **lime,** add 5 cc. of the fluid extract and immediately 45 cc. of **alcohol,** shake well for a minute or two to insure complete solution of the alkaloid, filter, render faintly acid with sulphuric acid filter once more, evaporate off the alcohol to procure an aqueous solution comparatively free from inert matter, from which the alkaloid may be extracted, after washing once or twice with ether, by rendering alkaline and shaking out in the usual way.

l. Add to the fluid extract a few drops of **acetic acid** then an equal volume of water, evaporate at a temperature not exceeding 50° C. (124°F.) to expel alcohol, remove chlorophyll, etc. by repeated washing with petroleum ether, recovering from this any alkaloid that may have been taken up by treating with a little dilute acid, mix the acid solutions, wash once with chloroform unless the alkaloid is one taken up by that solvent from acid solutions, in which case use ether, make alkaline and remove alkaloid with chloroform or other appropriate solvent

m. Introduce the fluid extract without previous treatment into a **perforator,** render acid and wash thoroughly with petroleum ether, then make alkaline and extract the alkaloid with the appropriate solvent.

132. Routine of Process (**131 a**) practiced by the author. The details will vary somewhat according to the solvent chosen. That which I find most generally useful is

the following: Measure into a one-ounce prescription vial having a square shoulder and a good lip exactly 5 cc. of the fluid extract. Put into the bottle 20 cc. of a mixture of chloroform one volume, ether three volumes. [Use pure ether preferably whenever that solvent can be relied upon to extract the alkaloid.] Add water of ammonia 10% five drops and immediately shake together, observing whether there is any tendency to emulsionize, in which case let the shaking be gentle. Continue the shaking 30 seconds, then let the fluids separate and pour off the ethereal fluid into a second vial containing 3 cc. of water and one drop of water of ammonia. Add to No. 1, 20 cc. of pure ether; if the contents of the vial seem inclined to become syrupy, add 1 cc. of dilute alcohol, but this is not often necessary. Shake both bottles a few seconds and let separate. After two minutes (longer if necessary) pour off the ethereal fluid of No. 2 into a third vial, that from No. 1 into No. 2; add to No. 1, 15 cc. of fresh ether, which should remove the last traces of alkaloid, but this must be ascertained by testing a drop of the residual fluid after acidifying, with Mayer's reagent.

133. Put into vial No. 3 three cc. of 3 per cent. hydrochloric acid, shake well 30 seconds, let separate and pour off the ethereal fluid into a fourth vial containing 2 cc. of water acidulated with hydrochloric acid. The ether which has been added to No. 1, is passed in succession through the series in regular order, and finally through a fifth containing 2 cc. of water; before being finally rejected. The result will be that we shall have in No. 1 and No. 2 nothing of value, in No. 3 nearly all the alkaloid in acid solution, in No. 4, an acid fluid containing a little alkaloid, in No. 5, water with possi-

bly traces of alkaloid. Now add to No. 4, twenty cc. of chloroform-ether (pure ether, if this will answer the purpose) and a few drops of water of ammonia, shake well, let separate and transfer the ethereal fluid to No. 3. Add also to No. 3 water of ammonia in excess (15 drops or more) shake 30 seconds, making sure that the ammonia is in excess (test the vapor with red litmus paper), let separate and transfer the ethereal fluid to No. 5., to which a drop or two of water of ammonia has been previously added.

134. Wash the contents of the vials No. 4 and No. 3 (in this order) in succession with two or three fresh portions of ether making sure that the whole of the alkaloid is extracted. Pass the ether in each case finally into No. 5, which serves merely as a wash bottle, and from this after complete separation into a tared beaker, in which it is evaporated to dryness on a water bath and finally weighed. Where chloroform has been used as a solvent, redissolve the residue in alcohol and evaporate again, to expel the last traces of chloroform. Finally the residue may be dissolved in ether or in alcohol, and the alkaloid in it determined by titration with standard acid. (84) or (85).

135. The above process I have given in minute detail because in my hands it has given almost always satisfactory results. Emulsionizing is not liable to occur, while the ether chloroform removes the alkaloids almost as certainly as pure chloroform does. It may be necessary sometimes to carry through the entire process with ether chloroform, or to vary it by using pure chloroform (10 cc.) in the first washing, adding immediately ether enough (20 to 25 cc.) to bring it to the surface. Petroleum ether may be used in the case of a

few drugs. A mixture of chloroform and petroleum ether may prove as generally useful a solvent as that above recommended. I have not yet given it a fair trial. Whatever solvent is used, the routine of the process will be the same.

136. Abbreviated Process. If the operator is willing to depend upon the alkalimetric test alone, the above process (133) and (134) may be abbreviated as follows. Proceed as before up to the point where the ethereal solution is treated with acid. Instead of decanting into a third vial, collect the several portions of ethereal solvent in a beaker, evaporate at gentle heat to dryness and determine the alkaloid by alkalimetry (84) or (85.) Subsequent separation and weighing of the alkaloid may be made if desired.

137. Use of the Perforator. Of course the acid solution obtained in vials No. 3 and 4 (133) can be transferred to a perforator, rendered alkaline and the alkaloid extracted (56) without any preliminary washing with petroleum ether.

138. Routine Alternative Process. (131 a) If the solvent has a higher specific gravity than the fluid extract (e. g. pure chloroform) a plan differing in detail must be adopted, as follows: Put into a small separator 10 cc. of the fluid extract (a smaller quantity of it is rich in alkaloid), add a few drops of solution of ammonia and 20 cc. of chloroform, agitate carefully, add a little water (3 to 8 cc.) agitate once more, let separate and draw off the chloroform 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 should now contain no trace of alka-

loid (prove this by evaporating a few drops on a watch crystal, adding a small drop of acidulated water and testing with Mayer's reagent.) Treat the contents of separator No. 1 with 10 cc. of fresh chloroform, which is to be passed through the series in succession, and the same process repeated as long as alkaloid is taken up from No. 1.

139. When the exhausted chloroform has been removed, [it is to be preserved for redistillation] put into separator No. 3, 15 cc. of fresh chloroform and a few drops of solution of ammonia, shake together and draw off both chloroform and aqueous fluid into No. 2; add more ammonia if necessary to make alkaline. Shake together, let separate and draw off the chloroform into No. 3, in which has been placed 5 cc. of water. Wash out the contents of No. 2 with several additional portions of chloroform, passing this through No. 3 and finally collecting it in a tared beaker. Evaporate the chloroform, dry moderately on the water bath, take up the residue with acid free alcohol, dry completely and weigh. Finally determine the alkaloid by titration with standard acid, (84) or (85.) Of course the modifications described in (136) and (137) are equally applicable to this alternative process.

140. Detail of **Process of J. U. Lloyd.** In a flat bottomed porcelain mortar with a good lip, mix 5 cc. of the fluid extract with 1 cc. solution perchloride of iron, add a mixture of equal parts of dry ferric hydrate and sodium bicarbonate with constant trituration until a stiff magma results. Abstract the magma by repeated trituration with chloroform, using first 20 cc. and then three portions of 10 cc. each, making up in all exactly 50 cc. Divide into two equal portions.

The first portion is placed in a separator with spherical bulb and extracted with three successive portions (10 cc) of dilute sulphuric acid (2%). Collect the acid solutions in a second separator, make alkaline with ammonia, wash out the alkaloid by **rotating** rather than shaking with three successive portions of chloroform (10 cc.) Evaporate the chloroform in a tared dish to constant weight and weigh. [The second portion of the original chloroformic extract may be used for an independant acidimetric determination.]

141. Detail of **Process of F. A. Thompson.** (131 e) Place in a capsule 5 to 7 grams of oak sawdust, pour gradually into this 10 cc. of the fluid extract. [If it contains more than one per cent. of a kaloid, dilute with 50% alcohol to reduce to approximately that strength and take 10 cc. of the diluted extract, noting of course exactly how much of the original extract this represents.] Mix thoroughly with the sawdust, which must be in sufficient quantity to absorb the fluid pretty fully, dry at a temperature not exceeding 45°C. (115° F), transfer to a 4 ounce prescription vial, add 100 cc. of modified Prollius' mixture (25), shake frequently during fifteen or twenty minutes, pour off exactly 50 cc. of the ethereal fluid and complete the assay exactly as described in (63) or (73). This method is certainly to be preferred to that of Steiglitz (131 d.), in which there is some difficulty in deciding just what quantity of the ethereal fluid should be taken as representing an aliquot part (e. g one-half) of the fluid extract taken.

142. I have myself sometimes adopted a plan similar to that of Steiglitz for rapid approximate work, as follows: To 5 cc. of the fluid extract add 5 cc. of ammoniated alcohol

(alcohol nine volumes, stronger ammonia, one volume) followed immediately by 45 cc. of ether, shake well two or three minutes, let separate and pour off the whole of the ether which may be assumed to contain 95% of the alkaloid present, and is to be treated as in the ordinary assay by Prollius' mixture (73.) I prefer, however, the plan described above (132) et seq., which does not in fact consume any more time.

143. Other absorbents besides sawdust have been employed, absorbent cotton by some, infusorial earth, absorbent paper, vegetable fiber in the form of twine cut in short pieces, etc. The sawdust seems to fill the requirement best of all for reasons that need not be enumerated. The choice of a sawdust rich in tannin does not commend itself to me. What is wanted is something as free as possible from soluble matter, particularly of a resinous nature. Hence pine sawdust is not to be used, unless first extracted with alcohol. Well washed sawdust from hard wood of any kind, (even oak) is good. It should not be too fine. Filter paper is liable to soften and mat together, but if cut in small pieces and used in quantity sufficient to absorb the moisture without being too much softened, it answers a good purpose, as does the chopped string or rope. Sand or broken glass cannot be recommended; pumice broken into fragments as large as a hemp seed is better, and is to be preferred to infusorial earth.

144. It is possible to combine the use of ferric chloride (Lloyd) or of lead acetate with that of sawdust or other absorbent. The ferric chloride is to be added to the fluid extract before it is mixed with the solvent. It may be necessary to add a little more ammonia than that contained in the Prollius' mixture. There must be enough to decompose both the alkaloidal salts and the iron or lead compound.

145. Absorbent cotton is used by F. B. Raynale in the following manner: Pack somewhat firmly in the bottom of a four ounce, round shouldered, wide mouthed prescription vial two grams of absorbent cotton. Into the center of this place by means of a pipette 10 cc. (or a smaller quantity, if rich in alkaloid) of the fluid extract. When this is completley absorbed by the cotton, add 100 cc. of Prollius' fluid modified (25) and shake occasionally during half an hour. Then take out an aliquot part of the ethereal fluid and treat as in (63) or (73)

146. Kebler as a routine process adds to 12 cc. of the fluid extract 12 cc. of water, shakes, adds chloroform 30 grams, ether 90 grams, shakes, adds ammonia in excess, shakes frequently during 30 minutes, then takes 50 grams, equivalent to 5 cc. of the fluid extract, and extracts the alkaloid in the usual way.

147. Routine of Process of J. Katz. (131 g.) Put into a separator 10 cc. of a fluid extract (25 cc. of a tincture) which should contain about 50% (by volume) of alcohol, add one cc. of a 33 per cent. solution of sodium hydrate *and 50cc. of ether and shake well five minutes and let separate. Draw off the aqueous fluid into a two ounce vial, add to the contents of the separator 3 cc. of water, shake, let separate, and draw off the water into the vial. Shake out the contents of the vial twice with ether containing 10% of alcohol (25 cc. each time.) The first portion is shaken in a second vial with 1.5 cc. of water and then decanted into a flask to which has been also transferred the ether from the separator. The ether

*This alkali is inadmissible in the case of solutions containing cephaeline. See chapter on assay of Ipecac.

is then deprived of traces of alkaline water by shaking with plaster of Paris (2—3 grams) and is then filtered into a glass stoppered flask containing 50 cc. of distilled water. The second portion of alcoholic ether is washed like the first with 1.5 cc. of water and then used to wash the flask containing the plaster of Paris, and the filter previously used, being finally mixed with the rest of the ether. The alkaloid is then titrated with centinormal acid, using as an indicator three drops of an alcoholic solution of iodeosin 1:250.

148. The method is recommended by its author on account of dispensing wholly with the application of heat. The weak point in it is the possibility that ammoniacal salts may be present in the tincture and so make it assay high in alkaloid. The ethereal solution can be evaporated surely without harming the alkaloid, unless it be a volatile one, and the titration after evaporation of the solvent would be much more conclusive, although this modification deprives the process of its one unique feature.

149. Tinctures containing chlorophyll, fat or fatty acids, must be deprived of these before extracting (as above) with ether, (the latter would otherwise appear in the result as alkaloid.) They are removed by mixing the tincture with water acidulated with sulphuric acid, shaking with talcum at intervals during several hours and filtering. The filtrate may be further washed once with petroleum ether to remove the last traces of the objectionable bodies

150. If the alkaloid is one not easily soluble in ether, it is necessary to use as a solvent a mixture of chloroform and ether. Three portions of the solvent are used of respectively 30,15 and 15 cc. but these are washed, not with pure water

but with a 30 per cent. solution of sodium chloride, since with pure water emulsification is liable to occur.

151. Tinctures, Wines and Elixirs are to be treated in general like fluid extracts, but the volume of fluid may be generally reduced considerably with advantage, by evapo ration at a low temperature as a preliminary step.

152. Syrups may, in some cases, be assayed by adding alkali and shaking out repeatedly with chloroform, ether being added to facilitate separation, but in many cases it is necessary to dilute the syrup, precipitate the alkaloid with Wagner's reagent and recover it from the precipitate (119).

153. Solid Extracts are to be converted into fluid extracts by dissolving in dilute alcohol where that is practicable. In many cases they cannot be dissolved as a whole and we must then adopt some especial procedure to obtain a solution. If the dilute alcohol yields a turbid mixture having only minute suspended particles of undissolved matter, we may simply add sawdust, dry, and follow out the process of (141).

154. If the extract is an aqueous one, heavily loaded with gummy matter, we may exhaust it with alcohol by the following treatment. Treat two grams of the extract in a capsule with dilute alcohol, 5 cc., add strong alcohol 15 cc. to precipitate gum, stir until the gummy matter separates, decant into a flask or vial; redissolve the gum in 2 or 3 cc. of water, add alcohol drop by drop, 3 cc. or to the point of incipient precipitation, then add 10 cc. of strong alcohol to reprecipitate the gum. Repeat this process once more if necessary [test the gummy residue for alkaloid with Mayer's reagent.] The united alcoholic solutions may now be concen-

trated by evaporation to obtain a solution suitable for extraction of the alkaloid.

155. In the case of extracts (alcoholic) heavily loaded with chlorophyll, fats or resinous matter, the following procedure is a good one: Soften the extract by warming in a capsule with a little dilute alcohol. Add to the syrupy extract a few drops of dilute sulphuric acid and treat with successive portions (5—10 cc.) of ether pouring these off into a separator until the most of the chlorophyll, etc., has been taken up. Put into the separator 3 cc. of water with five drops of dilute sulphuric acid (10%). Shake, let separate, draw off the acid fluid into the capsule containing the residue of the extract, which will be now mostly soluble in water. Transfer the solution to a vial, wash once or twice with fresh ether to remove the last traces of chlorophyll, etc., then make alkaline and shake out with ether or other appropriate solvent.

156. Method of L. Van Ittalie.* Dissolve 3 grams of the extract in exactly 20 cc. of water containing 5 drops of dilute sulphuric acid (10%), add 10 cc. of solution of lead acetate (10%) allow the precipitate to settle, filter. Measure into a suitable vial 16 cc. of the filtrate, add 40 cc. of a mixture of ether 25 volumes, chloroform 4 volumes and water of ammonia one volume. Shake well for one minute, make up the ether-chloroform layer to its original volume of 40 cc., shake again one minute and let separate. Evaporate 25 cc. of the clear ethereal fluid, representing one gram of the original extract, and determine the alkaloid in the residue by alkalimetry. (84).

*Rundş, f. Pharm. 21, p. 327; Pharm. Post, 1895. 236. See also Nieuw Tijds. Pharm. 1889. p. 6; Pharm. Centralh., 1889 p. 117.

157. Method of H. Beckurts. The original process* consisted in dissolving the extract. 2.5 grams, in a mixture of alcohol 3 cc. and water 6 cc., adding water of ammonia 1 cc., and shaking out with three successive portions of chloroform, 20, 10 and 10 cc., and determining the alkaloid by alkalimetry. In the case of extracts containing chlorophyll he now advises the following procedure.† Dissolve 5 grams of the extract in 50 cc., of alcohol, sp. gr. 0.892, add baryta water to 150 cc., allow to deposit, filter, precipitate excess of baryta with carbon dioxide. Filter, evaporate 75 cc. of the filtrate (equivalent to 2.5 grams of the extract) to a syrup, dissolve in a mixture of water 6 cc., alcohol 3 cc., and water of ammonia 1 cc.; shake out with chloroform as above, and determine alkaloid by alkalimetry.

158. Method of O. Schweissinger and G. Sarnow.‡ Dissolve 2 grams of the extract in 8 cc. of water, add water of ammonia, 2 cc., shake with 40 cc. of a mixture of chloroform 3 volumes, ether 5 volumes, and let separate. After half an hour, pour off exactly 20 cc. of the chloroform-ether, corresponding with one gram of the extract: evaporate and determine alkaloid by alkalimetry. Obviously the method cannot be used without modification in examining extracts containing chlorophyll. Otherwise it has the advantage of rapidity of execution.

159. Method of E. Dieterich.¶ Mix two grams of the extract (one gram in the case of nux vomica) with 3 cc.

*Pharm. Centralh. 1887, p. 508.

†Pharm. Rund. 1891, p. 240, Apoth. Zeitg. 1891, p. 538.

‡Pharm. Centralh, 1890, p. 583, 771.

¶Am. Journ. Pharm. 1887, p. 179, from Pharm. Centralh., 1887.

of water, add 10 grams of coarsely powdered lime forming a crumbly mass which is introduced into a displacement apparatus, and extract with ether, of which 30 grams may be used. The repercolation is continued 30 to 45 minutes in the case of aconite, belladonna and henbane, an hour and a half in that of nux vomica. The ethereal solution of the alkaloid is transferred to a tared porcelain capsule, the last portions being rinsed out with a little fresh ether, 1 cc. of distilled water is added and the ether evaporated at a temperature not exceeding 30°C. taking care to avoid proximity of volatile acids like hydrochloric, nitric or acetic. The residue, weighing 1.5 grams is dissolved in 0.5 cc. alcohol, sp. gr. 0.892, 10 cc. distilled water added, and the solution titrated with centinormal acid, using rosolic acid as indicator.

160. The method last described has been justly criticised owing to the liability to loss of alkaloid from the action of the lime. It certainly has no advantage over the other processes, in some of which we may, if we desire, employ the method of hot repercolation for exhausting the alkaloid.

161. All methods in which an aliquot part of an immiscible solvent is taken to represent the corresponding aliquot part of the original drug or extract must be regarded as only approximate; although they are not to be rejected as useless. The practical man even though a chemist, is willing to sacrifice scientific exactness when good approximate results can be reached without expenditure of time required for exact determinations. My personal preference is for the plan of dissolving the extract in dilute alcohol and if a reasonably clear solution results, treating it as in (132), otherwise adding sawdust, after the method of Thompson (141). When

the extract contains chlorophyll I prefer the method of (155), although extraction with acid water and filtration will often give a solution containing the whole of the alkaloid, which can be removed by rendering alkaline and shaking out with generous amounts of ether or other immiscible solvent.

ACONITE.

162. Active Constituents. There is as yet much to learn about the alkaloids of aconite. They are exceedingly unstable bodies, liable therefore, to undergo changes in the processes of extraction, even when these are conducted with extraordinary care. The most recent researches by Dunstan, Umney and others indicate that the root of Aconitum Napellus contains at least three alkaloids, aconitine, aconine and napelline, of which the first must be regarded as the active principle of the drug. The formula of aconitine is given by Dunstan as $C_{33}\ H_{45}\ N\ O_{12}$, by Freund as $C_{34}\ H_{47}\ N\ O_{11}$, but both authorities agree that it is acetyl-benzoyl-aconine, since it can be split so as to yield acetic and benzoic acids and aconine.

163. Practical Manufacture of Crystallized Aconitine. Of the various processes that have been proposed perhaps the best is the following:* Exhaust the powdered tuber with amylic alcohol (fusel oil) by maceration and percolation, extract the alkaloid by shaking repeatedly with highly dilute sulphuric acid, remove traces of amylic alcohol from the aqueous solution by shaking with ether, and warm to expel dissolved ether; precipitate the alkaloid with sodium carbonate, collect the precipitate and dry on blotting paper without heat, boil the crude alkaloid with pure (alcohol free) dry

*E. Richards and Ashley Roger, Chem. & Drug. 1891, p. 204, 242.

ether and set aside to crystallize. The crystals thus obtained must be washed with a little cold ether to remove amorphous alkaloid.

164. The aconitine thus prepared represents as nearly as possible the alkaloid upon which the activity of aconite depends, but it is a curious fact, apparently well established that if this alkaloid is converted into a nitrate, and from this a crystallized alkaloid is prepared, this last will be physiologically six times as active as before conversion into a nitrate.

165. Characteristic reaction of Aconitine. Dunstan and Carr* have recently called attention to a reaction by which aconitine may be distinguished from most other alkaloids. Potassium permanganate produces in its solutions a crystalline precipitate which appears even at a dilution of 1 : 4000. The other alkaloids of aconite produce also precipitates but only in much stronger solutions, aconine, 1 : 200. benzaconine, 1 : 100, so that it is possible to determine approxi ately in a mixture of the alkaloids the proportion of aconitine by diluting with water and finding the limit of precipitation. The only alkaloids besides those of aconite which produce precipitates with potassium permanganate are cocaine, hydrastine and papaverine, the first alone giving a crystalline precipitate and all requiring solutions as strong as 1 : 100.

166. Assay of Aconite Root may be easily made either by the author's method (72) or by that of C. Keller (50). Keller uses aconite root, No. 80 powder, 12 grams, ether 90 Grm., chloroform 30 Grm., water of ammonia 10 Grm., adding finally 20 cc. of water and pouring off 100

*Pharm. Journ. Trans. 1896. p. 122.

grams of the ether chloroform. This is extracted with hydrochloric acid, the alkaloid finally shaken out with pure ether. We may be satisfied with this determination of total alkaloid, since it consists in large part of aconitine, or we may purify it in the following manner: Allow the alkaloidal residue to stand a day or two to crystallize, then wash it with small portions of ether in the cold, the crystallized aconitine remaining undissolved, dry and weigh. Of course this gives us only an approximate result, since the aconitine itself is somewhat soluble in cold ether, and there is no certainty that all of the impurities will be thus removed.

167. The method suggested by Wright and Luff, and described in detail by A. H. Allen,* is more exact, although open to similar objections. The alkaloidal residue is dissolved in alcohol, sodium hydrate added, and the solution boiled until the alkaloid is completely "saponified". From the residue after evaporation the benzoic acid formed in the reaction is extracted with ether after acidifying with hydrochloric acid and determined by titration with baryta water N 1-50, phenolphthalein being used as indicator. Each molecule of benzoic acid represents one molecule of aconitine, therefore each cc. of the baryta water corresponds with 12.94 milligrams of aconitine.*

168. Mayer's Reagent in assays of Aconite. Titration with Mayer's reagent is not recommended in the case of an alkaloid so easily extracted and determined alkalimetrically as aconitine. If for any reason, the operator choose to

*Pharm. Journ. Trans. 1894, p. 2-0.

*More recently (1896) Dunstan and Tickle propose to determine aconitine by the acetic acid produced in its partial hydrolysis, but there seems to be no advantage in this over the earlier method.

employ this method he may proceed as described in (97), making up the solution for titration so that each cc. shall represent 0.62 gram of aconite root. Under these conditions each cc. of Mayer's reagent used will indicate nearly 15 milligrams of alkaloid.*

169. Physiological Test of Dr. Squibb. There is an easier way of arriving at the value of any preparation of aconite. It consists in testing by physiological experiment the actual effect it produces, under prescribed conditions, on the nerves of the tongue and lips. The test is applied in the following manner: Make an aqueous solution representing in 200 parts the alkaloidal contents of one part of drug. Thus a fluid extract would be diluted 1 : 200 with water (containing a little hydrochloric acid); the alkaloidal residue from 2½ grams of drug would be dissolved in a little alcohol, a few drops of dilute hydrochloric acid (to decided acid reaction) added and the solution made up with water to a volume of 500 cc.

170. The solution should be allowed to stand half an hour before proceeding to the actual test. Put into a 25 cc. measuring flask 8:3 cc. of the above solution, add water to the mark and mix by shaking. Rinse the mouth well with water, take into it one fluidrachm of the solution and hold it in the anterior part of the buccal cavity one minute by the

*According to Snow the titration equivalent of Mayer's reagent for solutions of aconitine is:

Dilution	1 cc. precipitates aconitine.
1 : 500	0.0129
1 : 450	0.0132
1 : 400	0.0136
1 : 350	0.0138
1 : 300	0.0140
1 : 250	0.0141

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.

171. 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 preparation can be thus judged after practice with a considerable degree of precision. Different persons of course differ in susceptibility so that each must work out his own personal equation as regards this test. To make the results quantitative, it would be necessary to have in the first place some pure crystallized aconitine, of which a solution containing one part in 60,000 should be made a basis of comparison.

172. Aconite leaves contain a much smaller proportion of alkaloid than the root, and the constituent alkaloids are probably different. The assay may be made in the same manner as that of the root, but a larger quantity of materal is required. Keller advises 25 grams; in the method of (72) the quantity should be not less than twelve grams. Keller states that the alkaloid obtained from the leaves has a much lower titration equivalent, 440 to 450, than that from the root, 645 to 647. I should be inclined to attribute this to impurities in the former, and in any case should supplement the chemical by the physiol gical test. The effect produced on the tongue by a solution prepared from aconite leaves is generally feeble at a dilution of 1 : 100, while that of aconite root should be well marked at a dilution of 1 : 600.

173. Galenical preparations of Aconite. Preparations made without heat like fluid extracts and tinctures may

be valued, with results as good as those obtained in the case of the drug, by general processes already given in detail, particularly by (132) et seq. or (137). Oleate of aconitine is to be dissolved in ether, and the alkaloid extracted from it by repeated washings with water containing hydrochloric or sulphuric acid. Solid extracts, however, must be judged wholly by the physiological test, since the alkaloids they contain are likely to have suffered such changes as to render them comparatively inert.

174. Per cent. of Alkaloid contained in Aconite. Aconite root contains from 0.6 to 1.25 per cent. of alkaloid, Keller reports out of five samples only one containing less than 0.9 and one containing more than 1.15% average about 1.05. The author, out of seventeen samples found five below 0.87 per cent., one as low as 0.56 per cent., average 0.93 per cent., one sample as high as 1.3 per cent. In aconite leaves the quantity of alkaloid is about 0.2 per cent.

175. Commercial aconitine may be judged by the physiological test. A solution 1 : 50.000 ought to produce a strong impression of tingling on the tongue. The test should be commenced with a solution. 1 : 75.000, and if in a solution 1 : 25.000 we fail to obtain a tingling comparable with that produced by a good sample of the root 1 : 600, we should conde n the article. Of course hydrolysis of the alkaloid and determination of the benzoic acid produced will give more exact, although still possibly fallacious results.

ALOES.

176. The several varieties of Aloes contain crystalline principles called aloins which are not identical in the different varieties but are evidently closely related chemically. Although these do not constitute exclusively the a tive principles of the aloes, they may be taken as indicating the quality of the drug, while we await fuller knowledge of the subject.

177. Determination of Aloin. Schaefer* has used the following process with satisfactory results: Dissolve 50 grams of the aloes in 300 cc. of hot water to which have been added a few drops of hydrochloric acid. Allow the solution to stand until resinous matter has separated, decant and mix with 50 cc. of solution of ammonia, 20 per cent. Add now a solution of calcium chloride 15 grams in water 30 cc. and stir vigorously. A compound of aloin with calcium separates, and after fifteen minutes may be collected on a filter and drained. The precipitate is then mixed in a mortar with a slight excess of hydrochloric acid, boiling water added just sufficient to dissolve the aloin, the solution filtered, the filter washed with a little boiling water, and the filtrate and concentrated washings e posed to a low temperature to crystallize. The crystals should be washed with a saturated aqueous solu-

*Pharm. Zeitschr. f. Russl., 1897, 65.

tion of aloin which has been prepared in the same manner from the same variety of aloes, dried at 100° C. and weighed.

178. Aloes in mixtures may be roughly estimated by the following method according to H. Hager:* Exhaust the powdered substance with a mixture of absolute alcohol 1 vol., chloroform 2 vols., benzol 3 vols., and dry the residue. Digest this with 80 per cent. alcohol at a temperature near 50° C. (122° F.) frequently shaking, evaporate the solution in a tared capsule and weigh the residue. Treat this with twelve times its weight of 2 per cent. solution of ammonia, which will dissolve any aloes present, add to the filtered solution lead acetate in slight excess with ammonia enough to restore a slight ammoniacal smell. Collect the precipitate, wash with a little distilled water, dry and weigh. The weight multiplied by 0.4256 will be approximately that of the aloes originally present, although other substances may be also present. By mixing the lead compound with an equal weight of ammonium sulphate, repeatedly moistening with water and drying, and finally extracting the mixture with 80 per cent. alcohol, the aloes is recovered with a loss of about twelve per cent. and will be recognizable by its physical properties.

179. Detection of aloes in Pharmaceutical Preparations. In addition to tests given in the dispensatories, of which that of Cripps and Dymond is the best, the following, by Pierre Apery† is worthy of notice. Extract the substance with alcohol of 80 per cent., filter, evaporate to dryness,

*Pharm. Centralh. No. 12 1885.

†Proc. Soc. Imp. de Med. Constantinople: Zeitschr. Oest. Apoth. Ver., 1896. 766.

extract the residue with water, precipitate the aqueous solution with lead acetate, filter, concentrate, remove excess of lead with sodium carbonate, filter once more and neutralize the filtrate with acetic or nitric acid. Upon adding a few drops of a dilute solution of ferric chloride a distinct red brown color is produced even in dilutions of one part of aloes in 2000 or 3000 of water.

BELLADONNA AND ALLIED DRUGS.

180. **Belladonna,** root and leaf, **Stramonium,** seed and leaf and **Hyoscyamus** may be considered under one head since they contain identical or nearly related alkaloids. The assay is easy if we bear in mind the fact that we are dealing with alkaloids easily destroyed or altered. Methods are to be chosen which avoid the application of heat, or the action, more than momentary, of such agents as the caustic alkalies. Henbane contains so little alkaloid that the assay is rather difficult. It is necessary to use a large quantity of the drug, and we can hardly hope to recover from tinctures or fluid extracts the whole of the alkaloid by the best methods we possess. That which will yield the largest proportion is the method of Thompson (141.)

181. For the respective drugs follow either the author's Short Process No. 2, (72) or the method of Keller (50). Keller uses for the assay of belladonna root 12 grams of drug, and ether 90 Grm., chloroform 30 Grm. water of ammonia 10 Grm., water 15 cc. to pour off 100 grams of the ethereal fluid. For the assay of belladonna or stramonium leaves the quantities are; powdered drug 25 grams, ether 100 Grm., chloroform 25 Grm., ammonia 10 Grm., water 40 cc. to pour off 100 grams. For hyoscyamus a still larger quantity of the drug would have to be used. In the Short Process No. 2 as applied to hyoscyamus, use 20 grams of drug.

182. It is best in all cases to determine the alkaloid by alkalimetry rather than by weighing, since, with the most careful manipulation, impurities will be carried over into the final product. However in a well conducted assay, gravimetric results should not be very far from the truth, and in the hands of the unskilled operator, the liability to serious error may be greater in the use of the burette than of the balance. The beginner should use both, and it is not a bad plan to make that a rule in any case.

183. Dr. Schwickerath* gives the following details of his method as applied to these drugs. (53) Twenty grams of powdered drug (40 Grm. hyoscyamus) are digested with 150 cc. of a weak Prollius' mixture (with stramonium seed only 100 cc. are requisite, with henbane 200 must be used) for twelve hours, shaking frequently (best continuously by a mechanical agitator.) Then remove of the clear fluid 75 cc. (with stramonium seed 50 cc. with henbane 100 cc.) corresponding with 10 grams (henbane 20 grams) of the drug. The assay is continued as described in (54) et seq

184. Determination of Atropine by the Periodide Method. Instead of titrating the alkaloidal residue with standard acid, or after so titrating it, we may determine the atropine by the method recently elaborated by Prescott and Gordin (114). Dissolve the alkaloidal residue in a little hydrochloric acid and make up with water to a weight 250 times that of the residue taken. Put in a small flask 10 cc. of decinormal iodine solution (U. S. P.), add 2.5 grams of the alkaloidal solution and proceed according to (115). If the quantity of material is large enough, use 25 cc. of the iodine

*Pharm. Rundsch. 1893, p. 282.

solution, with a proportionate quantity of the alkaloidal solution. It is necessary to be certain of the agreement between the solution of iodine and that of sodium thiosulphate, and these must, of course, be really of decinormal strength, which means that they must have been recently prepared and standardized. The method is not therefore to be recommend- except when assays are frequently made.

185. Titration w.th Mayer's Reagent is not to be advised with these drugs. The precipitate does not separate promptly and the end of the reaction is not sharp. If Mayer's reagent is used at all, it should be for a gravimetric determination. When so used, the result is quite as good as that obtained by separating and weighing the alkaloid. The method is as follows: Place in a tared beaker the acid solution containing the alkaloids from five grams of drug (ten of hyoscyamus) and add a moderate excess of Mayer's reagent. At the end of 12 to 24 hours, when the precipitate has completely subsided and adhered, for the most part, to the bottom and sides of the beaker, decant the fluid through a small filter, wash the precipiate in the beaker several times with distilled water (1 or 2 cc.)which is to be afterwards passed through the same filter, finally dissolve the precipitate remaining on the filter in strong alcohol, run the solution into the beaker, evaporate at 100°C. and weigh. Multiply the weight of the precipitate by 0.454 for the weight of the alkaloid it contains. (112).

ASSAY PROCESS OF DUNSTAN AND RANSOM.

186. (a) **Belladonna Root.*** Exhaust 20 grams of the dry and finely powdered drug by hot percolation, pre-

*Pharm. Journ and Trans. 1884, p. 923.

ferably in an extraction apparatus (9) 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, carrying with them the alkaloid. Mix the aqueous solutions and shake with more chloroform to remove adherent coloring matter. Separate the chloroform, add to the aqueous solution ammonia, and wash out the alkaloid by shaking twice with chloroform (25 cc.). Wash the chloroform with a little ammoniated water, transfer to a tared capsule and evaporate to constant weight, which usually requires a little less than one hour. The results reported by the authors are low as compared with those obtained by other processes. Personally I have no experience with the method, but I should certainly add some hydrochloric or sulphuric acid before attempting to wash out the alkaloid with water from the alcohol-chloroform, although the authors state that this is unnecessary.

187. (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 hydrochloric acid. From the slightly warmed liquid remove chlorophyll, 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.

188. Assay of Galenical preparations of belladonna,

*Pharm. Journ. and Trans. 1885, p. 237.

etc. Fluid extracts are to be assayed by (132) et seq.; using ether to extract the alkaloid, or the method of (138)(139) may be employed, making the final extraction of the alkaloid, however, with ether rather than with chloroform. The fluid extract of henbane and perhaps that of stramonium seed is best treated by (141). Tinctures may be assayed best by concentrating first in a vacuum, or by a very gentle heat, and treating as fluid extracts. Extracts may be assayed by methods described (153—155). Use for an assay 5 to 10 cc. of a fluid extract, except of henbane of which 15 cc. will be required; one gram of an alcoholic extract of belladonna, 2 grams of an ordinary solid or powdered extract, except of henbane of which 4 grams should be taken. In most cases the extract can be best treated by (155) or with the intervention of sawdust after dissolving in dilute alcohol.

189. British Pharmacopoeia method for assay of fluid extract of belladonna. Shake 10 cc of the fluid extract with 10 cc. of chloroform, 50 cc. of water and a decided excess of ammonia. Separate the chloroformic solution and twice repeat the agitation and separation with chloroform; shake the mixed chloroformic solutions with 5 cc. of diluted sulphuric acid, B. P., mixed with twice its volume of warm water, separate and repeat the operation. Wash the mixed acid liquids with 3 cc. of chloroform which is rejected, add ammonia in excess and agitate the solution with three successive portions (10 cc.) of chloroform. Wash the mixed chloroformic solutions with 5 cc of water containing one drop of solution of ammonia, evaporate on a water bath, dry at 100°C. and weigh. Then dissolve the residue in 10 cc. of decinormal hydrochloric acid, neutralize with centi-

normal sodium hydroxide, using cochineal as indicator, deduct the measure of soda solution used from 100 cc. and multiply the remainder by 0.00287, to obtain the weight in grams of the alkaloid present in 10 cc. of fluid extract.

190. Dr. Schwickerath advises for extract of belladonna (leaf or root); Treat 2 grams of the extract with three successive portions (3 cc.) of dilute sulphuric acid (2½ per cent.), working the extract well with a very small platinum spatula to insure solution of all the active principle. Filter the acid solution into a perforator (12), wash the filter with a little water, treat the acid solution one hour with ether then add ammonia and extract with fresh ether three hours (55). The latter solution will contain the alkaloid.

191. Dunstan and Ransom advise to dissolve the extract (of belladonna) in warm water acidulated with hydrochloric acid, filter through absorbent cotton into a separator wash the cotton with a little water, make alkaline with ammonia, shake with two portions of chloroform (5 and 3 cc.) which is to be drawn off into a second separator. Extract the alkaloid with two successive portions (5 and 3 cc.) of acid water, and treat the aqueous solution once more with ammonia and chloroform (5 and 3 cc.) obtaining thus finally a solution from which the alkaloid is procured by evaporation in sufficient purity to weigh. I should myself prefer to make the final extraction with ether rather than with chloroform. Otherwise the plan is a good one although difficulty may be found in getting a solution in the first instance which can be easily filtered. In such a case the operator can always resort to the sawdust expedient.

192. Per cent. of Alkaloid contained in Belladonna, etc. Belladonna root yields from 0.3 to 0.8 per cent. of alkaloid, average about 0.55 per cent.; Belladonna leaves, 0.2 to 0.5, average 0.35 per cent.; Stramonium seed, 0.2 to 0.45, average 0.33 per cent.; Stramonium leaves, 0.2 to 0.45, average 0.33 per cent.; Hyoscyamus, 0.05 to 0.2 per cent., data insufficient for any statement of an average. The above figures are based on determination of the alkaloid by acid titration, Results obtained by weighing the "purified" alkaloid have been generally 20 to 30 per cent. higher. Some recent assays of belladonna leaf by W. A. Puckner* show a range from 0.01 to 0.52 per cent. indicating the worthlessness of some of the drug in market and the need for an official standard.

*Pharm. Review, 1898, p. 324.

CACAO.

193. The characteristic alkaloid of Cacao (stated by some to be contained also in Kola) is theobromine, which is accompanied, however, by caffeine in small proportions. Theobromine is soluble in about 750 parts of cold and 136 parts of boiling water, in 818 parts of boiling absolute alcohol, 5800 parts of cold and 2710 parts of boiling chloroform and in 21000 parts of cold ether. The assay process of W. E. Kunze* provides for the determining of both alkaloids.

194. Assay Process. Boil 10 grams of the cacao 20 minutes with 150 cc. of 5 per cent. sulphuric acid, filter and exhaust the residue with boiling water. Add to the warm solution a large excess of phosphomolybdic acid; after 24 hours, collect the precipitate and wash with about a litre of 5 per cent. sulphuric acid. The filter containing the precipitate is transferred to a beaker where it is treated with an excess of baryta in the cold, and carbonic anhydride is then passed through the solution until all the baryta is thrown down. The whole is then dried on the water bath and extracted with boiling chloroform, the chloroform is distilled off and the alkoloids left as a perfectly white residue.

195. The residue is weighed, dissolved in ammonia, and the solution heated to boiling. A considerable excess of

*Zeitsch. Anal. Chem. 33, 1—29.

silver nitrate (about 1.3 parts of silver to one of theobromine) is added and the boiling is continued until ammonia no longer escapes, and the liquid is reduced to a few cubic centimeters. The caffeine remains in solution, while the theobromine combines with silver and is precipitated. The precipitate is collected, washed with boiling water, dissolved in nitric acid and treated with hydrochloric acid to precipitate the silver. One part of the ignited precipitate corresponds with 1.255 parts of theobromine. (The alkaloid can itself be recovered from the solution after removing the silver).

196. Instead of precipitating the silver, we may determine indirectly how much is present in the precipitate, a known quantity of silver solution having been taken in the first place, by estimating the excess in the filtrate volumetrically with thiocyanate solution N 1-10 (U. S. P.) One part of silver nitrate=1.06 parts theobromine. Or else the silver precipitate may be simply ignited and the residual silver weighed, one part of silver=1.667 parts theobromine. The caffeine may be recovered from the solution containing it by evaporating with addition of sodium chloride and extracting the residue with chloroform.

197. Alternative Assay Process of L. Maupy.* Extract 5 grams of finely powdered cacao with light petroleum (100 cc.) by boiling and leaving the two in contact for a day. Triturate the dried residue with 2 cc. of distilled water and heat one hour with 20 grams of a 15 per cent. solution of phenol in chloroform. When cold, filter and boil the residue twice with chloroform 15 grams. Distill off solvent from mixed solutions, heat the residue to 100°C. at least half an

*Journ. Pharm. 1897, [VI.] 5, 329.

hour. When cold add ether 40 grams, stir the mixture well and set aside six hours. Collect the theobromine on a pair of mutually counterpoised filters, wash with a little ether, dry and weigh. In the case of chocolate, the residue after extraction with light petroleum is triturated with 4 cc. of alcohol (70°) instead of with water.

198. Emminger* extracts (10 grams) with light petroleum, (150 grams) boils the residue with sulphuric acid, (100 cc., 3.4 per cent.) half an hour, neutralizes exactly with baryta water, evaporates and extracts residue five hours with chloroform (150 grams.) The dry residue is treated with carbon tetrachloride (100 cc.) to dissolve fat and caffeine, the residue is exhausted with boiling water, the solution filtered, evaporated and the residue of theobromine weighed. By this method, cacao of different kinds was found to contain from 1.08 to 2.34 per cent. of caffeine.

*Forschungs Berichte, 1896, 275.

CANTHARIDES.

199. Cantharidin, the active constituent of cantharides, is very readily soluble in chloroform, but nearly insoluble in water. When the drug is treated with chloroform, the cantharidin is removed, accompanied with fatty matter. If the chloroform is allowed to evaporate, the cantharidin crystallizes out and can then be freed from fat by washing with carbon disulphide.

200. Assay process of Nagelvoort.* The assay process of Proctor and Mordreux, based upon the facts above stated has been improved by Nagelvoort as follows: Moisten 10 grams of powdered cantharides with a ten per cent. solution of caustic soda, and set in a warm place six hours. Acidify the mass now with hydrochloric acid, transfer to a Soxhlet tube, exhaust with chloroform (about 90 cc.), evaporate off the chloroform, treat the crystalline residue with carbon disulphide to remove fat redissolve in chloroform, filter, wash the filter with chloroform, finally evaporate to dryness at a temperature not exceeding 79.5°C. (175°F.) and weigh.

201. Proportion of Cantharidin in the drug. Assays by Martin† in 1884 showed a percentage of cantharidin ranging from 0.25 to 1.06 (the last from a Chinese blistering fly, probably): F. A. Thompson‡ in 1892 reported on seven samples containing from a trace to 1.00 per cent., average of all but the worst sample 0.97 per cent.

*Chemist and Druggist, 1890, p. 154

†Am. Journ. Pharm. 1884, p. 570.

‡Proceedings A. Ph. A. 1892, p. 261.

CEVADILLA.

202. The total alkaloids of Cevadilla may be easily determined by the Prollius method. Keller* uses for the assay 15 grams of the finely powdered drug, 150 grams of ether, 10 cc. solution of ammonia. The ether should be allowed to act on the drug an hour, with occasional shaking, to extract fixed oil before the ammonia is added. After adding the ammonia, shake vigorously, let stand one hour with occasional shaking, add 30 cc. of water, shake and when well separated, draw off 100 grams of the ethereal solution which is to be extracted with hydrochloric acid as described in the general process (50). The alkaloid is to be finally extracted with ether. It consists of a mixture of alkaloids of which the titration equivalent may be taken as about 625. Keller reports the yield of alkaloid by his process to be about 4.25 per cent., a much larger portion than has heretofore been generally believed to exist in the drug.

*Schweiz. Wochenschr. f. Pharm. u. Chem., 1884; Apoth. Ztg., 1894 pp. 52. 133.

CINCHONA BARK.

ESTIMATION OF TOTAL ALKALOIDS.

203. The method of Prollius more or less modified, is now almost universally adopted for the determination of total alkaloids in cinchona bark. For rapid work, where great exactness is not demanded, the author employs for the assay 4 grams of the finely powdered drug which is digested with 100 cc. of the modified Prollius' mixture (25) following the routine of (73—75). If the drug is rich in alkaloid 25 cc of the ethereal fluid representing one gram of the drug, suffices for the assay, and a duplicate can be carried out with a second portion by the abbreviated process of (136)

204. Method of the German Pharmacopoeia. This is essentially the process of W. Haubensak* which was modified in 1892 by C. Kürsteiner.† The German Pharmacopoeia directs: Shake vigorously and repeatedly 20 grams of finely powdered bark with 10 cc. of solution of ammonia, 20 cc. alcohol and 170 cc. ether, and after twenty-four hours pour off 100 cc. of the clear solution (which seems to be taken to represent ten grams of drug.) Add to this 3 cc. normal hydrochloric acid and 27 cc. water, distill off the ether and alcohol, and, if necessary, add enough normal hydrochloric acid to make distinctly acid. Filter the solution, add to it

*Schwz. Wochenschr. f. Pharm., 1891. p. 147.

†Pharm. Ztg. 1892. p. 750, from Schwz. Wochenschr. 30, p. 473.

3.5 cc. normal caustic potassa or sufficient to show excess by reddening phenolphthalein. The precipitate is collected on a tared filter, washed repeatedly with small amounts of water until the washings no longer redden phenolphthalein, drained, moisture removed by pressing gently between blotting paper, dried, first over sulphuric acid, then on the water bath and weighed.

205. The method departs in the last stage from that of Haubensak, who dissolves out the alkaloid, after adding caustic soda, with chloroform, evaporates the solution, dries and weighs. Kürsteiner substitutes for the chloroform a mixture of chloroform and ether as less apt to emulsionize. Instead of weighing the precipitate on the tared filter, I should be inclined to dissolve it in alcohol, evaporate the solution, dry and weigh, or else to dissolve in alcohol and determine alkaloid by alkalimetry. Haematoxylin. Brazil wood or cochineal may be used as indicator. To these, compounds like the normal sulphate of quinine react neutral. To methyl orange,* on the other hand the acid sulphate reacts neutral, the normal sulphate alkaline, hence if this indicator is used, the titration equivalent for quinine is one-half what it is with the other indicators. See (88).

206. Short Method of H. 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

*A. H. Allen, Analyst; 1886, p. 85, H. W. Salomonson, Ned. Tydschr. Pharm. 7. pp. 195, 225.

†Handbuch der Pharmaceutischen Praxis, I. p. 828.

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 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. 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.

207. Assay by Petroleum Oil. E. Landrin* uses kerosene as a solvent in the assay of Java red bark, as follows: Mix 30 grams of the bark in fine powder with 7.5 grams of slaked lime suspended in water and 7.5 grams of a solution of caustic soda (40 per cent.) 200 cc. of Kerosene are added and the mixture heated on the water bath 20 minutes with frequent shaking. The petroleum is then decanted off, and the residue is treated in a similar manner with a second portion of 200 cc. of the solvent. The oil is then shaken with 20 cc. of normal sulphuric acid, the acid solution drawn off and the treatment repeated with two additional portions (20 and 10 cc.) of the acid by which the whole of the alkaloid is removed. The assay may be carried on from this point by any method the operator may choose. A large quantity of bark can be operated upon if the several alkaloids are to be separated, 100 grams or more being as easily treated as the smaller quantity and at a nominal cost for reagents.

*Compt. Rend., 108, p. 750; Rep. de Pharm., 1889, 212.

208. Method of the U. S. Pharmacopoeia. To 20 grams of cinchona in very fine powder, and contained in a bottle with accurately ground stopper, add 200 cc. of a previously prepared mixture of alcohol 19 volumes, chloroform 5 volumes, water of ammonia one volume, stopper the bottle and shake thoroughly and frequently during four hours. Then pour off through a funnel containing a pellet of absorbent cotton, 100 cc. of the solution (representing 10 grams of bark), and evaporate to dryness. Dissolve the residue in 10 cc. of water and 4 cc. of normal sulphuric acid with the aid of gentle heat, cool, filter into a separator, and wash beaker and filter until the filtrate is no longer acid, using the smallest practicable amount of water. Add 5 cc. of normal potassium hydrate to render decidedly alkaline and extract alkaloid by shaking the mixture first with 20 cc. then repeatedly with 10 cc. of chloroform, until a drop of the last chloroform extraction leaves no residue when evaporated on a watch glass. Evaporate in a tared beaker, dry at 100°C. and weigh.

209. The practical difficulty in the above process is in the tendency of the alkaline solution to emulsionize when shaken with chloroform. This is much less likely to occur if a mixture of chloroform one volume with ether four volumes is used for the extraction, and when a quantity of the solution representing not more than 2.5 grams of the drug is employed, this solvent does its work in a wholly satisfactory manner. If one wishes to work with the larger amount, it is better to precipitate the alkaloid from the acid aqueous solution with caustic soda or potassa as in (152). See also (153).

210. Method of the British Pharmacopoeia. Mix

15 grams of red cinchona bark, in No. 60 powder, with 4.5 grams of calcium hydrate, moisten with 16.5 cc. of water, mix intimately in a capsule, let stand an hour or two, when the mixture should be a moist, dark brown powder with no lumps or visible white particles. Transfer to a small flask, add 100 cc. of benzolated amyl alcohol (amyl alcohol, 1 volume, benzol, sp. gr. .850, 3 volumes;) connect the flask with an inverted condenser, boil half an hour, decant and drain off the liquid on to a filter, leaving the powder in the flask; repeat the boiling with two fresh portions of the same solvent, transfer the residue to the filter and exhaust by percolation with the benzolated amyl alcohol. Introduce the filtrate while still warm into a separator, add 1.3 cc. of diluted hydrochloric acid and 8 cc. of water. Shake well together, separate the acid solution, repeat the washing with successive small portions of acidulated water until all the alkaloid is removed.

211. Neutralize accurately the mixed acid solutions with ammonia, while warm, and concentrate to a volume of 12 cc. add one gram of sodium and potassium tartrate in powder, stir well with a glass rod and set by in a cool place. After an hour, collect the quinine and cinchonidine tartrates on a pair of mutually counterpoised filters, wash with several small portions of water, drain, press, dry and weigh. The weight multiplied by 0.8 will be that approximately of the quinine and cinchonidine present. The remainder of the alkaloids is contained in the filtrate and washings, to which solution of ammonia is to be added in slight excess. Collect the precipitate on a pair of mutually counterpoised filters, press, dry and weigh. The weight added to that of the quinine

and cinchonidine already obtained is that of the total alkaloids in 15 grams of bark. Of course the precipitation with sodium and potassium tartrate may be omitted if we desire only total alkaloids.

212. The Lime Process. The U. S. Pharmacopoeia of 1880 adopted for the assay of cinchona bark the lime process, which was that generally practiced at that date. It has been shown that a notable loss of alkaloid results from the prolonged action of lime. For this reason among others the method has fallen into disuse, but it has at least a historic importance. It was carried out in the following manner: Five grams of freshly slaked lime was mixed with 50 cc. of water, 20 grams of finely powdered bark thoroughly incorporated with the mixture and the whole then dried at a temperature not exceeding 80°C. (176°F.) The powder was then exhausted (best by hot repercolation) with strong alcohol and dilute sulphuric acid added to distinctly acid reaction as shown by litmus paper. The solution was filtered to separate calcium sulphate, the alcohol recovered by distillation from filtrate and washings, the residue treated with acidulated water to procure a solution from which, after filtration, the alkaloids were either precipitated by soda solution, or washed out with chloroform after addition of alkali.

213. Assay by Acid was also formerly much used. The method of **Dr. DeVrij** gives fairly good results, and is as follows: 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, then add more water, stirring thoroughly, to form a liquid which can be poured. Let stand until foam disappears. Introduce into a

cylindrical percolator, the orifice of which is closed with a loose plug of charpie (cheese cloth will answer equally well), as soon as the percolate runs clear it is collected, the turbid portion having been returned to the percolator. Continue the percolation with water until excess of caustic soda ceases to produce a precipitate, about 180 to 200 cc. being generally obtained. The author lays stress on the avoidance of heat in the extraction of bark with dilute acid, and declares that sulphuric acid will not completely exhaust the drug as hydrochloric acid certainly will.

214. From the acid solution above, the alkaloid may be separated (a) by precipitating with caustic soda in large excess, (add to weight obtained, as a correction for solubility of alkaloid, 0.000585 Grm. for each cc. of filtrate and washings) or (b.) by adding excess of caustic soda and immediately shaking out with benzol (1000, and 200 cc.) which must be given five minutes (not longer) to separate. From the benzol solution, the alkaloid may be withdrawn by shaking with very dilute nitric acid 30 cc., followed by water 20 cc., and from the acid solution finally by adding caustic soda in excess and shaking out with two portions, 200 and 100 cc., of ether.

ASSAY OF THE ALKALOIDS OF CINCHONA BARK.

215. For purposes of the pharmacist, it is sufficient generally to determine approximately the quinine in the sample in which total alkaloids have been determined. The most simple way to do this is to convert the mixed alkaloids into sulphates. Dilute the solution moderately, heat to about 85°C., neutralize carefully and cool, when the quinine sulphate nearly all crystallizes out, accompanied by a small proportion of the other sulphates. Another plan is to precipi-

tate together quinine and cinchonidine as tartrates, as in the method of the British Pharmacopoeia, (211.) In the case of red barks rich in cinchonine, this is not very satisfactory. A third plan, is to determine simply the total ether-soluble alkaloids (practically quinine and cinchonidine), or better the amount of alkaloid which is held in solution one hour by a limited amount of ether, cinchonidine, which is taken up freely when first precipitated, being soon deposited in crystals.

216. The Method of the U. S. Pharmacopoeia is an attempt to come still closer to the quantity of quinine. It is conducted as follows: The alkaloid from five grams of cinchona bark, by whatever process it may have been extracted, is brought into solution in chloroform (or alcohol) and poured little by little, over about five grams of powdered glass contained in a porcelain capsule over a water bath, causing the powder to absorb nearly the whole of the alkaloid. The solvent is wholly driven off, the capsule allowed to cool and the powder moistened with ether and transferred without loss by aid of successive small additions of ether to an ether-moistened filter, 7 cm. in diameter, in which it is treated with ether, added drop by drop until exactly 10 cc. of filtrate is procured. The receiver is then changed, and the treatment with ether continued until another 10 cc. has been obtained.

217. Evaporate in separate tared capsules the two portions of ether, dry to constant weight at 100°C. and weigh. The residue from the first portion will contain nearly all the quinine together with a portion of the less soluble alkaloids. The second portion will contain about as much of the less soluble alkaloids as the first, with almost no quinine. We

have then simply to deduct the weight of the residue from that of the first to obtain the quantity of quinine in the five grams of bark. The method is faulty in taking no account of amorphous ether-soluble alkaloids of which there may be a notable quantity present, reckoned by this method as quinine. The alkaloidal residue, dried at 100°C. will contain still one molecule of water; to convert into anhydrous alkaloid, multiply by 0.947.

218. Another mode of determining ether-soluble alkaloid is that of **Dr. Otto Kaspar.** Dissolve the alkaloids from ten grams of bark in hydrochloric acid in slight excess, make up to a volume of 10 cc., add caustic soda in excess (according to the author 15 grams of a ten per cent. solution) and shake immediately with 15 cc. of ether; separate the ether, shake again twice with fresh portions of ether (15 cc.), unite the ether solutions, let stand at least twelve hours for crystallization of the less soluble quinidine and cinchonidine, evaporate, dry at 100°C. and weigh. It is my impression that the quantity of ether as well as of soda used by Dr. Kaspar is needlessly large. I believe that a moderate excess of soda solution, and three portions of ether, 15, 10 and 10 cc., would be better. I am inclined to believe, also, that even better results would be obtained in the use of petroleum ether in place of ether, but experiment would be necessary to determine the quantity of this solvent required. Perhaps a still better plan would be to extract the bark direct with petroleum ether, or light petroleum oil, and ammonia, let stand twenty four hours for crystallization of the less soluble alkaloids, and recover the alkaloid in pure form by shaking out with acidulated water, rendering alkaline and shaking out with ether.

219. Improved Ether Method of A. Petit.* Dissolve the alkaloids from 30 grams of cinchona bark in slight excess of sulphuric acid, add 25 cc. of ether and 5 cc. of solution of ammonia and shake. Decant the ether into a vial, shake the alkaline solution once more with 10 cc. of ether. which is to be added to the first portion. Let the ether stand fifteen minutes. Decant the clear solution into a separator, add 10 cc. of dilute sulphuric acid (1 : 20), shake and separate. Wash the ether a second time with 5 cc. of the same dilute acid, and then with water enough to make in all 25 cc. of fluid. Heat this in a beaker to boiling, add cautiously dilute solution of ammonia until the fluid is faintly alkaline, cool to crystallize quinine sulphate, which is collected on a tared filter, washed with a saturated aqueous solution of quinine sulphate, dried at 115°C. to constant weight and weighed as anhydrous sulphate.

220. Method of De Vrij† by precipitation with **Chinoidine iodosulphate.** Dissolve the mixed alkaloids in 40 times their weight 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 alkaloid.] If the bark under examination contain a large proportion of cinchonidine, digest the crude alkaloid in powder 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

*Chemist and Druggist, 1884.

†Pharm. Journ. and Trans., 1875, p. 461; The Hague, July 5, 1880.

the ethereal solution, which will contain all the quinine, and employ this instead of the total alkaloids for the test.

221. To the solution in sulphuric acid and alcohol, add solution of chinoidine iodosulphate (23) from a pipette, drop by drop, stirring constantly, as long as the 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 precipitate at 100°C. (212° F.) and weigh. Add to the weight found, for each cc. of fluid previous to filtration, 0.0011 Grm. as a correction for solubility of herepathite in alcohol. Multiply the corrected result by 0.55055 to obtain anhydrous quinine, or by 0.7409 to obtain crystallized sulphate of quinine.

222. Oxalate Method of G. Shimoyama.* Dissolve the alkaloids (at least 0.5 Grm.) in a beaker in 30 or 40 cc. of water by aid of the smallest possible quantity of 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 grams; 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

*Arch. Pharm., (1885) 23, pp. 81, 209.

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 Grm. to obtain the weight of the quinine as oxalate. Multiply this by 0.878 for anhydrous quinine, or by 1.1815 for crystallized quinine sulphate.

223. According to DeVrij, the separation from cinchonidine cannot be effected by this last 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 herepathite method of DeVrij (220) is open to the same objection and has the added disadvantage of requiring the use of a special reagent somewhat troublesome to prepare.

FULL ASSAY OF CINCHONA ALKALOIDS.

224. John Muter* directs to dissolve the alkaloids, which have been weighed after drying at 116°C., 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 litre 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 in future operations. Evaporate alcohol 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.

*The Analyst, 1880, p. 223.

225. Cool the solution rapidly to 15°C. (59°F.), keep at that temperature one hour, filter through a pair of mutually counterpoised filters and wash the crystals with a little cold water (1.5 cc. for each cc. of volumetric acid used in the preliminary titration.) Drain the crystals, press, dry at 100°C., raising the temperature gradually at last to 116°C. (240°F.), and weigh as anhydrous quinine sulphate. (In the weighing bear in mind that this is exceedingly hygroscopic; it should be weighed in a weighing bottle securely stoppered.) Measure the filtrate and washings, and for each cc. of the fluid add to the weight actually obtained 0.000817 Grm. as correction for the solubility of quinine sulphate in water. Multiply by 0.8686 for quinine alkaloid.

226. To portion B. add dilute hydrochloric acid until it has a faint acid reaction, evaporate, and dissolve the residue in a minimum quantity of water at 38°C. (100°F.) Neutralize accurately with decinormal soda solution, add a saturated solution of sodium and potassium tartrate in excess, cool and keep for one hour at a temperature of 15°C. (59°F.), frequently stirring. Collect on a pair of mutually counterpoised filters, wash with 100 cc. of water at 15°C. dry at 104.4°C. (220°F.) and weigh. Add for each cc. of filtrate and washings 0.00083 Grm. to obtain weight of tartrates of quinine and cinchonidine. Deduct weight of quinine tartrate, found by multiplying the weight of anhydrous quinine sulphate by 0.915. The remainder multiplied by 0.804 will give cinchonidine alkaloid.

227. Concentrate the filtrate and washings from the tartrate to its original volume, cool, render faintly acid with acetic acid, and add with constant stirring an excess of a neu-

tral saturated solution of potassium iodide. After an hour or so collect, wash, dry and weigh precisely as in the case of the tartrate. Add 0.00077 Grm. for each cc. of filtrate and washings, and multiply by 0.7168 for quinidine alkaloid.

228. From the filtrate and washings precipitate the rest of the alkaloid with sodium hydrate, collect on a pair of filters, wash, dry at 104°C. and weigh. Heat with 40 per cent. alcohol to dissolve out amorphous alkaloid, dry the residue at 105°C. 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 grm., and for each cc. of filtrate from quinidine hydriodide 0.00052 grm.

229. Assay of Galenical Preparations of cinchona bark. For **fluid extracts:** put into a 25 cc. measuring flask 5 cc. of the fluid and fill to the mark with dilute alcohol. Use 5 cc. of the mixture, equivalent to 1 gram of bark for the assay. If the fluid is suspected to be poor in alkaloid, use 10 cc. instead of 5 to make the dilution. Assay according to detail in (132) to (134). Should there be any tendency to emulsionize, use Thompson's sawdust process (141). In my own experience the former process has been uniformly successful.

230. Schwickerath directs to put into a 50 cc. measuring flask 10 cc. of dilute hydrochloric acid, 10 per cent., add 5 cc. of the fluid extract (with Cinchona Comp., 10 cc.) shake, make up to 50 cc., shake well, allow to settle and filter. Put into a perforator (12) 10 cc. of the filtrate and extract one hour with petroleum ether to remove fatty sub-

stances, then add excess of caustic soda and extract three hours with a mixture of chloroform and petroleum ether. The results are good, but not as quickly reached as by the first process recommended.

231. Solid extracts are to be treated on the general principles stated in (153) *et seq.*

232. Proportion of alkaloid present in cinchona bark. The U. S. Pharmacopoeia requires that a bark shall contain five per cent. of alkaloids, and not less than 2.5 per cent. of quinine. The bark offered in the market during the past ten years has perhaps justified so high a standard. Assays reported by several analysts have shown for yellow bark from 4 to 7 per cent., the averages ranging from 4.4 to 6.1 per cent., of which we may assume that three fourths is quinine; for red bark the range has been from 4.5 to 9 per cent. and upwards, average about 6.5 per cent., of which probably not more than one third is quinine; for pale bark, unofficial, 2.5 to 4.5 per cent., the average not so high in recent years as it was ten years ago. The better qualities of bark are sold with statement of alkaloidal content, and it is not difficult to obtain bark of high grade at a moderate price.

ASSAY OF QUININE SULPHATE

233. Water of Crystallization. Altogether the most important determination to make in most samples of commercial quinine sulphate, is that of water of crystallization. As received from the manufacturers, the salt contains usually between 12 and 14.5* per cent. of water. This water has

*H. B. Parsons found in 1015 samples of quinine sulphate, fresh from the manufacturers, an average of 13.84 per cent. of water of crystallization, the highest average for one manufacturer 14.36 per cent. and these figures agree with my own somewhat extensive observation.

been paid for as quinine sulphate, under sanction of the U. S. Pharmacopoeia. Examine the stock of any apothecary, the probability is that you will find not more than 8 per cent. of water. Five per cent. more or less of the "quinine" has evaporated, and the apothecary's profits have been correspondingly cut down, although as a rule he is in blissful ignorance of his loss.

234. It is contrary to business principles to tolerate such a state of things. The remedy really lies with the revisers of the Pharmacopoeia, who may continue to recognize, if they must, "quinine sulphate, commercial," containing a maximum of 7 molecules (rather than 8) of water of crystallization, but should require that for dispensing and manufacturing only an effloresced quinine sulphate be permitted, this being prepared by exposing the commercial salt, spread out in a thin stratum, to a temperature of 50° C. (122°F.) until it ceases to lose weight, and keeping the product afterwards in tight containers. Under the present conditions, the pharmacist is justified in drying out his quinine as just explained, and then using it in the proportion of 90 grs. for 100, the only official salt being that containing 16.18 per cent. of water.

235. To determine water of crystallization dry two grams of the quinine sulphate three hours in the water oven, or better one hour at 105°C. (221°F.), cool in a desiccator and weigh quickly. The exsiccated quinine sulphate is exceedingly hygroscopic, so that it is better to weigh in a weighing tube. If the quinine sulphate is dried at 50°C. it retains just 2 molecules of water or 4.6 per cent. of its weight, and when a regulated temperature can be depended upon, this plan of determining water may be adopted.

236. Detection of Cinchonidine and other cinchona alkaloids in quinine sulphate. It is practically impossible for the manufacturers to exclude wholly from quinine salts the other alkaloids of cinchona (or Remigia) bark. The presence of a small proportion, even as much as five per cent. does not appreciably affect the therapeutic uses of the quinine salt, nor does it cheapen the product sufficiently to defraud the customer. No simple plan of determining accurately the proportion of such impurities present in quinine sulphate has been found, although many have been proposed. Most of the Pharmacopoeias have agreed to adopt Kerner's ammonia test as a means of guarding against excessive amount of these impurities, and for the present it is as well to let this remain as a conventional requirement that is sufficiently exacting.

237. The Ammonia Test of Kerner* is best carried out as follows: 1.794 grams of quinine sulphate, fully effloresced by heating to 50°C. are digested with frequent shaking 30 minutes with 20 cc. of distilled water in a water-bath maintained at a temperature of 60° to 65°C. (140° to 149°F.) The test tube containing it is then placed in water kept at a temperature of exactly 15°C. (59°F.) two hours, being frequently shaken in the meanwhile. It is then filtered through a small filter, 5 cc. of the filtrate placed in a small flask and while the temperature of 15°C. is maintained, 7 cc. of water of ammonia, sp. gr. 0.960, also at 15°C., is added, and the fluids mixed by closing the mouth of the tube with the thumb and inverting once or twice. A clear solution should result from which no deposit should be thrown down within 24 hours, if the tube be corked and set aside.

*Arch. Pharm. (3) 16, 186.

238. The quantity of quinine salt prescribed by the U. S. Pharmacopoeia, following Kerner's original process, is two grams of the crystallized salt. The German Pharmacopoeia calls for 2 grams of the effloresced salt, making the test considerably more rigorous. This authority is also in error in demanding that 4 cc. of water of ammonia shall produce a clear solution, unless the reagent be of sp. gr. 0.920, which was probably intended. It is better to use the fully effloresced salt, as prescribed by the German Pharmacopoeia, but to weigh out as above directed, not 2 grams, but 1.794 grams. The tests of the U. S. Pharmacopoeia for other quinine salts all start with 2 grams of the salt. As Prescott* and Ruddiman† have pointed out, the quantities ought to be such as to contain the same quantity of quinine. Thus we should take of quinine alkaloid 1.734 grams, of quinine bisulphate 2.514 grams, of quinine hydrobromate 2.910 grams, of quinine hydrochlorate 2.694 grams, following otherwise the directions of the Pharmacopoeia.

239. The Oxalate Process of Schaefer‡ is carried out as follows: Put into a small tared flask 2 grams of quinine sulphate and add 55 cc. of water. Heat to boiling and boil a few minutes, then add a solution of 0.6 gram of crystallized potassium oxalate (neutral) in 5 cc. of water, cool to 20°C. (68°F.), bring the weight of the contents of the flask to 62.5 grams, keep the temperature at 20°C. 30 minutes, shaking occasionally. Filter and to 10 cc. of the filtrate add a drop of solution of caustic soda, sp. gr. 1.16, which should

*Organic Analysis, p., 146.

†Pharm. Era., 1887, p. 244.

‡Arch. Pharm., (3) 25, 64 and 1033.

produce within a few minutes no turbidity. In case other alkaloids are present to the extent of one per cent. a precipitate is produced.

240. The Chromate test of DeVrij,* as modified by De Koningh.† Dissolve 2 grams of the quinine sulphate in 80 cc. of hot water, add 12 cc. of a five per cent. solution of potassium neutral chromate. Allow the solution to stand some hours to crystallize, filter, add to the filtrate 5 cc. of a ten per cent. solution of soda, when an immediate precipitate will indicate the presence of cinchonidine, quinidine, or hydroquinine. Filter, shake the filtrate twice with chloroform (10 and 5 cc.) to remove traces of these alkaloids, add 2 grams of ammonium chloride and shake out again with chloroform which will remove cupreine if that alkaloid is present. Cinchonine is not detected by this test, since its chromate is as little soluble as that of quinine, but cinchonine is not often present in quinine sulphate.

241. Barthes' test‡ depends upon the inferior solubility of quinine sulphate to that of the other sulphates. Shake one gram of the quinine sulphate with 100 cc. of water at frequent intervals during one hour. At the same time treat 5 grams of the salt in precisely the same way with 100 cc. of water. The two solutions will be saturated with quinine sulphate, but the second will contain five times as much of the impurities as the first. Filter both and titrate 50 cc. of each filtrate with decinormal potash using phenolthalein as an indicator. The difference between the quantities of

*Arch. Pharm., (3) 24 and 1073.

†Ned. Tydschr. Pharm., 1897, 97.

‡Compt. Rend., 115, 1085.

alkali required multiplied by 1.985 will give the precentage of impurity calculated as cinchonidine sulphate.

242. The bisulphate method is carried out in the following manner: Five grams of the quinine sulphate is dissolved in 12 cc. of normal sulphuric acid by warming, and the solution allowed to crystallize in a cool place. The mother liquor is separated and shaken with ammonia and ether (16 cc.). The ether is separated and left at rest 24 hours, when the greater portion of the cinchonidine will separate in crystals. These may be collected dried and weighed, but will contain some quinine. Howard accordingly advises to dissolve the crystals in absolute alcohol, add 2.1 cc. of 50 per cent. sulphuric acid for every gram of the alkaloid, collect the crystals of cinchonidine sulphate which separate, dissolve in water, extract the alkaloid in the usual manner, dry and weigh.

243. Ether Process of B. P., modified by S. J. Lewis.* Put into a tared flask 10 grams of the quinine sulphate, dried at 100°C. Add 250 grams of water, dissolve by heat and let cool, having made up any loss of water. When cold filter, place 125 cc. of the filtrate (representing 5 grams of the quinine) in a flask which it fills to the neck, add ether sufficient to produce after shaking a supernatant layer of about 5 cc., add ammonia in very slight excess, shake thoroughly so as to redissolve the quinine at first precipitated. Set aside 12 hours, then remove the ether with a pipette. Shake the residual fluid with two successive portions of fresh ether, 4 and 2 cc., removing this also with the pipette. Collect on a tared filter any crystals that may have

*Chem. and Drug. 1895, 134.

separated, dry the filter with the crystals, pour over them 5 cc. of ether, dry at 100°C. and weigh. Four parts of the alkaloid are said to correspond with five of cinchonidine (or cinchonine) sulphate, but in fact the crystals always contain much quinine, and some cinchonidine is always held in solution by the ether.

244. Water test and Carbonic Acid test of Kubli * For the water test which depends upon the solubility in water of quinine alkaloid: place in a tared flask 1.793 gram. of effloresced quinine, add 60 cc. of water and heat to boiling. After 5 minutes add water making up in all 62 grams, cool to 20°C. (68°F.) and keep at that temperature 30 minutes, then shake well and filter. Put into a test tube 5 cc. of the filtrate, add three drops (no more) of a solution 1 : 10 of sodium carbonate then add gradually from a burette with constant shaking, water until a clear fluid results. If the sample is pure 10 cc. will suffice: The amount of impurity is to be inferred from the additional quantity required, the temperature being maintained throughout the titration at 20°C. For every one per cent. of cinchonidine sulphate 0.4 cc. of water will be required.

245. The carbonic acid test is made in five cc. of a filtrate obtained exactly as in the water test; three drops of the solution of sodium carbonate are added, and the precipitate redissolved by adding 5 cc. of a solution of pure sodium bicarbonate. Carbon dioxide is then passed into the solution, 80 to 100 bubbles per minute, for 30 minutes at 15°C. (59°F.) it being essential that the gas should be free from atmospheric

*Pharm. Zeitsch. f. Russland, 1895, pp. 593, 609, 625, 641; Pharm. Centralh., 1896, p. 578.

air. The tube is then shaken gently until the volume of the precipitate is constant (this may occupy 30 minutes or more). The fluid is then transferred to a cylindrical graduate, left to stand an hour or two, and the volume of the precipitate then read off. For pure quinine sulphate, this should be 1.4 to 1.5 cc., with one per cent. impurity it is larger, but where the impurity exceeds 2 per cent. it is much diminished. The test is too circumstantial—granting its possible value—to be commended.

246. I have collected these various processes for convenient reference. There is much controversy about their relative value, and it is conceded that none of them give results that can be depended upon, quantitatively. As qualitative or limiting tests they are useful, and there may be the germs of good quantitative processes in some of them.

COCA LEAVES.

247. The Alkaloid Cocaine, which is the active principle of the drug is readily soluble in ether and in petroleum ether (also in kerosene oil) by which solvents it may be removed completely from alkaline aqueous solutions. It is, however, an exceedingly unstable body so that in assay processes we must avoid heat or prolonged contact with strong alkalies (particularly lime) and acids. Cocaine, which is itself chemically methyl-benzoyl-ecgonine, is accompanied by other kindred alkaloids, especially isatropyl-cocaine and cinnamyl-cocaine from which it is possible for the chemist to obtain ordinary cocaine. In the ordinary assay, we are content to extract the ether soluble (or benzin-soluble) alkaloid.

248. The assay may be made preferably by the short assay process No. 3 (76), which yields a particularly pure alkaloid. Otherwise Process No. 2, using ether alone instead of the mixture of ether and chloroform, is satisfactory, or Process No. 1, using the weaker Prollius' mixture. In the author's original assay process* with petroleum benzin, the quantity of ammonia used in the first step in the operation was needlessly stingy, the time of maceration was unnecessarily prolonged and the aliquot portion taken did not represent quite accurately the five grams of drug of which it was taken as the equivalent. The process, however, was based

*Manual of Pharmaceutical Assaying, p. 74.

upon a plan actually in successful use for the extraction on the manufacturing scale of cocaine from the leaves, and has been commended by nearly all who have given it a trial*.

249. Keller's Process of assay is a good one, but there is no advantage in using chloroform in this case. Schwickerath† extracts the drug (12 grams) with petroleum benzin 95 cc., ammonia (30%) 1 cc. and alcohol 4 cc., after 12 hours evaporates by an air current 50 cc. with 10 cc. of dilute sulphuric acid (0.5%) and extracts the acid solution one hour in the perforator with ether, and finally after rendering alkaline, 3 hours with fresh ether. The prolonged application of even a gentle heat is inadvisable, and seems quite unnecessary in the case of an alkaloid so easily removed by the shaking out process.

250. Assay by Percolation with Ammoniated Ether. A. Gunn‡ prefers percolation to maceration and proceeds as follows: Moisten 5 grams of the powdered leaves with weak solution of ammonia (2 per cent.). After half an hour place in a narrow tubular percolator (25×1.5 Cm.) and percolate with ammoniated ether to obtain 100 cc. From the percolate the alkaloid is washed out with three portions of dilute hydrochloric acid (2 per cent.) in all about 50 cc. from which in turn it is finally extracted with ether after addition of ammonia.

251. Kerosene process of Dr. Squibb.§ 100 grams

*Dr. Dohme (Proc. A. Ph. A., 1895, p. 258.) reports very low results in the use of this process, for which I can account only on the hypothesis that he used 10% ammonia instead of the 28 per cent. prescribed.

†Pharm. Rundsch., 1893, p. 282.

‡Journ. Pharm. Chem., 1893, [5], 152.

§Ephemeris, June 1888, p. 1101,—1106.

of the powdered leaves are moistened with 100 cc. of a 7 per cent. solution of crystallized sodium carbonate, packed at once in a percolator and percolated to about 700 cc. with water white kerosene. The percolate is shaken out thrice successively with 30 cc. of water containing 2 per cent. of hydrochloric acid. The acid solution is well washed with 30 cc. of ether to remove fatty substances, the ether solution being rejected. Then 20 cc. of ether are added, with 10 cc. of a solution of sodium carbonate containing 247 grams of the crystallized salt to the litre (Dr. Squibb directs to use sodium carbonate sufficient to exactly neutralize the acid that has been taken, and five per cent. additional; there must be a distinct but not large excess), and the fluids well shaken together, after the carbonic acid has escaped. The ether is separated and the treatment repeated twice with fresh ether, 20 cc. The ether is transferred to a tared beaker, evaporated to constant weight, the weight of the residue in grams being the per cent. of alkaloid.

252. The above procedure recommends itself by its directness and cheapness, and furthermore it gives us a quantity of alkaloid sufficient to use for a further determination of true cocaine. This may be carried out according to Grandval and Lajoux by adding water equal to ten times the weight of the alkaloid, and just enough hydrobromic acid to neutralize completely, heating the solution on a water bath and saturating rapidly with potassium bromide. On cooling we obtain a crystallized mass of the double bromide of potassium and cocaine. Percolate the crystals in a funnel having the tube rather firmly plugged with absorbent cotton, with a saturated solution of potassium bromide, which will remove ecgon

ine. Finally dissolve the crystals in hot water, cool and treat with ammonia and ether to recover the pure cocaine.

253. Other Processes of Assay. Koehler* mixes 50 grams of finely powdered leaves with 5 grams of dry carbonate of sodium and 15 grams of oxide of lead, macerates with 50 cc. of water and dries in vacuo at the temperature of the water bath. The mass is then twice macerated 24 hours with benzin, 250 cc. each time, the alkaloid withdrawn from the benzin with hydrochloric acid, which in turn is treated with sodium carbonate in excess and shaken out with ether. **Van der Marck**† mixes 50 grams of the powdered leaves with 20 grams of magnesia, dries at 60°C. and extracts with ether. The solvent is distilled off, the residue taken up with hydrochloric acid, 2 per cent. (about 30 cc.), the acid solution treated with ether to remove coloring matter, etc., made alkaline with ammonia and shaken out with three successive portions of ether (25 cc.). **Grandval and Lajoux** adopt the procedure of (247) and (248).

254. The Author's preference, in assays such as the pharmacist has occasion to make, is for the processes already recommended (248) of which the first yields the purer product of cocaine. For more exact determinations, the process of Dr. Squibb is best. Of course one can judge roughly of the quality of coca leaves by simply chewing a single leaf, or preferably 0.2 gram, (about 3 grains) of the powder, and observing attentively the benumbing effect upon the tongue.

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*Repert. de Pharm., March 1888; Am. Journ. Pharm., 1888, 238.

†Pharm. Ztschr. f. Russ., 1889, 349, and Ned. Tids. Pharm., 89, 116; Pharm. Ztg. 39, 282.

255. Proportion of alkaloid in coca leaves. Coca leaves of good quality should yield of total alkaloid as much as 0.6 per cent. When fresh they may contain one per cent., possibly more, but they deteriorate gradually under the most favorable conditions, and very rapidly if exposed to dampness or "sweated" in the bales. I have found from 0.40 to 0.92 per cent. in freshly imported leaves, and usually from 0.60 to 0.75 per cent. in commercial samples represented as of first quality. Thompson reports in 13 samples, probably directly imported, an average of 0.61 per cent. (max. 0.98, min. 0.39). Dr. Squibb in 1888 found in 26 lots (freshly imported, no doubt) only two having less than 0.5 per cent., four between 0.5 and 0.6, four between 0.6 and 0.7, nine between 0.7 and 0.8, four between 0.8 and 0.9 per cent. The extremes were 0.338 and 0.983, the average 0.716 per cent.

256. Galenical Preparations of Coca. For a fluid extract, follow the routine of (132) [or (136)] using for the first extraction pure ether instead of ether and chloroform; as an alterative the sawdust process, (141). Tinctures or wines must be evaporated at a low temperature, best in vacuo or under reduced pressure, and treated by (141). Solid extracts are treated on general principles (153). When sawdust is used, the solvent may be ether or petroleum benzin as in the assay of the leaves.

COLCHICUM.

257. The active principle of colchicum seed or colchicum root (corm) is the alkaloid colchicine, which differs from most alkaloids in the following particulars. 1st. It is removed from *acid* solutions by shaking with chloroform; 2nd. It is quite freely soluble in water; 3rd. 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. These are facts to be borne in mind in devising process for the assay of the drug.

258. The assay process which in the author's hands has given the most satisfactory results is the following, a modification of the short process No. 2: Place 10 grams of the powdered drug in a flask or bottle, add 100 cc. of Prollins' mixture modified (25%), cork securely and macerate with occasional shaking twelve hours (or place in a mechanical shaker four hours). Decant 50 cc. of the clear fluid, evaporate in a beaker by a very gentle heat nearly to dryness. Take up the residue with 10 cc. of ether, add 5 cc of dilute sulphuric acid (2.5 per cent.) and stir until the ether evaporates. Filter the acid fluid into a separator, retaining the insoluble residue as much as possible in the beaker, to which it is indeed mostly adherent. Redissolve the residue in a little ether, add

2 cc. of the dilute acid, stir as before, and filter the acid aqueous solution into the separator, wash the filter with a little of the acid, adding the washings to the contents of the separator.

259. Put into the separator 15 cc. of chloroform, shake carefully but continuously—a rotatory motion is best—during two minutes, let separate and draw off the chloroform into a tared beaker. Repeat the treatment with two portions of fresh chloroform (10 cc.) Test a drop of the aqueous solution remaining after chloroform has evaporated, with Mayer's reagent; if this shows presence of alkaloid, repeat the treatment with chloroform, after having rendered the solution nearly neutral with ammonia. Three washings, however, will generally extract the alkaloid even in presence of excess of acid. Finally evaporate the chloroformic solution to dryness, redissolve in a little dilute alcohol and dry again to constant weight. The residue is nearly pure colchicine. It may have retained still some chloroform, and therefore should be dissolved once more in dilute alcohol and dried.

260. Instead of extracting the alkaloid, we may determine it with reasonable precision by titration with Mayer's reagent. The acid solution of (258) is made up to a volume of 10 cc. for colchicum root or 15 cc. for colchicum seed with dilute sulphuric acid 2.5 per cent. and Mayer's reagent is added from a burette as long as it produces any cloud in the filtered fluid (99). The quantity of alkaloid indicated by the titration may be found approximately by the following empirical rule: Subtract from the quantity of Mayer's reagent N 1-20 (16) consumed, for each cc. of fluid present at the end of the titration, 0.08 cc. Each cc. of the remainder will

correspond with 14.7 milligrams of colchicine. Example: Taken, 10 cc. of acid solution; added, 5.4 cc. Mayer's reagent. $5.4-15.4\times0.08=5.4-1.23=4.17$ $4.17\times14.7=61.3$ mg.

261. It is better to make the gravimetric determination as above (259), dissolve the alkaloid in about 300 times its weight of dilute sulphuric acid, 2.5 per cent., and titrate as above. The result of titration, when the above arbitary rule is adopted, will be close if the alkaloidal solution has approximately an initial strength of 1 : 300: if weaker, the results are liable to be high, if stronger low. It is essential that the solution titrated contain not less than 2 per cent. of sulphuric acid; a little more will not materially affect the result; my own practice has been to make it as nearly as possible 3 per cent.

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

Quantity of Alkaloid present, Grams.	Quantity cc. 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.
0.010	1.15	.....	.....	.....
0.020	1.95	2.35	.....	.....
0.030	2.70	3.10	3.50	.....
0.040	.....	3.85	4.25	4.65
0.050	.....	4.70	5.00	5.40
0.060	.....	.....	5.80	6.15
0.070	.....	.....	6.60	7.00
0.080	.....	.....	7.40	7.70
0.090	.....	.....	.....	8.50

263. K. Schwickerath* modifies slightly the process of (234), using a modified Prollius' mixture containing about 15 cc. of alcohol and 3 cc. of stronger water of ammonia instead of 25 and 10 cc. respectively. (These quantities are no doubt sufficient) and extracting the alkaloid by the "perforator" (Fig. 10.) He uses 20 grams of drug in a moderately fine powder, with 120 cc. of the Prollius' mixture, places in a mechanical shaker twelve hours, filters off 60 cc. of the fluid to which he adds 8 cc. of water and acetic acid enough to render perceptibly acid, evaporates off the chloroform, etc., filters into the perforator, washes first with petroleum ether one hour to remove impurities, then adds sodium carbonate to alkaline reaction and extracts with a mixture of chloroform and ether, 1 : 3, taking care to insert just below the opening of the delivery tube a ring of absorbent cotton (55). The alkaloid is to be dried with the precautions mentioned in (259) and weighed.

264. Proportion of alkaloid in the drug. Colchicum seed yields 0.5 to 1.0 per cent. of colchicine, average not far from 0.7 per cent. Colchicum root is not quite so rich in alkaloid, but a drug of prime quality seldom falls short of 0.5 or exceeds 0.8 per cent., average about 0.55 per cent.

265. Assay of Galenical Preparations. From a fluid extract (5 cc.) the alkaloid can be easily removed by adding an equal volume of water and shaking out with chloroform, using several successive portions, (15, 10 and 10 cc.). Determine the alkaloid exactly as in the Prollius' mixture of (258.) As an alterative, dilute 10 cc. of the fluid extract with 85 cc. of water, add solution of lead subacetate in slight

*Pharm. Rundsch., 1893, 282.

excess (i. e. until the fluid has a distinctly sweetish taste) make up to exactly 100 cc. with water and filter. To the filtrate add sodium phosphate in powder, sufficient to throw down the excess of lead, filter once more. Put into a separator 50 cc. of the filtrate and shake out with chloroform, three (or more) portions, 15, 10 and 10 cc. Dry and weigh. See (259).

266. Solid extracts may be dissolved in dilute alcohol and treated like fluid extracts. Tinctures, wines, etc., may be concentrated at as low a temperature as possible (neutralized first if necessary), mixed with sawdust, dried and treated with the modified Prollius' fluid precisely as in (258).

CONIUM.

267. The volatile alkaloid coniine is the active constituent of poison hemlock, occuring in all parts of the plant, but most abundantly in the immature fruit which is the only part recognized by the U. S. Pharmacopoeia. Many specimens of conium leaves and not a few of conium fruit 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 solution of potassa, 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. The test serves at least to condemn a drug greatly deficient in alkaloid.

268. An assay of the Drug is practicable by the following process: Put into a 4 oz. prescription vial 12 grams of the finely powdered drug. Add 100 cc. of weaker Prollius' mixture (24), shake well at frequent intervals for four hours, or continuously during that time by aid of a mechanical shaker. Let settle and pour off of the clear fluid exactly 50 cc. add normal sulphuric acid drop by drop, just sufficient to render distinctly acid. Dissipate ether by a gentle heat, add alcohol 15 cc. and set aside in a cool place a few hours for separation of ammonia as sulphate. Filter, wash residue with a little alcohol, concentrate filtrate and washings (make sure

that free acid is nearly neutralized previously) by a very gentle heat, to 3 cc., add water 3 cc. and a few drops of dilute sulphuric acid. Wash twice with ether 10 cc. to remove traces of fatty matters, rejecting the ether.

269. The assay may now be completed by either the alkalimetric or the gravimetric method. In either case add sodium carbonate in excess and shake out with ether, three successive portions, 15, 10 and 10 cc. (I.) Treat the ethereal solution with exsiccated calcium sulphate (neutral) to remove minute droplets of alkaline water and titrate with standard acid, N 1-25 or N 1-100 using iodeosin as indicator (147). Calculate coniine from table on p. 55. See also (271) (II.) Add to the ethereal solution drop by drop sufficient hydrochloric acid, 5 per cent., to supersaturate, avoiding much excess; dissipate the ether by a gentle heat in a tared evaporating dish with flat bottom. Drive off the excess of acid by evaporating twice with a little alcohol (2—3 cc), dry a few minutes at a temperature not exceeding 60°C. (140° F.) and weigh the residue of coniine hydrochloride. After weighing, keep in a desiccator over sulphuric acid an hour and weigh again to make sure that the drying was complete. Multiply by 0.777 to obtain the weight of the alkaloid.

270. Alterative Method. Moisten the drug (12 grams) with an equal weight of a 5 per cent. solution of crystallized sodium carbonate, place at once in a suitable flask, add petroleum benzin 100 cc., shake well and macerate with occasional shaking four hours (or else pack in a percolator and percolate to 100 cc. with benzin). Pour off 50 cc. of the clear fluid. Extract the alkaloid by shaking out with three portions of acidulated water, 5, 3 and 3 cc., wash the acid solu-

tion twice with ether, 10 cc., to remove traces of fatty matters, then proceed as in (269.)

271. In absence of iodeosin indicator, the alkalimetric process may be carried out in the following manner: Wash out the alkaloid from the acid solution (269) with petroleum benzin instead of ether, using three portions of 25 cc. each. Wash each portion with 3 cc. of water, and unite all finally in a separator in which has been placed 10 cc. of standard acid N 1-25, together with a little cochineal or Brazil wood indicator. Shake together; if the reaction should not be acid, add standard acid sufficient to make it so persistently after shaking. Draw off the aqueous fluid, wash the benzin with two successive portions of water, 5 cc., which is to be added to the alkaloidal solution. Finally titrate back to the point of extract neutrality with standard alkali N 1-25 and subtract the quantity used from that of the standard acid.

272. Schwickerath* treats 10 grams of the drug with dilute Prollius' mixture in the usual way, filters 50 cc. of the fluid for the assay, adds 8 cc. of water and 2 or 3 cc. of dilute sulphuric acid, 2.5 per cent., and evaporates. The acid fluid is filtered into the perforator (12) and then washed one hour with ether, to remove impurities, the ether changed for fresh ether, the solution made alkaline with sodium carbonate and washed with the ether three hours. Finally 10 cc. of standard sulphuric acid N 1-20 is added, the ether dissipated by warming and the excess of acid titrated back with standard soda solution N 1-100. The extraction of the alkaloid would be much more speedily and perfectly accomplished by introducing into the flask the standard acid; 20 or 30 minutes

*Pharm. Record, 1893, 282.

would then undoubtedly suffice.† The process is faulty in that some ammonia must surely accompany the coniine.

273. R. A. Cripps‡ exhausts the drug (conium fruit) by percolating the powder (5 grams) with a mixture of alcohol 25 cc. chloroform 25 cc. and chloroform saturated with hydrochloric acid gas 10 cc. and then extracting in a Soxhlet tube two hours with the percolate. When cold, the solution is extracted in a separator with two successive portions (25 cc.) of water. The aqueous solution is treated with several small portions of chloroform to remove coloring matter etc., made alkaline with soda and the alkaloid washed out with chloroform used in three successive portions. The chloroform is washed with a little alkaline water (why not better pure water?) neutralized with a solution of hydrochloric acid in ether, evaporated at a temperature not above 90°C., and weighed. The weighing is checked by titration of the chlorine with silver nitrate N 1-100, a procedure which had been already suggested by the author.§

274. There is no satisfactory mode of titrating aqueous solutions containing salts of coniine. The alkaloid affects the indicator phenolphthalein, so that the alkalimetric process (87) cannot be resorted to. Mayer's reagent precipitates the alkaloid only in rather strong solutions and the precipitate runs through an ordinary filter, so that useful results cannot be obtained. Titrations with sodium phosphotungstate or of sodium phosphomolybdate (Zinoffsky) have been employed with somewhat better satisfaction, and they furnish an avail-

†G. Liljeström, Pharm. Ztg., 1894, 57.

‡Pharm. Journ. Trans., (3) 18, pp. 12, 511, 820 and 888.

§Manual of Pharmaceutical Assaying p. 85.

able means of judging rapidly the relative alkaloidal strength of samples of the drug or of its galenical preparations.

275. The titration by **Phosphomolybdate of Sodium** (20) is carried out in the acid solution of (268) or (270) which is brought to a volume of 10 cc. and titrated in the same manner as with Mayer's reagent. A solution of coniine hydrochloride 1 : 250 must be used as a means of standardizing the phosphomolybdate, but it will be found that the titration equivalent of pure coniine hydrochloride is considerably higher than that of the hydrochloride obtained in the assay of the drug.

276. Proportion of alkaloid in the drug. Conium fruit should contain not less than 0.5 per cent. of alkaloid. Commercial samples often contain less than 0.2 per cent., and the proportion may be as high as 0.9 per cent. There is more need in the case of this drug than of any other of an authoritative standard, which should be adopted in the next revison of the U. S. Pharmacopoeia.

277. Galenical Preparations of Conium. A fluid extract may be treated as follows: To 5 cc. of the fluid contained in a 2 ounce vial, add a little potassium carbonate (0.2 to 0.3 gram) and about 50 cc. of petroleum benzin. Shake well, let separate, decant the benzin into a vial containing 5 cc. of one per cent. hydrochloric acid. Shake, separate; return the benzin to the first vial, shake again, separate and transfer the benzin once more to vial No. 2. Shake, let separate, decant the benzin into a third vial containing 2 or 3 cc. of acidulated water. Shake, let separate and reject the benzin. Extract the alkaloid from the acid solutions with ether according to (269). Otherwise the fluid extract may

be mixed with sawdust after adding 0.1 gram of tartaric acid, dried, and the assay made according to (268) or (270).

278. Solid extracts, which generally are very deficient in alkaloid, are to be dissolved in dilute alcohol and titrated as above. Two and a half grams is a convenient quantity to operate upon.

CONVALLARIA.

279. Lily of the valley contains two glucosides, convallarin and convallamarin, the latter of which appears to be the principle to which the characteristic medicinal activity of the drug is solely due. The former is scarcely soluble in water, yet sufficiently so to impart to it its acrid taste and the property of frothing when shaken. It dissolves freely in alcohol. Convallamarin is soluble in water and in alcohol, but not in ether. It is characterized by a strongly bitter taste, afterwards sweetish, and has been assigned the formula $C_{23}H_{44}O_{12}$.

280. Preparation of Convallamarin. The valuation of the drug must involve a determination of convallamarin which may be attempted by Tanret's process for the isolation of the glucoside, as follows:* The drug is exhausted with alcohol, the tincture treated with lead subacetate and filtered; the excess of lead is removed with dilute sulphuric acid, of which no more should be used than is necessary. Filter, neutralize carefully, evaporate off the alcohol. The residue is to be taken up with water, filtered and precipitated with a solution of tannin, care being taken to keep the solution neutral with sodium carbonate. The precipitate is washed carefully, dissolved in 60 per cent. alcohol, decolorized with animal charcoal, decomposed with zinc oxide, filtered and evap-

*Journ. de Pharm., (6) iii. 355; Pharm. Jn. Trans., 1882, 423.

porated to dryness. To free it from saline impurities, redissolve in 90 per cent. alcohol, filter and again evaporate to dryness.

281. The yield being very small, only 0.2 per cent., it would be necessary to use at least 25 grams of drug (better 50) for an assay, and the process probably does not yield the whole of the glucoside, but no better assay process has been suggested.

DIGITALIS.

282. Active Constituents. There is much yet to learn about the chemistry of this drug. At least two of the numerous educts which chemists have obtained from it have physiological activity, but the relationship of these to one another has not been satisfactorily determined, and chemists are not agreed on the question whether these educts really exist in the drug, or whether many of them are not produced during the processes of their extraction. The recent researches of Keller* confirm in the main the conclusions of Schmiedeberg with regard to the existence of three principal glucosides, digitoxin, digitalin and digitonin.

283. Keller has recently published an assay process much simpler and easier of execution than those heretofore proposed. He believes that for practical purposes it will suffice to determine one constituent only, digitoxin. His procedure is as follows: Exhaust 20 grams of the powdered leaves with 70 per cent. alcohol by percolation. Evaporate, dissolve the residue in water and add solution of lead subacetate in slight excess. Remove the excess of lead from the filtrate with sodium sulphate, filter, make alkaline with ammonia and shake with several successive portions of chloroform. The chloroform solution on evaporation leaves a resi-

*Ber. Pharm. Ges., 1895, Heft. II.

*Ber. Pharm. Ges., 7, 125—136; Pharm. Centralh., 1897, i. 1211.

due of crude digitoxin which should be weighed as such, then dissolved in three grams of chloroform, the solution mixed with 7 grams of ether and poured into 50 grams of petroleum ether. The digitoxin seggregates in flakes which are caused to separate by vigorous stirring of the mixture and are then collected, dried and weighed as pure digitoxin.

284. Digitonin may be obtained from the aqueous solution after shaking with chloroform by expelling the ammonia, acidifying with hydrochloric acid, precipitating with tannin, dissolving the tannates in 50 per cent. alcohol, adding lead oxide, evaporating, extracting the residue with dilute alcohol, filtering and evaporating the filtrate to dryness. Digitonin however is of no medicinal importance.

285. Digitalin (one of the active constituents of the drug) is obtained from the filtrate from the digitonin tannate as follows: Add more tannin solution and some sulphuric acid, dissolve the precipitate in 70 per cent. alcohol, boil the solution with lead carbonate, decant, evaporate with addition of lead oxide, extract the residue with alcohol and evaporate the solution.

ELATERIUM.

286. The active principle of the drug is **Elaterin**, $C_{20}H_{28}O_5$, a readily crystallizable substance of a bitter, disagreeable taste. It is almost insoluble in water or glycerin and, according to the U. S. P. 1890, requires for solution 543 parts of ether, 337 parts cold alcohol or 34 parts of boiling alcohol. Its best solvent is chloroform. The drug is at best very variable in strength, and often contains a large proportion of mineral matter (calcium carbonate, alumina, terra alba, etc.)

287. Assay Process. (1.) Determine mineral matter by igniting in a porcelain or platinum crucible 0.25 grams until the ash is quite white. The ash ought not to weigh more than 0.02 gram.

288. (2.) **Determination 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 about 55°C. (131° F.) Transfer to a small filter, and when the chloroform has all passed, percolate on the filter with fresh chloroform to complete exhaustion. 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 tared 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.

289. After a few hours decant the ether into a second tared beaker, and allow the ether to evaporate until reduced to about 3 cc. Decant the remaining ether into a third beaker, leaving the crystals of elaterin which have formed. Wash these with a little ether by decantation. Allow the ether in the third beaker to evaporate entirely, add 3 cc. of ether and observe whether any crystalline residue remains undissolved. If so this must be carefully washed with a little ether. Finally dry all the crystals obtained and weigh. The weight of the elaterin is usually about one half that of the original chloroform extract. If it fall much short of that, loss of elaterin in the crystallization may be suspected. In any case it is safe to add an arbitrary correction of ten milligrams. Elaterium of good quality should yield 16 to 20 per cent. of crystallized elaterin.

ERGOT.

290. The most recent study of ergot is that made by C. C. Keller* who arrives at the conclusion that ergot contains but one alkaloid, and that this is the active principle of the drug. This alkaloid, which Keller believes to be identical with Tanret's ergotinine, Kobert's cornutine and Dragendorff's picrosclerotine, has the following characters: It is insoluble in petroleum ether, which precipitates it from its ethereal solution; it is readily soluble in alcohol and in chloroform, but dissolves only with difficulty in ether after it has been crystallized. The alcoholic solution has a strong bluish violet fluorescence especially after acidulation. The neutral salts are readily soluble in water, the acid salts only sparingly so. From a somewhat acid solution, chloroform extracts the alkaloid; ether removes but little.

291. Assay process of Keller. Pack lightly in a small percolator 25 grams of dry ergot in No. 60 powder, exhaust with petroleum ether until a few drops of the percolate leave no residue when evaporated on a watch crystal. Dry the powder at a gentle heat, transfer it to a tared cylinder of 250 cc. capacity, add 100 grams of ether and after ten minutes a mixture of magnesia 10 grams, water 20 cc. Shake the mixture well and repeat the shaking frequently during half an hour. Then pour off 80 grams of the ethereal solu-

*Schweiz. Wochensschr. Chem. u. Pharm., 1894.

tion or as much as is obtainable, four grams representing one gram of the ergot.

292. If the solution is not quite clear let it stand for some time. Pour off from any sediment and shake with three successive portions of water (25, 15 and 10 cc.) containing 0.5 per cent. of hydrochloric acid. [Wash with a fourth portion of acidulated water and test with Mayer's reagent to ascertain whether the alkaloid has all been removed.] The mixed acid solutions are made alkaline with ammonia and shaken out with three successive portions of ether (50, 25 and 25 cc.) Distill off the ether in a tared flask, treat the residue twice with a little fresh ether, finally dry to constant weight and weigh. Chloroform is a better solvent for the alkaloid than ether, but is liable to form obstinate emulsions, hence ether is preferred and in the quantities prescribed extracts the whole of the alkaloid.

293. Proportion of alkaloid in ergot. Different samples of ergot yielded in Keller's hands from 0.095 to 0.245 per cent. of alkaloid. From an old sample which had been kept in powdered condition 0.165 per cent. was obtained, showing that the drug may be kept for some time without becoming inert. Old samples may be distinguished from new, as Koster had already observed, by the color of the ether extract, which in the former is dark brown, in the latter of a bright yellowish color.

294. Physiological test of Ergot. Owing to the existing uncertainty concerning what is the real active principle of ergot, Kobert and Grünfeld resorted to a physiological test of the activity of the drug by feeding definite quantities of the sample to roosters. The effect of an active drug or

preparation is promptly shown in the comb and wattles of the fowl, which assume a characteristic dark color from the peculiar action of the drug upon the capillary circulation. Dr. Houghton* suggests the employment of this procedure for determining the value of any specimen of ergot or of its preparations, but he gives no details of his method, which in any case must leave much to be desired as a quantitative test. Jacobi, who has studied the drug carefully by the method of physiological experiment arrives at the conclusion that the true active principle of ergot is not its alkaloid, but the resinous sphacelotoxin (sphacelinic acid). The alkaloid, however, certainly posseses active properties, and an assay based upon its determination unquestionably has value.

*Therapeutic Gazette, July 15, 1898.

GELSEMIUM.

295. Yellow Jessamine contains at least one alkaloid capable of yielding crystallizable salts. It is sparingly soluble in petroleum benzin, freely soluble in ether and still more so in chloroform. This is accompanied 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 analagous to aesculin, but regarded as peculiar to gelsemium, and called gelseminic acid. This is removed from acid solutions by thorough washing with ether or chloroform, but a portion of it is almost sure to accompany the alkaloid when extracted in the usual manner.

296. The assay is best conducted by Short Assay Process No. 2, (72) to (75), the alternative of (73) being preferably followed. The alkaloid obtained is always of a dark color, and is of complex composition, so that alkalimetric titration rather than direct weighing is to be relied upon. The titration equivalent may be taken as that of the pure crystallizable alkaloid, hence one cc. of the N 1-25 acid ordinarily employed in alkalimetric titrations is assumed to neutralize 16.3 mg. of alkaloid.

297. Titration with Mayer's Reagent. The acid solution of (73) may be titrated with Mayer's reagent with results almost as satisfactory as those by acid titration, since

in either case the equivalent factor is arbitrarily assumed, and we know nothing as yet of the relative medicinal activity of the several alkaloids presumably present. Perhaps a gravimetric determination by weighing the precipitate (See p. 67) produced by Mayer's reagent in the acid solution just mentioned will give as good a practical measure as any we can reach, with our present knowledge, of the value of the sample.

298. Assay of Galenical Preparations of Gelsemium. The fluid extract may be easily assayed by the process of (132) et seq., the determination of alkaloid being finally made by alkalimetry as above. A purer alkaloid is obtained by the process of (131 b.), and for a gravimetric determination this plan (or that of Farr and Wright, below) should be adopted. Tinctures, solid extracts, etc. are to be treated on general principles. See (151) to (154).

299. Process of Farr and Wright*. Fifty cc. of a tincture or 10 to 20 cc. of a fluid extract are evaporated on a water bath to a small volume, adding water if necessary to remove all alcohol. To the solution, when cool, add 1 cc. seminormal sulphuric acid, mix well and filter through absorbent cotton into a small separator. Rinse dish and filter with a little acidulated water, wash the acid fluid with three portions of chloroform (10,5 and 5 cc.), which in turn are washed with water 5 cc., added afterwards to the contents of the separator. Render the aqueous solution alkaline with ammonia and shake out with three portions of chloroform (15,10 and 10 cc.) The chloroform is then washed with four successive portions (5 cc.) of water containing about 1.5 per cent. of sulphuric acid. The acid solutions are united, an

excess of Wagner's reagent (17) is added and the mixture set by until the precipitate has subsided leaving the fluid clear.

300. The fluid is decanted through a small filter, the filter washed with a little water, and then 5 cc. of a 5 per cent. solution of sulphurous acid is passed through it into the flask containing the alkaloidal precipitate. The flask is allowed to stand with occasional agitation until the alkaloidal periodides have been wholly decomposed, as shown by absence of dark-colored particles. The solution is then filtered, flask and filter washed, first with 2—3 cc. of sulphurous acid, then with water until the washings are no longer precipitated by Mayer's reagent. Filtrate and washings are made alkaline with ammonia and shaken with three successive portions of chloroform (10,10 and 5 cc.), the chloroform evaporated, the residue dried at 100°C. and weighed.

*Chem. and Drug., 1892. 261.

GRANATUM.

301. Pomegranate Bark owes its virtue as a taenicide to a liquid alkaloid pelletierine, to which is assigned the formula $C_8 H_{13} NO$. This is easily soluble in water, alcohol, ether and especially in chloroform. This alkaloid is accompained by several others, but the relative medicinal activity of these has not been studied, and it may suffice to determine the total alkaloids as an indication of the value of a given sample of drug.

302. On this principle W. Stoeder* has made a series of assays showing that the bark of the root is richer in alkaloids than other portions of the plant, containing 1. to 1.3 per cent. of alkaloid, while the root bark from wild varieties in Java yielded 1.7 to 3.75 per cent.

303. The assay may be conducted by extracting the drug with the modified Prollius' mixture as in Short Process No. 2 (72), evaporating the solvent at a very gentle heat, taking up the residue with fresh ether (acid free) and evaporating, then dissolving the residue in excess of standard acid, N 1-25 and titrating back with a corresponding standard alkali. The titration equivalent may be taken at 139, so that one cc. of the N 1-25 acid would neutralize 5.56 milligrams of alkaloid.

*Nederl. Tydsch. Pharm. 6, 39; Chem., Centrlb., 1894, 1, 606; also Neder. Tyd. Pharm., 1890.

GUARANA AND OTHER DRUGS CONTAINING CAFFEINE.

304. **The active constituents** of guarana, coffee and tea is caffeine (or theine) which is also a constituent of kola and of maté or Paraguay tea. The alkaloid is very feebly basic, so that it cannot be determined by alkalimetry, at least with any of the indicators ordinarily employed. It is not precipitated by Mayer's reagent, although a characteristic crystalline precipitate is produced by a solution of potassium iodide saturated with mercuric oxide. In an acid solution it is precipitated by Wagner's solution (17) and it is also precipitated by tannin.

305. Caffeine is soluble in 80 parts of cold water, in 9.5 parts of boiling water, also is freely soluble in cold aqueous solutions of certain salts, notably the salicylates and benzoates of the alkalies; it dissolves in 33 parts of alcohol, in 555 parts parts of ether and in 7 parts of chloroform, and is also readily soluble in benzol and in carbon tetra-chloride. It is removed from watery solutions even in presence of free acids by shaking with chloroform, althought not so readily as when the solution is neutral or alkaline. It crystallizes easily from its solutions in volatile solvents, and may be easily sublimed without alteration.

306. Moses Gomberg* has shown that the alkaloid can be determined with a fair degree of precision by a volumetric

*Journ. Am. Chem. Soc., 1896, 18 331—342.

process involving its conversion into a periodide. His procedure, applicable only in absence of other substances precipitated by iodine, is as follows: To 25 cc. of decinormal iodine solution U. S. P., add a solution of the caffeine acidulated with hydrochloric acid, the quantity of caffeine not to exceed 25 milligrams. See (115). Make up the volume with water to exactly 50 cc. Shake the mixture well and let stand until the precipitate separates so as to leave the supernatant fluid clear. [This must be of a deep iodine red color, otherwise the experiment must be repeated using a smaller quantity of the caffeine solution.] Decant 25 cc. of the clear solution and titrate with decinormal solution of sodium thiosulphate. Deduct twice the volume of the thiosulphate solution required from 25, and multiply the remainder by 4.85 to find the weight in milligrams of the caffeine present in the portion of solution used.

307. In many of the older processes for extracting caffeine, lime was used. It has been shown that this involves loss of a portion of the alkaloid, hence it is not admissible in an assay process. Magnesia seems not to be open to the same objection, and no direct loss is occasioned by the use of oxide of lead, oxide of iron, alumina or basic lead acetate, although some alkaloid may be withdrawn from a solution by occlusion in precipitates produced by these agents.

308. For extraction of caffeine from aqueous extracts or other liquid preparations not containing chlorophyll, the method of J. U. Lloyd (140) is well adapted. The detail of this method as applied to a fluid extract of guarana is as follows.* Put into a flat bottomed graduate 2.5 cc. of the fluid

*Proc. Am. Ph. Assoc., 1892, p. 453.

extract, add 2.5 grams of a mixture of equal parts of sodium bicarbonate and dry ferric hydrate, mix well, preferably with a mechanical stirrer, add 10 cc. of chloroform and stir well together. Should the magma remain floculent and the chloroform not separate therefrom, add a sufficient amount (10 to 20 drops) of a mixture of glucose and water equal parts, and incorporate thoroughly. Decant the chloroform into a tared beaker. Repeat the treatment with two or three fresh portions (5 cc.) of chloroform. [Should the magma collect on the sides of the graduate above the stirrer, scrape it down occasionally.] Finally evaporate off the chloroform, dry to constant weight and weigh.

309. Assay process of A. Petit and P. Terrat.* [Proposed for the assay of tea, but stated by the authors to be equally applicable to guarana or kola.] Treat 10 grams of powdered tea with 30 cc. of boiling water, macerate 15 minutes then evaporate on the water bath until the liquid has disappeared, leaving the powder visibly and uniformly moist. Transfer to a small percolator and exhaust with chloroform (continue percolation until a few drops of the percolate, evaporated and taken up with water, show no cloudiness on adding tannin.) Distil off chloroform, treat residue with boiling water, filter, wash filter with hot water, evaporate filtrate to obtain caffeine in condition generally pure enough for weighing. The authors affirm that chloroform extracts from the thoroughly moistened powder the whole of the caffeine, the use of lime, magnesia or ammonia being superfluous.

310. An obvious modification of the above would con-

*Journ. Pharm. Chim., 3, 529; Chem. Centralb., 1896, II, 214.

sist in macerating the moistened powder with 100 cc. of chloroform with frequent or constant shaking several hours, decanting 50 cc. of the chloroform, evaporating, taking up with boiling water as above and determining caffeine by the method of Gomberg (278). In applying the method to guarana or kola, maceration with warm water would, no doubt, be better than boiling in the first step of the process, owing to the presence of starch.

311. Keller's Assay process for Tea.† Place in a suitable separator 6 grams of the dried tea, unpowdered, add 120 grams of chloroform and shortly after 6 cc. of 10 per cent. solution of ammonia. Shake half an hour, then let stand 3 to 6 hours. Through a filter moistened with chloroform, filter (avoiding loss by evaporation) 100 grams of the chloroform, distill, treat the residue in the flask with 3 cc. of absolute alcohol, evaporate the alcohol by aid of a hand bellows, whereby the chlorophyll is caused to separate. Treat on the water bath with a mixture of water 7 cc., alcohol 3 cc., add water 20 cc., shake to cause chlorophyll to aggregate and filter. Wash flask and filter with 10 cc. of water. Evaporate the aqueous solution to dryness and weigh.

312. The caffeine may be further purified by dissolving in 5 cc. of boiling alcohol; when cool, add 50 cc. of solution of lead subacetate, after twelve hours separate the crystals, which may then be brought into solution in chloroform, the solvent evaporated and the crystals weighed. A better plan would be to determine caffeine in the final aqueous solution of (312) by Gomberg's method, or else to add to this solution a little lead subacetate, filter (washing filter well),

†Berich. d. Pharm. Ges., 7, 105; Chem. Centralh., 1897, i. 1134.

remove excess of lead from filtrate and washings with sodium phosphate, filter, concentrate filtrate and washings to 25 cc. and extract the pure caffeine by shaking out with chloroform (313).

313. The simplest plan perhaps for the assay of tea or coffee is the following: Place in a flask provided with a reflux condenser 6 grams of the sample in powder [Raw coffee must be in very fine powder] with 580 cc. of water; heat on a steam or water bath six or eight hours. Add lead subacetate 4 cc. or sufficient to impart to the mixture a perceptible sweet taste. When cool make up the volume to 600 cc., filter, add to the filtrate sufficient sodium phosphate or potassium carbonate (in powder) to remove excess of lead. Filter once more, evaporate 300 cc. of the filtrate, equivalent to 3 grams of the sample, to 40 cc. Shake out with chloroform, using at least three successive portions, 15,10 and 10 cc. (more if necessary). Evaporate to dryness and weigh the pure white crystals of caffeine.

314. As a control, determine caffeine in 25 or 50 cc. of the filtrate, equivalent to 0. 25 or 0.5 grams of the sample, by the method of Gomberg (306).

315. The extraction of the sample in the beginning may be made by boiling with successive portions of water, 145 cc. each, (30 minutes each time) making up finally about 580 cc. of the decoction which is to be treated as already described. Instead of shaking out with chloroform, we may extract in a perforator (12) either with chloroform, ether or benzol, or by a mixture of benzol with chloroform. We may evaporate an aliquot portion of the solution (perferably after treatment with lead subacetate and sodium phosphate) to a very

small volume and extract the caffeine by the method of Lloyd (140). Or finally, we may evaporate nearly to dryness, add calcined magnesia 0.3 gram and powdered pumice, dry and extract by percolation, or by repercolation with ether.

316. Guarana cannot be so conveniently exhausted with boiling water on account of the starch it contains. It is better to exhaust the powder, 10 grams, by percolation with a mixture of two volumes of alcohol and one of water, obtaining 100 cc. or more of percolate. Evaporate the percolate to a volume of 50 cc., pour into a 500 cc. measuring flask containing 425 cc. of water, add 8 cc. of solution lead subacetate, or sufficient to impart to the mixture a sweetish taste, make up to 500 cc. and proceed as in (313) remembering that of this solution 50 cc. represents one gram of the drug.

317. The following assay processes offer alternative methods which may be preferred by some to the foregoing. **Mulder,** whose method has been pronounced by more than one authority the best (for tea), proceeds as follows: Extract the tea (10 grams) with boiling water, evaporate the solution to a small volume, add calcined magnesia (2 grams), reduce to a syrupy consistence, extract with pure chloroform, evaporate and extract the residue with hot water.

318. A. Grandval and H. Lajoux* direct to stir in a porcelain capsule five grams of the finely powdered material (tea, coffee, kola) with a mixture of ether five grams, solution of ammonia, sp. gr. 0.925, one gram, transfer to an extraction apparatus and exhaust with chloroform. Distill off the chloroform and treat the residue with 1 cc. of dilute sulphuric acid, 10 per cent., then extract repeatedly with hot water,

*Journ. de Pharm. et de Chim. 1893, p. 545.

and filter the solution. In the case of roasted coffee, this solution is simply rendered alkaline with soda and shaken out with chloroform, which is evaporated and the residue weighed as caffeine. In other cases the aqueous solution is treated with excess of ammonia, evaporated, the residue extracted with chloroform, which is filtered and evaporated, leaving a residue of caffeine sufficiently pure to weigh. Here again caffeine might be determined conveniently by the method of Gomberg.

319. G. L. Spencer* adds to the decoction of tea while hot a considerable quantity of freshly prepared ferric hydroxide, digests one hour to detannate, cools the solution, and makes up to such a volume that 100 cc. represents one gram of the sample; filters and determines caffeine in an aliquot part (20 or 25 cc.) by the method of Gomberg. Obviously the alkaloid could be extracted also by concentrating an aliquot part (250 cc.) and shaking out with chloroform as in (313).

320. A. Hilger and A. Juckenack† give the following process as one yielding a very pure product of caffeine. Digest 20 grams of the finely powdered tea or coffee in 900 cc. of water several hours, then boil 1½ hours (2 hours in the case of unroasted coffee) in a flask provided with a reflux condenser. Cool to 60° or 80°, add 100 cc. of solution basic aluminum acetate of the German Pharmacopoeia‡ and then gradually 1.9 grams sodium bicarbonate stirring the mixture

*Journ. Am. Chem. Soc., 1897, 19, 279.

†Apoth. Zeitg., 1897, 12, 145 and 422.

‡Dissolve 30 parts of aluminum sulphate in 80 parts of water, add 36 parts of acetic acid of 24 per cent., add with constant stirring a mixture of 20 cc. of water with 20 grams of calcium carbonate, stir occasionally during 24 hours, strain, press out and filter.

during the addition. Boil 5 minutes, when cold make up to 1020 grams. Filter, and to 750 grams of the filtrate add 10 grams of dried and finely powdered aluminum hydroxide, and a little filter paper in pulp, dry and extract eight hours with carbon tetrachloride. Evaporate the solvent, when pure caffeine remains.

321. Dr. Squibb* mixes 10 grams of coarsely powdered tea or finely powdered coffee with 2 grams of calcined magnesia stirs into 100 cc. of boiling water, boils 5 minutes, filters and washes the residue with 50 cc. of hot water; repeats the boiling with 100 cc. of water, filters and percolates the residue to exhaustion with hot water. The liquid is evaporated to 20 cc. and shaken out with chloroform, In the case of coffee the direct shaking out is impracticable. The solution is therefore poured into alcohol, 100 cc., to precipitate albuminous matter, filtered, the precipitate washed well with alcohol, filtrate and washings evaporated, taken up with water, filtered and the aqueous solution finally shaken out with chloroform. Guarana he treats like coffee with magnesia, and boils once with water 100 cc., then adds 30 cc. of strong alcohol, filters, and percolates the residue with 100 cc. of a mixture of alcohol one volume, water two volumes, boils the residue with 100 cc. of the same alcoholic mixture, with which he finally percolates the residue to exhaustion. The alcoholic solution is finally evaporated to 20 cc. and shaken out with chloroform.

322. The following additional notes may furnish useful suggestions. **Paul and Cownley**† obtained very high results

*Ephemeris. 614, 637, 641.

†Pharm. Journ. Trans. 1887, p. 565.

by mixing finely powdered tea or coffee with moist lime, exhausting in an extracting apparatus with alcohol, evaporating, adding water and a few drops of dilute sulphuric acid, filtering and shaking out with chloroform, accepting the residue on evaporation as pure caffeine.

323. Forster and Riechelman* treat roasted coffee (20 grams) by boiling with four portions of water (200 cc.) [These authors extract the alkaloid with chloroform by the perforation method, after adding a little sodium hydroxide. They determine nitrogen in the residue by the method of Kjeldahl, and so estimate the caffeine.] Tassilly† treats 10 grams of coffee 10 minutes with 200 cc. of boiling water, repeats this three times, then boils twice more with the same quantity of water.

324. Georges‡ exhausts powdered tea (15 grams) at 100°C. with a solution of sodium salicylate in water, concentrates to 50 cc. and shakes out with chloroform. The method of **Herlunt§** (for coffee) is similar in principle, but involves the objectionable use of lime. The finely powdered coffee is mixed with slaked lime, and extracted with a 5 per cent. aqueous solution of sodium benzoate. The solution is made alkaline (?) with sodium carbonate, filtered and extracted with chloroform.

325. Kola nut contains in addition to caffeine a glucoside **Kolanin,** which by hydrolysis yields caffeine. According to Heckel and Schlagdenhauffen (1884) there is also

*Zeitschr. f. Offentl. Chem., 3, 129.

†Bull. Soc. Chim., (3), 17, 761.

‡Journ. Pharm. Chim., 1896, July 15th.; Chem. Centralb., 1897, ii, 506.

§Monit. de la Pharm., Feb. 1892, 1028.

present a small amount of theobromine, but the researches of Knox and Schlotterbeck (1895) do not confirm this. The activity of the drug may be assumed to depend upon the total caffeine which it contains actually (free) and potentially (in its kolanin.) The assay process therefore should be so conducted as to decompose the kolanin before extracting caffeine. If the powdered drug is macerated twelve hours with hydrochloric acid, 1 per cent., the kolanin will be hydrolysed and an assay may then be made for caffeine by processes already described. those of (309) (310) and (316) being especially applicable.

326. A. R. L. Dohme and H. Engelhardt* direct to boil the powdered kola with a mixture of alcohol one volume, water two volumes, three hours, in a flask provided with a reflux condenser. Filter, evaporate nearly to dryness on a water bath, mix with calcined magnesia and sand, dry completely and extract by boiling with chloroform. **Knox and Schlotterbeck†** simply extract the kola with chloroform ten hours in a Soxhlet apparatus. **Carles‡** directs to use a very fine powder, mix 10 grams of this with 1 gram of slaked lime and 20 grams of 80 per cent. alcohol, dry on a water bath to 14 grams, exhaust by boiling with four successive portions, (35, 35, 30 and 25 cc.) of a mixture of alcohol 20 grams, chloroform 100 grams. Evaporate and extract with slightly acidulated boiling water. The kolanin he determined by first exhausting with cold water to remove caffeine, then extracting with 70 per cent. alcohol. In the resi-

*Proc. A. Ph. A., 1896, p. 599.

†Proc. A. Ph. A., 1895, p. 335.

‡Annal. de Chim. Anal., i., 345; Proc. A. Ph. A., 1897, p. 533.

due caffeine, formed from hydrolysis of the kolanin, is determined by lime, chloroform and alcohol as above.

327. The proportion of caffeine in guarana is generally between 4 and 4.5 per cent., in tea varies from 1.5 to 4.25 per cent., average about 3 per cent., the black teas being in general richer than the green; in coffee about 1.2 per cent., the proportion varying little, and being nearly as great in roasted as in green coffee; in kola nut from 0.75 to 2 per cent., average about 1.25 per cent.

HYDRASTIS.

328. Active Constituents. The drug contains two principal alkaloids. The more abundant is that to which it owes its bitterness and its deep yellow color, berberine, a constituent of many plants besides golden seal. The other ,which is characteristic of this drug and distinctively its active principle, is hydrastine. The former is soluble in chloroform but practically insoluble in ether, and forms readily crystallizable salts, many of which are sparingly soluble in water particularly in presence of free acid. The latter is very freely soluble in ether, soluble also in petroleum benzin and in all the usual solvents for alkaloids, easily crystallizable, but is of very feeble alkalinity and forms salts which cannot be easily crystallized.

329. The method of Keller* (50) is the best yet devised for the assay of hydrastis, where we desire merely to determine hydrastine. Use powdered hydrastis, 12 grams; ether 120 grams, (no chloroform); water of ammonia, 10 grams; water 15 grams; decant 100 grams to represent 10 grams of drug; extract with hydrochloric acid and finally with ether. The alkaloid procured by this process is not strictly pure. It may be obtained in a purer form by washing the acid solution well with petroleum benzin, rendering alkaline with ammonia and washing out promptly with several

*Schweitz. Wochenschr. f. Chem. u. Pharm., 1894; Apoth. Ztg,, 1894, 52, 133.

successive portions of hot benzin. Keller proposes to crystallize it by dissolving the alkaloidal residue (from 20 grams of drug) in 8 cc. of alcohol, adding 4 cc. of ether and, gradually, 20 cc. of water and letting stand 24 hours. In my experience the yield of hydrastine by this plan is far too low. A better plan is to determine the alkaloid by alkalimetry (331), or we may adopt the method of Schwickerath.* The acid solution [second stage of assay above] is washed one hour in a perforator with ether, the ether rejected, the solution made alkaline with ammonia and extracted three hours with ether.

330. Alternative Process. Treat 5 grams of the finely powdered drug with 100 cc. of Prollius' mixture as in (63). Decant 50 cc. of the clear fluid for the assay and follow (73) to (75), or else treat the acid solution of (73) by the perforator method as described in the preceding paragraph.

331. On page 55 the statement is made that hydrastine cannot be satisfactorily determined by the alkalimetric method when the ordinary indicators are used. I find, however, no difficulty in the titration using as indicator haematoxylin, cochineal or methyl orange. The titration equivalent will be found on page 55. A residual titration may be made, indeed, in a sufficiently concentrated aqueous solution without using any indicator, owing to the very sparing solubility of the alkaloid. In a solution containing less than 700 mg. of hydrastine to the litre, a dilute soda solution produces no precipitate. If to a solution of the alkaloid in standard acid, an equivalent quantity of standard alkali is added and at the same time distilled water just enough to redissolve the pre-

*Pharm. Rundsch., 1893, p. 282.

cipitated alkaloid, it will be found that 75 cc. of the solution will contain very nearly 50 mg. of hydrastine.

332. If an alkaloidal residue is dissolved in standard acid N 1-25, using 1 cc. for each 10 mg. of the residue, and without further dilution, standard soda N 1-25 is added until a permanent precipitate is produced, it will be found that there is held in solution very nearly one milligram of hydrastine for each cc. of the solution. If we deduct therefore the quantity (in cc.) of soda solution used from that of the acid taken, multiply by 15.88 and add one for every cc. of the solution at the end of the titration, we shall have approximately the quantity of hydrastine present, but the direct weighing has given already an approximation equally close.

333. **A more complete assay** of the drug may be made by the method of F. A. Thompson.* Exhaust 10 grams of the drug in moderately fine powder in an extraction apparatus with strong alcohol, dilute the extract to 100 cc. with alcohol; to 25 cc. of the mixture, add 1.3 cc. hydrochloric acid (U. S. P.), 0.2 cc. strong sulphuric acid and 12.5 cc. of ether [this is after the method of J. U. Lloyd.†] Let stand in a refrigerator 24 hours, then transfer the crystals of berberine hydrochloride to a pair of mutually counterpoised filters, wash with a mixture of equal volumes of alcohol and ether until all free acid is removed, press, dry at 105°C. and weigh. Multiply weight by 0.9018 to obtain the weight of the alkaloid.

334. Thompson proceeds to extract the hydrastine from

*Proc. Mich. State Pharm. Assoc., 1893; Am. Journ. Pharm., 1893, 371.

†Druggists Circular, Feb. 1885, p. 22.

the acid solution above by the sawdust process, but it is better to determine this alkaloid in 25 cc. of the original alcoholic tincture by reducing to a small bulk and either mixing with sawdust and extracting with a weak Prollius' mixture, or else shaking out direct (from a solution measuring 5 cc. and containing about 50 per cent. of alcohol) with ether, washing out alkaloid with hydrochloric acid and from the aqueous solution after adding ammonia with ether.

335. A fluid extract of golden seal if made with dilute alcohol, may be assayed for hydrastine* by simply shaking out 2 cc. of the fluid with three successive portions (15 cc.) of ether following details of assay process (132) et seq., *but using no chloroform.* **Berberine** may be determined approximately in the residue by diluting with water, filtering if necessary, acidulating with hydrochloric acid and precipitating with Mayer's reagent in slight excess, collecting on a pair of mutually counterpoised filters, drying and weighing. One-half the weight may be taken as approximately the weight of the berberine.

336. Berberine may also be determined in a fluid extract as follows: Mix 5 cc. of the fluid extract with 15 cc. of strong alcohol. If no precipitate is produced, dilute to 50 cc. with alcohol, add 17 cc. of ether, 1.25 cc. hydrochloric acid (U. S. P.), 0.3 cc. concentrated sulphuric acid, and proceed as in (333). If a precipitate forms as will usually happen, allow it to subside and decant the clear fluid. Dissolve the precipitate by adding a few drops of water and reprecipitate with strong alcohol. Repeat this operation until 50 cc. of alcoholic solution has been obtained and to this add ether and acids as above.

*The Author in Pharm. Era, April 7th, 1898.

337. E. G. Eberhardt* determines hydrastine in a fluid extract by warming 25 cc., adding 10 cc. of ether, 20 cc. of water and 5 cc. of solution of ammonia (10%), rotating briskly, setting aside 12 hours with occasional rotating during the first two or three hours, collecting the crystals, washing with water, drying and weighing. The alkaloid may be first washed out of the fluid extract with ether and ammonia, shaken out with three successive portions of two per cent. sulphuric acid, addition made of 5 cc. of water, 10 cc. of alcohol, 3 cc. of ether, ammonia sufficient to make alkaline, the mixture shaken frequently during six hours, and the crystals of hydrastine separated and weighed. The results are of course only approximative, but near enough to be of value.†

*Am. Journ. Pharm., 1893, 374.

†For other methods of assaying hydrastis and its preparations see Pharm. Centralh., 1895, No. 25 (Linde), and Chem. Centralb., 1896, II, 454, from Nederl. Tyds. Pharm., 8, 197, (J. M. A. Hegland).

IPECACUANHA.

338. Until recently Ipecac has been credited with but one alkaloid, called emetine. The researches of Paul and Cownley* have, however, shown that the emetine heretofore known consists of a mixture of two distinct alkaloids, differing (although analagous) in physiological and therapeutic action. To these alkaloids the names emetine ($C_{15}\ H_{22}\ NO_2$) and cephaeline ($C_{14}\ H_{20}\ NO_2$) have been given, the former being regarded as the expectorant, the later as the emetic principle of the drug. The same authors have announced (1895) the discovery of a third alkaloid which is present, however, in small quantities, and is not known to have important medicinal properties. Further it appears that the antidysenteric virtue of ipecac is not dependent upon the alkaloids of the drug.

339. The following† is the method employed by **Paul and Cownley** for the complete assay of ipecac: Mix 50 grams of the powdered drug with 10 grams of freshly slaked lime, moisten with water and extract by percolation with amylic alcohol. Extract the alkaloids by shaking out with dilute sulphuric acid, make alkaline with ammonia and shake out with ether. The third alkaloid will remain in the aqueous solution, from which it can be taken out with chloroform.

*Pharm. Journ. Trans., 1894, 396; See also Ibid. 1895, (1) 61 and 1895, (2) 111, 373, 641 and 690.

†Pharm. Journ, Trans. 1896, p. 321.

The ethereal solution is to be evaporated and the residue titrated with seminormal hydrochloric acid, of which one cc. will neutralize 124 milligrams of emetine or 117 mg. of cephaeline.

340. The hydrochloride solution is mixed with caustic soda in excess and shaken out with ether to remove emetine. Some cephaeline, however, accompanies it, so that it is necessary to bring the emetine again into acid aqueous solution and repeat the treatment with soda and ether until the residual alkaline solution is no longer clouded by adding ammonium chloride. The purified emetine is finally determined by titration with standard acid. The cephaeline is obtained by adding to the residues containing it ammonium chloride and shaking out with ether, and is also determined by alkalimetry.

341. The further studies of Paul and Cownley show that there is a marked difference between Brazilian and Carthagena ipecac in the relative proportion of the several alkaloids they contain. In the former the emetine constitutes about 70 per cent. of the whole, in the latter only 40 per cent. We cannot therefore consider the two drugs as therapeutically identical.

342. For practical purposes, in the present state of our knowledge, it is sufficient to determine the total content of alkaloid in a sample, the variety, Brazilian or Columbian, being always specified. The assay is easily made either by Short Assay Process No. 2, following (73) etc., by Keller's method (50), by that of Cripps and Whitby or by that of Grandval and Lajoux (47). In the Short Process No. 2, ether alone may be used instead of the mixture of ether and

chloroform. In following Keller's process, use ipecac 12 grams, ether 90 grams, chloroform 30 grams, water of ammonia 10 grams, finally water 10 grams; pour off 100 grams, extract with hydrochloric acid and again with ammonia and a mixture of chloroform 3, ether 2 parts. Whichever process we adopt we may finally determine the alkaloid without purification either by titration with Mayer's reagent (348) or by alkalimetry, taking as the titration equivalent the conventional figure 254,* from (88), or the presumably more correct equivalent 244, required by the molecular weights given above. Statement should of course be made which of the equivalents is adopted.

343. R. A. Cripps and A. Whitby† use as the initial solvent either acetic ether alone, acetic ether containing one per cent. of glacial acetic acid or a mixture of acetic ether chloroform and acetic acid, and adopt the following procedure: Place 2.5 grams of the powdered ipecac in a small cylindrical percolator, shake down lightly, drench with the menstruum, cork the upper end of the percolator and let macerate over night. Then percolate to complete exhaustion (about 50 cc.) and wash out the alkaloid with four successive portions (8 cc.) of acidulated water. Wash the acid solution once with ether, make alkaline with ammonia and shake out with three portions (6 cc.) of ether, followed by two portions (6 cc.) of chloroform. Evaporate in a current of air, dry 6 hours over sulphuric acid and weigh. Finally determine alka-

*Simonson (Proc. A. Ph. A., 1893, 194.) empirically verifies this factor, which has been widely adopted.

†Pharm. Journ. Trans., 1889, 721.

loid volumetrically with Mayer's reagent [or by alkalimetry.]

344. Other methods of assay. G. Kottmayer* directs to digest 15 grams of powdered ipecac (air dried) with 148 cc. of 90% alcohol and 2 cc. of hydrochloric acid, sp. gr. 1.12, four days at 40°C. with frequent agitation; after cooling measure out 100 cc. of the fluid, mix with 20 cc. of a one per cent. solution of lead acetate in dilute alcohol, add 1.5 grams of slaked lime, evaporate to a pasty consistence; incorporate 5 grams of powdered glass, dry on water bath with constant stirring, extract the alkaloid by hot repercolation (10 hours) with chloroform. The crude alkaloid left on evaporation of the solvent is dried at 100°C. and weighed and then treated with two cc. of normal hydrochloric acid, the insoluble residue of resinous matter washed on a filter, dried and weighed, and its weight deducted from that of the crude alkaloid.

345. The method of E. M. Arndt† is interesting as a foreshadowing of the general method proposed by J. U. Lloyd (140). Ten grams of the powdered ipecac are intimately mixed with 5 grams of sodium carbonate and one gram of crystallized ferric chloride, the mixture digested on a water bath one hour with 100 grams of 60 per cent. methyl alcohol, in a flask fitted with a reflux condenser. The solution is then filtered, evaporated to remove alcohol, the residue taken up with 50 cc. of a very dilute solution of ammonia, and the mixture shaken out with chloroform.

346. Method of Dragendorff as modified by the author.* Place in a flask 50 cc. of distilled water, then 10

*Pharm. Post., 1892, 913, 933.

†Apoth. Ztg., 1890, 781.

*Am. Journ. Pharm., 1885, p. 531.

grams of ipecac in fine powder. Mix, cork and set by in a moderately warm place shaking occasionally, at the end of 24 hours add 52 cc. of strong alcohol (making a total of 100 cc. of menstruum, allowing for condensation of volume.) Cork and macerate three days, occasionally shaking. Measure out 25 cc. of the clear fluid for the assay, put this in a capsule with 5 drops of a highly dilute sulphuric acid (6%), evaporate alcohol by a gentle heat, make up the volume with water to 20 cc., digest a few minutes on the water bath, cool and titrate with Mayer's reagent. [If the solution is filtered and the filtrate and washings precipitated with a small excess of Mayer's reagent the determination may be made a gravimetric one (102).]

347. While the foregoing lacks scientific exactness, it is simple and easy of execution, and, although the results are too high, it enables us to pronounce correctly on the relative value of different samples of ipecac (of the same variety). W. H. Snow* has modified the method making it gravimetric and reasonably exact. After evaporating the alcohol, he treats the residue with a little dilute hydrochloric acid, filters, washes well and precipitates the filtrate and washings with a solution of platinum chloride. This is collected, washed, dried, ignited (91) and the residual platinum weighed. Mr. Snow found that 100 parts of the dry precipitate yielded only about 21 parts of platinum, instead of 29.7 as Dragendorff states. See (92.) This harmonizes with the statement of Paul and Cownley, that the platinum residue from emetine is 21.63 per cent., that from cephaeline 22.38 per cent. of the respective precipitates. The corresponding factors would be

*Proc. Mich. State Pharm. Assoc., 1887; Pharm. Era, 1887, 400.

2.54 and 2.40, and we should be safe in substituting for the figures in the table on p. 58, 22 per cent. in column three and 2.50 as the factor in column four, and so in multiplying the weight of the platinum residue above by 2.5 to obtain the weight of the emetine.

348. Mayer's Reagent has been much used in determining "emetine," but without discrimination of the several alkaloids present. Practically, no doubt, when the titration is made in a properly prepared solution, the results are as trustworthy as those obtained by other methods which determine only total alkaloid. The figures given in the table on p. 67 may be adopted as giving a sufficiently close approximation to the truth, if the fluid titrated contains 2 to 5 parts of alkaloid in 1000.

349. Galenical preparations of ipecac may be assayed easily by the usual methods. See especially (132) to (135). In the case of a "soluble" fluid extract of ipecac, we may advantageously dilute 5 cc. with water up to 25 cc. Take 5 cc. of the diluted extract for the assay, add a little hydrochloric acid and wash twice with ether, 25 and 20 cc. Then make alkaline with ammonia and shake out twice with ether, 25 and 20 cc., once with a mixture of ether 15, chloroform 5 cc., and finally with ether 15 cc.., to obtain an alkaloid pure enough to weigh. Of course it is a quicker way to make alkaline at once and shake out with ether and chloroform, and determine alkaloid by acid titration. If emulsions form resort to the sawdust process.

350. The British Pharmacopoeia directs for the assay of fluid extract of ipecac, the process outlined in (131 b.), which is perhaps as good as any, although I have generally

found notable loss of alkaloid in following this process unless the solution is made quite dilute before adding the lead subacetate.

351. The proportion of total alkaloid in good ipecac is probably not less than two per cent. Dr. Dohme has shown that the "wiry" root contains more alkaloid then the "bold" or "fancy" root; the woody part of the root contains very little. The results of assay by acid titration seem to show that the alkaloid in the condition in which it is ordinarily weighed is far from pure. The analyses reported by a number of independant workers, which make the percentage of alkaloid between 1.5 and 3. (or even higher) have been generally made by gravimetric methods or by titration with Mayer's reagent.

JALAP.

352. The Pharmacopoeial test for jalap requires that the drug yield twelve per cent. of resin of which not more than one tenth shall be soluble in ether. The requirement is too exacting, jalap in these days seldom yielding even 10 per cent. of resin. The assay process may be conducted exactly as in the official method for making resin of jalap, using 25 grams of the powdered drug.

353. F. H. Alcock* proposes the following as a simple and inexpensive assay process: Put into a dry bottle one gram of jalap in fine powder, add 20 cc. of amylic alcohol and shake occasionally. After four hours pass the liquid through a little absorbent cotton into a small separator, wash out the bottle with 5 cc. of amylic alcohol, and place the washings on the marc in the funnel; repeat with 5 cc. more if necessary, so as to ensure solution of the whole of the resin. Now shake the amylic alcohol with several successive small portions of water at 50°C. which must be allowed to separate and drawn off, removing thus every thing of a non-resinous nature. Transfer the amylic alcohol to a tared capsule containing 10 cc. of distilled water (to prevent "creeping" of the amylic alcohol during evaporation), dry on the water bath and weigh.

*Pharm. Journ. Trans., 1892, 107.

LOBELIA.

354. While our knowledge of the active constituents of lobelia is imperfect, an assay based on the amount of alkaloid contained in the drug is better than nothing. Such an assay method has been adopted by Farr and Wright* for the valuation of tinctures of lobelia. The following are the details of the process: Place in a porcelain capsule 50 cc. of the tincture with 5 drops of 33 per cent. acetic acid and 20 or 30 cc. of water. Evaporate on the water bath to 25 or 30 cc., filter through absorbent cotton into a separator, rinsing dish and cotton with a little acidulated water. Add ammonia in distinct excess and shake out with three successive portions of chloroform, 10,5 and 5 cc. Evaporate chloroform by a gentle heat, treat the residue first with 10 cc. then with 5 cc. of 1 per cent. hydrochloric acid and filter into a separator. Again render alkaline with ammonia and shake out with three successive portions of anhydrous ether 15, 5 and 5 cc. Evaporate the ether, dry the residue one hour at 100°C. and weigh.

355. Assay of the drug may be readily made by the Short Assay Process No. 2., (72) to (75), using ether for the final extraction of the alkaloid. After evaporation of the ether by a gentle heat, the alkaloidal residue may be dried best in a desiccator over sulphuric acid, in view of the possi-

*Chemist and Druggist, 1893, 454; Proc. A. Ph. A., 1893, p. 462.

ble volatility of the alkaloid or of some of the alkaloids present. I believe that the alkaloid can be determined by titration with acid, but am not prepared to state what titration equivalent should be adopted. One experiment gave the equivalent as high as 33.3 mg. as the equivalent of 1 cc. of decinormal acid, but I believe this is much too large a figure. Titration with Mayer's reagent gives very satisfactory results, the end of the reaction being sharply defined, but here again the titration equivalent remains to be fixed. In one experiment, one cc. of the Mayer's solution N 1-20 precipitated very nearly 10 milligrams of the impure alkaloid.

356. E. L. Patch communicates the following assay process; Macerate 10 grams of the drug (lobelia herb or seed) with 100 cc. of weaker Prollius' mixture (24) [one-half strength of ammonia], four hours with frequent shaking. Decant 50 cc. of clear liquid, wash out alkaloid with dilute sulphuric acid (1—2%), separate the acid washings, make alkaline with ammonia, wash out alkaloid with three successive portions of ether, 15 cc., evaporate ether spontaneously, dry residue in desiccator over sulphuric acid and weigh. By this method, he has obtained from lobelia herb 0.54 to 0.6 per cent. of alkaloid, from the seed 0.36 to 0.42 per cent.

357. Fluid extracts may be easily assayed by the general method of (132) to (134). Mr. Patch employs a somewhat shorter method, as follows: Put into a separator 5 cc. of the fluid extract with 5 cc. of water, add 10 cc. of ether and dilute sulphuric acid (2%) to strong acid reaction, shake and separate; wash the acid solution with two additional portions of ether, make alkaline with ammonia and wash out alkaloid with ether as above.

NUX VOMICA AND IGNATIA BEAN.

358. 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 upward of 50 per cent. of the total alkaloid. In ignatia, the proportion is larger. The medicinal action of the two alkaloids is believed to be similar, but strychnine is very much more powerful—according to Falk, 38 times as active.

359. Assay of the drug may be made by the author's Short Assay Process No. 2, using five grams of the drug in as fine a powder as can be made, and increasing the proportion of chloroform (ether three volumes, chloroform one volume), following perferably the method of (73) The method of the following paragraph differs from this in some details and is perhaps preferable.

360. Alternative Process. Put into a 4 oz. prescription vial 5 grams of the drug in as fine a powder as can be easily made (No. 40 will answer), add 100 cc. of a mixture of ether three volumes and chloroform one volume [cool to the room temperature before measuring], and 1 cc. of a 50 per cent. solution of ferric chloride; shake well and let macerate one hour. Then add 5 cc. of a saturated solution of sodium chloride and 10 drops of ten per cent. hydrochloric

acid; shake occasionally during one hour: Add solution of ammonia (10%) 5 cc., shake vigorously and macerate with frequent or continuous shaking four to six hours. Complete the assay by (73) to (75). The alkaloid obtained is nearly white and contains very little impurity, but may be titrated with standard acid if desired see (362).

361. Keller* proceeds as follows: Put into a small tubular percolator (12 cm. long, 2.5 cm. wide) 15 grams of the thoroughly dried drug in fine powder, exhaust by percolation with ether[to remove fat, but this preliminary step seems quite unnecessary] of which about 100 cc. will be required. The ethereal solution will contain a part of the alkaloid which must be removed by shaking out first with 5 cc. of decinormal hydrochloric acid, then with three successive portions of acidulated water (6 cc. each). The drug in the percolator is transferred with the aid of ether to a tared flask, and sufficient ether added to make in all 100 grams (allowing 0.5 gram for the fat removed by the ether). Fifty grams of chloroform are then added, followed by 10 cc. of solution of ammonia (10%) and the flask is well shaken at frequent intervals during half an hour. The acid solution containing the alkaloids removed from the ethereal percolate is then added, the mixture well shaken, and when the ethereal solution is well separated, 100 grams of it are transferred to an Erlenmeyer flask, the solvent distilled off, the residue treated repeatedly with a little pure alcohol to remove traces of chloroform, dried at 100°C. and weighed.

362. The alkaloid in the residues of either of the foregoing processes may be determined approximately by alkali-

*Schweiz. Wochensch., 33, 452.

metry. Keller directs to dissolve the residue in 5 cc. of chloroform with the aid of a little heat, add ether 40 cc., water 10 cc., iodeosin indicator (1% alcoholic solution) one drop, then decinormal hydrochloric acid 10 cc. Titrate back with decinormal ammonia until a permanent red color appears in the aqueous solution. [Cork the flask and agitate after each addition of ammonia.] Each cc. of the decinormal acid corresponds with 36.4 mg. of alkaloid, assuming that nearly 50 per cent. of it is strychnine. The exact determination can only be made after determining strychnine (366). The titration may also be made according to (84) or (85).

363. C. E. Smith* exhausts the drug with acetic acid. The following is his procedure: Place in a bottle 10 grams of the powdered drug with 100 cc. of 10% acetic acid, and shake frequently or continuously during 12 hours. Filter the solution and wash the residue with cold water until the washings are nearly tasteless. Evaporate to dryness in a shallow dish, while warm add 6 cc. of a mixture of strong alcohol and solution of ammonia (10%) equal parts, rub to a uniform thick syrup with a rubber-tipped glass rod. Transfer to a separator, add 10 cc. of ether and 45 cc. of chloroform, wash the capsule with 6 cc. of ammoniated alcohol (see above) in three or four successive portions, shake five minutes, let stand an hour or longer to separate. Filter the ethereal solution through a small dry filter which is to be afterwards washed with a mixture of ether and chloroform in equal volumes. Finally distill off the ether-chloroform and determine alkaloid in the residue by alkalimetry.

364. By the following procedure, very fair results may

*Am. Journ. Pharm., 1896, p. 189.

be obtained in a somewhat shorter time. Macerate 5 cc. of nux vomica in the finest practicable powder two hours with a mixture of 40 per cent. acetic acid 5 cc. and a saturated solution of sodium chloride 5 cc. Add 90 cc. of 50 per cent. alcohol, macerate with frequent or constant shaking 10 hours. Take 50 cc. of the clear solution for the assay. Evaporate nearly to dryness on the water bath, transfer to a suitable vial by aid of a sufficient amount of dilute alcohol containing 10% of solution of ammonia. Make sure that ammonia is in excess and shake out with ether-chloroform (3:1) 20 cc. followed by ether, two or three portions 15 cc. each to obtain an alkaloid sufficiently pure for titration with standard acid. If it is desired to weigh the alkaloid, it must be shaken out from the ethereal solution with acid water, and extracted a second time as above with ether-chloroform and ether.

365. Dragendorff's method* is efficient although it consumes a good deal of time. Exhaust 15 grams of the powdered drug by boiling with three successive portions (150 cc.) of distilled water containing sulphuric acid (10 drops of the strong acid in each portion.) After each boiling strain and press the residue. The marc should 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 $2\frac{1}{2}$ times its volume of alcohol of 90° Tralles, heat to boiling, filter while hot and wash the residue on the filter thoroughly with hot alcohol of 65° Tralles. Distill off the spirit, add some sulphuric acid, filter, wash repeatedly with benzol to remove fatty and waxy matter, finally add ammonia in excess and shake out with several successive

*Chemische Werthbestimmung, etc., p., 61.

portions (25 cc.) of chloroform, evaporate chloroform to obtain an alkaloidal residue which may be weighed and treated with standard acid as above.

DETERMINATION OF STRYCHNINE.

366. Unquestionably assays of nux vomica and of ignatia are incomplete and of little value unless they ascertain the quantity of strychnine as well as of total alkaloids. The British Pharmacopoeia has already adopted the strychnine basis and our own will probably do the same in its next revision. The determination of strychnine is not difficult. It may be made either by oxidizing the brucine and determining the residual strychnine (Gerlock, Keller, Nagelvoort), by precipitating the strychnine as ferrocyanide (Dunstan and Short,)or simply by washing out the brucine with a saturated solution of strychnine in dilute alcohol (40%) collecting the residual strychnine on a filter, dissolving in strong alcohol and evaporating the solution to dryness (method practiced by the author).

367. Keller's method* (for determining the strychnine) is perhaps the simplest. Dissolve 0.25 gram of the (purified) alkaloidal residue in 10 cc. of dilute sulphuric acid (10%), add 1 cc. of nitric acid (sp. gr. 1.42), mix well and set aside two hours. Then add 40 grams of chloroform, 40 grams of ether, 10 cc. of solution of ammonia (10%). Shake well several minutes, put into a flask 40 grams of the filtered ethereal solution and distill off the solvent, dry at 95° to 100° C. and weigh. The distillation should be discontinued when crystallization of the strychnine begins, and the remainder of the solvent driven off by an air current. The purity of

*Proc. Am. Ph. Assoc., 1894, p. 531.

the strychnine must be established by dissolving a portion in concentrated sulphuric acid and adding a small crystal of potassium nitrate which should not produce any red color.

368. The assay process for nux vomica may obviously be simplified (strychnine alone determined) by dissolving the whole of the unweighed alkaloidal residue obtained by any of the above assay processes, and representing 2.5 grams of drug, in dilute sulphuric acid and treating as above. Observe, however, that the residue so treated should be pure enough to dissolve to a tolerably clear solution in the dilute acid. (If necessary the residue must be purified by dissolving in acid with aid of heat, filtering and shaking out with chloroform and ether.) It is better to extract the whole of the strychnine at last by shaking out with successive portions of solvent than to follow Keller's procedure, which assumes that the whole of the alkaloid has been abstracted by a single washing, and which involves danger of loss of volatile solvent during the required filtration and weighing.

369. Nagelvoort,* operating upon a crude alkaloid obtained by washing out with ether, and adopting a modification of the procedure of Gerlock,† directs to dissolve the alkaloid (from 5 grams of nux vomica) in 5 cc. (more if necessary) of decinormal sulphuric acid, add decinormal soda to render nearly neutral, then a saturated aqueous solution of picric acid. Add ice-water to make 100 cc., set in a refrigerator until the precipitate has settled well (ocasional stirring facilitates this), test supernatant fluid with more picric acid to make sure of excess, if necessary add more. Decant the

*Proc. A. Ph. A., 1893, p. 165.

†Arch. d. Pharm., 1889, p. 158; Am. Journ. Pharm., 1889, p. 180.

clear fluid as closely as possible, and wash the precipitate four times by decantation with water. Dry in the beaker at 105° C. and weigh.

370. Treat the crystals with 25 cc. of nitric acid, sp. gr. 1.056, keep at 60°C. half an hour, stirring several times. Neutralize carefully with ammonia; the fluid will become darker when the neutralization is complete. Acidulate again immediately with acetic acid. Let the strychnine picrate settle in a cold place, wash by decantation as before four times, dry to constant weight at 105°C. and weigh. The weight multiplied by 0.5932 will be that of the strychnine present.

371. The Ferrocyanide Process of Dunstan and Short, as adopted by the British Pharmacopoeia is as follows: [Having brought the alkaloids from 10 cc. of a fluid extract of nux vomica into solution in 30 cc. of chloroform by the shaking out process,] Shake out the chloroform with three portions (10 cc. each) of dilute sulphuric acid containing 3 per cent. H_2SO_4 by weight, dilute the acid solution to 175 cc., add 25 cc. of a five per cent. solution of potassium ferrocyanide, shake well and frequently during half an hour in a stoppered flask. Transfer the precipitate which forms to a filter, rinse the flask with water containing one twenty-fifth its volume of dilute sulphuric acid (U. S. P.) and wash the precipitate until the washings are free from bitterness. [Probably such washing is excessive; wash, at any rate, well]. Next shake the precipitate in a separator with 5 cc. of solution of ammonia, add 15 cc. of chloroform in two successive portions, evaporate in a tared beaker in a current of warm air, the beaker being covered to avoid loss of strychnine by de-

crepitation. Dry the crystals one hour on the water bath and weigh.

372. The periodide method of Prescott and Gordin (114) and (115) may be employed in determining the combined strychnine and brucine in an aliquot portion of the alkaloidal solution obtained in assaying nux vomica or its galenical preparations, while from a second portion the strychnine can be separated by (367) or (371) and determined also by the periodide method. There seems to be no advantage in this over acid titration of the alkaloids.

373. Galenical Preparations of nux vomica. Measure 5 cc. of a fluid extract or 25 cc. of a tincture into a capsule. Add 1 cc. of dilute sulphuric acid 10% and 10 cc. of water. Evaporate to expel alcohol, adding if necessary more water. Pour the watery solution, measuring about 10 cc., into a 2 oz. vial having 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 last washing a mixture of chloroform one volume and ether three volumes. Having decanted this, add ammonia to strong alkaline reaction and wash out the alkaloid with four successive portions (15,10,10 and 10 cc.) of the same mixture of chloroform and ether. Bring the ethereal solution to a volume of 50 cc., evaporate 25 cc. to determine total alkaloid. In the remainder, after evaporation, determine strychnine by (367) or (371).

374. For solid extracts the plan of H. Beckurts* is commonly followed. The extract (2 grams) is dissolved in a mixture of 5 cc. of water, 10 cc. of alcohol and 5 cc. of water

*Pharm. Centralh., 1887, p. 508.

of ammonia and shaken out with several successive portions of chloroform or of a mixture of chloroform and ether. It is as well to dissolve the extract as above and make up a volume of 25 cc. and of this take for the assay two portions, each of 5 cc., in one of which total alkaloids are determined, in the other the strychnine, by the method of Keller (367).

375. The extract may be dissolved in acid instead of by the aid of ammonia. Nagelvoort* directs to weigh in a small flask a quantity of the extract approximating one gram (this is easier than to attempt to get exactly one gram.) In case some of the extract adheres to the neck of the flask, no attention need be paid to this. Put into the flask five cc. of dilute sulphuric acid (10%), cover the flask with a small beaker, inverted, and warm on a sand bath until the extract is all dissolved, or reduced to a homogeneous semi-fluid. Wash the acid solution three times with alcohol-free ether, then render alkaline with ammonia and wash out the alkaloid. This method is preferable to the preceding. Here also however, we may advantageously make up the acid solution to 20 cc. and take two aliquot portions for the assay as suggested in the last paragraph.

376. The method of Lloyd (140) may be adopted for the assay of the extract with satisfactory results if a sufficient quantity of chloroform is used in extracting the alkaloid. The sawdust method is also available, but I have never found any difficulty in washing out the alkaloid by a direct process where the quantity present does not exceed 100 mg.

377. Nux vomica contains from 2 to 3.25 per cent. of alkaloids of which about 42 per cent. is strychnine. A fluid

*Proc. A. Ph. A., 1893, p. 167.

extract, however, cannot be easily made to contain more than 1.5 per cent. of total alkaloids and this standard has been generally adopted by manufacturing pharmacists. Ignatia contains about the same quantity of total alkaloid as nux vomica, but the proportion of strychnine is larger.

OPIUM.

378. Of the opium alkaloids, morphine is the most abundant and by far the most important, and an assay of the drug or of its galenical preparations calls usually for a determination of this alkaloid alone. Morphine exists in opium in the form of a salt readily soluble in water, which is indeed a better menstruum than alcohol for exhausting the drug. The narcotine is mostly left behind in the aqueous extraction of the opium, although it is more certainly excluded by first treating the drug with ether. Otherwise, the separation is made either by precipitating the morphine with ammonia in presence of ether, which holds narcotine in solution, or else by the use of lime or baryta, which hold in solution morphine but render still less soluble narcotine.

379. Morphine (the alkaloid) is nearly insoluble in cold water. It requires for solution 500 parts of boiling water, 100 parts of cold or 36 parts of boiling alcohol (90%), 60 parts of cold or 13 parts of boiling absolute alcohol. When crystallized, it requires over 6000 parts of ether, nearly 9000 of benzol and 4400 of chloroform to dissolve it, but if the solvent is applied at the moment that the alkaloid is set free by an alkali, it requires of ether about 1000 parts, of benzol 2000 and of chloroform 860 parts. Hence the alkaloid may be partially removed from an aqueous solution by shaking out with ether immediately after alkali is added, but much of it will in a short time crystallize out of the ethereal solution.

In assay work, the only solvents suitable for a shaking out process are acetic ether, amylic alcohol or (less objectionable in odor and toxic effect on the operator) isobutylic alcohol. Mixtures of one or other of these with chloroform are sometimes employed; chloroform alone abstracts the alkaloid very imperfectly.

380. Separation of Opium Alkaloids. The following scheme* for the separation of the principal alkaloids of opium may contain suggestions useful to some. To an aqueous solution containing narcotine, papaverine, narceine, thebaine, codeine and morphine, add a concentrated solution of sodium acetate and let stand 24 hours. Narcotine and papaverine are precipitated. Dissolve the washed precipitate in dilute hydrochloric acid and dilute to four hundred times its weight, add potassium ferrocyanide to precipitate (in 24 hours) papaverine. From the filtrate, narcotine is precipitated by neutralizing with ammonia. Concentrate the solution from which narcotine and papaverine have been removed and let stand 24 hours. Narceine will separate. Filter and add sodium salicylate. After 24 hours filter out thebaine salicylate. Acidulate the filtrate with hydrochloric acid and shake out repeatedly with chloroform to remove remaining salicylic acid, narceine and thebaine. Drive off dissolved chloroform by a gentle heat and add potassium sulphocyanate to precipitate codeine. After 24 hours filter and add to the filtrate ammonia to precipitate morphine.

381. U. S. P. Process of Assay. The U. S. Pharmacopoeia of 1890 discarded the lime process in favor of Dr. Squibb's method of assay, which, therefore, it is hardly neces-

*P. C. Plugge, Arch. de Pharm., 1887, 345.

sary to give here in detail. Full particulars will be found in Ephemeris July 1889, pp. 1150—1161.

382. Sampling the Opium for Assay. Where assay has to be made of a case of opium, some systematic method must be adopted for sampling. Dr. Squibb directs: From every fifth larger lump and every tenth smaller one cut a cone shaped piece, the apex of the cone at the center of the lump. From each cone take off a slice which shall represent in due proportion the moist center and the drier exterior portion. Collect these slices of approximately equal weight into a mass working rapidly so as to avoid evaporation of moisture. Rol into a cylinder, fold on itself and roll again into a cylinder. Repeat this process six times to obtain a perfectly homogeneous mass, a weighed quantity of starch being used for rolling out if the opium is moist and sticky. Two portions, each representing exactly 10 grams of the opium (allowance being made for the starch that has been added) are then weighed off. One of these is flattened into a thin disc and dried a 100°C. to constant weight to determine moisture. The other is to be used for the assay.

383. Method of J. Howard Wainwright* of the U. S. Laboratory attached to the Custom House. [This assay process differs only in some details from that of Dr. Squibb.] Put into a 4 oz. wide mouth bottle 10 grams of the opium with 100 cc. of boiling water. Cork securely and allow to stand, with frequent hard shaking, 12 to 24 hours. Decant the clear liquid upon a filter of convenient size and collec the filtrate in a beaker. To the residue in the bottle add 30 t 50 cc. of boiling water. Agitate well and transfer the mas

*Journ. Am. Chem. Soc., VII, p. 48.

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.

384. 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 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. [Observe whether this produces any tendency to form a precipitate. If so, add 10 cc. more of alcohol, let stand an hour, filter, reduce filtrate and washings once more to 20 cc. and again add 10 cc. of alcohol.] Add a volume of ether equal to that of the mixture, cork and shake well. Immediately add 4 cc. [better 3 cc.] of solution of ammonia, 10%, cork again and shake until crystals of morphine begin to separate. Set aside in a cool place, or else shake continuously half an hour or more. [The best plan is to place in a mechanical shaker at least six hours. I prefer to allow 24 to 48 hours for complete crystallization of the morphine, although the alkaloid thus obtained is less pure. In any case the morphine in the deposit must be determined. See (387.)]

385. 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, shake once, let separate and decant this also through the filter which is to be then 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. [My own practice is to separate the ether in the first place by aid of a small funnel, the orifice of which is closed with a finger until the ethereal layer comes to the surface, when the aqueous fluid is allowed to flow into a small beaker, in which it is washed with the second portion of ether.]

386. When the ether has drained through completely, pour on the filter the hydro-alcoholic solution, containing the crystals of morphine. Wash the crystals from the flask [and beaker] by aid of portions of the filtered fluid. When all the fluid has passed through the filter, rinse the flask [and beaker] with a little distilled water. [A saturated aqueous solution of morphine is better.] Wash the crystals on the filter with this and then with pure distilled water until this comes through colorless. [I prefer to wash the crystals finally with a saturated solution of morphine in alcohol.] Drain the filter thoroughly, fold, press between dry filters, finally dry at 100°C. to constant weight and weigh. If any crystals have adhered to the flask this also must be dried and weighed.

387. The morphine thus obtained is always impure. If dried at 100°C. it retains water of crystallization. To deprive it wholly of this, it should be dried at least an hour at 110°C. I prefer however to dry at about 60°C. and weigh the crude morphine, which is afterwards to be dissolved in volumetric hydrochloric or sulphuric acid and titrated back with volumetric soda solution. Each cc. of decinormal acid indicates 30.23 mg. of monohydrated morphine, which is commonly reported in assays as "morphine," or 28.44 mg. of the

anhydrous alkaloid. The morphine may be determined also volumetrically by the periodide method. See (397).

388. Unquestionably the acid titration furnishes the best single test of the purity of the morphine obtained in assays. Another mode of testing is to treat the powdered morphine with lime water in excess, collect the residue, dry and weigh, deducting the weight from that of the crude product. Still another mode consists in igniting a weighed portion of the crude alkaloid and weighing the residue. In this case the weight of the residue is probably less than that of the total impurity. However, the test is so quickly made that it may well be included in the routine of an assay. Finally we are to remember that precipitation of the morphine is never complete, so that if we exclude, as we properly should, all that is not morphine in the crude product, we ought also to add to our result a correction for imperfect precipitation of morphine, which may be taken in the foregoing assay process as 50 milligrams.

389. Fluckiger's Assay Process in its most recent form* is as follows: Place in a plaited filter, 12 cm. in diameter, 8 grams of powdered opium, tap the funnel slightly to settle the powder in the filter, dry half an hour in a water oven. Then pour on the powder a mixture of 10 cc. of chloroform and 10 cc. of ether and tap the funnel frequently (it should be kept covered). When the ether-chloroform has passed, add 10 cc. of chloroform, drain and spread out the filter and its contents to dry. Transfer the powder to a flask, add 80 grams of water, in which 0.2 gram of ammonium oxalate has been dissolved, shake well and forcibly and macerate

*Arch. Pharm., 27, 721, 769; Am. Journ. Pharm., 1880, 14.

with frequent or continuous shaking two hours. Filter, put into a tared flask 42.5 grams of the filtrate, representing four grams of the opium, add 7.5 cc. of alcohol, 15 cc. of ether and 1 cc. of solution of ammonia (10%). Shake the flask well and frequently and after six hours collect the crystals on a pair of mutually counterpoised filters, wash flask and morphine with 10 cc. of a saturated aqueous solution of morphine, dry at 100°C., transfer again to the flask, dry at 100°C. to constant weight and weigh. (See 387.)

390. Nagelvoort* modifies the above process in some particulars. He dries the opium 3 hours at 100°C. noting loss of weight, then pulverizes in a dry mortar and weighs out 10 grams for the assay. He treats the opium as above with ether and chloroform but adds to the dried powder 100 cc. of water, and after the two hours maceration with frequent shaking, filters and introduces into a salt mouth bottle 50 cc. of the filtrate, supposed to represent 4 grams of the opium, adds 10 cc. of strong alcohol, 20 cc. of ether and 1 cc. of solution of ammonia (10%) and proceeds otherwise as in Flückiger's method.

391. Method of E. Dieterich as adopted by the German Pharmacopoeia. Rub up in a mortar 6 grams of powdered opium with 6 cc. of water, dilute and wash the mixture with water into a weighed flask and make up to a total weight of 54 grams. Shake repeatedly during one hour and filter through a plaited filter 10 cm. in diameter. To 42 grams of the filtrate add 2 cc. of normal ammonia, mix well, but avoid active shaking, and filter at once through a plaited filter. Place in a tared flask 36 grams of the filtrate, representing

*Am. Journ. Pharm., 1890, 407.

four grams of opium, add 10 grams of ether, mix by a rotary movement, add 4 cc. normal ammonia, continue the rotary movement until the liquid becomes clear. Allow to stand 6 hours, pour off the ether as completely as possible on to a plain filter 8 cm. in diameter, add to the contents of the flask 10 cc. more of ether, rotate and pour the ether on the filter. When this has passed, pour the remaining solution on the filter, wash the flask and the crystals on the filter with two successive portions of water saturated with ether, dry filter and flask as in (386.)

392. Hager's lime Process as modified by Beckurts* is as follows: Macerate 8 grams of powdered opium for half an hour with 77 cc. of water in a well stoppered flask, add 3 grams of freshly slaked lime and, after frequent shaking during one hour filter off 15.5 cc., representing 5 grams of opium. Pour upon it so as to form a layer 30 cc. of a mixture of alcohol 1 volume and ether 5 volumes, which mixture has been saturated with opium, add 6 cc. of a saturated solution of ammonium chloride and shake vigorously. After 6 to 8 hours pour off the ether-alcohol on to a filter moistened with ether, shake the mixture again with 10 cc. of ether-alcohol, transfer the ethereal liquid to the filter, and, after it has passed through, pour on the aqueous liquid together with the morphine crystals; wash flask and crystals with a morphine-saturated mixture of equal parts of ether-alcohol and water, dissolve in boiling 90 per cent. alcohol, filter from the insoluble calcium meconate, to the filtrate add 25 cc. of decinormal hydrochloric acid and titrate back with centinormal soda, using cochineal as indicator. See (387.)

*Apoth. Zeitg. 1891, p. 526.

393. E. H. Farr and R. Wright recommended* the ollowing modificaion of the lime process which has been adopted in the British Pharmacopoeia, for the assay of tincture of opium: [Prepare an aqueous solution of opium of which 30 cc. represent 8 grams of opium], mix this with 3 grams of freshly slaked lime, add water to make 85 cc., set aside half an hour, stirring occasionally. Put into a 4 ounce bottle having a wide mouth, fitted with an accurately ground stopper 50 cc. of the filtered fluid, add 5 cc. of 90 per cent. alcohol, 30 cc. of ether and 2 grams of ammonium chloride. Shake well at intervals during half an hour, set aside 12 hours for the morphine to separate, filter, wash with morphinated water and dry the crystals first by pressing between filter paper, then at 60°C. and *finally for two hours at 110°C.* Weigh the crystals and titrate with decinormal sulphuric acid until the liquid, after boiling, slightly reddens sensitive blue litmus paper. Add to the weight of the morphine thus indicated 0.05 Gm. to represent the average loss of morphine in the assay.

394. Method of D. B. Dott.† Digest 10 grams of powdered opium with 25 cc. of water, add a solution of 1.8 Gm. of barium chloride in 12 cc. of water. Make up the mixture to 50 cc., mix well and in a short time filter. To 22 cc., equivalent to 5 grams of opium, add dilute sulphuric acid just sufficient to precipitate the excess of barium (about 1 cc.), warm the solution to cause the precipitate to subside, and filter. Wash the residue: to filtrate and washings add dilute ammonia to nearly neutralize free acid, and concent-

*Pharm. Journ. 1897, 202.

†Briz. and Col. Drugg., 1894, 372.

rate to 6 or 7 cc. Add 1 cc. alcohol, 1 cc. ether and ammonia in slight excess, stirring well. There should be a distinct odor of ammonia. After three hours (ammonia being still preceptibly present) collect the morphine on a pair of mutually counterpoised filters, wash with morphinated water, dry, wash with benzol, dry and weigh; finally titrate with decinormal acid as in (387).

395. 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 not measure more than 400 cc. Pass through the solution a current of carbon dioxide to precipitate excess of barium. Evaporate rapidly on the water bath to dryness. Moisten the residue with absolute alcohol, transfer to an Erlenmeyer flask and exhaust by repeatedly boiling with absolute alcohol of which 200 to 300 cc. will be required. Distill off the alcohol, add 15 cc. of water containing some ammonia, and allow to stand some time; stir well with a glass rod, collect the morphine on a tared filter, dry at 40° C. (104° F.) and treat repeatedly with chloroform. The product may be weighed as morphine (mono-hydrated) and titrated with standard acid.

396. Method of C. Montemartini and D. Trasciatti.† After testing many methods for the assay of opium, the authors approve those of Perger and Squibb, but offer the

*Journ. pr. Chemie, (2), 29, 97, 110; Journ. Chem. Soc., Nov. 1884, p. 1217.

†Gazzetta Chim. Ital., 27, ii, 302; Journ. Chem. Soc., 1898, ii, 271.

following as equally good: Macerate 10 grams of the powdered opium in a mortar with 90 cc. of a 20 per cent. solution of sodium chloride for one hour and filter. Treat the residue one hour with 60 cc. of the sodium chloride solution, filter, and treat the residue further with sodium chloride solution until a drop gives no reaction with Froehde's reagent (22). Evaporate the mixed filtrates to dryness on the water bath, extract the powdered residue with boiling absolute alcohol (300—350 cc.) until the fluid ceases to give a reaction with Froehde's reagent. Evaporate or distill, cover the residue with 15 cc. of a very dilute solution of ammonia and let stand 24 hours. Collect the separated morphine on a tared filter, wash with water saturated with morphine until the washings are colorless, and dry at 100°C. Treat the dried morphine on the filter with chloroform until, on evaporating a few drops, taking up the residue with hydrochloric acid and adding soda solution, no turbidity is obtained. The morphine is then dried at 100°C. and weighed.

397. Assay by Periodide method of Prescott and Gordin.* One gram of the opium in very fine powder is put into a small mortar which can be well covered with admission of a small pestle. [A 4 oz. screw-top ointment jar with rounded bottom answers very well, the cover being perforated to admit a pestle.] A mixture is made of stronger solution of ammonia 5 cc., alcohol 5 cc., chloroform 10 cc., ether, 20 cc. The opium is rubbed up with two or three cc. of this mixture to make a uniform paste, the covered dish is put aside for three hours being gently moved from time to time; 15 grams of well dried and finely powdered sodium chloride is

*Journ. of Am. Chem. Soc. 1898, p. 725.

now added and carefully mixed with the mass. The dish is then left open in a warm place 2 or 3 hours, when it is placed 12 hours in a desiccator containing sulphuric acid and a dish of paraffin, a vacuum desiccator being perferable, when the mixture should be perfectly dry and dusty. The powder is next transferred carefully to a very small percolator (the barrel of a one-ounce glass syringe), the dish being cleaned with two successive small portions of the sodium chloride. The powder is percolated with pure benzene (benzol) until, upon evaporating two drops on a watch glass, dissolving in 4 drops of acidulated water and adding a drop of Wagner's reagent (17) no precipitate is produced.

398. The benzol is rejected and the percolation is continued with acetone (boiling point 54° to 58°C.) to complete exhaustion; the percolate being received in a small capsule. [The percolation is carried on until 10 drops of the percolate evaporated on a watch glass and taken up with 4 drops of acidulated water give no turbidity with a drop of Wagner's reagent.] Usually as much as 200 cc. of acetone will be required. The acetone is evaporated off at a temperature not exceeding 45°C. (113°F.) The dry residue, when cold, is treated with several successive portions of freshly prepared lime water, the quantity of solution being finally made up to a volume of 100 cc. with lime water. The turbid fluid is shaken during 30 minutes and filtered. Of the filtrate 50 cc. is measured into a stoppered flask, dilute hydrochloric acid is added sufficient to render just perceptibly acid and 25 cc. of decinormal iodine solution is run in from a burette, the flask being gently shaken during the process. Water is now added to make up a volume of exactly 100 cc. and the mix-

ture is then shaken vigorously and continuously until, on standing, the supernatant liquid is perfectly clear and transparent (about 20 minutes.)

399. The color of the solution must be of a pronounced iodine red; if it is not, add 5 cc. more (or a sufficient quantity) of the decinormal iodine solution. Filter, titrate 50 cc. of the filtrate (or one-half its volume before filtration) with decinormal solution of sodium thio-sulphate, using starch as an indicator. The number of cubic centimeters of the thiosulphate solution is multiplied by two and the product subtracted from 25 [or from total volume of decinormal iodine used] and the remainder multiplied by 1.896 to obtain per cent. of morphine in the opium assayed. The precautions to observe in the assay are: (1) to make sure that the opium residue is thoroughly dry before the percolation, (2) to use an acetone conforming strictly to the stated requirement as to boiling point, (3) to continue the shaking after addition of the iodine solution until the fluid is quite clear.

400. Morphine may be determined in the crude product obtained by other processes of assay (free from other alkaloids) by dissolving 150 milligrams in very dilute hydrochloric acid, rendering nearly but but not quite neutral with solution of soda and making up with water to a volume of 75 cc. To this is to be added 25 cc. of decinormal iodine solution and the operation continued exactly as in (398) and (399). Each cc. of decinormal iodine solution corresponds with 9.479 milligrams of the alkaloid.

401. Tincture of Opium (160 cc.) is to be evaporated to about 30 cc. and poured in a thin stream with constant stirring into 115 cc. of cold water, the dish well rinsed, first

with portions of the mixture, then with water sufficient to make in all exactly 150 cc. Let the mixture stand an hour, then filter, evaporate 100 cc. of the filtrate to 20 cc., transfer to a tared Erlenmeyer flask and proceed with the assay as in (348) et seq. As an alternative process, evaporate 80 cc. of the tincture to 30 cc. and proceed as in (393.)

402. Solid extract of opium (10 grams) is to be dissolved in water 25 cc., the solution poured into 115 cc. of cold water, and the assay carried on exactly as in the first process of (401). Otherwise 5 grams of the extract may be dissolved as far as possible in 25 cc. of water and the solution treated as in (393).

403. Detection of morphine in mixtures, syrups, pills, etc. The detection of morphine in mixtures is not always easy; its quantitative separation must be left to experts. From mixtures containing much morphine, it is possible to separate enough of the alkaloid for identification (unless other alkaloids are also present) by simply adding ammonia and shaking out with amylic, or better, isobutylic* alcohol. To obtain it in sufficient purity for tests, the solution should be shaken with water containing sulphuric acid, and the acid solution should be again extracted with the same solvent, which on evaporation will leave a residue that may be tested qualitatively for morphine. Complex mixtures should be submitted to dialysis for 24 hours, and the dialysate concentrated and tested as above. Good reactions should be obtained where morphine is present in anything like ordinary medicinal doses.

*Nagelvoort; Proc. A. Ph. A., 1894, p. 274.

PHYSOSTIGMA.

404. Calabar bean contains two alkaloids, physostigmine, also called eserine, and calabarine or eseridine. It is the former of these that we may regard as the active principle of the drug. It is readily removed from its aqueous solutions by adding ammonia and shaking out with ether which leaves behind the second alkaloid.

405. Assay Process. Put into a 4 oz. prescription vial 15 grams of calabar bean in No. 60 powder, with 105 cc. of ether, shake and let stand one hour. Add 7.5 cc. of a saturated solution of common salt and 1 cc. of dilute hydrochloric acid, 5 per cent., shake well and macerate one hour with occasional shaking. Then add solution of ammonia, 10 per cent., 5 cc. Shake well and macerate four hours with frequent or continuous shaking. Take for the assay 70 cc. of the ethereal fluid, corresponding with 10 grams of drug and follow the routine of (73) to (75) to obtain the alkaloid, using ether as the solvent. This must be used rather liberally. Chloroform will still remove a little alkaloid, which can be weighed separately, the drug valuation being based on the quantity of ether-soluble alkaloid present. Short Assay Process No. 2, may be followed, but the yield of alkaloid I have found smaller than that obtained by the above modified procedure.

406. Alternative Process. Digest 20 grams of the finely powdered drug in a flask twelve hours with 50 cc. of

alcohol and 1 cc. of 40% acetic acid, add 50 cc. of alcohol and heat to boiling. Decant the alcohol, add 50 cc. of alcohol, boil 20 minutes, connecting the flask with a reflux condenser. Repeat the boiling with two additional portions of alcohol, unite the alcoholic solutions, distill off alcohol and 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, wash the aqueous solution with two or three successive portions (10 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 beaker. Treat the alkaline fluid with two or three successive portions 15 and 10 cc. of fresh ether until no more alkaloid is taken up. Evaporate the mixed ethereal solutions at a gentle heat in a place sheltered from strong light, dry at 100°C. to constant weight and weigh.

407. Solid extracts of calabar bean must be dissolved in water and ether as described above, one gram sufficing for the assay. Fluid extracts or tinctures are to be evaporated to expel alcohol and the residue treated in the same manner.

408. The yield of ether-soluble alkaloid from calabar bean is generally between 0.15 and 0.25 per cent., sometimes as low as 0.1 per cent.

PILOCARPUS.

409. The leaves of the several species of Pilocarpus which are imported as jaborandi, contain variable amounts of pilocarpine and an accompanying alkaloid called pilocarpidine. The former may be considered to be the active principle of the drug, but the latter is believed to have similar medicinal properties, and we have not as yet any satisfactory method of separating them. Indeed, both alkaloids are present in the commercial salts of pilocarpine. Leaves formerly imported contained nearly two per cent. of alkaloid. Those now in the market yield hardly 0.2 per cent. We must be content in an assay to determine merely the total amount of alkaloid present.

410. Assay Process. I have employed for the assay of jaborandi leaves the Short Assay Process No. 2 (72) and Process No. 1, (63) et seq., with practically identical results. It is necessary to use at least 12 grams, better 15, of the finely powdered leaves for the assay.

411. Poehl* gives the following method of assay: Extract 10 grams of the leaves with 100 cc. of water containing one per cent. of hydrochloric acid. Precipitate the infusion with lead subacetate, remove excess of lead with hydrochloric acid, filter; precipitate the filtrate with sodium phosphomolybdate, collect the precipitate, wash with water containing hydrochloric acid, dry at 100°C. and weigh. Multiply by 0.4566 to obtain the weight of the alkaloid.

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

PIPER NIGRUM.

412. W. Johnstone* offers the following scheme for the assay of black pepper. 1. Determine volatile oil by distilling 20 grams with water; the distillate is shaken with ether, the ethereal solution evaporated at a very low temperature and the residue dried over sulphuric acid. 2. Determine free piperidine by distilling as for volatile oil and titrating the distillate with decinormal sulphuric acid. Each cc. of the acid neutralizes 8.5 mg. of piperidine.

413. 3. Determine piperine by digesting at 100°C. in a closed bottle, 10 grams of the powdered drug with 3 grams of potassium hydroxide, dissolved in 25 cc. of water and 25 cc. of alcohol. The bottle (4 oz.) should have the top ground flat and be closed by a plate of caoutchouc pressed tightly upon it by a screw frame. After 4 to 6 hours digestion the bottle is cooled, the contents washed into a large flask and distilled as long as the distillate is alkaline. The piperidine in the distillate is determined by titrating with decinormal sulphuric acid, of which one cc. corresponds with 28.5 mg. of piperine. Further determinations may be made, in the usual manner, of moisture, ash (soluble and insoluble in hydrochloric acid; the latter is important as indicating mineral sophistication), alcoholic extract, nitrogen, crude fibre and starch. The moisture in 13 samples examined was 12 to 15 per cent., ash 1.07 to 4.46 per cent., oil 0.53 to 1.87 per cent., free piperidine 0.21 to 0.77 per cent., piperine 5.21 to 13.03 per cent., fibre 4.2 to 15.05 per cent., starch 29.6 to 53.5 per cent.

*Analyst, 14, 41

PULSATILLA.

414. **H. A. Bishop** communicates the following assay process for pulsatilla, based on the assumption that the active principle of the drug is the neutral principle anemonin. This is a crystallizable substance, nearly insoluble in water and glycerin, soluble in hot alcohol, but only slightly so in cold alcohol, easily dissolved by ether and especially by chloroform.

415. The following is the proposed process of assay: Put into a suitable still 500 grams of the fresh drug cut into small pieces, with enough water to cover, macerate at 60° C. (140° F.) twenty four hours, then raise the temperature to the boiling point and distill as long as the distillate gives an acid reaction. About 1000 cc. of distillate will be obtained, which is to be put into a stoppered flask and set by in a warm place several days. The fluid is to be then shaken with several successive portions (50 cc.) of ether which will remove anemonin together with anemonic acid. The ether is to be evaporated or distilled off and the residue treated with several successive portions of hot alcohol. From the concentrated alcoholic solution the anemonin crystallizes out on cooling, and at the end of 24 hours may be collected on a filter, dried and weighed.

QUEBRACHO BARK.

416. Aspidosperma contains several distinct alkaloids, differing materially in medicinal action. Our knowledge of these alkaloids is, however, as yet so imperfect that we must be content to determine the total alkaloidal content of the bark as an empirical measure of its value.

417. The assay of Quebracho Bark may be conducted in precisely the same manner as that of cinchona bark, the Short Assay Process No. 2, (72) to (75), giving quite satisfactory results. The percentage of alkaloid found ranges from 0.8 to 1.8 per cent. E. L. Patch uses the lime process formerly directed by the U. S. P. (1880) for the assay of cinchona bark. See (212).

418. A fluid extract can usually be assayed by the process of (132) to (135). Schwickerath evaporates to dispel alcohol, takes up the residue with 2 cc. of dilute sulphuric acid, 2.5 per cent., filters into the perforator and treats one hour with ether to remove impurities, then, after rendering alkaline, extracts again three hours with fresh ether. [Care must be taken that alkaloid is not lost in the treatment of the residue with acidulated water. If much residue remain, this should be redissolved in a little dilute alcohol, a little acidulated water added, the alcohol again dissipated by a gentle heat and the aqueous solution added to that first obtained. It may be necessary to repeat this once more.] Solid extracts are treated on general principles. See (153) to (155).

SANGUINARIA.

419. Bloodroot contains several alkaloids, differing probably in medicinal as they do in physical properties. The largest part of the alkaloid is soluble in petroleum benzin, and an assay based on benzin-soluble alkaloid, like that of C. H. LaWall (424), is perhaps the best that can be advised. Ether-soluble alkaloid may also be adopted as a basis for the assay. If a fluid extract is made alkaline with ammonia and shaken out with ether repeatedly, and is then treated with chloroform, the latter solvent becomes colored more or less deeply of an amethyst purple, as was also the ether to a less degree. The chloroform solution contains alkaloid which, after having been brought into acid aqueous solution, can be partially extracted by ether after addition of ammonia. The purple substance, in contrast with the ether-soluble alkaloid, dissolves easily in alcohol and appears to be a compound easily dissociated and yielding by dissociation ether-soluble alkaloid. Probably such dissociation could be easily effected as an initial step in the assay, but I have not yet succeeded in accomplishing this. At present I know no better plan than repeated solution in chloroform, extraction with acid and then with ammonia and ether.

420. From solutions containing excess of hydrochloric acid, chloroform removes a portion of the ether-soluble alkaloid, so that it is impossible to wash out all of the alkaloid from a chloroform solution with water acidulated with this

acid. Sulphuric acid is better, but even when this is used it is necessary, after washing out the chloroform in the usual manner, to evaporate it to a very small volume and again wash out with acidulated water. It is noticeable that the purple compound is removed completely from a chloroform solution by treating with a little dilute sulphuric acid, the ether-soluble alkaloid being taken out much more slowly. When a concentrated chloroformic solution of the crude alkaloid is mixed with a large excess of petroleum benzin, the purple compound is thrown out of solution, and with it also a small amount of ether-soluble alkaloid. These facts are stated as explanatory of the assay process of (421) to (423).

421. The Assay of Bloodroot is best made by a modification of Short Process No. 2. as follows: Put into a 4 oz. prescription vial 5 grams of the drug in fine powder. Pour in exactly 100 cc. (measured after cooling) of a mixture of ether 3 volumes, chloroform one volume, cork securely and shake well. After ten minutes shake once more, add 5 cc. of solution of ammonia (10%), cork and immediately shake vigorously, repeating this several times at intervals of a minute or two. Place in the mechanical shaker four hours, or else shake every five or ten minutes during that period, then decant of the clear solution 50 cc., equivalent to 2.5 grams of the drug. Evaporate this solution to a volume of about 1.5 cc., pour into it all at once 25 cc. of commercial petroleum benzin, previously warmed to about 45°C. (113° F.), filter, and wash the residue on the filter with warm benzin.

422. Unite the benzin solutions in a 2 oz. prescription

vial, add 2.5 cc. of dilute sulphuric acid (1%), shake carefully 60 seconds, let separate, transfer the benzin to a second vial containing 1.5 cc. of the dilute acid. Repeat the washing with several successive small portions of acid as long as these are colored, unite the acid solutions in a one ounce prescription vial, add ammonia in excess and wash out the alkaloid with three successive portions (15, 10 and 10 cc.) of ether. Finally evaporate in a tared beaker to constant weight and weigh.

423. The precipitate left on the filter after washing with benzin may be dissolved in alcohol, evaporated nearly to dryness, rendered acid with sulphuric acid, water added, and the solution rendered alkaline and shaken out with ether. The residue after evaporation and drying may be weighed, redissolved in a little chloroform and precipitated as before with petroleum benzin, to obtain a small additional quantity of benzin-soluble alkaloid, and we may, if we choose, work out from the residues quantitatively the ether-soluble alkaloid, but these are refinements that cannot be made part of the ordinary routine of assay. The yield of benzin-soluble alkaloid by the method of (421) and (422) is about 2 per cent.

424. C. H. LaWall* adopts a simple plan of assay, by which, however, he obtains a smaller yield of benzin-soluble alkaloid, as follows: Macerate 10 grams of the drug with 100 grams of petroleum benzin and 10 grams of solution of ammonia, 10%, separate the benzin, evaporate, and extract the alkaloid with a mixture of chloroform three parts, ether one part. By this method he obtains from the drug about 1.5 per cent. of benzin-soluble alkaloid.

425. E. L. Patch adopts for the assay of bloodroot

*Am. Journ. Pharm., 1896, 305.

Lloyd's method (140), the drug being first exhausted by percolation with an appropriate alcoholic menstruum, and the solution concentrated to the strength of a fluid extract. Five cc. of this solution (or of a fluid extract of the drug) are treated, best in a wide bottomed cylindrical graduate or a specially constructed "mechanical abstractor," with 10 cc. of ether and 8 grams of Lloyd's mixture (sodium bicarbonate and dry ferric hydrate, equal parts); add 5 cc. of a mixture of equal parts of glucose and water, agitate until the ether separates, decant the dark purple ethereal solution into a deep beaker into which has been previously poured 15 cc. of semi-normal sulphuric acid, rotate and then evaporate the ether on a steam bath.

426. Wash the magma in the abstractor with two additional portions (10 cc.) of fresh ether, which is to be evaporated as before in the beaker. Cool the deep red solution, rotate a few times to curdle the resin and then filter through a small wet filter into a separator, wash the residue in the beaker twice with a little of the dilute acid and filter into the separator. Make the solution alkaline with ammonia, add 10 cc. of ether and rotate until the nearly white precipitate of alkaloid is taken up, leaving undissolved only a brown resinous sediment. Draw out the aqueous fluid into another separator, decant the ether into a tared beaker and evaporate the ether. Treat the fluid in the second separator with 10 cc. of ether, draw off the aqueous solution, decant the ether into the first separator, rotate to wash the brown residue therein, finally decant into the beaker and evaporate. Return the aqueous solution to separator No. 2, treat again with 10 cc. of ether precisely as before, using the ether for a final washing of the resinous residue in separator No. 1. Evaporate the

ether, dry the residue to constant weight, and weigh. By this process, bloodroot yields 1.6 to 2 per cent. of alkaloid.

427. Galenical Preparations of Bloodroot. A fluid extract of bloodroot may be assayed by the general method detailed (132) et seq., but there is almost sure to be loss of alkaloid in the process. A better plan is the following: Put into a separator 2 cc. of the fluid extract, add 5 cc. of alcohol, 15 cc. of chloroform, 5 drops of solution of ammonia, shake carefully, add 3 cc. of water and shake again, not too violently, for perhaps 20 seconds. After complete separation draw off the amethyst colored solution which will contain practically all of the alkaloid. [The aqueous residue may be washed once or twice with heavy ether chloroform if it is desired to extract the last traces of alkaloid, the washings to be added to the chloroform solution.]. Evaporate the chloroform solution just to dryness, add 1.5 cc. of chloroform to redissolve and proceed exactly as in (421) and (422).

428. Instead of precipitating with benzin, we may proceed as follows, to obtain ether-soluble alkaloid: Dissolve the residue as above in a little chloroform, add immediately 2 cc. of water containing about 0.5 per cent. of sulphuric acid. Cause the alkaloid to enter into combination with the acid by rotating the beaker, heat gently to expel the chloroform. Resinous matter will separate, and it may be necessary after decanting the aqueous solution to take up the residue once more with a few drops of chloroform and repeat the treatment with dilute sulphuric acid (1 cc.). Place the mixed acid solutions in a one ounce prescription vial, add ether 15 cc., shake, let separate and reject the ether. Add ammonia in excess and shake out with three successive portions of ether (15 cc.). Evaporate ether, dry to constant

weight and weigh residue of crude ether-soluble alkaloid.

429. The ether fails to remove the whole of the alkaloid from the alkaline solution, but this is perhaps fully compensated by impurities that inevitably accompany the alkaloid. In the ordinary routine it may be as well to allow these errors to compensate one another. An additional amount of alkaloid may be obtained by treating the alkaline residue twice with chloroform, evaporating the chloroform solution and treating the residue as before. A little ether-soluble alkaloid will be thus obtained to be added to that already weighed. The whole of the ether-soluble alkaloid may be then dissolved once more in dilute sulphuric acid, the solution filtered unless it can be decanted clear, and the alkaloid again extracted with ether to obtain a purified alkaloid. A still better procedure is to treat the whole of the ether-soluble alkaloid with chloroform and benzin, as in (421) and (422), weigh the benzin-soluble alkaloid, dissolve the residue in dilute sulphuric acid (best after washing from the filter with alcohol). Wash the (alcohol-free) acid solution with ether, then make alkaline and shake out remaining ether-soluble alkaloid with ether.

430. Tinctures are to be concentrated by careful evaporation, with addition of a little acetic acid, and treated as in (427). If there is much separation of insoluble matter during the concentration, this should be treated with dilute alcohol and a little acetic acid. The solution thus obtained after suitable concentration is to be added to the principal solution, or else the sawdust expedient of (141) may be resorted to. **Solid extracts** are to be dissolved in dilute alcohol containing acetic acid and the solution treated like a fluid extract.

STROPHANTHUS

431. The active principle of Strophanthus is the glucoside strophanthin, which is freely soluble in water, less readily in alcohol, and scarcely at all in chloroform or ether. It is precipitated from aqueous solutions by tannin. Mineral acids, and even the stronger organic acids decompose it, producing strophanthidin and glucose. Strophanthidin is nearly insoluble in water, but dissolves in alcohol and in chloroform. It seems to have toxic properties similar to those of strophanthin. An alkaloid is present in the drug, but is believed to have no important medicinal properties.

432. The direct determination of strophanthin is not very easy. The following is Elborne's* method of isolating the glucoside: Digest the finely powdered drug (one part) 12 hours with water (four parts) containing ten per cent. of alcohol, add absolute alcohol (52 parts by volume), shake and macerate six hours. Filter, wash residue with rectified spirit and distill off four fifths of the volume of the solution. Add lead subacetate in excess, heat to the boiling point, filter, wash the residue with a little water and allow the filtrate and washings to cool. Treat the cold solution with hydrogen sulphide to remove lead, filter, and shake out with three volumes of amylic alcohol. On distilling off the amylic alcohol, strophanthin remains nearly pure. The process is open to objection for assay work, since hydrogen

*Yearbook of Pharm. 1887, 423.

sulphide, according to Dr. Frazer, converts some of the strophanthin into strophanthidin: this fault might be remedied by using a neutral salt, sodium sulphate or phosphate, to remove the excess of lead.

433. Pure Strophanthin may be obtained from an aqueous solution according to T. R. Frazer by adding a solution of tannin as long as it produces a precipitate, avoiding large excess, mixing the moist precipitate, after slight washing, with oxide of lead and extracting with rectified spirit. The solution is evaporated, taken up again with a small quantity of rectified spirit and the solution poured into ether which precipitates the strophanthin. The precipitate is dissolved in weak alcohol and through this solution carbonic anhydride is passed to remove traces of lead. After filtration the solution is evaporated at a low temperature and dried in vacuo over sulphuric acid. The process is liable to be attended with loss, so that it is not to be recommended in assay work.

434. John Barclay* proposes an indirect mode of determining strophanthin, by converting it into strophanthidin. The drug having been exhausted with alcohol, most of the alcohol is to be recovered by distillation. Water is added and the remainder of the alcohol driven off by a gentle heat, the aqueous solution is shaken with chloroform to remove fatty substances, a little dilute sulphuric acid is added and the mixture heated one hour on the water bath. The turbid solution is then shaken out with three successive portions of chloroform, which takes up the strophanthidin. The chloroform is distilled off and the residue dried at a temperature

*Pharm. Journ. and Trans., Nov. 28, 1896, p. 463.

not exceeding 65.5°C. (150° F.). The weight multiplied by the factor 2.74 may be taken as that of the strophanthin originally present. Mr. Barclay found that if an alcoholic extract of the drug is exhausted with water, and the aqueous extract thus obtained is in turn exhausted with absolute alcohol, the product consists almost wholly of strophanthin.

435. Assay process based on the foregoing. Mix 13,333 grams of the finely powdered drug with 40 cc. of water containing ten per cent. of alcohol, macerate with occasional shaking twelve hours, add 161 cc. of absolute alcohol, shake and macerate six hours. Pour off 150 cc. of the tincture, evaporate on a water bath until all alcohol is driven off. Having reduced the volume to 15 cc., transfer to a separator with aid of a little water. Shake with two successive portions of chloroform (10 cc.). Wash the chloroform with water 5 cc., which is to be added to the aqueous solution in the separator. Transfer this to a small flask, rinsing with a little water, add 1 cc. of dilute sulphuric acid, 10%. Connect the flask with a reflux condenser and heat on a water bath one hour. Transfer the turbid solution to a separator and extract with three successive portions of chloroform (10 cc.), evaporate the chloroform, dry at 65°C. (150° F.), weigh, and multiply by the factor 2.74 to find the amount of strophanthin in ten grams of drug.

TOBACCO.

436. The determination of Nicotine in Tobacco is conveniently made by the method of **R. Kissling.*** The tobacco is stripped, cut, dried for one or two hours at 50° to 60°C. (122° to 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 hydroxide (6 grams of sodium hydroxide, 40 cc. of water and 60 cc. of alcohol); the moist powder is enveloped without delay in filter paper, introduced into an extraction tube and extracted with 100 cc. of anhydrous ether for two or three hours. The ether is carefully and not quite completely distilled off leaving the nicotine practically free from ammonia. The residue is mixed with 50 cc. of a dilute aqueous solution of sodium hydroxide (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 decinormal sulphuric acid, rosolic acid being used as indicator.

437. It has been objected to this process that some ammonia may remain, or else some nicotine be lost in the distillation of the ether, and that soda may be carried over in

*Zeitschr. f. Anal. Chem., XXI. No. I; Proc. A. Ph. A., 1882, p. 167.

the final distillation giving results too high. It seems indeed unnecessary to add sodium hydroxide at all in this distillation. If an alkali be necessary, magnesia might be employed. While I have had personally no experience in this assay, I should expect good results from the method of (268) for assay of conium.

VERATRUM.

438. Active Principles of the drug. The several species of veratrum contain a number of alkaloids which are as yet imperfectly known. In veratrum album, **Wright and Luff*** in 1879 found, besides jervine, two crystallizable alkaloids, which they named rubijervine and pseudojervine, also an amorphous base which they called veratralbine. In 1891 Salzberger† reported that he had discovered two more crystallizable alkaloids, which he named protoveratrine and protoveratridine, the former of which he found to be an exceedingly active poison. Veratrum viride is hardly distinct specifically from veratrum album, and the same alkaloids are found in both, although in different proportions. In the present state of our knowledge, we cannot determine by an assay the therapeutic value of a sample of the drug. We can simply determine the total content of alkaloid, and assume that this will indicate roughly the quality of the drug.

439. The most characteristic alkaloid of veratrum, especially abundant in V. album, is jervine, which forms with the mineral acids very sparingly soluble salts. This peculiarity of the alkaloid must be borne in mind in assaying the drug, or we shall be in danger of losing some of the alkaloid. It is best to use acetic acid in place of hydrochloric in abstracting the alkaloids from an ethereal solution.

*Pharm. Journ. and Trans., May 31, 1879.

†Arch. de Pharm., 228, 462; Med. Chron., April, 1891.

440. An Assay of veratrum viride to determine the total alkaloid present may be easily made by the Short Process No. 2 (72), using as the primary solvent a mixture of chloroform one volume and ether four volumes, and remembering to substitute acetic for hydrochloric acid to extract the alkaloid from the ethereal solution in (73). It is necessary to wash the ethereal solution repeatedly with the acidulated water to remove the whole of the alkaloid. My own practice is to wash three times, then evaporate the ethereal solution to dryness and take up simultaneously with ether and acidulated water, repeating this operation as long as Mayer's reagent shows the presence of alkaloid. For the final extraction of the alkaloid, chloroform should be used. The drug should yield about one per cent. of alkaloids, but commercial samples vary greatly in strength, judged by this mode of assay.

441. Kremel* exhausts the drug by hot repercolation with a mixture of chloroform and absolute alcohol, equal volumes, agitates the solution with several successive portions of water acidified with hydrochloric acid, filters the acid solution [This certainly is not to be recommended where hydrochloric acid has been used, and in any case seems unnecessary], renders alkaline with potassium hydroxide, and shakes out with three successive portions of chloroform. He reports a yield of 1.3 to 1.5 per cent. of alkaloid from veratrum album, in the form of white scales and microscopic prisms.

442. Jervine, which is the most abundant alkaloid, although possibly not the most important, therapeutically, may be easily determined by taking advantage of the insolu-

*Pharm. Post, 1889, p. 227.

bility of its nitrate in a solution of potassium nitrate.* The process is carried out in the following manner: Dissolve the crude alkaloid obtained from the drug or from one of its preparations in dilute acetic acid, filter the solution and add an equal volume of a saturated solution of potassium nitrate. Set by for twelve hours, collect the jervine nitrate on a pair of mutually counterpoised filters, wash with solution of potassium nitrate and finally with a little water, drain, 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 reckon that 89 per cent. of the weight of the salt is alkaloid.

443. Assay of Galenical Preparations. A fluid extract may be assayed by the process of (132) et seq., but it will be found difficult to obtain the whole of the alkaloid. It is advisable, after extracting several times with the mixture of chloroform and ether to employ pure chloroform, adding at the same time a little alcohol to prevent the formation of an emulsion. If an emulsion should form, resort to the saw dust expedient (141), which may indeed be adopted in the outset, although not with our usual confidence that the whole of the alkaloid will be thus extracted. It is possible that Lloyd's method (140) will give good results; I have not given it a trial. The method of (131) k., p. 79, may also be tried, using, however, acetic instead of sulphuric acid.

444. H. W. Snow,† after a good many experiments gave preference to the following process, which he admits

*Chas. Bullock, Am. Journ. Pharm., 1879. p. 338.

†Proc. Mich. State Pharm. Assoc., 1886; Pharm. Era, 1887, p. 12.

is not wholly satisfactory: 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 hydrogen sulphide, evaporate to expel alcohol, add water and a little acetic acid to make up a volume of 10 cc. and titrate with Mayer's reagent (N 1-20) of which each cc. will indicate about 15 milligrams of alkaloid. Instead of estimating the alkaloid by titration, we may extract it with chloroform after addition of alkali, but, as long as knowledge of what is the real active principle of the drug is so imperfect, the roughly approximate results obtained by titration have as much value as the possibly more exact ones secured by the gravimetric process.

WILD CHERRY BARK.

445. The medicinal value of wild cherry bark depends on the hydrocyanic acid which it yields when treated with water. An assay process based upon this principle has been employed by Dr. A. R. L. Dohme,* and in a simplified form has been recently described by A. B. Stevens.† The procedure adopted by the latter is as follows: Place in an 800 cc. flask ten grams of the ground bark with 100 cc. of water. Cork securely and let macerate 24 hours. Place the flask in a water bath and connect with a good condenser, arrange a second flask to deliver steam into the bottom of the first; when the apparatus has been connected, bring the water bath to boiling and then deliver a brisk current of steam through its contents for 20 minutes.

446. The delivery tube of the condenser is carried to the bottom of a small flask in which has been previously placed 10 cc. of a decinormal solution of silver nitrate (U. S. P.) and 20 cc. of distilled water. Any uncondensed vapor is conducted from this flask by a tube reaching to the bottom of a second flask also containing a silver solution similar to that in the first flask. The hydrocyanic acid is absorbed by the silver solutions, which, at the close of the process, are united and the residual silver determined by titration with a decinormal solution of potassium sulphocyanate (U. S. P.).

*Pharm. Rundsch. XIII, 260.

†Proc. A. Ph. A., 1896, p. 216.

Subtract the quantity of decinormal sulphocyanate required from 20 cc., and multiply the remainder by .02698 to obtain per cent. of potential hydrocyanic acid in the sample.

447. The plan adopted by Dr. Dohme differed from the above in the use of decinormal solution of potassa to receive the hydrocyanic acid, the quantity of which is determined by titration with decinormal silver nitrate. The method seems to give somewhat higher results than the preceeding, possibly from the more certain capture of the vapor of hydrocyanic acid by the alkaline solution.

448. Results of assays by A. B. Stevens show that the bark of the root contains the largest proportion of glucoside yielding hydrocyanic acid, the bark of young trees more than that of old ones, bark from the twigs more than that from the trunk of the tree. The percentage of hydrocyanic acid to be expected from freshly gathered bark would be, for bark of the root, 0.2 to 0.25 per cent., for bark of the twigs, 0.12 to 0.16 per cent., for bark of the trunk, 0.08 to 0.12 per cent.

ADDENDUM.

General Assay Process for Tinctures. E. H. Farr and R. Wright determine total alkaloid in tinctures by the following procedure, which should be added to the list under (131): Evaporate 50 cc. of the tincture over a water bath to a low bulk, adding water if necessary until all alcohol is removed. When cool, add 1 cc. of seminormal sulphuric acid and filter through absorbent cotton into a separator. Rinse dish and funnel with a little acidulated water, then with 15 cc. of chloroform, add rinsings to the contents of the separator, shake well, let separate, draw off the chloroform, and repeat the washing with 10 cc. of fresh chloroform. Wash out the chloroform with three successive small portions of acidulated water to recover traces of alkaloid, add the washings to the original acid solution, render alkaline with ammonia and shake out with three successive portions (15 cc.) of chloroform.

To obtain the alkaloids in a pure condition, they are again washed out from the chloroform with acidulated water, the solution made alkaline, and the alkaloid once more shaken out with chloroform. The chloroform is washed once with ammoniated water, evaporated on the water bath and the residue dried to constant weight at 100°C. In some cases, it is advisable to purify the alkaloid by precipitating the acid solution with Wagner's reagent (17), and treating the precipitate as described in (299) and (300).

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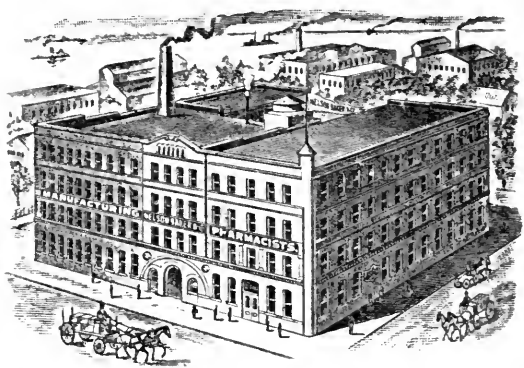

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