UNITED STATES PHARMACOPŒIA.

TENTH REVISION.

ABSTRACT OF PROPOSED CHANGES WITH NEW STANDARDS AND DESCRIPTIONS.*

BIO-ASSAYS AND PROXIMATE ASSAYS.

PART VII.

BIO-ASSAYS.

The Pharmacopœial Convention of 1920 recommended that abstracts of changes proposed for the U. S. P. X 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 of the text do not necessarily represent that to be finally adopted and doses have not been appended.

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

E. Fullerton Cook,
636 South Franklin Square,
Philadelphia, Pa.

GENERAL NOTE.

The following Bio-Assays are proposed as requirements for the U. S. P. X, as authorized by the 1920 Convention. In order to facilitate the adoption of these standards and to provide a greater degree of uniformity in the application of these assays, the Bureau of Chemistry of the U. S. Department of Agriculture at Washington, have indicated their willingness to supply substances conforming to the new pharmacopœial standards.

The assays are published in sufficient detail to permit those who are familiar with Bio-Assays to critically examine the processes. The wording is not necessarily that of the final texts.

Aconitina.

Aconitine, administered subcutaneously to guinea-pigs, has a minimum lethal dose of not less than 0.000,000,055 Gm. and not more than 0.000,000,065 Gm. for each gram of body weight of guinea-pig.

Assay.—Dissolve the Aconitine in distilled water with the aid of a little acetic acid and proceed as directed under *Tinctura Aconiti.*

Aconitum.

Aconite, in the form of the tincture, administered subcutaneously to guinea-pigs, has a minimum lethal dose not exceeding 0.000,04 Gm. for each gram of body weight of guinea-pig.

Assay.—Prepare a Tincture according to the official process, and assay as directed under *Tinctura Aconiti.*

Tinctura Aconiti.

Tincture of Aconite, administered subcutaneously to guinea-pigs, has a minimum lethal dose of not less than 0.000,35 cc. and not more than 0.000,45 cc. for each gram of body weight of guinea-pig.

Assay.—Use guinea-pigs weighing from 275 Gm. to 325 Gm. each, and in good health. Dilute the Tincture with distilled water to make the dose about 1 cc. and inject into the guinea-pig, under the skin of the abdomen. The standard dose must kill within six hours at least two of every three guinea-pigs injected.

Cannabis.

Cannabis, in the form of the fluidextract. administered by the mouth to dogs in doses not exceeding 0.1 cc. for each kilogram of body weight of dog, produces a degree of incoördination equivalent to that caused by the same dose of the standard fluidextract of cannabis, prepared as directed below.

Standard Fluidextract of Cannabis.

Prepare a composite fluidextract, representing at least ten different lots of cannabis, conforming to the official botanical description, and administer this fluidextract in gelatin capsules to dogs by the mouth. This standard fluidextract must be so adjusted that it will produce incoördination in dogs which have been found to be susceptible to the action of cannabis when administered in doses of 0.03 cc. for each kilogram of body weight of dog.

Assay.—Use adult dogs which weigh less than 15 kilograms and which are susceptible to the action of cannabis. The dogs must not be fed for twelve hours before being used and observations should be made within one hour after administration. The same animal must not be used for testing purposes at shorter intervals than three days. Administer the fluidextract in gelatin capsules by the mouth.

Extractum Cannabis.

Extract of cannabis, administered by the mouth to dogs, in doses not exceeding 0.004 Gm. for each kilogram of body weight of dog, produces a degree of incoördination equivalent to that caused by 0.03 cc., for each kilogram of body weight of dog, of the standard fluidextract of cannabis prepared as directed under *Cannabis*.

Assay.—Administer the Extract in gelatin capsules, proceeding as directed under *Cannabis*.

Fluidextractum Cannabis.

Fluidextract of cannabis, administered by the mouth to dogs in doses not exceeding 0.1 cc. per kilogram of body weight of dog produces the same degree of incoördination as that produced by an equivalent dose of the standard fluidextract of cannabis, prepared as directed under *Cannabis*.

Assay.—Proceed as directed under *Cannabis*.

Digitalis.

Digitalis, in the form of the tincture, properly diluted and injected into the ventral lymph sac of a frog, has a minimum systolic dose (the minimum dose producing in one hour a stoppage of the ventricle of the heart in systole) not exceeding 0.006 cc. of tincture, equivalent to 0.000,000,5 Gm. of ouabain, for each gram of body weight of frog.

Assay.—Prepare a tincture according to the official process, and proceed as directed under *Tinctura Digitalis*.

Tinctura Digitalis.

Tincture of Digitalis, injected into the ventral lymph sac of a frog, has a minimum systolic dose of not less than 0.0055 cc. and not more than 0.0065 cc. equivalent to not less than 0.000,000,46 Gm. and not more then 0.000,000,54 Gm. of ouabain, for each gram of body weight of frog.

Assay.—Use healthy frogs of the same species (*Rana pipiens Schreber*) and of a fairly uniform size (20 to 30 Gm.).

Storage.—Before use, the frogs should be stored in constant temperature tanks, preferably where the temperature does not rise above 15° C. The bottom of the tanks should be covered with running water. The day before the frogs are to be used, a sufficient number should be taken from the storage tanks and placed in a tank, the temperature of which is approximately 20° C. One hour before the assay, they are weighed to within 0.5 Gm. and placed in wire cages or containers in a tank containing water to the depth of about 1 cm. ($^1/_2$ inch), the water being kept at a uniform temperature of 20° C. during the assay.

Dosage.—The doses of the tincture to be given the frogs are calculated according to the weights of the frogs and are injected into the ventral lymph sac by means of a pipette or glass syringe which is graduated in hundredths of a cubic centimeter. The injection is made into the lymph sac through the floor of the mouth, care being taken not to puncture the skin. The amount of fluid to be injected into the different frogs should be as uniform in quantity as possible, approximately 0.015 cc. for each gram of body weight of frog. In case the alcohol content in any preparation after dilution is higher than 20 per cent. the preparation may be subjected to careful evapo-

ration and subsequent addition of distilled water until the original volume is restored and the alcohol content is not above the per cent. named. The animal is replaced in its cage in the tank after injection, the temperature being maintained at 20° C.

About fifty-eight minutes from the time of injection, each frog is pithed, the heart is exposed and its condition examined. For the correct end-reaction, at the expiration of one hour from the time of injection, the ventricle must be in systolic standstill, while the auricles are widely dilated. Following mechanical stimulation, feeble contractions may occur in the auricles and localized contractions in the ventricle, but no general contraction is allowable.

If, when the lymph sac is opened to expose the heart, any of the injected drug is found unabsorbed, the animal must be discarded and not considered in the results obtained.

Liquor Epinephrini Chloridi.

Solution of epinephrine hydrochloride, diluted with physiological solution of sodium chloride and injected intravenously into dogs by the method described below, produces a rise in the systolic blood pressure of the dog, corresponding to that produced by an equivalent amount of standard solution of epinephrine hydrochloride prepared as directed below.

Standard Solution of Epinephrine Hydrochloride.

Prepare a standard solution of epinephrine hydrochloride from epinephrine by dissolving 0.050 Gm. of epinephrine in 5 cc. of tenth-normal hydrochloric acid and dilute this to 50 cc. by the addition of distilled water to make a 1 in 1000 solution. On account of the possibility of deterioration, this standard solution must have been recently prepared. It will keep for a short time (two or three weeks) if preserved in amber-colored bottles and stored in a refrigerator but it must be discarded if any signs of deterioration, such as discoloration, are observed.

Assay.—For the assay, the standard 1 in 1000 solution must be diluted by adding 1 cc. of the said solution to 99 cc. of physiological solution of sodium chloride. This dilute solution (1 in 100,000) must not be used if more than eight hours old.

The solution of epinephrine hydrochloride to be tested is diluted by adding 1 cc. of the solution to 99 cc. of physiological solution of sodium chloride and thoroughly mixing the same.

Dogs.—The dogs to be used should be of medium size and be anesthetized with a suitable anesthetic. They are prepared for blood pressure estimations by the insertion of a cannula into a carotid artery, connecting the same with a mercury manometer. The trachea may also be exposed and a cannula inserted so that the animals may receive artificial respiration during the course of the experiment if necessary. The injections are made into the exposed femoral vein.

Before a test is made, in case any muscular movement such as twitching is present, the dog should receive by intravenous injection a sufficient dose of curare (page —), but if the animal is deeply anesthetized, this is not necessary. The dog should also receive a sufficient dose of atropine sulphate (from 0.001 Gm. to 0.002 Gm.) to paralyze the vagi, this paralysis being proved by electrical stimulation. The blood pressure tracing is recorded on a kymograph.

Injections must be made at regular intervals of approximately five minutes. The rise of blood pressure must be submaximal (from 30 to 60 mm.). In case this figure is much exceeded, a second test injection of a small amount may be made five minutes later. After a satisfactory dose has been ascertained, the uniformity of reaction should be tested by the injection of two or more doses of equal size, if these injections produce approximately equal increases in blood pressure, alternate injections of the solution of unknown strength and of the standard are made, varying the amount of the unknown, until two or more successive injections raise the blood pressure to the same height, indicating that the amount of active agent is the same in the doses used. From the results thus obtained the strength of the unknown solution may be determined and adjusted.

Ergota.

Ergot, in the form of the fluidextract, administered by intramuscular injection to single-comb, white Leghorn cocks, in doses not exceeding 0.5 cc. for each kilogram of body weight of cock, produces a darkening of the comb, corresponding in intensity to that caused by the same dose of a standard fluidextract of ergot, prepared as directed below.

Standard Fluidextract of Ergot.

Prepare a composite fluidextract, representing at least ten different lots of ergot, conforming to the official botanical description.

This standard fluidextract, which must be aged for at least six months before being standardized and must be preserved in a vacuum, when administered by intramuscular injection in doses not exceeding 0.5 cc. per kilogram of body weight of cock produces darkening of the comb, of a single-comb, white leghorn cock, which is less than eighteen months of age, and which weighs approximately 2 kilograms.

Assay.—Use single-comb, white Leghorn cocks, which are less than eighteen months of age, and weigh approximately 2 kilograms. Injections are made deeply into the breast muscles, and the effects are observed within one hour to one hour and a half after the administration of the drug. The same cock must not be used for testing purposes at shorter intervals than two weeks.

Fluidextractum Ergotæ.

Fluidextract of ergot, administered by intramuscular injection to single-comb, white Leghorn cocks, in doses not exceeding 0.5 cc. for each kilogram of body weight of cock, produces a darkening of the comb, corresponding in intensity to that caused by the same dose of the standard fluidextract of ergot prepared as directed under *Ergota*.

Assay.—Proceed as directed under *Ergota*.

Liquor Pituitarii.

Solution of pituitary contains the water-soluble, principle or principles from the fresh posterior lobe of the pituitary body of cattle, 1 cc. having an activity upon the isolated uterus of the virgin guinea-pig, corresponding to not less than 80 per cent. and not more than 120 per cent. of that produced by 0.005 Gm. of the standard, dried, defatted powdered gland, prepared as directed below.

Standard Powdered Pituitary Gland.

Prepare the standard in the following manner: Select a number of absolutely fresh posterior lobes (at least 25), of the pituitary body of cattle, carefully dissect them free from all extraneous material, and drop them into not less than 150 cc. of acetone. After three hours cut the glands into small bits with scissors, place the cut glands in fresh acetone, and allow to stand over night. Then dry them in a vacuum desiccator over calcium chloride at a temperature not above 50° C. for five hours. Grind the material in a mortar until it will pass through a No. 40 sieve and again dry for at least twelve hours in a vacuum desiccator over calcium chloride. Extract this material in a small Sohxlet extraction apparatus with acetone for three hours, and again dry in a desiccator over calcium chloride for twelve hours. The powder is now ready for use. It should be preserved in the dark in sealed ampuls *in vacuo*, or in vacuum desiccators over calcium chloride.

Assay.—Prepare the solution for use in the assay as follows: Weigh a suitable amount of the dry standard powder very carefully. Place it in a small agate mortar, and moisten with a few drops of 0.25 per cent. acetic acid, added from a burette. Triturate the moistened powder thoroughly until of an impalpable frothy consistence. Add a few cc. of 0.25 per cent. acetic acid and stir the mixture thoroughly. Wash it into a hard glass test-tube or beaker, adding enough of the dilute acetic acid from the burette to make the final volume correspond in number of cc. to the number of milligrams of the dry powder taken. Heat the mixture to the boiling point and filter. The filtrate contains in each cc. the active principles of 1 milligram of dry powder. Preserve this solution in hard glass ampuls and sterilize by fractional sterilization at a temperature not exceeding 100° C.

The apparatus used for making the test may be any modification of the general type for studying the activity of the isolated smooth muscle of mammals. It must be provided with an accurate temperature-regulating device, and the chamber in which the uterus is suspended must have a capacity of not less than 100 cc.

The Locke-Ringer solution should have the following composition:

Sodium chloride	0.9	Gm.
Potassium chloride	0.042	Gm.
Calcium chloride crystals	0.024	Gm.
Magnesium chloride	0.0005	Gm.
Sodium bicarbonate	0.05	Gm.
Dextrose	0.05	Gm.
Freshly glass-distilled water	100	cc.

All of the salts and the dextrose must be of the highest purity obtainable. The solution must be made up fresh each day. The individual constituents (except the dextrose) may be made up in more concentrated stock solutions and diluted as needed.

Use healthy guinea-pigs weighing between 175 Gm. and 350 Gm. They should not have been pregnant and should not be in heat. It is recommended that young female pigs be segregated at the time of weaning and kept thereafter out of sight and smell of the males.

Kill the guinea-pig by a blow on the head, or by decapitation, and remove the entire uterus from the body. Suspend a part or all of one horn in the chamber of warm oxygenated Locke-Ringer solution, (page —), and weighted as necessary. A temperature of the bath of between 37° and 38° C. is satisfactory, but a uniform temperature throughout an assay is essential. When relaxation is complete, add alternate doses of the standard and the unknown extract to the muscle chamber until quantities of the two are found which give submaximal contractions of the same amplitude in at least two successive sets of contractions. The strengths of the standard and the unknown extracts are then in inverse ratio to the quantities necessary to produce equal contractions.

Scilla.

Squill, in the form of the tincture, properly diluted and injected into the ventral lymph sac of a frog has a minimum systolic dose (minimum dose producing in one hour a stoppage of the ventricle of the heart in systole) not exceeding 0.006 cc. of tincture, equivalent to 0.000,000,5 Gm. of ouabain, for each gram of body weight of frog.

Assay.—Prepare a tincture according to the official process and proceed as directed under *Tinctura Digitalis.*

Tinctura Scillæ.

Tincture of Squill, injected into the ventral lymph sac of a frog, has a minimum systolic dose of not less than 0.0055 cc., and not more than 0.0065 cc. equivalent to not less than 0.000,000,46 Gm. and not more than 0.000,000,54 Gm. of ouabain for each Gm. of body weight of frog.

Assay.—Proceed as directed under *Tinctura Digitalis.*

Strophanthus.

Strophanthus, in the form of the tincture, properly diluted and injected into the ventral lymph sac of a frog, has a minimum systolic dose (the minimum dose producing in one hour a stoppage of the ventricle of the heart in systole) not exceeding 0.000,06 cc. of tincture, equivalent to 0.000,000,5 Gm. of ouabain, for each gram of body weight of frog.

Assay.—Prepare a tincture according to the official process and proceed as directed under *Tinctura Digitalis.*

Tinctura Strophanthi.

Tincture of Strophanthus, injected into the ventral lymph sac of a frog, has a minimum systolic dose of not less than 0.000,055 cc. and not more than 0.000,065 cc., equivalent to not less than 0.000,000,46 Gm. and not more than 0.000,000,54 Gm. of ouabain, for each Gm. of body weight of frog.

Assay.—Proceed as directed under *Tinctura Digitalis.*

FOOD AND NUTRITION BULLETINS READY.

Following the expansion and development of the Bureau of Home Economics, it is hoped that new bulletins dealing with phases of home problems will be issued by the U. S. Department of Agriculture. At present bulletins on food and nutrition far outnumber those on other home economics subjects. Over 100 popular and technical bulletins on foods and nutrition resulting from the research work of this department are now available free or by purchase for nominal sums. These represent the work now organized under the Bureau of Home Economics as well as of the Bureaus of Chemistry and of Animal Industry.

The Bureau of Roads has contributed through its engineering division several bulletins on household heating and farm sewage disposal. A few other publications have been issued on household equipment, textiles, and some of the economic problems of the home.

PROXIMATE ASSAYS.

GENERAL DIRECTIONS FOR ALKALOIDAL ASSAYS.

Accurate results in alkaloidal assays are dependent upon familiarity with the principles involved and experience in the technique.

Fineness of Powders.

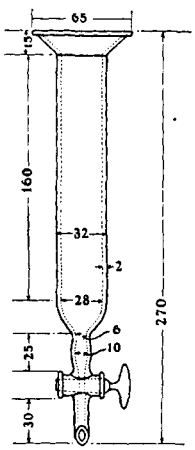
The portion to be assayed, which must represent the entire drug, is to be in powder of specific fineness. This is interpreted to mean that the drug powder must not be coarser than the size specified, but finer powders may also be used.

Extraction of the Alkaloids.

Aliquot Part Method.—For the extraction of the powdered drug, macerate the quantity directed in the specified quantity of solvent, contained in a cork-stoppered flask or bottle of excess capacity in the presence of an alkali, for a definite time with intermittent or continuous shaking. The time for maceration is one hour of continuous or two hours of intermittent shaking in which latter case agitate the container vigorously for one minute at intervals of not more than fifteen minutes. Then allow the drug to macerate over night, again shake it intermittently during one-half hour and allow to settle until the supernatant liquid is clear. This clarification may be hastened by vigorous agitation after the addition of a few cc. of water.

This method involves the taking of an aliquot part of the solvent and requires that the solvent shall have been accurately measured and that the aliquot portion taken shall have also been measured with equal care at approximately the same temperature. Loss by evaporation or leakage must be avoided by the use of tightly-fitting cork stoppers.

Total Extraction Method.—The extraction of the powdered drug is accomplished by percolating the quantity directed with the specified solvent in the presence of an alkali. Place the drug, thoroughly moistened with the solvent, in a small cylindrical percolator (see illustration), and allow to stand for not less than five minutes. Then add the alkali, mix it with the drug, and macerate for one hour. Now pack the drug firmly and percolate with the solvent until completely extracted. Any suitable percolator may be used, but one similar in form and size to that illustrated is recommended.



Percolator recommended for Type Process B (measurements are given in millimeters).

Extraction with Immiscible Solvents.—For the extraction of an ethereal or chloroformic solution of the alkaloids, use dilute acid. The strength of the acid and the quantity employed are left to the discretion of the operator, but it is advisable to use as small quantities as can be conveniently handled. Shaking should not be violent, but should be sufficient to mix the liquids thoroughly. The extraction with acid must be repeated until the alkaloid is completely removed from the ethereal or chloroformic liquids. Each portion of acid solution as drawn off should be filtered through cotton or filter paper. If preferred, the combined acid solutions may be washed with a little chloroform or ether, this, in turn, washed with distilled water and the water added to the acid solution.

In shaking out an alkaline aqueous solution with an immiscible solvent, the process must be repeated until the extraction of the alkaloid is complete. Each portion of solvent as drawn off should be filtered through cotton or filter paper.

Care must be taken to avoid loss of alkaloidal solutions through imperfect separation, creeping, or other similar conditions.

Ether-Chloroform Mixture.—When a mixture of ether and chloroform is employed, the proportion of the mixture is to be three volumes of ether and one volume of chloroform cooled to working temperature after being mixed.

Evaporation of Solvents.—The evaporation of the final alkaloidal solution may be conducted by distillation, by evaporation on a water-bath, or by means of a current of warm air. In the case of chloroform solutions, the residue should be treated with ether or alcohol which is in turn evaporated to carry off the last traces of chloroform.

Titration of the Alkaloid.—When the alkaloid is to be estimated by titration, the residue is dissolved in neutralized alcohol, the volumetric acid, accurately measured, is added, and followed

by a convenient quantity of distilled water. The excess of acid is determined by titration. If preferred the residue is dissolved in ether or chloroform, the volumetric acid and distilled water added, and the ether or chloroform expelled by gentle heat before titrating.

Indicators.—The indicators to be employed in the titration of alkaloids are cochineal T. S. and methyl red T. S. These are interchangeable and either may be used at the discretion of the operator.

Type Process For Alkaloidal Assays.

TYPE PROCESS A FOR DRUGS—ALIQUOT PART METHOD.

Extraction of the Drug.—Place the accurately weighed drug in a dry flask or bottle of suitable capacity, add the required volume of solvent, accurately measured, stopper securely with a well-fitting cork stopper and shake. Allow to stand for at least five minutes, add 10 cc. of ammonia T. S., stopper tightly and shake for one hour in a mechanical shaker or intermittently during two hours. In case of intermittent shaking, agitation must be vigorous for at least one minute and be repeated at intervals of not more than fifteen minutes. Allow the mixture to stand over night, again shake intermittently during one-half hour and then allow the drug to settle. If the supernatant liquid is not clear, add a few cc. of distilled water, shake vigorously and again allow to settle.

Decant an aliquot part of the liquid, accurately measured, at approximately the same temperature at which the original solvent was measured. Transfer the liquid to a separator, rinsing the measuring container with a small quantity of the solvent and adding the rinsing to the separator.

Shaking out with Acid.—Completely extract the alkaloid from the ethereal or chloroformic solution by shaking with successive portions of dilute sulphuric acid, filtering each portion drawn off or washing with ether or chloroform if preferred. After extracting with several portions of acid, test a few drops of the last portion used for the presence of alkaloid. Extraction must be continued until not more than a very faint cloudiness results upon the addition of a drop of mercuric potassium iodide T. S. or, in the case of hydrastis and colchicum, upon the addition of a drop of iodine T. S.

Shaking out with Immiscible Solvent.—Add a portion of the specified solvent to the combined acid solutions and make the mixture alkaline with ammonia T. S. Completely extract the alkaloid from the aqueous liquid with successive portions of the solvent, filtering each portion as drawn off. After extracting with several portions of the solvent, evaporate about 1 cc. of the last portion used, dissolve the residue in a few drops of dilute acid and test for the presence of alkaloid. Extraction must be continued until not more than a very faint cloudiness results upon the addition of a drop of mercuric potassium iodide T. S. or, in the case of hydrastis and colchicum, upon the addition of a drop of iodine T. S.

Determination of Alkaloidal Content.—Determine the alkaloid volumetrically or gravimetrically as directed.

Volumetric Determination.—Evaporate the solvent and dissolve the residue in neutralized alcohol, chloroform, or ether. Add an excess of tenth-normal sulphuric acid and a convenient quantity of distilled water and if chloroform or ether were used, expel it by gentle heat. Determine the excess of acid by titration with fiftieth-normal sodium hydroxide, using cochineal T. S. or methyl red T. S. as indicator.

Gravimetric Determination.—Evaporate the solvent in a tared flask or beaker and if the solvent were chloroform, treat the residue with two small portions of neutral alcohol, and evaporate to dryness after the addition of each portion. Dry the residue to constant weight at 100° C. and weigh.

TYPE PROCESS B FOR DRUGS—TOTAL EXTRACTION METHOD.

Extraction of the Drug.—Place an accurately weighed quantity of the ground drug in a small cylindrical percolator (page —), previously prepared by packing the outlet with cotton. Add a sufficient amount of the solvent to saturate the drug completely and mix thoroughly. Cover the percolator and allow it to stand at least five minutes, then add the specified amount of ammonia T. S. and mix this thoroughly with the drug. After macerating for one hour pack the drug firmly, place a pledget of cotton above the drug and percolate slowly with the solvent

until the drug is completely extracted. The percolation must be continued until, upon evaporating 3 or 4 cc. of the percolate, dissolving the residue in a few drops of dilute acid and adding a drop of mercuric potassium iodide T. S., only a faint cloudiness results.

From this point proceed as directed under *Type Process A*—beginning with the words "Shaking out with Acid," page —.

TYPE PROCESS C FOR GALENICAL PREPARATIONS—ALIQUOT PART METHOD.

Preparation of the Sample. *Powdered Extracts.*—When extracts are assayed by the aliquot method, they are to be treated as directed under the powdered drug. Place an accurately-weighed quantity of the powdered extract in a dry flask or bottle, cover it with the specified volume of solvent and diffuse by gentle shaking. After standing at least five minutes, add a 10-cc. portion of ammonia T. S., stopper the container tightly with a well-fitting cork, previously wetted with water and shake the mixture continuously or intermittently for the time specified. Decant an aliquot portion and treat as directed in Type Process A, beginning with the words "Shaking out with acid." If the extract has a tendency to form lumps in the solvent, mix it with an equal bulk of clean sand or powdered pumice. If lumps form after the addition of the ammonia, they should be disintegrated by the addition of a little water.

Pilular Extracts.—Liquefy an amount of extract, accurately weighed, approximating the quantity directed, and warm with sufficient diluted alcohol, or with the menstruum used in making the extract, stirring with a glass rod. To the thoroughly liquefied extract, contained in a small evaporating dish, add sufficient sawdust or other suitable material (page —) to absorb the liquid, and dry at a temperature not exceeding 60° C. Transfer the dry mixture to a flask or bottle containing the specified quantity of solvent, and remove any traces of material adhering to the dish by rinsing with successive small portions of ammonia T. S. using a total of 10 cc., and adding the ammoniacal rinsings to the solvent. Continue the process as directed under Type Process A.

Fluidextracts.—Pipette fluidextracts directly upon the sawdust, or other suitable material (page —), contained in a small evaporating dish, dry at a temperature not exceeding 60° C. and transfer as directed under *Pilular Extracts* to a flask or bottle containing the specified quantity of solvent. Then proceed as directed under Type Process A, beginning with the words "Shaking out with acid."

Tinctures.—Concentrate the specified quantity of tincture, by evaporation at a temperature not exceeding 60° C., to a volume of about 10 to 15 cc., add sufficient sawdust or other suitable material (page —) to absorb the liquid, and continue the heat until dry. Transfer the dry mixture as directed under *Pilular Extracts* to a flask or bottle, containing the specified quantity. Then proceed as directed under Type Process A, beginning with the words "Shaking out with acid."

Absorbents.—The absorbents employed may be sawdust, gauze, cheesecloth, paper pulp, asbesto fibre, absorbent cotton, or any similar suitable substance. Sawdust should be clean, not resinous, and must not show the presence of alkaloids when tested as follows: Macerate a 5 Gm. portion in 50 cc. of an ether-chloroform mixture, containing 10 cc. of ammonia T. S., shaking frequently during two hours. Decant 10 cc. of the clear ethereal liquid, evaporate to dryness on a watch glass, and treat the residue with 1 cc. of diluted sulphuric acid. Test portions of the acid liquid for alkaloids with mercuric potassium iodide T. S. and with iodine T. S.

If necessary, sawdust may be purified for use in assays by extracting it in a percolator, first with a 1 per cent. solution of sodium hydroxide, then with a 1 per cent. solution of hydrochloric acid, finally washing with distilled water until free from acid and soluble salts, and drying. The last portions of the acid percolate must give no test for alkaloid with mercuric potassium iodide T. S. or with iodine T. S.

TYPE PROCESS D FOR GALENICAL PREPARATIONS—TOTAL EXTRACTION METHOD.

Preparation of the Sample. *Powdered and Pilular Extracts.*—Liquefy an amount of extract, accurately weighed, approximating the quantity directed, and digest in about 10 cc. of diluted alcohol, or in the menstruum used in making the extract. Transfer the liquid to a separator containing a portion of the specified solvent, and rinse all traces of the extract from the dish, using small portions of diluted alcohol, or menstruum, and adding the rinsings to the separator. Dilute the alcoholic liquid with an equal quantity of distilled water, add sufficient ammonia T. S. to make it decidedly alkaline, and completely extract the alkaloids by shaking with successive

portions of the solvent. Complete the assay as directed under Type Process A, beginning with the words "Shaking out with acid."

Fluidextracts.—Pipette fluidextracts directly into a separator containing a portion of the specified solvent, add a volume of distilled water about equal to that of the fluidextract taken, render the liquid alkaline by the addition of a few drops of ammonia T. S., and completely extract the alkaloids by shaking out with successive portions of the solvent. Complete the assay as directed under Type Process A, beginning with the words "Shaking out with acid."

Tinctures.—Concentrate the specified quantity of tincture, by evaporation at a temperature not exceeding 60° C., to a volume of about 10 to 20 cc. Transfer the concentrated liquid to a separator containing a portion of the specified solvent, and rinse all traces of the liquid from the dish, using small portions of diluted alcohol, and adding the rinsings to the separator. Add a volume of distilled water equal to that of the alcoholic liquid, render the liquid alkaline by the addition of a few drops of ammonia T. S., and completely extract the alkaloids by shaking out with successive portions of the solvent. Complete the assay as directed under Type Process A, beginning with the words "Shaking out with acid."

Aconite.—The chemical assay is to be omitted, bio-assay retained.

Tinctura Aconiti.—The chemical assay is to be omitted, bio-assay retained.

Asafœtida.—No change in U. S. P. IX assay, except to dry filters and residue at 100° C. instead of 115° C.

Belladonna Folia. *Assay.*—Proceed by Type Process B using 10 Gm. of Belladonna Leaves, in fine powder, ether-chloroform mixture for percolating the drug, and chloroform as the immiscible solvent for extracting the alkaloid from the aqueous liquid. Determine the alkaloids volumetrically. Each cc. of tenth-normal sulphuric acid corresponds to 0.02893 Gm. of the total alkaloids of Belladonna Leaves (Proximate Assays, page —).

Extractum Belladonnæ (Pilular). *Assay.*—Proceed by Type Process D, taking 2 Gm. of Pilular Extract of Belladonna, using chloroform as the solvent, and determine the alkaloids volumetrically. Each cc. of tenth-normal sulphuric acid corresponds to 0.02893 Gm. of the total alkaloids of belladonna leaves (Proximate Assays, page —).

Extractum Belledonnæ (Powdered). *Assay.*—Proceed as directed under *Pilular Extract of Belladonna.*

Tinctura Belladonnæ. *Assay.*—Proceed by Type Process D, taking 100 cc. of Tincture of Belladonna, using chloroform as the solvent, and determine the alkaloids volumetrically. Each cc. of tenth-normal sulphuric acid consumed corresponds to 0.02893 Gm. of the total alkaloids of belladonna leaves (Proximate Assays, page —).

Belladonnæ Radix. *Assay.*—Proceed by Type Process B, using 10 Gm. of Belladonna Root in fine powder, ether-chloroform mixture for percolating the drug, and chloroform as the immiscible solvent for extracting the alkaloid from the aqueous liquid. Determine the alkaloids volumetrically. Each cc. of tenth-normal sulphuric acid corresponds to 0.02893 Gm. of the total alkaloids of belladonna root (Proximate Assays, page —).

Fluidextractum Belladonnæ Foliorum. *Assay.*—Proceed by Type Process D, taking 10 cc. of Fluidextract of Belladonna Leaves, using chloroform as the solvent, and determine the alkaloids volumetrically. Each cc. of tenth-normal acid corresponds to 0.02893 Gm. of the alkaloids of belladonna leaves (Proximate Assays, page —).

Fluidextractum Belladonnæ Radicis. *Assay.*—Proceed by Type Process D, taking 10 cc. of Fluidextract of Belladonna Root, using chloroform as the solvent, and determine the alkaloids volumetrically. Each cc. of tenth-normal acid corresponds to 0.02893 Gm. of the alkaloids of belladonna root (Proximate Assays, page —).

Emplastrum Belladonnæ. *Assay.*—Introduce 10 Gm. of Belladonna Plaster into a 100-cc. flask. (If the plaster is spread on fabric, cut the portion to be assayed into strips, weigh accurately, and introduce it into the flask.) Now add 50 cc. of chloroform, and shake the mixture until the plaster is dissolved. Pour the chloroform solution into a 250-cc. beaker and wash the cloth upon which the plaster was spread with two portions of 25 cc. each of chloroform, adding the washings to the chloroform solutions in the beaker. Then wash this cloth with 80 cc. of alcohol containing 1 cc. of ammonia T. S. and pour the washings into the chloroform solution in the beaker. Stir the mixture gently and allow it to stand until the rubber has separated into a compact mass. Dry the cloth upon which the plaster was spread, weigh it and subtract its weight from the original

weight of the plaster. Pour the chloroform-alcohol solution into a 250-cc. separator, rinse the beaker and rubber with 10 cc. of alcohol and add the rinsing to the separator. Completely extract the alkaloids from the chloroform-alcohol solution by shaking it out repeatedly with weak sulphuric acid. Collect the cold washings in a separator and add ammonia T. S. until the solution is decidedly alkaline to litmus paper, and completely extract the alkaloids by shaking out repeatedly with chloroform. Filter the chloroform solution through a pledget of purified cotton, evaporate it to dryness and dissolve the alkaloids from the residue in exactly 5 cc. of tenth-normal sulphuric acid, and titrate the excess of acid with fiftieth-normal sodium hydroxide, using cochineal T. S. as indicator. Each cc. of tenth-normal sulphuric acid consumed corresponds to 0.02893 Gm. of the alkaloids from belladonna leaves.

Cinchona. *Assay.*—Heat 5 Gm. of Cinchona, in fine powder, for one hour, in a 500-cc. flask on a water-bath, with 5 cc. of diluted hydrochloric acid and 10 cc. of distilled water. Cool, add 200 cc. of ether-chloroform mixture, and 10 cc. of stronger ammonia T. S. Proceed by Type Process A, decanting an aliquot portion of 160 cc. representing 4 Gm. of Cinchona. Use chloroform for the final extraction and determine the alkaloids gravimetrically (Proximate Assays, page —).

Fluidextractum Cinchonæ. *Assay.*—Proceed by Type Process C, taking 5 cc. of Fluidextract of Cinchona with 1 cc. of hydrochloric acid, and using 200 cc. of ether-chloroform mixture for maceration. Decant an aliquot portion of 160 cc., representing 4 cc. of Fluidextract of Cinchona, using chloroform for the final extraction, and determine the alkaloids gravimetrically (Proximate Assays, page —).

Tinctura Cinchonæ. *Assay.*—Proceed by Type Process C, taking 25 cc. of Tincture of Cinchona, using 200 cc. of ether-chloroform mixture and decanting an aliquot portion of 160 cc. of the ethereal solution representing 20 cc. of Tincture. Use chloroform for the final extraction and determine the alkaloids gravimetrically (Proximate Assays, page —).

Tinctura Cinchonæ Compositæ. *Assay.*—Proceed by Type Process C, taking 50 cc. of Compound Tincture of Cinchona, using 200 cc. of ether-chloroform mixture and decanting an aliquot portion of 160 cc. representing 40 cc. of the Tincture. Use chloroform for the final extraction and determine the alkaloids gravimetrically (Proximate Assays, page —).

Extractum Colchici. Place 6 Gm. of Extract of Colchicum in a 500-cc. flask, containing 275 cc. of distilled water, mix well, add 10 cc. of solution of lead subacetate, and agitate the mixture frequently during one hour. Filter off 200 cc. and proceed as directed under *Colchici Semen*, page —, seventh line of the assay, beginning with the word "Shake."

Colchici Semon. *Assay.*—Place 15 Gm. of Colchicum Seed, in fine powder, in a 500-cc. flask, and add 290 cc. of distilled water and 10 cc. of solution of lead subacetate. Weigh the flask and contents, and digest the mixture at from 60° to 70° C. for three hours, with occasional agitation. Cool, add distilled water to restore the original weight and filter off 200 cc. Add 2 Gm. of sodium phosphate to the clear filtrate, or sufficient to completely precipitate the lead, shake the mixture frequently during half an hour, and filter off 100 cc., representing 5 Gm. of Colchicum Seed. Shake out the alkaloid from the filtrate with chloroform until completely extracted, as shown by testing with iodine T. S., and evaporate the chloroform solution. Add about 1 cc. of alcohol and again evaporate. Repeat this operation once more and dry the residue to constant weight at 100° C. To this weighed residue contained in a flask add 5 cc. of tenth-normal sulphuric acid and 5 cc. of distilled water and heat the mixture for ten minutes at 70° C. Filter the liquid through a pledget of purified cotton, wash the flask and cotton with distilled water, reject the filtrate and washings and remove as much of the water from the cotton as possible. Dissolve any insoluble residue that may remain on the cotton by washing it first with a little alcohol and then with ether; collect the alcohol ether washings in the flask, evaporate, and dry the residue to constant weight at 100° C. Deduct this weight from the weight of residue previously obtained. The difference will be the weight of colchicine obtained from 5 Gm. of Colchicum Seed (Proximate Assay, page —).

Fluidextractum Colchici. *Assay.*—Place 15 cc. of Fluidextract of Colchicum in a 500-cc. flask, containing 275 cc. of distilled water, mix well, add 10 cc. of solution of lead subacetate, and agitate the mixture frequently during one hour. Filter off 200 cc. and proceed as directed under *Colchici Semen*, page —, seventh line of the assay, beginning with the word "Shake."

Tincture Colchici. *Assay.*—Evaporate 150 cc. of Tincture of Colchicum on a water-bath

to about 15 cc., cool, and dissolve the residue in sufficient distilled water to make 290 cc. Add 10 cc. of solution of lead subacetate, and agitate the mixture frequently during one hour. Filter off 200 cc. and proceed as directed under *Colchici Semen*, page —, seventh line of the assay, beginning with the word "Shake."

Hydrastis. *Assay.*—Proceed by Type Process A, taking 10 Gm. of Hydrastis, in fine powder, using 100 cc. of ether and decanting an aliquot portion of 50 cc., representing 5 Gm. of Hydrastis. Use ether for the final extraction and determine the hydrastine gravimetrically (Proximate Assays, page —).

Fluidextractum Hydrastis. *Assay.*—Proceed by Type Process D, taking 5 cc. of Fluidextract of Hydrastis, using ether as the solvent and determine the hydrastine gravimetrically (Proximate Assays, page —).

Hyoscyamus. *Assay.*—Proceed by Type Process B, taking 25 Gm. of Hyoscyamus, in fine powder, using ether-chloroform for percolating the drug and chloroform for the final extraction of the alkaloids. Determine the alkaloids volumetrically. Each cc. of tenth-normal acid corresponds to 0.02893 Gm. of the alkaloids of hyoscyamus (Proximate Assays, page —).

Extractum Hyoscyamus (Pilular). *Assay.*—Proceed by Type Process D, taking 5 Gm. of Extract of Hyoscyamus, using chloroform as the solvent and determine the alkaloids volumetrically. Each cc. of tenth-normal acid corresponds to 0.02893 Gm. of the alkaloids of hyoscyamus (Proximate Assays, page —).

Extractum Hyoscyamus (Powdered). *Assay.*—Proceed as directed under *Pilular Extract of Hyoscyamus*.

Fluidextractum Hyoscyamus. *Assay.*—Proceed by Type Process D, taking 25 cc. of Fluidextract of Hyoscyamus, using chloroform as the solvent, and determine the alkaloids volumetrically. Each cc. of tenth-normal acid corresponds to 0.02893 Gm. of the alkaloids of hyoscyamus.

Tinctura Hyoscyami. *Assay.*—Proceed by Type Process D, taking 250 cc. of Tincture of Hyoscyamus, evaporating to about 25 cc., using chloroform as the solvent, and determine the alkaloids volumetrically. Each cc. of tenth-normal sulphuric acid corresponds to 0.02893 Gm. of the total alkaloids of hyoscyamus (Proximate Assays, page —).

Jalapa. *Assay.*—Place 10 Gm. of Jalap in fine powder in a dry flask, add 50 cc. of alcohol and stopper the flask with a perforated cork holding a reflux condenser. (An open glass tube of not less than 0.6 M (2 ft.) in length will suffice.) Place the flask on a water-bath and digest for three hours with occasional shaking. Then transfer to a small percolator, allow to drain and percolate with alcohol until the percolate measures 100 cc. Allow to cool to room temperature and add alcohol to make exactly 100 cc. Mix well.

Transfer 20 cc. of this Tincture, accurately measured, representing 2 Gm. of Jalap to a separator, add 10 cc. of chloroform and 20 cc. of saturated solution of potassium citrate (20 Gm. of potassium citrate dissolved in 12 cc. of distilled water). Shake well during two minutes, then set aside for not less than ten hours or over night. Draw off and discard the lower aqueous liquid and filter the alcohol-chloroform solution through a small filter, wetted with alcohol-chloroform, into a tared flask or beaker. Rinse the separator with a mixture of 10 cc. of alcohol and 5 cc. of chloroform and pass the rinsing through the filter. Mix the chloroformic liquids, evaporate on a water-bath, dry at 100° C. and weigh.

Ipecacuanha. *Assay.*—Proceed by Type Process A, taking 10 Gm. of Ipecac, using 100 cc. of ether and decanting an aliquot portion of 50 cc., representing 5 Gm. of Ipecac. Use ether for the final extraction, make the acid solution of the alkaloids strongly alkaline, and determine the alkaloids volumetrically. Each cc. of tenth-normal acid corresponds to 0.024 Gm. (Proximate Assays, page —).

Fluidextractum Ipecacuanhæ. *Assay.*—Proceed by Type Process D, taking 5 cc. of Fluidextract of Ipecac, using ether as solvent, make the acid solution of the alkaloids strongly alkaline, and determine the alkaloids volumetrically. Each cc. of tenth-normal acid corresponds to 0.024 Gm. of the alkaloids of ipecac (Proximate Assays, page —).

Ipomœa. *Assay.*—Proceed as directed under *Jalapa*.

Linimentum Camphoræ. *Assay.*—Place 5 cc. of Liniment of Camphor in a tared, porcelain dish, having a diameter of about 75 mm. and heat it at about 110° C. for ninety minutes, or until

the odor of camphor is no longer discernible. Cool and weigh. The loss in weight is not less than 0.95 Gm. nor more than 1.05 Gm.

Nux Vomica. *Assay.*—Proceed by Type Process A, taking 10 Gm. of Nux Vomica, in fine powder, using 100 cc. of ether-chloroform mixture and decanting an aliquot portion of 50 cc., representing 5 Gm. of Nux Vomica. Use chloroform for the final extraction and determine the alkaloids volumetrically. Each cc. of tenth-normal sulphuric acid corresponds to 0.0364 Gm. of the total alkaloids of nux vomica (Proximate Assays, page —).

Extractum Nucis Vomicæ. *Assay.*—Proceed by Type Process D, taking 1 Gm. of Extract of Nux Vomica, using chloroform as the solvent, and determine the alkaloids volumetrically. Each cc. of tenth-normal acid corresponds to 0.0364 Gm. of the alkaloids of nux vomica (Proximate Assays, page —).

Tinctura Nucis Vomicæ. *Assay.*—Proceed by Type Process D, taking 50 cc. of Tincture of Nux Vomica, using chloroform as the solvent, and determine the alkaloids volumetrically. Each cc. of tenth-normal sulphuric acid corresponds to 0.0364 Gm. of the total alkaloids of nux vomica (Proximate Assays, page —).

Opium and Its Preparations.—No change in the U. S. P. IX assays.

Podophyllum. *Assay.*—Proceed as directed under *Jalapa*, page —, using 10 Gm. of Podophyllum in fine powder.

Stramonium. *Assay.*—Proceed by Type Process B, taking 10 Gm. of Stramonium in fine powder, using ether-chloroform mixture for percolating the drug, and chloroform for the final extraction. Determine the alkaloids volumetrically. Each cc. of tenth-normal sulphuric acid corresponds to 0.02893 Gm. of the total alkaloids of stramonium (Proximate Assays, page —).

Extractum Stramonii (Pilular). *Assay.*—Proceed by Type Process D, taking 2 Gm. of Extract of Stramonium, using chloroform as the solvent and determine the alkaloids volumetrically. Each cc. of tenth-normal acid corresponds to 0.02893 Gm. of the alkaloids of stramonium (Proximate Assays, page —).

Extractum Stramonii (Powdered). *Assay.*—Proceed as directed under *Pilular Extract of Stramonium.*

Tinctura Stramonii. *Assay.*—Proceed by Type Process D, taking 100 cc. of Tincture of Stramonium, using chloroform as the solvent, and determine the alkaloids volumetrically. Each cc. of tenth-normal sulphuric acid corresponds to 0.02893 Gm. of the alkaloids of stramonium (Proximate Assays, page —).

DRUG-MEDICATION OF THE FUTURE.

The following passage occurs in an article in the *New Statesman* of August 9, on "The Science of Medicine," by Dr. Harry Roberts, the well-known medical writer: "Many of us have gone through a stage of sneering at the potentialities of drugs and medicine. It was a healthy stage, but it did not represent the last word. There is every reason to believe that drug-medication has a future which will bear a relation to its past, comparable with that of modern chemistry to the alchemy of the darkest ages. When we contemplate the enormous part which minute quantities of self-manufactured drugs—ferments and hormones and the rest—play in the regulating, modifying and harmonizing work of the body, it is clearly more than plausible that with greater knowledge physicians will be able, as they are just beginning to be able, to produce corresponding modifications by chemical substances introduced from without. At present, the most potent of these substances are derived directly from the bodies of other animals; but, with fuller insight into their nature, and more minute and accurate knowledge of their physical and chemical composition, there is good reason for expecting that before long the creation of all these drugs will be within the capacity of the synthetic chemist."—Through *Pharmaceutical Journal and Pharmacist.*

THE BOYCE-THOMPSON INSTITUTE FOR PLANT RESEARCH OPENS.

There were present about 200 representatives of American universities and scientific institutions at the formal opening of the Boyce-Thompson Institute for plant research in Yonkers, September 24. The speakers of the occasion were Prof. John M. Coulter of the University of Chicago, Dr. William Croker, Yonkers, Prof. Vernon H. Blackman, London, Prof. Louis R. Jones, Madison, Wis., and Dr. Raymond F. Bacon, New York.