UNITED STATES PHARMACOPŒIA.

ABSTRACT OF PROPOSED CHANGES WITH NEW STANDARDS AND DESCRIPTIONS. ELEVENTH REVISION.

PART II—PROXIMATE ASSAYS.

The Pharmacopœial Convention of 1930 recommended that "abstracts of changes proposed for the U. S. P. XI and new standards and descriptions" be published before final adoption, that those who are not members of the Revision Committee may have an opportunity for comment and criticism.

In compliance with this recommendation, the following abstracts are submitted. The nomenclature and the exact wording to not necessarily represent that to be finally adopted.

Comments should be sent to the chairman of the Revision Committee.

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The most important changes proposed for the proximate assays of the U. S. P. XI are as follows:

1. Instead of using type processes for assays as was done in the U. S. P. X, the detailed assay process will accompany each drug.

2. The general statement for proximate assays has been rewritten and will be found below.

3. The process for the assay of mydriatic drugs has been changed. This change was made necessary by the research work of Dr. H. G. DeKay, who showed that, in the case of hyoscyamus at least, volatile amines were present and therefore it was necessary to heat the residues to eliminate them. It was proved conclusively that mydriatic alkaloids in the absence of moisture and alkalies can be subjected to the temperature of a water-bath for an hour or more without decomposition. The proposed change will be indicated under the *Assay of Hyoscyamus*.

4. It is proposed to assay *Nux Vomica* for strychnine content instead of for total alkaloids.

5. An assay process is proposed for *Camphorated Tincture of Opium*, a preparation which heretofore has not been officially assayed.

6. Minor changes in the assay processes of a number of drugs are indicated on the following pages.

The General Directions on Proximate Assays, to appear in the back of the Pharmacopœia, are as follows:

GENERAL DIRECTIONS FOR THE ASSAY OF DRUGS.

Most alkaloids are practically insoluble in water, but are soluble in certain organic solvents which are immiscible with water, such as chloroform, ether, amyl alcohol, benzene, petroleum benzin or mixtures of these. The salts of the alkaloids, however, are usually soluble in water, but in most cases insoluble in nearly all of the above-mentioned solvents. The process of assay by immiscible solvents, which is generally known as the "shaking out" process, is based on this property of alkaloids and their salts, and is carried out by treating the drug or a concentrated liquid extract with a solvent immiscible with water, in the presence of an excess of alkali which liberates the alkaloid. The free alkaloid is dissolved by the immiscible solvent which is then transferred to a separator and extracted with an excess of acid which has been diluted with distilled water. The acid portions are then extracted with an immiscible solvent in the presence of a slight excess of alkali, and the immiscible solvent evaporated to obtain the alkaloids.

Weighing of Portions for Assay.—In the preparation of drugs for assay, all portions directed to be weighed should be weighed accurately, but with crude drugs, accuracy to the second decimal point is sufficient. Portions of pilular extracts may be weighed on a piece of waxed or parch-

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mentized paper, the surplus paper cut away, and the extract and adhering paper dropped into a separator, beaker or dish containing the solvent, and the extract dissolved. In transferring weighed portions to a separator, the dish containing the material to be assayed should be thoroughly rinsed and the rinsings added to the separator.

Extraction of the Drug.—The drug to be extracted should be ground to a powder of the fineness designated (from a No. 40 to a No. 80 powder) and a representative portion selected for the assay and accurately weighed. The drug must not be coarser than the size directed, although a finer powder may be used. The definition of powders will be given. Care should be taken to avoid the loss of water during the powdering of the drug. If it be impossible to avoid this loss, the drug should be dried at a low temperature before powdering, the loss of water noted and a correction made in the final calculations.

METHODS OF EXTRACTION.

A. By Maceration.—An accurately weighed portion of the ground drug is treated with the specified solvent or mixture of solvents made alkaline with ammonia T.S. and thoroughly mixed and allowed to macerate for from twelve to twenty-four hours with occasional agitation, or for a shorter period with continuous agitation. At the end of this period, the drug is allowed to settle and then an aliquot portion of the solvent decanted and used for the extraction of alkaloids.

B. By Percolation.—An accurately weighed quantity of the ground drug is placed in a suitable container and completely saturated with the specified solvent or mixture of solvents and allowed to stand for five minutes. A sufficient quantity of ammonia T.S. to make the drug alkaline is added and thoroughly mixed with the drug. The moistened drug is transferred to a cylindrical percolator, previously prepared by packing the outlet with purified cotton. A small amount of the solvent may be used to rinse the container and the rinsing added to the percolator. The drug is allowed to macerate for a suitable period of time (from one to twelve hours or over night, depending upon the drug to be assayed), then the drug is firmly packed, a pledget of purified cotton placed above it and percolated slowly until the drug is completely extracted. Complete extraction of the alkaloid is determined by evaporating about 4 cc. of the last percolate to dryness, dissolving the residue in dilute acid and adding a drop of mercuric potassium iodide T.S. or, when testing for caffeine or colchicine, a drop of iodine T.S. There should be no turbidity produced by these reagents. The percolate is then treated for the extraction of the alkaloids.

C. By Continuous Extraction.—An accurately weighed portion of the ground drug is placed in an extraction thimble and the thimble transferred to a suitable extractor (a Soxhlet extractor of appropriate size is satisfactory). The drug is moistened with the specified solvent and mixed by means of a stirring rod and allowed to stand about five minutes. It is then made alkaline with the specified quantity of ammonia T.S. and thoroughly mixed. The stirring rod is rinsed with a small portion of the solvent and the drug allowed to macerate for from six to twelve hours or over night. The drug is covered with a pledget of purified cotton and packed in the thimble, a sufficient quantity of solvent is added and the drug extracted for a specified period of time. The solvent remaining in the extraction chamber is transferred to the receiving flask and the liquid extract treated for the extraction of alkaloids.

Extraction of Alkaloids.—The volume and strength of the acid to be used is usually left to the discretion of the operator. It is best, however, to keep the total volume as small as possible. For the first extraction, it is advisable to use at least 10 cc. of normal acid or sufficient to render the mixture distinctly acid; for succeeding extractions, it is preferable to use 5 cc. of normal acid and 5 cc. of distilled water. In all assays, the extraction should be continued until 0.5 cc. of the acid washings show only a very faint cloudiness on the addition of a drop of mercuric potassium iodide T.S., or, in the case of caffeine and colchicine, on the addition of a drop of iodine T.S.

The combined acid solutions containing the alkaloid are shaken thoroughly with the appropriate immiscible solvent and allowed to stand until the mixture has completely separated. The acid solution is then drawn off into a second separator, the immiscible solvent washed with a little distilled water and this wash water added to the acid solution. The acid solution is then made alkaline, in most cases with ammonia T.S., and then extracted with several successive portions of the appropriate immiscible solvent. The volume of the latter to be used in each operation is not less than half that of the aqueous solution, and this operation must be repeated

as long as any alkaloid is extracted by the immiscible solvent. To determine the completion of extraction, evaporate 1 cc. of the last washing and dissolve the residue in a few drops of diluted hydrochloric acid; the resulting solution should show no turbidity on the addition of a drop of mercuric potassium iodide T.S., or, in the case of caffeine and colchicine, on the addition of a drop of iodine T.S. The number of extractions required depends largely on the character of the alkaloid. With most alkaloids it is advisable to extract three or four times before testing. Physostigmine and pilocarpine require about twice as many extractions as other alkaloids.

Determination of Alkaloids.—An alkaloidal residue to be determined volumetrically should be softened by the addition of about 1 cc. of alcohol or ether made neutral to the indicator used in the titration, the required amount of standard acid added, and the mixture gently warmed to insure the complete solution of the alkaloid. If preferred, the alkaloidal residue may be dissolved in chloroform, the standard acid added and the chloroform removed by evaporation. Before titrating add a sufficient quantity of distilled water to make the volume of the mixture measure about 25 cc. When the residue is to be weighed, if the final solvent has been chloroform, the last traces of that solvent should be removed by the addition of a little ether or alcohol made neutral to the indicator used in the titration followed by evaporation. Care must be taken to avoid loss by decrepitation, especially when evaporating chloroformic solutions of nux vomica or cinchona alkaloids. Decrepitation may usually be prevented by the addition of a little alcohol made neutral to the indicator used in the titration after the solution has been reduced to a volume of 1 or 2 cc., evaporating at a low temperature, and rotating the container during the process.

Adsorbent.—In assaying fluidextracts, tinctures and other preparations of alkaloidal-bearing drugs, it is often necessary to evaporate these to dryness and, to avoid loss and to aid in the evaporation, they are usually added to some adsorbent material. Paper pulp or asbestos fibre should be used for this purpose. Such adsorbent material must be acid and alkali washed and then rendered neutral by washing with distilled water and dried before being used.

Indicators.—Methyl red T.S. is to be used as the indicator in volumetric estimations. The same lot of indicator used in titrating the alkaloids should also be used in evaluating the standard solutions.

Apparatus for Proximate Assays.—When a container of definite size and shape is recommended in a proximate assay process, it is understood that this is advisory and not obligatory, except when volumetric flasks, measuring burettes or other exact measuring apparatus are specified.

Aliquot Parts.—When using "aliquot parts," the solvent and the aliquot part should be measured at the same temperature. When handling volatile liquids, a lower temperature and a more quickly conducted operation reduces the loss from evaporation.

Emulsions.—The shaking or rotation of an aqueous solution with an immiscible solvent, in a separator, should ordinarily be continued for about one minute. Long or violent agitation should be avoided as emulsions are likely to form. Hyoscyamus, belladonna and stramonium leaves sometimes contain saponins which cause troublesome emulsions. Should emulsions prove persistent, draw off the emulsified portion and add an excess of either solvent. This usually breaks the emulsion and permits a complete separation. It is sometimes preferable to break the separated emulsion by the addition of a small amount of anhydrous sodium sulfate. If this is done it becomes necessary to wash the residue with additional solvent to completely remove the alkaloids.

Emulsification is sometimes prevented by increasing the volume of the aqueous or of the immiscible solvent. Chloroform or ether solutions of drugs which contain large proportions of fat may form troublesome emulsions. In such cases it is advisable to add sufficient normal sulfuric acid to assure acidity, and evaporate the volatile solvent, while stirring with a rubber-tipped glass rod. When the resinous and fatty matter has been agglutinated, cool the acid solution and filter it through a small, wetted filter into a separator. Redissolve the residue in 15 cc. of ether, add 5 cc. of tenth-normal acid, evaporate the ether as before, with continued stirring, and pour the acid solution through the filter into the separator. Repeat the extraction of the fatty residue with dilute acid two or three times and finally wash the filter free from alkaloids.

Washing.—The stems of separators and funnels and the lips of flasks, separators and graduates, from which volatile solvents containing alkaloids, have been drawn or poured, should be carefully washed with some of the solvent to prevent loss and to remove any of the alkaloids left by evaporation. These washings should be added to the other extractions.

MOISTURE DETERMINATION BY THE TOLUENE DISTILLATION METHOD.

The following method has been adopted for the determination of moisture in Asafetida and similar drugs:

Apparatus.—Use a 250- to 500-cc. flask of resistance glass, a 20-inch, sealed-in, straight-tube, Liebig condenser and a distilling tube receiver.

The condenser and receiving tube must be chemically clean to prevent an undue quantity of water from sticking to them. Clean them with chromic-sulfuric acid solution or similar oxidizing cleaner, rinse with distilled water, then with alcohol and dry in an oven.

Determination.—Place in the flask an accurately weighed amount of the drug to be tested, which it is estimated will yield from 2 to 4 cc. of water. If the drug is likely to cause bumping, add enough dry sand to cover the bottom of the flask. Add sufficient toluene to cover the drug completely, usually about 75 cc., and connect the apparatus. Fill the receiving tube with toluene by pouring it through the top of the condenser. Heat the toluene in the flask until it boils, and distil slowly, about two drops per second, until most of the water has passed over; then increase the rate of distillation to about four drops per second. When the water is apparently all over, wash down the condenser by pouring toluene in at the top, continuing the distillation a short time to ascertain whether any more water will distil and if it does, repeat the washing of the condenser with toluene. If any water remains in the condenser, remove it by brushing it down into the tube receiver with a tube brush attached to a copper wire and saturated with toluene, at the same time washing the condenser with toluene. Allow the receiving tube to stand until of room temperature and if any drops of water still adhere to the sides of the tube they can be forced down by a rubber band wrapped around a copper wire. Finally read the volume of water and calculate to determine the percentage.

Assay of Asafetida.—To yield not less than 50 per cent of alcohol-soluble extractive.

Place about 2 Gm. of Asafetida, accurately weighed, in a tared extraction thimble and extract with alcohol in a Soxhlet apparatus or other suitable extraction apparatus for three hours or until completely extracted. Dry the insoluble residue at 100° C. for thirty minutes and weigh. Determine the amount of moisture in the drug by the toluene distillation method, calculate the weight of moisture in the Asafetida and subtract this weight of moisture from the original weight of the Asafetida taken for the assay. The difference between this result and the weight of the residue determined above represents the alcohol-soluble extractive.

Assay of Aloe.—This drug has received a great deal of careful study, both from the chemical assay and from the physiological effect on daphnia. However, no satisfactory method has yet been devised for assaying this drug.

Assay of Aspidium.—Prepare an ethereal extract as directed under Oleoresin of Aspidium, using 125 Gm. of the drug. Assay as directed under Oleoresin of Aspidium. Aspidium yields not less than 6.5 per cent of oleoresin and not less than 1.5 per cent of crude filicin.

Assay of Oleoresin of Aspidium.—Warm the Oleoresin on a water-bath and stir until it is thoroughly mixed. Transfer about 3 Gm. of it, accurately weighed, to a 250-cc. flask, dissolve it in 40 cc. of ether, add 75 cc. of a 3 per cent aqueous solution of barium hydroxide, and shake the mixture vigorously for five minutes. Transfer the mixture to a separator, allow the liquids to completely separate, and draw off and filter the barium hydroxide layer. Rinse the 250-cc. flask with two 25-cc. portions of a 3 per cent aqueous barium hydroxide solution. After each rinsing, transfer the barium hydroxide solution to the separator, shake the mixture for one minute, allow the liquids to separate completely and draw off and filter the barium hydroxide layer. Transfer the combined filtered barium hydroxide solutions to a separator, render distinctly acid to litmus paper by the addition of hydrochloric acid, and extract with three successive portions of 30 cc., 20 cc. and 15 cc. of ether. Filter the combined ethereal solutions, wash the filter with ether, evaporate and dry the residue to constant weight at 100° C. This residue is calculated as crude filicin and its weight should be not less than 24 per cent of the weight of Oleoresin taken for the assay.

Assay of Belladonna Leaves.—Proceed as directed under Assay of Hyoscyamus.

Assay of Extract of Belladonna Leaves.—A. Pilular.—Proceed as directed under Assay of Pilular Extract of Hyoscyamus. B. Powdered.—Proceed as directed under Assay of Powdered Extract of Hyoscyamus.

Assay of Tincture of Belladonna.—Proceed as directed under Assay of Tincture of Hyoscyamus, with slight modifications.

Assay of Belladonna Root.—Proceed as directed under Assay of Hyoscyamus.

Assay of Fluidextract of Belladonna Root.—To 10 cc. of fluidextract of belladonna root add 10 cc. of approximately tenth-normal acid and 10 cc. of distilled water and evaporate on a water-bath to about 10 cc. Then add 10 cc. of distilled water and proceed as directed under Assay of Hyoscyamus, beginning with the words "filter this mixture."

Assay and Standard of Belladonna Ointment.—Ointment of Belladonna yields not less than 0.118 per cent and not more than 0.132 per cent of the alkaloids of belladonna leaves.

Assay. Accurately weigh about 25 Gm. of the well-mixed Ointment and transfer it completely to a 250-cc. separator having a pledget of purified cotton packed loosely in the stem. Add 100 cc. of ether-chloroform mixture (ether, four parts and chloroform, one part) and shake the mixture vigorously until all of the fats have been dissolved. Extract the alkaloids from the mixture with five successive 20-cc. portions of 2 per cent sulfuric acid. Draw off each portion of the clear acid solution into a small separator containing 10 cc. of ether. Wash each acid-extraction successively through this same 10 cc. of ether and draw off the acid solutions into another 250-cc. separator. Render the combined acidified solutions alkaline with ammonia T.S. and extract the alkaloids completely by shaking out with successive, 25-cc. portions of chloroform, testing for complete extraction as directed under *Proximate Assays*. Allow each portion to settle, and then filter through purified cotton, wetted with chloroform, into a 250-cc. beaker, finally washing the stem of the separator and the filter with a little chloroform. Evaporate the chloroform from the combined solutions by warming at a moderate heat on a water-bath until it is reduced to a volume of about 10 cc. Add a measured excess (about 10 cc.) of fiftieth-normal sulfuric acid, stir the mixture, and continue the evaporation until all of the chloroform has been expelled. Add 20 cc. of recently boiled and cooled distilled water and one drop of methyl red T.S. and titrate the excess of acid with fiftieth-normal sodium hydroxide. Each cc. of fiftieth-normal sulfuric acid is equivalent to 0.00578 Gm. of the alkaloids of belladonna leaves.

Assay of Belladonna Plaster.—Belladonna Plaster is a mixture of adhesive plaster mass and an extract prepared from belladonna root, spread evenly upon fine cotton cloth or other suitable backing material. The plaster mass yields not less than 0.25 per cent and not more than 0.30 per cent of the alkaloids of belladonna.

Each 100 square centimeters of the spread plaster contains at least 2.5 Gm. of the belladonna plaster mass.

Assay. Same as in U. S. P. X except for the following changes: An extra washing of the cloth and beaker, the precipitated rubber is kneaded with a glass rod to force out any chloroform-alcohol solution, the cotton pledget is pressed to remove the rest of the solvent, the residue is dissolved in 5 cc. of chloroform made neutral to methyl red T.S., 10 cc. of fiftieth-normal acid added and the chloroform evaporated on a water-bath and then the excess of acid titrated with a fiftieth-normal alkali.

Assay of Benzoinum.—

Assay. Place about 2 Gm. of Benzoin, accurately weighed, in a tared extraction thimble and extract with alcohol, containing 0.5 per cent of sodium hydroxide, in a Soxhlet apparatus or other suitable extraction apparatus for five hours, or until completely extracted. Dry the insoluble residue at 100° C. for thirty minutes and weigh. Determine the amount of moisture in the drug by the toluene distillation method, calculate the weight of moisture in the Benzoin and subtract this weight of moisture from the original weight of the Benzoin taken for the assay. The difference between this result and the weight of the residue determined above represents the alcohol-soluble extractive.

Assay of Cinchona.—Place 5 Gm. of Cinchona, in "fine powder," and 15 cc. of 3 per cent hydrochloric acid in a 500-cc. flask and heat the mixture on a water-bath for one hour. Cool and add 200 cc. of ether-chloroform solution (ether, 4 volumes, chloroform, 1 volume) and 10 cc. of stronger ammonia T.S. Stopper the flask tightly and shake it for one hour in a mechanical shaker. Allow the mixture to stand over night, again shake it for one-half hour, and then allow the drug to settle. (If the supernatant liquid is not clear, add a few cc. of distilled water, again shake the contents of the flask vigorously and allow the drug to settle.)

Quickly decant 160 cc. of the clear, ether-chloroform solution, measured at approximately

the same temperature as the original menstruum and representing 4 Gm. of the drug. Transfer the solution to a separator, rinse the measuring vessel with a small quantity of the ether-chloroform solution and add the rinsings to the separator. Completely extract the alkaloids with approximately 5 per cent sulfuric acid and collect the acid solution of the alkaloids in a second separator.

Make the acid solution strongly alkaline with ammonia T.S. and completely extract the alkaloids with chloroform. Evaporate or distil the chloroform in a tared beaker or flask and dry the alkaloidal residue to constant weight at 100° C. The weight obtained, multiplied by 25, represents the per cent of the alkaloids of cinchona in the drug.

Assay of Compound Tincture of Cinchona.—Evaporate 50 cc., accurately measured, of Compound Tincture of Cinchona, to about 10 cc. at a temperature not exceeding 100° C. Add sufficient asbestos fiber or paper pulp to absorb the liquid and continue the evaporation to dryness. Transfer the residue to a flask or bottle, add 200 cc., accurately measured and at room temperature, of ether-chloroform mixture (ether 4 volumes, chloroform 1 volume) and sufficient ammonia T.S. (which may be used to rinse out the adhering portions of the Tincture from the evaporating dish) to render the mixture strongly alkaline. Securely stopper the container and shake it mechanically during one hour, or intermittently during two hours and then allow the mixture to stand over night. Again shake the mixture intermittently for half an hour; allow it to settle; quickly decant 160 cc. (representing 40 cc. of the Tincture) of the approximately clear liquid. Filter this into a separator and wash the measuring vessel with sufficient of the original menstruum, adding the rinsings to the separator. Extract the alkaloids from the clear liquid with acidulated water, using sufficient dilute sulfuric acid to render the contents of the separatory funnel and each extract distinctly acid to litmus paper. Pass the acid extracts in succession through a wetted, double filter into a second separator. Render the combined liquids distinctly alkaline with stronger ammonia T.S. and extract with chloroform. Pass the chloroformic extracts through a double filter, which is kept saturated with chloroform, into a suitable, tared receptacle. Evaporate the chloroform on a water-bath, dry the residue to constant weight at 100° C. and weigh. The weight multiplied by 2.5 represents the weight of alkaloids in 100 cc. of the Compound Tincture of Cinchona.

Assay of Colchicum Seed.—U. S. P. X process is continued with only minor changes in the wording.

Assay of Ginger and Fluidextract of Ginger.—The standards and assays are as follows:

Ginger contains not less than 4.5 per cent of ether-soluble extractive.

Assay. Place 20 Gm. of Ginger, in moderately coarse powder, in an extraction thimble in a Soxhlet or similar extractor. Extract with ether for six hours, evaporate the liquid on a water-bath until the odor of ether is no longer perceptible, and place the container in a desiccator for twelve hours or over night and then weigh. The weight of the extract should be not less than 0.90 Gm.

Only Jamaica Ginger will be recognized in the forthcoming pharmacopœia.

Fluidextract of Ginger contains, in each 100 cc., not less than 4.5 Gm. of ether-soluble extractive.

Assay. Place 20 cc. of Fluidextract of Ginger in a 200-cc. beaker. Place this on a water-bath and evaporate the liquid until there is no longer any odor of alcohol. Remove it from the bath and add 50 cc. of ether. Stir the contents of the beaker with a stirring rod to dissolve the soluble resin and decant the ether through a dry, 9-cm. filter into a tared 200-cc. beaker. Repeat the extraction two or three times, using 50-cc. portions of ether. Finally wash the filter with a small amount of ether and evaporate the combined ethereal extractions on a water-bath until the odor of ether is no longer perceptible. Place the container in a desiccator for twelve hours or over night and then weigh. The weight of residue shall not be less than 0.90 Gm.

Assay of Hyoscyamus.—To yield not less than 0.040 per cent of the alkaloids of hyoscyamus by the new assay. The U. S. P. X assay gave a larger yield but the substance estimated was not all alkaloidal.

As indicated above it was necessary to change the assay process of this drug because of the volatile amines present and this assay process is made the basis for the assay of all mydriatic drugs and their preparations. The process is as follows:

Place 25 Gm. of Hyoscyamus, in fine powder, in an extraction thimble, insert the thimble

in a Soxhlet extractor, moisten the drug with a mixture of 8 cc. of stronger ammonia T.S., 10 cc. of alcohol and 20 cc. of ether and mix thoroughly. Macerate the mixture over night, then extract it for not less than three hours, on a water bath, using ether as the solvent. The following alternative process may be used: Moisten 25 Gm. of Hyoscyamus, in fine powder, with a mixture of 8 cc. of stronger ammonia T.S., 20 cc. of ether and 10 cc. of chloroform, in a small percolator, previously prepared by packing the outlet with a pledget of purified cotton. Macerate the mixture over night, pack it in the percolator and extract the drug by slowly percolating with a mixture of 3 parts of ether and 1 part of chloroform. Continue the percolation until the 3 or 4 cc. of percolate last passed, when evaporated to dryness and the residue dissolved in dilute sulfuric acid, fails to become turbid when treated with mercuric potassium iodide T.S. Evaporate the extractive, obtained by either method, to about 15 cc., then add 10 cc. of approximately tenth-normal sulfuric acid and 10 cc. of distilled water and continue the evaporation until the volatile solvents are removed. Filter this mixture, collecting the filtrate in a separator, dissolve the chlorophyll residue in chloroform, add acidulated water, evaporate on a water-bath until the chloroform is removed and filter into the same separator through the filter previously used. Render the mixed filtrates alkaline with ammonia T.S. and remove the alkaloids by extracting with chloroform, testing for the complete extraction of the alkaloids. Evaporate or distil the chloroform from the combined extractions until reduced to a small volume, then evaporate to dryness on a water-bath and keep at this temperature for fifteen minutes. Dissolve the residue in chloroform, evaporate to dryness on a water-bath and continue the heating for fifteen minutes. Repeat this treatment for the third time. Dissolve the resulting residue in chloroform, add 15 cc. of fiftieth-normal sulfuric acid, remove the chloroform by evaporation and titrate the excess acid with fiftieth-normal sodium hydroxide, using methyl red T.S. as the indicator. Each cc. of fiftieth-normal acid is equivalent to 0.00578 Gm. of the alkaloids of Hyoscyamus.

Assay of Pilular Extract of Hyoscyamus.—To yield not less than 0.135 per cent and not more than 0.175 per cent of the alkaloids of hyoscyamus.

Dissolve approximately 5 Gm. of Pilular Extract of Hyoscyamus, accurately weighed (see Proximate Assays, for method of weighing), in 10 cc. of chloroform, add 10 cc. of approximately tenth-normal sulfuric acid and 10 cc. of distilled water and evaporate the mixture on a water-bath until the chloroform is removed. Complete the assay as directed under *Hyoscyamus*, beginning with the words "Filter this mixture, collecting the filtrate in a separator." Each cc. of fiftieth-normal acid is equivalent to 0.00578 Gm. of the alkaloids of hyoscyamus.

Assay of Powdered Extract of Hyoscyamus.—To yield not less than 0.135 per cent and not more than 0.175 per cent of the alkaloids of hyoscyamus.

Dissolve 5 Gm. of Powdered Extract of Hyoscyamus in 10 cc. of chloroform, add 10 cc. of approximately tenth-normal sulfuric acid and 10 cc. of distilled water and evaporate the mixture on a water-bath, until the chloroform is removed. Complete the assay as directed under *Hyoscyamus*, beginning with the words "Filter this mixture, collecting the filtrate in a separator." Each cc. of fiftieth-normal acid is equivalent to 0.00578 Gm. of the alkaloids of hyoscyamus.

Assay of Tincture of Hyoscyamus.—Each 100 cc. to yield not less than 0.0034 Gm. and not more than 0.0046 Gm. of the alkaloid of hyoscyamus.

Evaporate approximately 250 cc., accurately measured, of Tincture of Hyoscyamus, at a temperature not exceeding 80° C., to about 25 cc. Add 10 cc. of approximately tenth-normal sulfuric acid and 10 cc. of distilled water and complete the assay as directed under *Hyoscyamus*, beginning with the words "Filter this mixture, collecting the filtrate in a separator."

Each cc. of fiftieth-normal acid is equivalent to 0.00578 Gm. of alkaloids of Hyoscyamus.

Assay of Ipecac.—Place 10 Gm. of Ipecac in "fine powder" in a dry, 250-cc. flask. Add 100 cc. of ether, which is free from peroxide, stopper the flask, shake the mixture thoroughly and allow it to stand for five minutes; then add 10 cc. of ammonia T.S. Again stopper the flask tightly and shake it for one hour in a mechanical shaker, or intermittently during two hours. Allow the mixture to stand over night, again shake it intermittently during one-half hour and then allow the drug to settle. Decant into a separator 50 cc., accurately measured, of the clear, supernatant liquid (representing 5 Gm. of drug), and rinse the vessel with a small quantity of ether.

Completely extract the alkaloids from this ethereal solution with approximately normal sulfuric acid, preferably using 15 cc. the first time and 10 cc. on each succeeding extraction, and

filtering each portion into a second separator. Continue the extraction until no reaction can be detected in the sulfuric acid solution when tested as directed in the General Article on Proximate Assays.

To the combined acid solutions add about an equal volume of peroxide-free ether, render the mixture alkaline by the addition of ammonia T.S. and extract with successive portions of the ether until no visible reaction takes place when tested as directed above. Filter each portion of the ethereal extract into a 200-cc. flask or beaker and carefully evaporate the combined ethereal solutions on a steam-bath, until nearly but not quite dry. Add 5 cc. of the peroxide-free ether and again evaporate nearly to dryness. Add 10 cc. of tenth-normal sulfuric acid and heat on a steam-bath to effect complete solution and to remove all of the ether. Cool and titrate the excess of acid with tenth-normal sodium hydroxide, using methyl red T.S. as the indicator. Each cc. of tenth-normal sulfuric acid is equivalent to 0.0240 Gm. of the ether-soluble alkaloids of Ipecac.

Assay of Fluidextract of Ipecac.—Each 100 cc. to yield not less than 1.55 Gm. and not more than 1.90 Gm. of the ether-soluble alkaloids of ipecac.

Transfer 10 cc. of Fluidextract of Ipecac, accurately measured, to an evaporating dish containing either absorbent paper or asbestos and dry at a temperature not exceeding 60° C. Transfer the absorbent to a flask containing 100 cc. of peroxide-free ether, stopper the flask, shake well and allow the mixture to stand for five minutes. Then add 10 cc. of ammonia T.S. using a portion of the ammonia T.S. to rinse traces of the absorbent from the evaporating dish. Stopper the flask tightly and shake the mixture during one hour in a mechanical shaker, or, occasionally, by hand, during a period of about two hours. Allow the mixture to stand over night and again shake it occasionally during a one-hour period. Allow the absorbent to settle and decant exactly 50 cc. (representing 5 cc. of the Fluidextract) of the clear supernatant liquid into a separator. Completely extract the alkaloids from the ethereal solution with approximately normal sulfuric acid, filtering each portion into a second separator and test for complete extraction as directed under Proximate Assays. Render the combined acid solution alkaline with ammonia T.S., extract with successive portions of peroxide-free ether and again test for complete extraction. Filter the ethereal extracts into a flask or beaker and evaporate them carefully on a steam-bath, nearly, but not quite, to dryness. Add 5 cc. of ether and again evaporate nearly to dryness. Add 10 cc. of tenth-normal sulfuric acid and heat on a steam-bath to effect complete solution and to remove all of the ether. Cool, and titrate the excess of acid with tenth-normal sodium hydroxide, using methyl red T.S. as the indicator. Each cc. of tenth-normal sulfuric acid is equivalent to 0.0240 Gm. of the ether-soluble alkaloids of ipecac.

Assay of Nux Vomica.—To yield not less than 1.15 per cent of strychnine.

Place 15 Gm. of Nux Vomica, in coarse powder, in a flask or bottle, add 150 cc. of a mixture of 3 volumes of ether and 1 volume of chloroform, agitate the mixture and allow it to stand for about two minutes. Then add 10 cc. of stronger ammonia T.S., agitate thoroughly, stopper the container securely and shake frequently, but gently, during one hour. Now allow the mixture to stand for twelve hours or over night in a cool place. At the expiration of this period, shake the container gently for fifteen minutes, and then allow it to separate. Decant 100 cc. of the liquid (representing 10 Gm. of Nux Vomica), and transfer it to a separator, rinsing the container with a little chloroform and adding the rinsings to the separator. Now add about 40 cc. of approximately normal sulfuric acid to the separator and shake the mixture gently for five minutes, then allow the liquids to separate and draw off the acid layer into another separator and repeat with successive portions of the acid, until the drug is completely extracted. (Test for the complete extraction of the alkaloids.)

To the combined acid solutions in the separator, add a small piece of red litmus paper and 50 cc. of chloroform, and follow with sufficient ammonia T.S. to render the aqueous layer alkaline and, after gently shaking, add 2 or 3 cc. more of the ammonia T.S. Now shake the mixture thoroughly, but gently, for about ten minutes, and allow the liquids to separate. Draw off the chloroform into a container and repeat, with additional portions of chloroform, until all of the alkaloid is extracted.

Carefully evaporate the combined chloroformic extracts to dryness on a steam-bath, dissolve the residue by warming with 15 cc. of approximately 3 per cent sulfuric acid, cool and then add 3 cc. of a mixture of equal parts of nitric acid and a 5 per cent solution of sodium nitrite

in distilled water, stir well and allow to stand for exactly ten minutes at room temperature. At the expiration of this period, pour the red solution into a separator containing 50 cc. of chloroform, rinse the flask with distilled water and add the rinsings to the separator. Now immediately add sufficient 10 per cent sodium hydroxide solution to make the contents of the separator distinctly alkaline to litmus paper, and then add a few cc. more of the hydroxide solution. Shake the mixture gently for ten minutes and allow the liquids to separate. Draw off the chloroformic layer into another separator and repeat the shaking out with additional portions of chloroform until the alkaloids are completely extracted. Add 10 cc. of distilled water to the combined chloroformic extract, shake the mixture gently and add a small piece of red litmus paper. The litmus paper should indicate not more than a slight alkalinity. Draw off the chloroform, passing it through a filter paper, moistened with chloroform, into a container. Shake the residual water with 5 cc. more of chloroform, separate this chloroform and add it to that previously separated. Wash the filter paper with warm chloroform and add it also to the container. If the water, after shaking with the chloroform, is strongly alkaline, draw off the chloroform into another separator and shake it with another 10 cc. of distilled water. Now shake out the combined water extract with 5 cc. of chloroform and draw off all of the chloroform through a chloroform-moistened filter paper as before.

Evaporate the combined chloroform very carefully on a steam-bath nearly, but not quite, to dryness. Add to the moist residue 6 cc. of tenth-normal sulfuric acid and follow by 30 cc. of distilled water. Heat the mixture on a steam-bath until the alkaloid is dissolved and the odor of chloroform is dissipated. Cool to room temperature and titrate the excess of acid with tenth-normal sodium hydroxide, using one drop of methyl red T.S. as the indicator. Each cc. of tenth-normal sulfuric acid is equivalent to 0.03342 Gm. of strychnine.

Assay of Extract of Nux Vomica.—To yield not less than 7.0 per cent and not more than 7.75 per cent of strychnine.

Place about 1.5 Gm. of Extract of Nux Vomica, accurately weighed, in a dish and digest it on a water-bath with about 10 cc. of diluted alcohol, acidulated with acetic acid, until the extract has liquefied. Transfer the solution to a separator containing 25 cc. of chloroform and wash the dish with successive small portions of diluted alcohol, adding the rinsings to the separator. Dilute the alcoholic liquid with an equal amount of distilled water, render it alkaline with ammonia T.S., and completely extract the alkaloids with successive portions of chloroform. Then proceed as directed under the *Assay for Nux Vomica*, beginning with the words "Carefully evaporate the combined chloroform extracts." Each cc. of tenth-normal sulfuric acid is equivalent to 0.03342 Gm. of strychnine.

Assay of Tincture of Nux Vomica.—Concentrate 100 cc. of Tincture of Nux Vomica to about 10 to 20 cc. by evaporating it at a temperature not exceeding 60° C. Transfer the concentrated liquid to a separator containing 25 cc. of chloroform and rinse all traces of liquid from the dish, using small portions of diluted alcohol and adding the rinsing to the separator. Add a volume of distilled water equal to that of the alcoholic liquid, render the solution alkaline with ammonia T.S. and completely extract the alkaloids by shaking out with successive portions of chloroform. Then proceed as directed under the Assay for Nux Vomica beginning with the words "Carefully evaporate the combined chloroform extracts," etc.

The number of cc. of tenth-normal sulfuric acid consumed, multiplied by 0.03342, indicates in grams, the amount of strychnine in 100 cc. of the Tincture.

Assay of Opium, Granulated Opium and Powdered Opium.—This is essentially the U. S. P. X assay process except that the morphine crystals are washed with "morphinated" water instead of distilled water.

Assay of Tincture of Opium.—Essentially the same as the U. S. P. X assay process except as indicated above in the Assay of Opium.

Assay of Camphorated Tincture of Opium.—This is the first time that an assay process has been included in the U. S. P. for this preparation. The process is as follows:

Each 100 cc. to yield not less than 0.035 Gm. and not more than 0.045 Gm. of anhydrous morphine.

To 100 cc. of the Tincture add 2 cc. of approximately normal sulfuric acid, evaporate the mixture on a water-bath to about 10 cc. and transfer the residue to a separator. Wash the evaporating dish with portions of about 10 cc. of a mixture of equal volumes of normal sulfuric

acid and distilled water, and add the washings to the separator. If necessary, wash the dish with several cc. of a mixture of 85 volumes of chloroform and 15 volumes of alcohol, adding these washings to the liquid in the separator. To this mixture add about 9 Gm. of sodium chloride and carefully neutralize to litmus paper by adding stronger ammonia water, and then add several drops in excess. Add 130 cc. of a mixture of 85 volumes of chloroform and 15 volumes of alcohol, shake the contents of the separator and then allow the mixture to completely separate.

Transfer the immiscible solvent portion to a second separator and extract the remaining aqueous solution with successive portions of the chloroform-alcohol mixture until a negative test for morphine is obtained with sulfuric acid formaldehyde T.S. Collect the extractions in the second separator. If more than four extractions are required, increase the quantities and volumes of all subsequent reagents so as to maintain the proportions here prescribed.

Dissolve 25 Gm. of sodium hydroxide in 1000 cc. of distilled water, saturate the solution with sodium chloride, filter and add 15 cc. of this alkaline salt solution to the chloroformic-alcohol extract just prepared. Remove the morphine by shaking with several successive portions of alkaline salt solution, collecting the latter. Wash the combined alkaline salt solutions with 10 cc. of chloroform and discard the chloroform. Exactly neutralize the alkaline salt solution to litmus paper by adding hydrochloric acid, and finally add a slight excess of acid. Cool the solution to 25° C., shake it with 10 cc. of chloroform. Remove the chloroform to another separator and shake it with 5 cc. of saturated sodium chloride solution to which a few drops of hydrochloric acid have been added. Discard the chloroform and add the acid salt solution to the combined salt solutions.

Now add stronger ammonia T.S. to the combined salt solutions until it is neutral to litmus paper, and then add a slight excess of the ammonia. Cool the solution to 25° C. and immediately extract the alkaloids with successive portions of the chloroform-alcohol mixture. Filter each extraction into a container, through purified cotton wetted with the chloroform-alcohol mixture and, when completely extracted, discard the liquid in the separator.

Evaporate the combined chloroformic solutions on a water-bath to a volume between 1 cc. and 1.5 cc. Add 10 cc. of alcohol neutral to methyl red T.S. to the residue and warm the mixture to dissolve the alkaloids and to remove the last traces of chloroform. Add 1 drop of methyl red T.S. and then a measured excess of fiftieth-normal sulfuric acid. *Guard against the presence of undissolved particles.* Cool and add 15 to 20 cc. of recently boiled and cooled distilled water. Titrate the excess of acid with fiftieth-normal sodium hydroxide which is sufficiently free from carbonate to insure a sharp end-point with methyl red T.S. as the indicator. Each cc. of fiftieth-normal sulfuric acid corresponds to 0.00571 Gm. of anhydrous morphine.

Assay of Podophyllym.—Considerable study was given to the assay process of podophyllum and the following process is recommended:

To yield not less than 4 per cent of resin of podophyllum.

Place 10 Gm. of Podophyllum, in fine powder, in a 125-cc. Erlenmeyer flask and add 35 cc. of alcohol. Fit a stopper with a glass tube for refluxing (a reflux condenser may be substituted) and heat on a water-bath for three hours. Transfer the mixture to a small percolator and percolate slowly with warm alcohol until the percolate measures 95 cc. Cool, add sufficient alcohol to make the volume exactly 100 cc. and mix thoroughly.

Transfer 10 cc. of this percolate to a separator, and add 10 cc. of chloroform and 10 cc of 0.6 per cent hydrochloric acid. Shake the mixture, allow it to separate, draw off the alcohol-chloroform layer into a second separator, and then wash the acid layer three times with successive, 15-cc. portions, of an alcohol-chloroform mixture prepared from one volume alcohol and two volumes of chloroform, adding the washings to the second separator. Add 10 cc. of 0.6 per cent hydrochloric acid to the combined extracts and washings, again shake the mixture, allow it to separate and draw off the alcohol-chloroform layer into a tared vessel. Wash the acid layer three times with 15-cc. portions of the alcohol-chloroform mixture, adding the washings to the tared vessel. Evaporate the combined extractions on a water-bath to apparent dryness, add 1 cc. of dehydrated alcohol and again evaporate to dryness and then to constant weight at 80° C. The weight of this residue, multiplied by 100, indicates the per cent of resin in the drug.

Assay of Stramonium.—To yield not less than 0.30 per cent of the alkaloids of stramonium.

Proceed as directed under Assay of Hyoscyamus.

Assay of Extract of Stramonium.—To yield not less than 1.10 per cent and not more than 1.30 per cent of alkaloids of stramonium.

Pilular. Proceed as directed under Assay of Pilular Extract of Hyoscyamus.

Powdered. Proceed as directed under Assay of Powdered Extract of Hyoscyamus.

Assay of Tincture of Stramonium.—Each 100 cc. to yield not less than 0.027 Gm. and not more than 0.033 Gm. of alkaloids of stramonium.

Proceed as directed under Assay of Tincture of Hyoscyamus.

A CORRECTION.

An error was made in transcribing the notes on our calomel article, JOUR. A. PH. A., 24, 97–102 (1935).

On pages 101 and 102 wherever the μ sign is used, it should read 1/1000 inch.

Will you please make this correction some time this year so that the correction will appear in the same volume as the article?

(Signed) CHARLES H. LAWALL.

CANCER RESEARCH.

Report was made at the American Chemical Society meeting in San Francisco, of apparent success in treating cancer by means of lead phosphate administration to a degree producing lead intoxication.

BLINDNESS CAUSED BY USE OF DINITROPHENOL.

W. G. Campbell, Chief of the Food and Drug Administration, has issued a Bulletin on the above subject. The statement is made that eye cataracts observed in dinitrophenol poisoning develop with rapidity and result in total blindness within a comparatively short time. The symptoms of the poisoning are nausea, stomach and intestine disturbance, high fever, rapid breathing and muscular rigor, followed by death.

Mr. Campbell states that the cases of progressive blindness recently reported in California are the result of medication with dinitrophenol. The statement is also made that dinitrophenol is sold under fanciful names and the statement is made that if the preparation contains the drugs it may not be known to the seller nor to the buyer. Purchaser should be advised regarding the dangers which follow the use of the drug.

BANG'S DISEASE IN CATTLE.

Testing to eradicate Bang's Disease in cattle is under way in nearly all states. The work is conducted by the Bureau of Animal Industry. A total of 250 herds showed one or more reactors and retesting is now under way.

PHARMACOLOGICAL AND VITAMIN LABORATORY.

Dr. Walter G. Campbell, chief of the Food and Drug Administration, has stated that the work of the Pharmacological and Vitamin Laboratory will be expanded. The work will include bioassay and other drug studies, investigation of the toxic effects of fruit-spray residues and examination of food and drug vitamin products.

MORPHINE ADDICTION.

Drs. O. H. Plant and D. Slaughter, of the State University of Iowa, reported to the American Society for Pharmacology and Experimental Therapeutics that the development of tolerance is one of the tests for judging the morphine substitutes. Dinitrophenol stimulates oxidation, the process by which the body burns food or other fuel to get energy; it increased the burning of morphine in the bodies of dogs that had no tolerance for the latter drug. In morphine-tolerant dogs, the general burning of oxidation process was speeded up by dinitrophenol, but judging from the fact that there was no decrease in the amount of morphine excreted, it appears that the burning of morphine itself was not affected by dinitrophenol in tolerant dogs. Consequently the investigators assume that the dog's body handles morphine differently when it has become used to the narcotic.

BRITISH PHYSICIANS VISIT UNITED STATES.

Fifty-five British physicians, with members of their families making a group of 110, visited New York, Washington, Chicago, Albuquerque, the Grand Canyon, Los Angeles and San Francisco between August 4th and 14th, on their way to the annual session of the British Medical Association in Melbourne, Australia, in September.