A laboratory manual of general agricultural chemistry. 1911

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A Laboratory Manual

OF

General Agricultural Chemistry

BY

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OF

Agricultural Chemistry

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Soils, fertilizers, feeding stuffs, insecticides, milk and the **various agricultural products** are complex materials and require special treatment for chemical analysis. This special treatment is the province of Agricultural Chemical Analysis, which may be considered as the application of methods of qualitative and quantitative analysis to materials of agricultural importance. Before proceeding to the analysis of any specific sample the student should know the source of the material at hand. He should also be familiar with the general properties of its constituents and understand the chemical processes involved in its production. To that end the following notes are provided. They consist of brief descriptive text, preliminary qualitative tests, and directions for quantitative analysis of the materials discussed. The notes for quantitative analysis have been adapted for the most part from the *Methods of Analysis of the Association of Official Agricultural Chemists.*

I. THE SOIL.

The soil is a product of the decay and disintegration of organic and inorganic matter and therefore contains those chemical elements which occur in rocks and in plant and animal tissues. The chemical elements of the soil which are essential to plant life and which therefore particularly concern the agricultural chemist are comparatively few in number. They include nitrogen, phosphorus, sulphur, potassium, calcium, iron, magnesium and chlorine. Carbon, hydrogen and oxygen are also of importance in the soil as constituents of organic matter but they do not serve higher plants as food elements when they exist in this form.

Minerals in great number contribute to soil formation. Of these, quartz, feldspar, mica, apatite and limestone may be taken as typical examples. The first consists of silica, or silicon-dioxide. It is extremely resistant to weathering agencies and chemical solvents and is the

chief constituent of poor, sandy soils. Feldspar may be either potassium-aluminum silicate or sodium-aluminum silicate, the latter containing also, in some cases, calcium. Feldspar is rather easily decomposed and in the case of orthoclase, the potassium feldspar, yields potassium to the soil-water, leaving a residue known as clay. Mica contains iron and magnesium in addition to the constituents of the feldspars and is both more complex and less easily disintegrated than the latter. Apatite is an example of the simpler soil constituents which are comparatively rich in plant food and readily dissolved by weathering agencies and strong chemical solvents. This mineral is a double salt of calcium with phosphoric acid and hydrochloric or hydrofluoric acid. It is an important source of phosphorus. Limestone is calcium carbonate, a compound readily decomposed by acids with liberation of CO_2. It thus possesses value in controlling soil acidity as well as in supplying calcium. It is apparent, from what has preceded, that an extraction of the soil with strong acid removes some minerals completely but others only in part or hardly at all.

The organic constituents of the soil, a complex mixture classified as humus material, contain most of the soil supply of nitrogen. This organic matter has been derived largely from proteins and other organic structures furnished by plant residues, bacterial cells and such added materials as manures and fertilizers. Its constituents are mostly insoluble in water, but by fermentation processes the organic nitrogen is gradually converted to ammonia which is finally oxidized by special bacteria to nitrites and nitrates. All of these latter forms are soluble in water and thus available to the plants. The nitrogen content and absorptive capacity of soils are greatly dependent upon the amount of organic matter in them.

ANALYTICAL METHODS.

Chemical study of the soil may be considered under,—

A. Methods for measuring temporary supply of plant food.

B. Methods for measuring permanent or potential supply of plant food.

Temporary supply of plant food is measured by the amount of plant food in soils supposedly of immediate availability to plants. It is the expression of results obtained in an attempt to duplicate the action of natural agencies (such as root acidity, bacterial flora, plant decay, etc.) on the soil. Various weak solvents including water, carbonated water, carbonated water and ammonium chlorid, and dilute acetic acid have been used for the purpose. Solutions of ammonium citrate and ammonium oxalate, a 1 per cent solution of aspartic acid, and solutions of mineral acids, such as N/200 HCl, N/5 HCl and N/5 HNO_3, have been used also for extracting the soil. In 1894 Dyer of England, after extensive study of the acidity of plant-root sap, recommended a 1 per cent solution of citric acid as an approximation to the natural solvent agents in plant feeding. Of all methods proposed this appears to be most substantiated by field tests. An analysis of the extracts obtained with

these various solvents has been considered an index to the store of readily available plant food in the soil.

Potential supply of plant food is an expression covering an attempt to determine the amount of food in a given soil that may become available to plants over a long period of time. To measure this capacity of the soil an attempt is made to simulate the action of the most vigorous weathering agencies. For this purpose strong reagents must be employed. Concentrated mineral acids heated under pressure may dissolve silica and its complexes. The same result may be reached by the use of hydrofluoric acid or by fusion of the soil with alkalies or alkaline earths and subsequent solution in acids. These reagents proceed further, however, than do the natural agencies within reasonable limits of time. Finding that a solution of HCl attained and maintained under distillation a density of 1.115 (nearly 23% strength), Owen was led to use it as an easily controlled reagent in soil analysis; and his method has been adopted for soil analysis by the Official Agricultural Chemists. This reagent effects only partial decomposition of the soil minerals.

Complete analysis of the soil can be made by resorting to fusion methods with alkalies or alkaline earths, excluding a given alkali from the fusion in which it is to be determined. This procedure converts all soil constituents to compounds which can be readily dissolved with mineral acids and gives the total invoice of the soil's supply of plant food.

PRELIMINARY TESTS.

These tests should be conducted by the use of table reagents unless other strengths are specified. The table reagent consists of one part of the concentrated reagent diluted with four parts of water. Express the reactions of these tests by equations, so far as possible.

1. To about 5 c. c. of HCl add a nearly equal amount of water glass (Na_2SiO_3. X. H_2O.). What precipitate forms? Filter it off and compare it with quartz sand for solubility in water, and in dilute and concentrated acids and alkalies. What is the difference in chemical composition between your precipitate and the quartz sand?

Dry a little of the precipitate thoroughly in an air oven at 110° C. Now test its solubilities in the reagents previously used.

How would you estimate silica in a soluble silicate? In an insoluble silicate?

2. To 1 c. c. of sodium di-hydrogen phosphate solution (10%) slightly acidified with HCl add 2 c. c. each of a saturated solution of ferric chloride and of alum. Now add an excess of NH_4OH, filter, wash with water and test the filtrate and washings for P_2O_5 by making slightly acid with HNO_3, adding 5 c. c. of clear molybdic reagent and heating until perceptibly hot to the touch. The solutions used in this experiment should be carefully measured. Explain the result.

Iron and aluminum are in excess over phosphoric acid in practically all soils. What is the nature of the precipitate formed when NH_4OH is added in excess to an acid extract of the soil?

3. Shake about 10 grams of loam soil in a small flask with about 40 c. c. H_2O and 10 c. c. of HCl, warming a little. Filter and make alkaline with NH_4OH. Now filter again and add to the filtrate a few drops of saturated $(NH_4)_2C_2O_4$ solution. What precipitate, if any, falls? What compound readily soluble in acids and giving this test is likely to be commonly found in soils? With what analogous compound and from what parent rock is it sometimes derived?

Let the test for lime stand warm a few minutes, then filter off the precipitate, add a few drops of sodium phosphate solution (used for test 2) and make slightly alkaline with NH_4OH. What precipitate appears on standing?

4. Shake up about 10 grams of a rich garden soil with 25 or 30 c. c. of warm distilled water. Filter and concentrate to about 5 c. c. Cool and add a bright crystal of $FeSO_4.7H_2O$ in a test tube. Now carefully pour about 3 c. c. of strongest H_2SO_4 down the inclined t. t. so as to form a layer below the solution. Watch for the appearance of a brown ring between the acid and the solution, due to $(FeSO_4)_2.NO$. This is a test for nitrates. What nitrate is common to fertile soils? How is it formed? Where do massive accumulations of nitrates occur? Why do they not accumulate in soils of humid regions?

5. Heat a thin layer of fertile soil gently in a covered crucible over a Bunsen flame. Charring indicates the presence of organic matter. Carbon, oxygen, hydrogen, nitrogen, sulphur, phosphorus and other elements are constituents of this organic matter. Which of these elements produces the char? What happens to them in the charring process? What are the sources of the organic matter of the soil? What important soil constituent is composed of organic matter? By what processes is the organic matter of cultivated soils exhausted?

6. Thoroughly mix in a mortar 5 to 10 grams of soil rich in organic matter with about an equal weight of soda-lime (approximately 1 part NaOH + 2 parts CaO). Transfer to a test-tube, insert a loose wad of asbestos near the mouth of the t. t., and incline in a clamp. Close the t. t. with a one-holed stopper bearing a glass tube bent so that its distant end may be readily immersed in distilled water in a tall 100 c. c. beaker. Test the water with red litmus paper. Now heat the t. t., gently at first, along the soil layer. Air will be expelled rapidly at first and water will collect in the asbestos, which will prevent it from running back and breaking the hot tube. What is the source of this water? Heat for some time and then remove the beaker before allowing the tube to cool. Test the water as before. To what is the alkaline reaction due? What is the nature of the compounds from which this alkali is derived and in what constituent of the soil do they occur? What sources have they?

METHODS FOR THE ANALYSIS OF SOILS.

1. Directions for Taking Samples.

Remove surface accumulations of decaying leaves, etc., and take samples with a soil tube or augur to the desired depth. If the tract to be studied is not of uniform character, divide into smaller tracts, that each

may be uniform, and from such tracts take five or six representative samples to the depth of 6 inches, or to the change between the surface soil and the subsoil, in case such change occurs between the depth of 6 and 12 inches. In no case is the sample to be taken to a greater depth than 12 inches. If the surface soil extend to a greater depth, a separate sample below the depth of 12 inches is to be obtained. If the surface soil extend to a depth of less than 6 inches, and the difference between it and the subsoil is unusually great, a separate sample of the surface soil should be secured, besides the one to the depth of 6 inches. Mix the samples of each depth thoroughly and take subsamples of 2 to 4 pounds, drying the latter in a well-aired, cool place.

The depth to which the sample of subsoil should be taken will depend on circumstances. It is always necessary to know what constitutes the foundation of a soil to the depth of 3 feet at least, since the question of drainage, resistance to drought, etc., will depend essentially upon the nature of the substratum. But in ordinary cases 10 or 12 inches of subsoil will be sufficient for the purposes of examination in the laboratory. The sample should be obtained in other respects precisely like that of the surface soil, while that of the material underlying this subsoil may be taken with less exactness, perhaps at some ditch or other easily accessible point. Mix and subsample as above. The sampling should be done preferably when the soil is reasonably dry.

It is recommended that the weight of a given volume of soil as it lies in the field be taken for calculating the percentage results obtained by analysis to pounds per given area of the soil.

2. Quantitative Analysis of the Soil.

Great care should be observed in preparing substances for this and all succeeding analytical work as it is very important to have homogenous and representative samples. Soils should be air dried and pulverized by rubbing lightly with a rubber covered pestle. After sifting through perforations of 1 millimeter diameter and thoroughly mixing, samples should be drawn and placed in tightly stoppered jars and carefully labelled.

a. Moisture.

Weigh out 5 grams of the air-dried soil into a suitable weighed dish, dry at 100° C. for 5 hours, cool in a dessicator and weigh. This determination should be made in dishes having covers fitted by ground glass edges. A pair of watch glasses with ground edges and provided with a clamp forms a suitable apparatus. During cooling and weighing the glasses should be clamped face to face to lessen absorption of moisture by the sample.

Record data in duplicate as follows:

Weight of dish and soil	.....	.....
Weight of dish and dried soil	.....	.....
Loss of weight	.....	.....
Per cent of moisture	.....	.....

Record all succeeding analytical data in this form.

b. Strong Acid Digestion of the Soil.

Method of Preparing Soil Solution.

Digest a quantity equivalent to 10 grams of moisture free soil (this facilitates the subsequent calculations). Make the digestion preferably in an Erlenmeyer flask of nonsoluble glass of 200 or 300 cc capacity. The flask should have a ground-glass stopper terminating in a reflux tube 20 inches or more in length. A rubber stopper carrying a tube may be substituted if glass-stoppered flasks are not available. Use 100 cc of hydrochloric acid of a constant boiling point (sp. gr. 1.115), made approximately by diluting 1,350 cc of ordinary acid (sp. gr. 1.20), with 1,000 cc of water. Digest continuously for 10 hours on a steam or water bath, shaking the flask every hour. After settling, decant the solution into a porcelain dish or hard glass beaker. Very small quantities of the sediment passing over will do no harm. Wash the insoluble residue onto a 15 cm filter with hot water and continue the washing until free from chlorids, adding the washings to the original solution for evaporation. Oxidize the organic matter present in the solution with a few drops of nitric acid and evaporate to dryness on a water bath. Take up with hot water and a few cubic centimeters of hydrochloric acid and again evaporate to complete dryness. When the final evaporation is complete and the dish cooled, add a few drops of strong hydrochloric acid, sufficient only to saturate the residue. Add 10 to 20 cc of water, warm on the bath to secure complete solution, and filter, washing until free from chlorids. Again evaporate this solution to dryness to render insoluble any silica that may yet be in solution, and treat as above. The filtrate constitutes the acid extract freed of soluble silica, and is made up to a volume of 500 cc, designated as solution A and stored in a heavy bottle.

Combine the two filters and the main residue and after drying ignite, preferably in a small dish over a Bunsen flame for an hour or more, then complete by igniting over a blast until it ceases to lose weight. Weigh as the insoluble residue and report as per cent of the soil.

With soils rich in organic matter the nitric acid treatment of the above solution may require several evaporations with nitric acid, using 2 or 3 c. c. each time, to destroy the organic matter. Why is the organic matter removed from solution? Why is this done by means of nitric acid rather than by ignition? What is the source of the SiO_2 separated from the soil extract? What is the nature of the insoluble residue from the acid digestion?

Analysis of Soil Solution.

1. Removal of Ferric and Aluminum Oxides and Phosphates Collectively.

To 100 c. c. of solution A add ammonium hydroxid, drop by drop, until the precipitate formed requires several seconds to dissolve, thus leaving the solution but faintly acid. Heat nearly to the boiling point, add sufficient ammonium hydroxid to precipitate all of the iron, alumina, etc. Allow the covered beaker to boil for about one minute, remove,

and if no ammonia is given off (detect by smelling), more is to be added drop by drop until it can be detected. Do not allow the precipitate to settle, but stir and pour onto the filter. Wash immediately with hot water, using a fine jet which is played around the edge of the precipitate, thus cutting it free from the paper in order to produce rapid filtration. Wash the precipitate several times and return it to the original beaker, dissolve with a few drops of hydrochloric acid and warm. Reprecipitate the iron, alumina and phosphoric acid as above and wash until free from chlorids. Reject the precipitate. The filtrate is designated as Solution B.

2. Calcium.

Evaporate Solution B to about 50 cc, make slightly alkaline with ammonium hydroxid, and add, while still hot, ammonium oxalate solution, drop by drop, so long as any precipitate is produced, adding a few cubic centimeters in excess to convert the magnesium also into oxalate. Heat to boiling, allow to stand for 3 hours or longer, decant the clear solution on a filter, pour from 15 to 20 cc of hot distilled water on the precipitate, and again decant the clear solution onto the filter, saving the filtrate. Dissolve the precipitate in the beaker with a few drops of hydrochloric acid, add a little water, and reprecipitate, boiling hot, by adding ammonium hydroxid, to a slight alkalinity, and a little ammonium oxalate solution; allow to stand as before and filter through the same filter; transfer the precipitate to the filter and wash it free from chlorids with hot water. The combined filtrates and washings become Solution D. Dry and ignite the precipitate, pulverizing if of considerable amount, and continue the blast until it ceases to lose weight. Report as per cent of CaO.

3. Magnesium.

Evaporate Solution D on the water bath to dryness and carefully heat to expel ammonium salts. Take up the residue, with 20 or 25 cc hot water and about 5 cc hydrochloric acid, filter, and wash. Concentrate to about 50 cc, cool and add sufficient acid-sodium-phosphate to precipitate the magnesium; then add gradually ammonium hydroxid, with constant stirring, until the solution is distinctly alkaline. Test with acid-sodium-phosphate to be sure that sufficient has been added. Allow to stand one-half hour, then add gradually 10 cc of strong ammonium hydroxid, cover closely to prevent escape of ammonia, and let stand in the cold. Filter after 12 hours, wash the precipitate free from chlorids, using 2.5 per cent ammonia water, dry thoroughly, burn at first at a moderate heat, then ignite intensely, and weigh as magnesium-pyrophosphate ($Mg_2P_2O_7$). Report as per cent of MgO.

Should the ammonium salts be present in large amounts and give trouble by decrepitation they may be partially oxidized by boiling in a concentrated solution with addition of strong nitric acid. This treatment should be followed by the usual evaporation and ignition.

4. Phosphoric Acid.

Evaporate 200 cc of Solution A to about 25 or 30 cc, neutralize with ammonium hydroxid and add about 10 cc additional, neutralize the

excess of ammonium hydroxid with nitric acid, gradually add at once about 20 cc of molybdate solution and place the beaker in a water bath at a temperature of about 60° C. When the precipitate has settled sufficiently, draw out with a pipette about 5 cc of the clear liquid and test by allowing it to run into 5 cc of warm molybdate solution. If any precipitate be produced, return the test liquid to the main portion, add more molybdate solution, and repeat the operation until all the phosphoric acid is precipitated. After standing several hours at a temperature not above 60° C., filter off the ammonium phosphomolybdate. Wash the precipitate thoroughly with cold water, dissolve with ammonium hydroxid, and determine the phosphoric acid as magnesium pyrophosphate, as directed under total phosphoric acid in fertilizers, page 20.

Do not allow the evaporation in this determination to go to dryness, as compounds may be formed which dissolve with difficulty.

5. Sulphuric Acid.

Evaporate 100 cc of Solution A nearly to dryness on a water bath to expel the excess of acid; then add 50 cc of distilled water; heat to boiling and add drop by drop a 10 per cent barium chlorid solution until no further precipitation occurs. Continue the boiling for about 5 minutes; allow to stand for 5 hours or longer in a warm place, pour the liquid on a tared gooch or on an ashless filter, treat the precipitate with from 15 to 20 cc of boiling water, transfer to the filter and wash with boiling water until the filtrate is free from chlorids. Dry the filter, ignite over a Bunsen burner, and weigh as barium sulphate. From the weight of $BaSO_4$ report the per cent of SO_3.

6. Potassium and Sodium.

Treat the filtrate from the sulphuric acid determination (5) with ammonium hydroxid exactly as in (1), page 6. Evaporate the filtrate and washings to dryness, heat below redness until ammonium salts are expelled, dissolve in hot water, add 5 cc of barium hydroxid solution, and heat to boiling; let settle for a few minutes, and test a little of the clear liquid with more barium hydroxid solution to be sure that enough has been added. When no further precipitate is produced, filter and wash thoroughly with hot water. Heat the filtrate to boiling, add ammonium hydroxid and ammonium carbonate to complete precipitation of the barium, calcium, etc., let stand a short time on the water bath, filter, and wash the precipitate thoroughly with hot water; evaporate filtrate and washings to complete dryness, expel ammonium salts by heat below redness, take up with a little hot water, add a few drops of ammonium hydroxid, a drop or two of ammonium carbonate, and a few drops of ammonium oxalate; let stand a few minutes on the water bath, set aside for a few hours, filter, evaporate to complete dryness on the water bath, and heat to dull redness until all ammonium salts are expelled and the residue is nearly or quite white. Dissolve in a minimum amount of water, filter into a tared platinum dish, add a few drops of hydrochloric acid, evaporate to dryness on the water bath, heat to dull redness, cool in a desiccator, and weigh as potassium and

sodium chlorids. Repeat heating until constant weight is obtained. Dissolve in a small amount of water; if any residue remains, the separation must be repeated until the residue of potassium and sodium chlorids is entirely soluble.

Dissolve the residue with water, add an excess of platinum chlorid solution (see instructor) and determine K_2O by the method given for fertilizers, page 28, carefully avoiding presence of NH_3 fumes in the process.

From the per cent of K_2O calculate the weight of KCl, deduct from the weight of KCl and NaCl recorded above, and calculate the per cent of Na_2O.

Humus Determination.

Place 10 grams of the air dry soil (5 grams in case of peat soil) in a gooch crucible, extract with 1 per cent hydrochloric acid until the filtrate gives no precipitate with ammonium hydroxid and ammonium oxalate, and remove the acid by washing with water. In the case of clay soil the washing should be done chiefly by decantation from a cylinder or tall beaker. This avoids clogging of the filter. Wash the contents of the crucible (including the asbestos filter) into a *glass-stoppered* cylinder, with 500 cc of 4 per cent ammonium hydroxid, and allow to remain, with occasional shaking, for 24 hours. During this time the cylinder is inclined as much as possible without bringing the contents in contact with the stopper, thus allowing the soil to settle on the side of the cylinder and exposing a very large surface to the action of the ammonium hydroxid. Place the cylinder in a vertical position and leave for 12 hours, to allow the sediment to settle.

Draw off 300 c. c. of the supernatant liquid with a pipette, without stirring of the sediment, place in a stoppered 500 c. c. flask and let stand for 48 hours. Carefully pipetteoff 200 c. c. of the liquid, now rid of a large part of the clay, into a 250 c. c. beaker, evaporate on the steam bath and let the residue heat on the bath for two hours. Dissolve the humus out with 200 c. c. of 4 per cent ammonium hydroxid and filter through paper to separate flocculated clay. Evaporate 50 c. c. aliquots, dry at 100° C., and weigh. Then ignite the residue and again weigh. Calculate the humus from the difference in weights between the dried and ignited residues. Report as per cent of the *dry* soil.

Carbon Dioxide Determination.

Make the determination by the absorption method, checking the accuracy of the apparatus by means of pure calcite in 0.5 gram samples.

Place 10 grams of the air-dry soil (5 grams, in case of soils rich in limestone) in the distilling flask of the apparatus. This flask should be fitted with a dropping-funnel and a delivery arm leading into an inverted condenser. The absorption train should consist of a sulfuric-acid absorption bulb, a bulb of 40 per cent KOH solution, a second sulfuric-acid bulb and a soda-lime tube, arranged in successive order from the condenser. The potash and second sulphuric-acid bulbs are weighed at the beginning of the experiment.

Having the apparatus carefully fitted, add 20 c. c. of 20 per cent HCl

(sp. gr. 1.1) to the soil from the dropping funnel and close the stopper. Heat gently so that bubbles do not pass through the absorption bulbs at a greater rate than two per second. Continue boiling for a few minutes after water appears in the condenser. The dropping funnel should now be fitted with a soda-lime tube, the valve of the funnel opened, and air gently aspirated through the system until the apparatus is cool. Remove the bulbs previously weighed and weigh again. Report the increase in weight as per cent of CO_2 on the *dry* soil.

Total Phosphorus Determination.

SODIUM PEROXID FUSION METHOD.

Weigh 10 grams of sodium peroxid into an iron or porcelain crucible and thoroughly mix with it 5 grams of the soil. If the soil is very low in organic matter, add a little starch to hasten the action. Heat the mixture carefully by applying the flame of a Bunsen burner directly upon the surface of the charge and the sides of the crucible until the action starts. Cover crucible until reaction is over and keep at a low red heat for fifteen minutes. Do not allow fusion to take place. By means of a large funnel and a stream of hot water, transfer the charge to a 500 cc measuring flask. Acidify with hydrochloric acid and boil. Let cool and make up to the mark. If the action has taken place properly there should be no particles of undecomposed soil in the bottom of the flask. Allow the silica to settle and draw off 200 cc of the clear solution.

Precipitate the iron, alumina, and phosphorus with ammonium hydroxid; filter, wash several times with hot water, return the precipitate to the beaker with a stream of hot water, holding the funnel over the beaker, and dissolve the precipitate in hot hydrochloric acid, pouring the acid upon the filter to dissolve any precipitate remaining. Evaporate the solution and washings to complete dryness on a water bath. Take up with dilute hydrochloric acid, heating if necessary, and filter out the silica. Evaporate filtrate and washings to about 10 cc, add 2 cc of concentrated nitric acid, and just neutralize with ammonium hydroxid. Clear up with nitric acid, avoiding an excess. Heat at 40° to 50° on water bath, add 15 cc of molybdic solution, keeping at this temperature for from one to two hours. Let stand over night, filter, and wash free of acid with a one-tenth per cent solution of ammonium nitrate and, finally, once or twice with cold water. Transfer filter to beaker, and dissolve in standard potassium hydroxid (see p. 21, 1 b.), titrate the excess of potassium hydroxid with standard nitric acid, using phenolphthalein as indicator. Report as total per cent P_2O_5 on dry soil.

Total Sulfur Determination.

FUSION WITH SODIUM PEROXIDE.

Ten grams of soil are placed in a 100 cc nickel crucible, moistened with water, about ten grams of a weighed twenty grams portion of sodium peroxide added, and the mixture thoroughly stirred with a

platinum rod. The crucible is placed over an alcohol flame and heated moderately until the mass is dry. The remainder of the sodium peroxide is then added, the cover placed on the crucible, strong heat applied until the mass melts, and kept in this condition for ten minutes. It is then allowed to stand over a lower flame for one hour.

The crucible is removed, cooled, placed in a 600 cc casserole, hot water added and the fused mass removed. It is neutralized with hydrochloric acid and then further acidified with 10 cc of hydrochloric acid. The volume is made up to about 450 cc and boiled for 15 minutes, or until no undecomposed soil particles remain on the bottom. The covered casserole is allowed to stand in the steam bath over night, filtered through a Nutche and the residue thoroughly washed with successive small portions of hot water. The filtrate and washings if over 500 cc are evaporated below that volume, refiltered and the volume made up to 500 cc. Aliquots of 250 cc each are heated to boiling, barium chloride added, boiled for five minutes and set aside on a steam bath for 24 hours. The volume should not be allowed to decrease as silicic acid may precipitate out if much evaporation takes place. After standing for this length of time the barium sulphate is filtered off, washed, ignited and weighed. Report as total per cent SO_3.

Total Nitrogen Determination.

Place 10 grams of the soil (3 grams in case of a peaty soil) in a 300 c. c. Kjeldahl digesting flask with 30 c. c. of strongest sulfuric acid and 0.7 grams of mercuric oxide and boil for 3 hours, or longer in the case of peats, etc. Oxidize the residue by adding potassium permanganate in small amounts (with stirring) until the liquid retains a greenish color. After cooling, add 50 c. c. of water, shake vigorously, allow the heavy matter to settle and pour the supernatant liquid into an 800 c. c. Kjeldahl flask. Repeat this process with four 50 c. c. portions of water. Conduct the distillation and remaining determination according to the Kjeldahl method (p. 23) for nitrogen in fertilizers and report the *per cent of nitrogen on the dry soil.*

Nitrification Experiment.

To 100 c. c. of an exactly N/20 solution of ammonium sulfate, add about 0.3 gram of KH_2PO_4, 0.5 gm. $CaCO_3$ and 2 gms. of rich garden soil. Mix well and *calculate* the milligrams of nitrogen in this mixture, neglecting the traces which may be added in the soil.

Fill a percolator ¼ full with washed (use water freed from ammonia by boiling off 1-3 its volume) and dried sand. Upon this pour the 100 cc of nutrient solution and place the percolator in the desk for 3 weeks. Do not let the sand become dry (nor absorb ammonia or nitric acid) during this time. At the end of this time percolate with 500 c. c. or more of distilled water in 100 c. c. portions, make to one liter and determine the nitrogen in 10 c. c. by the colorimetric method for nitrates in soil extracts (page 13). From the parts per million of nitrogen in this extract calculate the milligrams of nitrogen as nitrate re-

covered from the percolator and express as per cent nitrification on the Nutrient Solution as follows:

Milligrams N in Nutrient Solution =
" N " 10 c. c. percolate =
" N " total " =
Per cent Nitrification = ...

State the purpose of the following substances used in the above experiment: Potassium phosphate, garden soil and calcium carbonate.

Explain what has happened in the course of this experiment.

Of what compound has the nitrified nitrogen become a constituent? Write equations for the reactions in which NH_3 and $CaCO_3$ are involved.

Determination of Nitric Acid (Nitrates) in Soils.

a. Preparation of sample.

The moist sample from the field is well broken up and mixed and two 100-gram samples drawn from it. These are dried in the usual manner for a moisture determination in duplicate. At the same time a 250-gram sample of the moist soil is transferred to a tight, 1-quart Mason jar with 500 c. c. of distilled water and shaken at intervals for three minutes. Allow to settle for 20 minutes and then decant into the chamber of a pressure apparatus fitted with a well-washed Pasteur-Chamberland filter tube. Filter under pressure, rejecting the first fifty c. c. of filtrate.

In preparing the soil extract and reagents and throughout the manipulation of this determination the presence of nitric acid fumes must be carefully avoided.

Chlorides when present in considerable quantities interfere quite markedly with the determination of nitrates and must be previously removed. This is best accomplished by means of silver sulphate free from nitrates. This can be added in the solid form, thus avoiding dilution of the original solution. The silver sulphate is tested for nitrates by treating some of the solid salt with the phenoldisulphonic acid reagent, diluting with water and adding ammonia water. No yellow color should be produced. The silver sulphate as found in the market frequently contains nitrates in amounts sufficient to vitiate all results, and it is therefore advisable to prepare it specially for this work.

The presence of some kinds of organic matter also interferes seriously with the determination of nitrates by this method. In some cases it is the foreign color only which is produced by the strong acid, but often the action is of more vital importance, as a considerable loss of nitrates occurs, possibly due to oxidation of the organic matter by the nitrate instead of nitration of the phenoldisulphonic acid. In some cases it is advisable to reduce the nitrates to ammonia by means of the copper-zinc couple. The ammonia is distilled off and determined colorimetrically. The ammonia originally present in the solution must be determined separately and deducted. Nitrates are likewise reduced to ammonia and must be taken into consideration, if present.

b. Preparation of Reagents.

1. Phenoldisulphonic acid reagent.—This is prepared by mixing 3 grams of pure crystallized phenol with 37 grams (20.1 c. c.) of concentrated sulphuric acid (sp. gr. 1.84) and heating for six hours at 100° C. by setting the lightly-stoppered flask in boiling water.

The acid thus prepared may crystallize out on standing, especially during the cold season. It may be brought into solution by heat, but the addition of water to effect solution is to be avoided.

2. Ammonium hydroxide.—Dilute strong ammonium hydroxide solution (sp. gr. 0.9) with an equal volume of water.

3. Standard nitrate solution.—Dissolve 0.1631 gram of pure, dry potassium nitrate in water and make up to 1 liter. Of this stronger solution 100 cc are diluted to 1 liter. This constitutes the standard nitrate solution and contains 0.01 of a milligram NO_3 in each cubic centimeter.

4. Standard colorimetric solution.—Evaporate 10 c. c. of the standard nitrate solution (3) to dryness in a porcelain dish on a water or steam bath and treat as described under "Analytical Process" below, finally diluting the solution to 100 c. c. This standard colorimetric solution has the strength of 1 part of NO_3 per million.

c. Analytical Process.

Evaporate 50 c. c. of the filtered soil extract to dryness in a porcelain dish on a water bath, removing the dish as soon as it is completely dry. Add 1 cc of the phenoldisulphonic acid reagent (1) and stir thoroughly with the rounded end of a glass rod so as to loosen the residue and bring the acid well in contact with every portion of it. The time of action on the nitrate should be about 10 minutes. At the end of this time the acid is diluted with about 15 cc of water and made alkaline with ammonium hydroxide (2), a yellow color being developed when the solution becomes alkaline. This is then diluted to 50 c. c. and compared with the standard colorimetric solution by means of a colorimeter. If the color is too intense for direct comparison with this standard, an aliquot portion may be taken and diluted to definite volume and the strength of this determined. The result should be expressed as *parts per million* of the *dry* soil.

II. FERTILIZERS OR MANURES.

Fertilizers or manures are substances which, when added to the soil, either increase the supply of such elements as may be lacking for the growth of crops, or otherwise bring about changes favorable to their growth. These substances when furnishing food in themselves are termed direct fertilizers; when acting in other ways, they are classed as indirect fertilizers. In analytical work we shall deal mostly with direct fertilizers.

The soil elements most drawn upon by the plant and so most frequently exhausted are *Nitrogen*, *Phosphorus*, and *Potassium;* consequently, our commercial fertilizers contain compounds of these elements either singly or in mixtures, a mixture of all three being termed a "complete fertilizer." Farmyard manure is a natural, complete fertilizer.

A great variety of materials is used in the production of commercial fertilizers, many industries turning their by-products to account in this direction.

To a basal or "filling" material, nitrogen may be added in the form of chili saltpetre (sodium-nitrate) from deposits in arid regions, potassium as sulphate or chlorid from the Stassfurt salt deposits of Central Germany, and phosphorus in the form of apatite from Canada, or the rock and pebble ("river") phosphates from our southeastern states. The packing houses supply nitrogenous materials in the form of green-bone, tankage, meat scraps, and dried blood, these materials yielding from 2% up to 12% or 14% of nitrogen in organic forms. Hair, horn, and hoof, which are sometimes worked into fertilizers, contain much nitrogen, but decompose so slowly as to be of slight value as available forms of that element. Bones contain phosphorus equivalent to from 20 to 25% P_2O_5 and, like the rock phosphates, are mostly converted to acid-phosphates for the fertilizer trade. The slag and waste lime lining of furnaces used for removing phosphorus from iron in the Bessemer steel process (the so-called "Thomas Basic Slag") contains as high as 18% of P_2O_5 as a calcium-phosphorus-silicon complex soluble in ammonium citrate solution. The oil-seed industries furnish residues rich in phosphorus as well as nitrogen.

Until the discovery of the Stassfurt salts, wood ashes (containing 5-15% K_2CO_3) were the main source of potash, and potash still has its highest market value in the form of carbonate. The threatened early exhaustion of the Chilean nitrate beds has given impetus to the search for methods to "fix" atmospheric nitrogen with the result that "lime-nitrogen" (calcium cyanamide) and "calc-nitre" (calcium-nitrate) are becoming economic fertilizers carrying about 20% and 10% of nitrogen respectively.

AVAILABILITY.

The value of a fertilizer is usually estimated by the solubility or apparent availability of its constituents. In ammonium salts, nitrates and cyanamide we have soluble, active sources of nitrogen, while the more slowly decomposing organic forms represent varying degrees of availability. Potassium is usually found in available forms, nearly all of its salts being freely soluble in water. The organic compounds in which it occurs are apparently readily decomposed in the soil and hence also available. With phosphoric acid there are three degrees of solubility as expressed in the following table:

Total P_2O_5	Available P_2O_5	*Soluble* — Sol. in H_2O, dil. acids, etc.
		Reverted — Insol. in water. Sol. in dil. acids, salt solutions, etc.
	Unavailable P_2O_5	Sol. only in the strongest acids.

When the form insoluble in acids is converted to the form soluble in water this tends to become insoluble again (reverts) and we have a form no longer soluble in water, but soluble in saline reagents and supposedly still available to the plant. The following equations illustrate the production of acid phosphate and the process of reversion:

$$\underset{\textit{Insol.}}{Ca_3(PO_4)_2} + 2H_2SO_4 = \underset{\textit{Sol. in water}}{CaH_4(PO_4)_2} + 2CaSO_4$$

$$CaH_4(PO_4)_2 + Ca_3(PO_4)_2 = \underset{\textit{Reverted}}{2Ca_2H_2(PO_4)_2}$$

ANALYTICAL METHODS.

Three methods have found general application for the determination of nitrogen in organic matter.

The "Absolute Method" of Dumas has been used longest, being applicable to nitrogen in any form and consisting in complete combustion of the substance and reduction of the oxides so as to measure the element volumetrically in the gaseous state. Varentrapp and Will introduced a method based on the conversion of nitrogen to ammonia by means of soda-lime mixture and absorption of the ammonia in standard acid. The "moist combustion" process which Kjeldahl developed is the most rapid and convenient for this purpose.

In this process sulphur trioxide is the oxidizing agent, produced by heating sulphuric acid. Addition of potassium sulphate raises the temperature of digestion and promotes formation of SO_3. Compounds of mercury or copper, substances easily oxidized and reduced, facilitate the process as "carriers" of oxygen. With the various modifications

proposed by Gunning (mercury in digestion and potassium sulfid in distilling), Dyer, Jodlbauer (salicylic mixture for digestion) and others, this method has become generally applicable. The most important modificaton was that which insured the fixation of NO_3 by salicylic acid and reduction from this to ammonia by sodium-thiosulphate or zinc dust. Without this precaution nitrogen of nitrates would be lost in the process of digestion. The total nitrogen when converted to ammonia is fixed by the acid of the digesting liquor, from which it is finally freed by fixed alkali and distilled into standard acid solution. Since mercury and ammonia form double compounds not readily decomposed by potassium or sodium hydroxide, the mercury must be precipitated by potassium sulfid before distillation, in order to render certain the complete liberation of ammonia.

Nitrogen of ammonium salts may be estimated by direct distillation. Magnesia should be used as the liberating agent when organic matter is present, as caustic alkalies produce NH_3 from organic nitrogenous compounds. (See prelim. test No. 6 on soils.)

To estimate nitrogen from NO_3 compounds alone, reduction and distillation may be completed in one operation, caustic-soda and zinc forming the agent for reducing NO_3 to NH_3, the latter being expelled from the alkaline solution as rapidly as it forms. Reduction and distillation may also be conducted separately, employing an acid medium for the former process, which is much more rapid under these conditions.

In the determination of phosphoric acid, the yellow precipitate of ammonium-phospho-molybdate furnishes a ready means of separation from bases. They remain dissolved in the strong nitric acid of the molybdic solution. This precipitate is subject to slight variation in composition and although a volumetric method for estimating phosphorus has been based on its solubility in alkalies, it requires careful manipulation. The general procedure is to precipitate from ammoniacal solution by magnesia mixture. This forms magnesium-ammonium-phosphate, a body of definite composition.

The method for estimating potassium as finally perfected by Gladding (Lindo-Gladding Method) depends upon its conversion to sulphate from which ammonium salts can be separated by volatilization and foreign organic matter by incineration. The potassium is isolated as the platinic-chloride salt by washing out magnesium and calcium salts with ammonium chloride and sodium salts with 80% alcohol.

These are the general principles of the methods. Experience will prove that they cannot be carried out with the same facility on all sorts of fertilizers and fertilizer materials. The analyst soon finds, as in treating other agricultural materials, that various modifications and precautions must be introduced into the methods and that the procedure must be varied continually to suit the sample at hand.

PRELIMINARY TESTS.

1. Volatilization of Ammonium Salts.

Place about 0.1 gram each of ammonium carbonate and ammonium sulphate in separate test tubes. Apply gentle heat to each. Observe the sublimate which forms in the cool part of the tube. Do you observe any odor given off? What is it due to? What is the sublimate? Notice the liquid which collects on the walls of the tube in one case. What is it?

Ammonium carbonate is formed from urea by the addition of water. $(NH_2)_2CO + 2H_2O = (NH_4)_2CO_3$. Urea is the chief nitrogenous compound of urine. Fermentations of the manure pile, especially of the drier horse manure, produce ammonium carbonate and the heat produced by other reactions decomposes it to CO_2, H_2O and NH_3. How can loss of nitrogen by volatilization be prevented?

2. Fermentation of Manure.

Procure a sample of *fresh* horse manure. Place portions of the manure in two jars (1 quart or larger) filling one rather loosely and thoroughly wetting and compacting the contents of the other. Maintain these conditions in the samples, covering the jars loosely and suspending moist red litmus paper above the manure. The litmus paper should be kept moist. Examine frequently for bluing of the litmus or the appearance of odor and record your observations. What methods does this experiment suggest by which to preserve the nitrogen of manure heaps from losses by bacterial action?

3. Solubility of Phosphoric Acid in Bone-ash.

Transfer 0.5 grams of bone-ash to a test tube with 20 c. c. H_2O and heat. Filter and add 5 c. c. molybdic solution to the filtrate. Observe the result on standing. To what is it due? Save this test.

Repeat the test substituting dilute HNO_3 for the H_2O. What do you conclude from the result?

4. Preparation of Acid-phosphate (Superphosphate).

Place 100 grams of bone-ash in a 6-inch porcelain dish. Add slowly, and with constant stirring, 80 grams of strongest sulfuric acid, using a heavy glass rod for the purpose. The mixing should be done at the hood. Transfer the mixture to a wooden box, label it and set aside for about three days.

Then test 0.5 gram for water-soluble phosphates as in the case of bone-ash (Expt. 3) but without heating. Compare the precipitate with that obtained from the latter. (Save the tests and the acid-phosphate.) Describe your observations fully.

What is the irritating gas liberated in this experiment? Write the equation of reaction between the chief constituent of bone-ash and the sulfuric acid.

5. Solubility of Di-Calcium (Reverted) Phosphate.

Test 0.5 gram of di-calcium phosphate for solubility in water as in the case of acid-phosphate and compare the result with the corresponding extracts from bone-ash and acid-phosphate.

Compare the readiness with which small particles of powdered di-calcium phosphate are dissolved in 5 c. c. water and in 5 c. c. ammonium-citrate solution (sp. gr. 1.09) when the solvents are just hot to the touch.

What solvent in the soil might assume the power of the citrate solution observed here? Why could molybdic solution not be used for testing the citrate solution for phosphoric acid?

6. "Availability" of Nitrogen in Organic Forms.

Prepare a pepsin solution by dissolving 5 grams of commercial pepsin in a liter of water and adding 1 c. c. of strongest HCl. Place 0.5 gram samples each of ground leather, dried blood and bone-ash in separate 200 c. c. Erlenmeyer flasks. (Horn or hoof may be substituted for leather.) Add 100 c. c. of pepsin solution to each flask and incubate about 24 hours with occasional stirring. At the end of this time, observe the comparative amounts of insoluble matter remaining in the flasks and also the appearance of the solution in each case. Record your observations carefully.

What is the source of pepsin and what is its function there? Do soils or crops contain any substances related to it? How does solubility in pepsin extract indicate the value of the above materials as fertilizers? What do you conclude as to the relations of enzymes to inorganic sources of plant food?

7. Preparation of a Fertilizer.

Thoroughly mix in a box or porcelain dish the following substances:

20 grams acid-phosphate (from Exp. 4).
5 " kainit.
2 " sodium nitrate.
3 " dried blood.

Assuming the substances to be pure, calculate the percentage composition of this fertilizer and its trade value per ton, with N at 19c in blood and 17c in nitrates, P_2O_5 at 4c and K_2O at 4.5c per pound.

8. Water Soluble Materials in a Commercial Fertilizer.

Place two grams of a dry sample of commercial fertilizer (specially provided) in a filter paper previously fitted to a funnel. Pass 500 c. c. of distilled water through the sample in small portions at a time, saving the entire filtrate. Evaporate the filtrate to dryness in a weighed porcelain dish, dry for at least two hours in the steam oven, cool and weigh. Report the per cent of material dissolved. From 40 to 80 per cent of a high-grade, complete fertilizer will dissolve in water. What is the nature of the insoluble residue in your test?

METHODS FOR THE ANALYSIS OF FERTILIZERS.

1. Preparation of Sample.

If sampled from a large bulk of material, several small portions should be taken from different parts of the mass, thoroughly mixed and subsampled. The final sample should be well intermixed, finely ground, and passed through a sieve having circular perforations 1 mm in diameter. The grinding and sifting should be performed as rapidly as possible, to avoid loss or gain of moisture during the operation, and the sample should be stored immediately in a tightly stoppered jar.

2. Determination of Moisture.

In potash salts, sodium nitrate and ammonium sulphate heat 2 grams at 130° until the weight is constant. The loss in weight is considered as moisture. In all other fertilizers heat 2 grams for 5 hours at 100° in a steam oven.

3. Determination of Phosphoric Acid.

GRAVIMETRIC METHOD.

(1) Preparation of Reagents.

(a) Ammonium citrate solution.—Dissolve 370 grams of commercial citric acid in 1,500 cc of water; nearly neutralize with commercial ammonium hydroxide; cool; add ammonium hydroxide until exactly neutral (testing with saturated alcoholic solution of corallin), and dilute to a volume of 2 liters. Determine the specific gravity, which should be 1.09 at 20°.

(b) Molybdate solution.—Dissolve 100 grams of molybdic acid in 144 cc of ammonium hydroxid, specific gravity 0.90, and 271 cc of water; slowly and with constant stirring, pour the solution thus obtained into 489 cc of nitric acid (specific gravity 1.42), and 1,148 cc of water. Keep the mixture in a warm place for several days, or until a portion heated to 40° C. deposits no yellow precipitate of ammonium phosphomolybdate. Decant the solution from any sediment and preserve in glass-stoppered vessels.

(c) Magnesia mixture.—Dissolve 22 grams of recently ignited calcined magnesia in dilute hydrochloric acid, avoiding an excess of the latter. Add a little calcined magnesia in excess, and boil a few minutes to precipitate iron, alumina, and phosphoric acid; filter; add 280 grams of ammonium chlorid, 261 cc of ammonium hydroxid of specific gravity 0.90, and water enough to make the volume 2 liters. Instead of the solution of 22 grams of calcined magnesia, 110 grams of crystallized magnesium chlorid ($MgCl_2 6H_2O$) may be used.

(d) Dilute ammonium hydroxid for washing.—This solution should contain 2.5 per cent of ammonia (NH_3). Strongest NH_4OH contains about 28.0 per cent NH_3.

(e) Magnesium nitrate solution.—Dissolve 320 grams of calcined magnesia in nitric acid, avoiding an excess of the latter; then add a little calcined magnesia in excess; boil; filter from the excess of magnesia, ferric oxid, etc., and dilute with water to 2 liters.

(2) *Total Phosphoric Acid.*

(*a*) *Methods of making solution.*—Treat 2 grams of the sample by one of the methods given below. After solution, cool, dilute to 250 cc, mix, and pour on a dry filter.

(a_1) Ignite and dissolve in hydrochloric acid. This method is recommended for samples rich in organic matter.

(a_2) Evaporate with 5 cc of magnesium nitrate, ignite, and dissolve in hydrochloric acid.

(a_3) Boil with from 20 to 30 cc of strong sulphuric acid, adding from 2 to 4 grams of sodium or potassium nitrate at the beginning of the digestion and a small quantity after the solution has become nearly colorless, or adding the nitrate in small portions from time to time. A Kjeldahl flask marked at 250 cc is recommended. After the solution is colorless add 150 cc of water and boil for a few minutes, cool, and make up to mark.

(a_4) Dissolve in 30 cc of concentrated nitric acid and a small quantity of hydrochloric acid and boil until organic matter is destroyed.

(a_5) Dissolve in from 15 to 30 cc of strong hydrochloric acid and from 3 to 10 cc of nitric acid. This method is recommended for fertilizers containing much iron or aluminum phosphate.

(*b*) *Determination.*—Obtain from an instructor the approximate per cent of P_2O_5 in your sample. Take an aliquot portion of the solution prepared above corresponding to 0.4 gram, neutralize with ammonium hydroxid, and clear with a few drops of nitric acid. In case hydrochloric or sulphuric acid has been used as solvent, add about 15 grams of dry ammonium nitrate. To the hot solution add 50 cc of molybdate solution for every decigram of phosphoric acid (P_2O_5) that is present. Digest at about 65° C. for an hour, filter, and wash with cold water or, preferably, ammonium nitrate solution. Test the filtrate for phosphoric acid by renewed digestion and addition of more molybdate solution. Dissolve the precipitate on the filter with ammonium hydroxid and hot water and wash into a beaker to a bulk of not more than 100 cc. Nearly neutralize with hydrochloric acid, cool, and add magnesia mixture from a burette; add slowly, stirring vigorously. After fifteen minutes add 12 cc of strongest ammonium hydroxid solution (specific gravity 0.90). Let stand for some time—two hours is usually enough; filter, wash with 2.5 per cent ammonia (NH_3) until practically free from chlorids; ignite to whiteness or to a grayish white and weigh as $Mg_2P_2O_7$. Calculate and report the per cent of P_2O_5.

The yellow precipitate of this determination may be washed with a solution of about 5 per cent nitric acid. It should be washed finally well into the point of the filter. Small amounts of precipitate clinging to the beaker need not be transferred to the filter if thoroughly rinsed. Solution of the precipitate is readily accomplished by two or three applications of a few cubic centimeters of strong NH_4OH followed immediately by additions of hot water. The solution is received in the same beaker used for the original precipitation. If the magnesium precipitate stands for a long time in ammoniacal solution, more conc. NH_4OH should be

added some time before filtering to throw out phosphoric acid which may have redissolved in the weakened alkaline liquor.

(3) *Water-soluble Phosphoric Acid.*

Place 2 grams of the sample on a 9-cm filter, wash with successive small portions of water, allowing each portion to pass through before adding more, until the filtrate measures nearly 250 cc. If the filtrate be turbid, add a little nitric acid. If much organic matter is in solution, evaporate to 25 or 30 c. c., add 5 to 10 c. c. conc. HNO_3 and boil until clear. Make up to any convenient definite volume, mix well, use an aliquot, and proceed as under total phosphoric acid (b).

(4) *Citrate-insoluble Phosphoric Acid.*

Heat 100 cc of strictly neutral ammonium citrate solution of 1.09 specific gravity to 65° C. in a 200 cc Erlenmeyer flask placed in a warm-water bath, keeping the flask loosely stoppered to prevent evaporation. When the citrate solution in the flask has reached 65° C., drop into it the filter containing the washed residue from the water-soluble phosphoric acid determination close tightly with a smooth rubber stopper, and shake vigorously (bracing the bottom of the flask) until the filter paper is reduced to a pulp. Place the flask in the bath and maintain it at such a temperature that the contents of the flask will stand at exactly 65° C. Shake the flask every five minutes, uncorked at first so as to liberate excess of steam. At the expiration of exactly thirty minutes from the time the filter and residue are introduced remove the flask from the bath and immediately filter the contents as rapidly as possible on a 15 cm filter. Wash thoroughly with water at 65° C. Transfer the filter and its contents to a crucible, ignite until all organic matter is destroyed, add from 10 to 15 cc of strong hydrochloric acid, and digest until all phosphate is dissolved. Dilute the solution to 250 cc. Mix well, filter through a dry filter; take a definite portion of the filtrate and proceed as under total phosphoric acid (b).

(5) *Citrate Soluble Phosphoric Acid.*

The sum of the water-soluble and citrate-insoluble subtracted from the total gives the citrate soluble phosphoric acid. Add this to the water-soluble and report as per cent of *available* P_2O_5.

VOLUMETRIC METHOD.

1. Preparation of reagents.

(*a*) *Molybdate solution.*—To 100 c. c. of molybdate solution, prepared as directed on page 19, add 5 c. c. of nitric acid, sp. gr. 1.42. This solution should be filtered each time before using.

(*b*) *Standard solution of sodium or potassium hydroxid.*—Dilute 323.81 c. c. of normal alkali, which has been freed from carbonates to 1 liter. One hundred cubic centimeters of the solution should neutralize

32.38 c. c. of normal acid; 1 c. c. is equal to 1 milligram of P_2O_5 (1 per cent P_2O_5 on a basis of 0.1 gram of substance).

(*c*) *Standard acid solution.*—The strength of this solution is the same as, or one-half of, the standard alkali solution, and is determined by titrating against that solution, using phenolphthalein as indicator. Any mineral acid may be used.

(*d*) *Phenolphthalein solution.*—Dissolve one gram of phenolphthalein in 10 c. c. of 95% alcohol.

2. Total Phosphoric Acid.

(*a*) *Methods of making solution.*—

Dissolve according to methods (a_2), (a_4), or (a_5) used in the gravimetric method. Method (a_4) is preferred when these acids form a suitable solvent. Dilute to 250 c. c. with water.

(*b*) *Determination.*

For percentages of 5 or below use an aliquot corresponding to 0.4 gram of substance, for percentages between 5 and 20 use an aliquot corresponding to 0.2 gram substance, and for percentages above 20 use an aliquot corresponding to 0.1 gram substance. Add from 5 to 10 cc of nitric acid, depending on the method of solution (or the equivalent in ammonium nitrate), nearly neutralize with ammonia, dilute to 100 cc, heat in water bath to 65°, and for percentages below 5 add from 20 to 25 cc of freshly filtered molybdate solution. For percentages between 5 and 20 add from 30 to 35 cc molybdate solution; stir, let stand about 15 minutes, filter *at once*, wash once or twice with water by decantation, using from 25 to 30 cc each time, agitating the precipitate thoroughly and allowing to settle; transfer to filter and wash with cold water until two fillings of the filter do not greatly diminish the color produced with phenolphthalein by one drop of the standard alkali. Transfer the precipitate and filter to a beaker, dissolve in small excess of standard alkali, add a few drops of phenolphthalein solution, and titrate with standard acid. From the cubic centimeters of standard alkali neutralized by the precipitate calculate the per cent of *total* P_2O_5.

For the reactions involved in this determination see "Pemberton's Volumetric Method" in Wiley's "Fertilizers and Insecticides."

3. Water-soluble Phosphoric Acid.

Treat the sample according to the directions given for the corresponding determination under "Gravimetric method."

To an aliquot portion of the solution corresponding to 0.4 gram of fertilizer, add 10 c. c. of conc. nitric acid. Add ammonium hydroxid until a slight permanent precipitate is formed, bring to a volume of about 60 c. c. and proceed as with the solution for total P_2O_5.

4. Citrate-insoluble Phosphoric Acid.

Make the solution according to the method used in Gravimetric analysis and determine the phosphoric acid in an aliquot corresponding to 0.4 gram, as directed for total P_2O_5 by the volumetric method.

5. Citrate-soluble Phosphoric Acid.

This is calculated as in the Gravimetric method.

4. Determination of Nitrogen.

1. TESTING FOR THE PRESENCE OF NITRATES.

Mix 5 grams of the fertilizer with 25 cc of hot water and filter. To a portion of this solution add two volumes of concentrated sulphuric acid, free from nitric acid and oxids of nitrogen, and allow the mixture to cool. Add cautiously a few drops of a concentrated solution of ferrous sulphate, so that the fluids do not mix. If nitrates are present the junction shows at first a purple, afterwards a brown color, or if only a very minute quantity be present, a reddish color. To another portion of the solution add 1 c. c. of a dilute solution of nitrate of soda (3 grams to 300 c. c.) and test as before to determine whether sufficient sulphuric acid was added in the first test.

a. Modified Kjeldahl Method.

(*This method is to be used when nitrates are present.*)

1. Preparation of Reagents.

(*a*) *Standard acid solution.*

Hydrochloric acid, the absolute strength of which has been determined by precipitation from an aliquot portion of the solution and weighing as AgCl, or

Sulphuric acid, the absolute strength of which has been determined by precipitation and weighing as $BaSO_4$, or by evaporation of an aliquot portion with excess of ammonium hydroxid, drying and weighing as $(NH_4)_2SO_4$.

It is recommended that these solutions be used in tenth normal strength, but they may be employed with use of an N/10 factor.

(*b*) *Standard alkali solution.*—The strength of this solution relative to the acid used must be accurately determined. An approximately N/10 solution of NaOH is recommended.

(*c*) *Sulfuric acid for digestion.*—This should have a sp. gr. of 1.84 and be free from nitrates and also from ammonium sulphate.

(*d*) *Metallic mercury, or mercuric oxide.*—If the latter is used it should be prepared in the wet way, but not from mercuric nitrate.

(*e*) *Potassium permanganate.*—This reagent should be finely pulverized.

(*f*) *Granulated zinc or pumice stone.*—One of these reagents is added to the contents of the distillation flasks, when found necessary, in order to prevent bumping.

(*g*) *Potassium sulphid solution.*—A solution of 40 grams commercial potassium sulphid in 1 liter of water.

(*h*) *Sodium hydroxid solution.*—A saturated solution of sodium hydroxide free from nitrates.

(*i*) *Indicator.*—A solution of cochineal is prepared by digesting and frequently agitating 3 grams of pulverized cochineal in a mixture of 50 cc strong alcohol and 200 cc of distilled water for a day or two at ordinary temperatures. The filtered solution is employed as indicator and should be used when NH_4OH is used in the titration. The phenolphthalein used in the P_2O_5 determination may be used here provided the distillate is free from CO_2. The latter may be driven off by boiling for a few minutes, and the distillate should then be cooled.

(*j*) *Zinc dust.*

(*k*) *Sodium thiosulphate.*

(*l*) *Commercial salicylic acid.*

2. *Apparatus.*

(*a*) *Kjeldahl flasks for both digestion and distillation.*—These are pear-shaped flasks of about 800 c. c. capacity. They should be of moderately thick Jena glass, which permits use over a naked flame in the digesting hood. For distillation, the flasks are fitted with rubber stoppers and short-armed bulb-tubes bent so as to connect with the tubes of the condensers by means of collars fitted from rubber tubing. The bulb tube prevents sodium hydrate being carried over mechanically during distillation.

(*b*) *Receiving flasks.*—These should be strong flasks or bottles of clear, colorless glass, having a capacity of about 500 c. c. They should be so arranged at the still that the delivery tubes of the condensers will dip below the surface of the standard acid which they contain.

(3) DETERMINATION.

Place 2 grams of the substance to be analyzed in a Kjeldahl flask, add 30 cc of sulphuric acid containing 1 gram of salicylic acid, and shake until thoroughly mixed, then add 5 grams of crystallized sodium thiosulphate; or add to the substance 30 cc of sulphuric acid containing 2 grams of salicylic acid, then add gradually 2 grams of zinc dust, shaking the contents of the flask at the same time. Finally, place the flask on the stand for holding the digestion flasks, where it is heated over a low flame until all danger from frothing has passed. The heat is then raised until the acid boils briskly and the boiling continued until white fumes no longer escape from the flask. This requires about five or ten minutes. Add approximately 0.7 gram of mercuric oxid, or its equivalent in metallic mercury, and continue the boiling until the liquid in the flask is colorless, or nearly so. In case the contents of the flask are likely to become solid before this point is reached, add 10 cc more of sulphuric acid. Remove the flask from the flame, hold it upright, and while still hot drop potassium permanganate in carefully and in small quantities at a time until, after shaking, the liquid remains of a green or purple color.

After cooling dilute with about 200 cc of water, allow to cool again, add a few pieces of granulated zinc or pumice stone in order to keep the contents of the flask from bumping, and 25 cc of potassium sulphid solu-

tion with shaking. Next add 100 cc of the soda solution, or sufficient to make the reaction strongly alkaline, pouring it down the side of the flask so that it does not mix at once with the acid solution. Connect the flask with the condenser, mix the contents *thoroughly* by shaking, and distill until all ammonia has passed over into the standard acid. The first two-thirds of the distillate will contain all the ammonia. The distillate is then titrated with standard alkali and from the cubic centimeters of standard acid neutralized by the distillate the weight of nitrogen liberated as NH_3 is calculated. Report as *per cent of nitrogen on the dry fertilizer.*

In applying this method to fertilizers rich in organic nitrogen (as meat scraps) and to cotton-seed meal, cheese and other materials also rich in protein it is necessary to digest at least 4 hours in all. Troublesome frothing at the start may be somewhat remedied by adding a small lump of paraffin.

Do not add water to the acid residue from digestion until it is perfectly cool. The receiving flask should then be made ready at the still before alkali is added to the distilling flask to liberate the ammonia. After mixing, a red litmus paper should be dropped into the flask to make sure that the contents are alkaline. *Do not* allow the distilling flask to cool during distillation because the resultant contraction of volume may cause "sucking-back" from the receiving to the distilling flask. *Always* remove the distillate at the end of the process before turning off the gas flame. In case no nitrates are present, the unmodified Kjeldahl process (i. e., without the use of salicylic acid and zinc dust or sodium thiosulphate in the digestion) is used. Condensation of the acid may be facilitated and needless heavy loss of this reagent in digesting may be avoided, by boiling only gently. When the acid boils low there is danger of losing ammonium sulfate by volatilization.

b. Gunning Method.

(Not applicable in the presence of nitrates unless modified as in case of Kjeldahl method.)

(1) PREPARATION OF REAGENTS.

(*a*) *Potassium sulphate.*—This reagent should be pulverized before using.

The other standard solutions and reagents used are the same as those described under the Kjeldahl method.

(2) APPARATUS.

The apparatus used is the same as that employed in the Kjeldahl method.

(3) DETERMINATION.

Place the substance to be analyzed in a digestion flask, employing .6 grams or 1 gram according to its proportion of nitrogen. Add 10

grams of powdered potassium sulphate, 0.5 gram copper sulfate crystals, and 25 cc of sulphuric acid. Conduct the digestion as in the Kjeldahl process, starting with a temperature below boiling point and increasing the heat gradually until frothing ceases. Digest for a time after the mixture is colorless or nearly so, or until oxidation is complete. Do not add either potassium permanganate or potassium sulphid. Dilute, neutralize, distill, and titrate as in the Kjeldahl method. In neutralizing it is convenient to add a few drops of phenolphthalein indicator, by which one can tell when the acid is completely neutralized, remembering that the pink color, which indicates an alkaline reaction, is destroyed by a considerable excess of strong fixed alkali. The change of the copper sulfate to the deep blue hydroxid is also a good indication of alkalinity here.

This method may be modified to include nitrogen of nitrates as in the Kjeldahl method. In all cases, *blank determinations* of nitrogen should be made by digesting the reagents of a given method without the fertilizer. The latter should be replaced by 0.5 gram pure sucrose, which will assist in reducing any nitrates present, and the nitrogen found should be deducted from that found in the fertilizer, before calculating the per cent.

(c) ULSCH-STREET METHOD FOR DETERMINING NITRATE AND AMMONIACAL NITROGEN.

Place 1 gram of the sample in a half-liter flask. Add about 30 cc of water and 2 to 3 grams of reduced iron, and after standing sufficiently long to insure solution of the soluble nitrates and ammonia salts add 10 cc of a mixture of strong sulphuric acid with an equal volume of water; shake thoroughly and allow to stand for a short time until the violence of the reaction has moderated. Place a long-stemmed funnel in the neck of the flask to prevent mechanical loss. Heat the solution slowly, boiling it for five minutes, and cool. Transfer to a Kjeldahl flask with about 150 cc of water, add a little paraffin and an excess of strong NaOH. Connect with a condenser, such as is used in the Kjeldahl method, and boil the mixture for forty minutes, nearly to dryness; collect the ammonia in a known amount of standard acid and titrate in the usual manner. The nitrogen obtained represents the nitrates, plus the ammonia salts, contained in the sample. Report as *per cent of nitrogen in nitrates.*

In the *analysis of nitrate salts* proceed as above, except that 5 grams of the salt are dissolved in water, made to 500 c. c., and 25 c. c. of the nitrate solution, equivalent to 0.25 gram of sample, are employed with 5 grams of reduced iron. After boiling add 75 cc of water and an excess of sodium hydrate and complete the determination as above.

Zinc and iron in strong alkaline solution reduce nitrate to ammonia and by using these reagents, reduction and distillation may be carried out synchronously. This method requires somewhat more time, however, than the Ulsch method.

(d) MAGNESIUM OXID METHOD FOR THE DETERMINATION OF AMMONIACAL NITROGEN.

Place 3 grams of the fertilizer in a Kjeldahl flask, add 200 c. c. of water and 5 grams of magnesium oxide free of carbon dioxide. Distil into standard acid and titrate the residual acid as in the Kjeldahl method. Report as *per cent of nitrogen in ammonium compounds.*

In dealing with ammonium salts or substances rich in them, treat 5 grams of the sample with water and make to a volume of 250 c. c. Proceed with 25 c. c. of the solution, equivalent to 0.5 gram of sample, as in the case of the mixed fertilizer.

5. Potash.

LINDO-GLADDING METHOD.

(1) PREPARATION OF REAGENTS.

(*a*) *Ammonium chlorid solution.*—Dissolve 100 grams of ammonium chlorid in 500 cc of water, add from 5 to 10 grams of pulverized potassium-platinic chlorid, and shake at intervals for six or eight hours. Allow the mixture to settle over night and filter. The residue may be used for the preparation of a fresh supply.

(*b*) *Platinum solution.*—The platinum solution used contains 1 gram of metallic platinum (2.1 grams of H_2PtCl_6) in every 10 cc.

(2) METHODS OF MAKING SOLUTION.

(*a*) *With potash salts and mixed fertilizers.*—Boil 10 grams of the sample with 300 cc of water thirty minutes. In case of mixed fertilizers, add to the hot solution a slight excess of ammonium hydroxid and then sufficient powdered ammonium oxalate to precipitate all the lime present. Cool, dilute to 500 cc, mix and pass through a dry filter. In the case of potassium salts free from calcium, dissolve and dilute to 500 cc without the addition of ammonium hydroxid and ammonium oxalate.

(*b*) *With organic compounds.*—When it is desired to determine the total amount of potash in organic substances, such as cottonseed meal, tobacco stems, etc., saturate 10 grams with strong sulphuric acid and ignite in a muffle at a low red heat to destroy organic matter. Add a little strong hydrochloric acid, warm slightly in order to loosen the mass from the dish, and take up with water, make to a volume of 500 cc, and proceed as directed in the next section.

(3) DETERMINATION.

(*a*) *In mixed fertilizers.*—Evaporate 50 cc of the solution, corresponding to 1 gram of the sample, nearly to dryness, add 1 cc of dilute sulphuric acid (1 to 1), evaporate to dryness, and ignite to whiteness. As all the potash is in the form of sulphate, no loss need be apprehended by volatilization of potash, and a full red heat must be maintained un-

til the residue is perfectly white. Dissolve the residue in hot water, using at least 20 cc for each decigram of potassium oxid. Filter from any insoluble residue and wash. Add a few drops of hydrochloric acid, and platinum solution in excess. Evaporate on a water bath to a thick paste and treat the residue with 80 per cent alcohol, sp. gr. 0.8645, avoiding the absorption of ammonia. Wash the precipitate thoroughly with 80 per cent alcohol both by decantation and on the filter, continuing the washing after the filtrate is colorless. The filtering should be done on a fairly thick asbestos felt in a gooch crucible. This should be thoroughly dry and its weight recorded. Wash finally with 10 cc of the ammonium chlorid solution to remove impurities from the precipitate and repeat this washing five or six times. Wash again thoroughly with 80 per cent alcohol and dry the precipitate to constant weight at 100° C. The precipitate should be perfectly soluble in water.

In the case of potash salts, use 25 c. c. of solution, prepared according to (2a), add 25 c. c. of water, filter from any insoluble residue, acidify with a few drops of hydrochloric acid, add 10 c. c. platinum solution and proceed as above except in the case of sulphate-of-potash compounds and kainite. The platinum salts obtained from the latter should be washed with 30 c. c. portions of ammonium chlorid solution.

Absorption of ammonia should be carefully avoided in this determination after that used to precipitate calcium has been removed, because it forms a platinum chlorid salt similar in solubility to the potassium salt. For the same reason all excess of platinum reagent must be washed from the filter previous to washing with NH_4Cl solution. The presence of excess of platinum reagent is assured if the first washing by 80 per cent alcohol takes on a yellow color.

III. FEEDING STUFFS.

While the composition of fertilizers is for most purposes sufficiently indicated by determining a comparatively few simple constituents, on the other hand, a commercial valuation of feeding stuffs cannot be based upon their ultimate compositon. That is, they cannot be classified by an *ultimate* analysis, which measures their content of specific elements such as carbon, hydrogen and oxygen.

The average feeding stuff is a complex substance and its value to the feeder depends largely upon the amounts and kinds of groupings of the chemical elements which it contains. Carbohydrates, fats and proteins are compounds of feeding stuffs and are formed from such groupings. The two former classes of compounds contain carbon, hydrogen and oxygen, while protein is still more complex and contains nitrogen and sulphur, in addition to the other elements named. A third class of compounds, nucleins, contains phosphorus in addition to the other elements mentioned. In most cases the individual compounds of the feeding stuff cannot be isolated in a condition of purity and the analyst therefore separates his sample into groups of chemically related substances and submits his report as a *proximate* analysis. That is, he determines the percentage of groups of compounds similar to the humus extracted in soil analysis instead of measuring the amount of single specific elements such as phosphorus or nitrogen. Such an analysis is commonly restricted to the following groups of substances:

Moisture.
Ash.
Crude protein.
Ether extract.
Crude fiber.

In addition, 100 per cent minus the sum of the percentages of the above constituents is reported as percentage of nitrogen-free extract.

Feeding-stuff laws, which control the manufacture and sale of these materials, require a statement of guaranteed content of ether extract and crude protein, and these have been generally considered the most important nutrient substances. A determination of crude fiber is also required by law in some states, and this constituent is of significance both because of its own indigestibility and its suppressive action upon the digestibility of other constituents of the feeding stuff. An ash determination is often of value in indicating the source and worth of a feeding-stuff, especially in the case of condimental stock foods.

Moisture, as expressed by loss of weight of the sample may include a greater or less loss of volatile bodies such as essential oils. On the other hand, oxidation may decrease the true value for moisture, as a

result of increase in weight of fats and other bodies in the process of drying. Errors of the latter sort may be avoided by drying in hydrogen or other inert gases. These factors are usually so small, however, that they may be disregarded.

The ash of a feeding stuff is derived from such inorganic compounds as may be present in the sap and tissues of the plant. Silicon dioxide, calcium phosphate and potassium chloride are examples in point. The ash may also be formed from acid- and base-forming elements, such as K, Na, Ca, Mg, P and S, which occur in organic compounds and which are oxidized by ignition and combine to form salts. The basic elements are usually in excess and this excess will occur as carbonates in the ash, as in the case of K_2CO_3 in wood ashes.

Crude protein is an expression for the nitrogenous bodies of feeding stuffs. It is estimated by multiplying the nitrogen content (determined by the Kjeldahl method) by 6.25. This factor is based upon the average nitrogen content of pure proteins, which is about 16 per cent ($16 \times 6.25 = 100$).

The nitrogen estimated as crude protein in green plants (young clover, for example) may be derived largely from amides and related comparatively simple compounds, while the nitrogen of the mature seed of cereal plants exists almost entirely in the form of protein compounds. Since amides are much simpler and lower in molecular weight than the proteins, it is apparent that the crude protein determination in some cases may yield results considerably above the true protein percentage.

Ether extract is a term including all substances soluble in warm ether. In the case of feeding stuffs derived from pure cereal grains such as corn this material will be nearly pure fat, while in the case of hays and other feeding stuffs it may contain considerable amounts of waxes, resins and other non-fatty compounds. Chlorophyll is soluble in ether and will be extracted in this determination.

Crude fiber consists chiefly of cellulose, either free or in combination with poorly defined bodies closely related to it, as in the ligno-celluloses. A part of the cellulose which is free as such in the crude fiber may have been split off from compounds less resistant than the ligno-celluloses. No single definite body, but rather a proximate group of bodies is separated in this determination.

Nitrogen-free extract, which forms the remainder of the feeding stuff as determined by difference, is perhaps the most widely variant in amount of all the constituents considered, ranging from 17 per cent in cured corn stalks to over 70 per cent in cereal grains. It is composed chiefly of starch with varying amounts of sugars, dextrins, organic acids, pectins and allied compounds.

From the preceding brief survey it should be apparent that a given proximate constituent of feeding stuffs may vary widely in composition in samples from different sources.

ANALYTICAL METHODS.

Feeding materials containing much sugar or other oxidizable compounds should be dried in a circulating atmosphere of hydrogen for the moisture determination. The common drying oven, however, is suitable for most feeding stuffs.

The ash determination is simple in principle but often difficult in practice. It is not always possible to burn the carbon off readily without employing temperatures which may be high enough to volatilize some of the ash constituents. Various aids to oxidation in the way of chemicals and apparatus have been suggested. With care and patience, however, the ash can be determined by the use of a common Bunsen burner and open dish.

The ether extraction can be made thorough only upon perfectly dry samples of feeding-stuffs because ether and water are immiscible and the extracting agent therefore cannot penetrate wet plant tissue. Ether has been selected as the agent most convenient and effective for the purpose. Other solvents, such as benzine (a hydrocarbon oil closely related to gasoline) and carbon-tetra-chlorid, have been used. The extraction should be conducted with heat from another source than direct flames, on account of the great inflammability of the solvents commonly used.

The crude protein determination is conducted according to the unmodified Kjeldahl method, since plants and feeding stuffs produced therefrom contain no appreciable quantity of nitrates.

Stutzer's method for determining protein is frequently of value in discriminating between compounds of this class and amides. It depends upon the formation of insoluble compounds of proteins with certain metals. Copper is the metal in this case, and a suspension of copper-hydroxide in glycerine-water solution forms a reagent quite readily measured in uniform strength.

The crude fiber method now in use was worked out by Henneberg at Weende, Germany, succeeding several unsatisfactory methods. The present method yields a crude residue which is a complex mixture of substances. The treatment is supposed to remove most compounds except cellulose and its more resistant complexes, which appear to be combinations with lignin and pentosans. Excess of fat is first removed by an ether extraction and the subsequent boiling with dilute acid hydrolyzes dextrins, starch, pentosans and hemicelluloses to soluble sugars. The latter treatment also liberates more or less so-called "lignic-acids" from the cellulose.

Boiling with dilute alkali removes proteins and lignic-acids and related bodies, probably by dissolving them in the form of soluble salts or salt-like compounds.

The nature of the nitrogen free extract is of importance in some cases.

Reduction of Fehling's solution by the water extract of feeding stuffs is therefore taken to indicate the amount of reducing sugars present. This is only an approximate determination since maltose, dextrins and

other bodies possess reducing properties which are stated by the analyst in terms of glucose.

Starch is only approximately measured by the acid hydrolysis of the residue insoluble in fater, since pentosans, galactans and other hydrolyzable carbohydrates may be included in this determination. Starch may also be determined by digesting this residue of the feeding stuff with malt extract (diastase), washing out the resultant maltose and dextrins and hydrolyzing with dilute acid.

While the methods of feeding-stuff analysis must be understood as giving more or less indefinite results, they nevertheless have great value when properly interpreted.

PRELIMINARY TESTS.

Ash. 1. Burn 2 or 3 grams of alfalfa or clover hay to a gray ash in a porcelain dish. Add 50 c. c. of water and 5 c. c. HNO_3 and transfer to a beaker. Bring to boiling, filter, and divide the filtrate into three portions.

To one portion add ammonium hydrate in slight excess, make distinctly acid with acetic acid and then add a few drops of saturated solution of ammonium oxalate. What is the precipitate?

Filter, make the filtrate alkaline with NH_4OH and add a few c. c. of sodium-hydrogen-phosphate solution. What ash constituent gives a test here?

To a second portion of the filtrate add 2 or 3 c. c. of $BaCl_2$ solution. What is the precipitate?

To a third portion of the ash extract add a few drops of $AgNO_3$ solution. A positive test here demonstrates the presence of what ash constituent in the hay?

2. Repeat the above experiment using corn meal. Note the much smaller proportion of ash than was found in the hay. How does the strength of each test compare with the corresponding test of the previous experiment.

What do you conclude as to the value of these two feeding stuffs for supplying ash constituents to a ration?

3. Crush a small sample of wheat in a mortar. Ignite in a porcelain dish or crucible, transfer to a beaker, add about 40 c. c. H_2O and 10 c. c. HNO_3 and boil for a few minutes, breaking up charred particles with a stirring rod. Add water if necessary, filter and add 5 c. c. of molybdate solution. Heat till hot to the touch and let stand a few minutes. What constituent of the wheat gives this test? Is it abundant in this grain?

Name some sources in the plant of each ash constituent which you have detected. Phytin, which is a calcium, potassium, magnesium salt of an organic acid containing phosphorus, is a good example of a plant compound which contributes to the formation of ash.

Fats. 4. To 10 c. c. of ether in a test tube add a drop of cotton-seed oil by means of a pipette and shake, warming gently at the steam bath. (*In using ether be very careful to avoid the vicinity of flames.*) Add a few drops of the clear liquid to a clean filter paper, spread in a dry place and renew the solution as the ether evaporates. Thoroughly

dry the paper. Do you observe any residue on the paper? How does its appearance differ from a water spot? To what is it due?

5. Place a little cottonseed oil in a porcelain dish and rub up with a little potassium bisulphate by means of a stirring rod. Heat cautiously at the hood. Do you notice an irritating odor? This is due to acrolein, an aldehyde formed by dehydration of the glycerine in the fat. Glycerine is an alcohol or organic base.

6. To another small portion of oil in a small Erlenmeyer flask add a few c. c. of glycerol-soda solution and boil until frothing ceases. When cool add several times its volume of water. Does the product dissolve? What is it? Now acidify with sulfuric acid. What is the new substance which separates out? What is the general composition of fats and to what group of inorganic compounds do they correspond?

Carbohydrates. 7. Add a little dextrose to about 25 c. c. of water. Bring to boiling, add 5 c. c. of Fehling's solution and continue boiling for 2 or 3 minutes. What is the precipitate?

Make a similar solution of sucrose in about 50 c. c. H_2O and divide it into two parts. Boil one part and test with Fehling's solution as in the previous test. Do you get a precipitate?

To the remaining sucrose solution, which should be of about 25 c. c. volume, add 5 c. c. of strong HCl and heat nearly to boiling for several minutes. Neutralize with dilute solution of NaOH and test with Fehling's solution. Do you get a precipitate? What is it? Explain the change which sucrose has undergone and write an equation for it.

8. Add 1 or 2 grams of starch to 100 c. c. of water and boil for a short time. Cool, and add a few drops of iodine solution to a little of the starch preparation. Explain the result. Test another portion with Fehling's solution using the proportion of solution to reagent which obtained in previous tests. Do you get a precipitate?

To about 50 c. c. of the remaining starch "paste" add 10 c. c. conc. HCl and boil gently for some time. Neutralize and cool as in the case of sucrose. To separate portions apply iodine and Fehling's solutions as before. Record your observations and explain the changes to which the results are due.

9. Add a 9 cm. filter paper to 10 c. c. strongest sulphuric acid in a dry beaker. As soon as the paper has thoroughly disintegrated, dilute to 500 c. c. and mix well. Neutralize a 25 c. c. portion with dil. NaOH, bring to a boil, add 5 c. c. Fehling's solution and continue boiling for 2 or 3 minutes. Save this test. Boil another 25 c. c. portion of the filter paper preparation for several minutes, keeping the volume nearly constant, cool, neutralize as before and test with Fehling's solution. Compare with the preceding test. What is the precipitate? To what is it due? How do you explain the difference in results of these two tests? (Filter paper consists chiefly of a carbohydrate called cellulose.)

10. Place about 2 grams of wheat bran in a 200 c. c. Erlenmeyer flask with 10 c. c. strongest HCl and 30 c. c. water and boil. Lay over the mouth of the flask a piece of filter paper moistened with freshly-prepared anilin acetate solution. The pink coloration which appears after a time is due to furfurol, an aldehyde produced from pentosans. Test

sucrose and starch in the same way. These and related carbohydrates form traces of furfurol under the preceding condition. If the reaction from an unknown substance is much stronger that from sucrose or starch, the presence of either pentose sugars or pentosans is indicated. The pentosans are anhydrides of the pentose sugars and both occur for the most part combined, rather than free, in feeding stuffs.

The following are the general formulas for the carbohydrates just tested.

Dextrose (also called glucose or grape sugar).....$C_6H_{12}O_6$
Sucrose (also called cane or beet sugar).....$C_{12}H_{22}O_{11}$
Starch.....$(C_6H_{10}O_5)x$
Cellulose.....$(C_6H_{10}O_5)y$
Pentose.....$C_5H_{10}O_5$
Pentosan.....$C_5H_8O_4$

What do you conclude from your tests as to the general relation of the first four carbohydrates tested?

Proteins. 11. Place 100 grams of wheat flour in a porcelain dish, moisten with water and knead to a dough. Knead and wash the dough or gluten under the water tap until quite free from starch. Wheat gluten consists principally of two proteins, glutenin and gliadin.

Suspend a small portion of the gluten in 5 c. c. of water. Add an equal volume of concentrated nitric acid and heat. Observe any change of color, cool, add excess of ammonium hydroxide and note the change of color. This is the xantho-proteic reaction for proteins. It is frequently evident in the laboratory as a brownish stain upon the hands.

To another portion of gluten in 5 c. c. water add an equal volume conc. NaOH solution and boil. Add carefully 2 or 3 drops of very dilute $CuSO_4$ solution, letting it run down the sides of the test tube. This gives a light blue curd at the point of contact, from which a violet color should spread through the clear solution. This is the biuret reaction of proteins. It derives its name from the particular chemical grouping in the protein molecule to which it is due.

12. Dissolve a little egg albumen and urea (the former should be finely powdered, shaken well with H_2O, let stand a few minutes and filtered) in separate small portions of water. (Albumen is a protein, while urea is a comparatively simple derivative of proteins and belongs to a class of compounds called amides.) To separate portions of albumen and of urea solution add a few drops of the following reagents in concentrated solution: $HgCl_2$ and $CuSO_4$. Let stand some time. Record your observations. How would you separate proteins from amides where the two occur in the same solution, as in a water extract of some feeding stuffs?

METHODS FOR THE ANALYSIS OF FEEDING STUFFS.

1. Preparation of sample.

This is carried out as described for fertilizers.

2. Determination of Moisture.

Dry 2 grams of the substance in the steam oven for 5 hours. Cool and weigh as usual.

3. Determination of Ash.

Char 2 grams of the substance in a small porcelain dish and burn to whiteness, or a light gray color, at the lowest possible heat. Press the charred material upon the porcelain surface to ignite the last particles of carbon. Temperatures above dull red heating of the porcelain are to be avoided because of the volatility of some ash constituents. If a white ash cannot be obtained in this manner, exhaust the charred mass with warm water and collect the insoluble residue on an ashless filter. Dry this and ignite thoroughly in the dish first used, add the water extract, evaporate to dryness and heat to low redness till the ash is white or nearly so. Cool in a dessicator and weigh. Report as *per cent of ash* on the *dry* feeding stuff.

4. Determination of "ether extract."

Transfer 3 grams of the substance to a fat-free paper capsule by means of a wide-necked metal funnel. Plug in loosely with a wad of fat-free absorbent cotton and dry for 3 hours in the steam oven. Carefully clean a 50 c. c. Erlenmeyer flask, dry in the oven for ½ hour, cool in dessicator and weigh. Now drop the capsule of dried feeding stuff in an extraction tube, connect the weighed flask, pour 20 c. c. anhydrous ether into the apparatus and connect with a reflux condenser, allowing the flask to rest partly submerged in a bath of water kept at 50 to 55° C. The bath should be heated by steam or electricity, thus avoiding ignition of the ether. Extract for 16 hours, remove the flask, evaporate remaining ether, clean the outer surface and dry for 2 hours or to a minimum weight. Cool and weigh as before. Deduct the weight of flask and report as per cent of ether extract on dry sample.

(The thimble and residue should be saved. When they are dry, the residue may be thrown out and the thimble returned for credit or used again.)

5. Determination of crude protein.

Determine nitrogen on a 1 gram sample by the unmodified Gunning method as given for fertilizers (p. 25) and multiply the result by 6.25.

6. Determination of Protein Nitrogen (True Protein) by Stutzer's Method.

a. Preparation of Reagent.

Prepare cupric hydrate as follows: Dissolve 100 grams of pure cupric sulphate in 5 liters of water, add 25 cc of glycerol, and then a dilute solution of sodium hydrate until the liquid is alkaline; filter; rub the precipitate up with water containing 5 cc of glycerol per liter, and wash by decantation or filtration until the washings are no longer alkaline. Rub the precipitate up again in a mortar with water containing 10 per cent of glycerol, thus preparing a uniform gelatinous mass that can be measured out with a pipette. Determine the quantity of cupric hydrate per cubic centimeter of this mixture.

b. Determination.

Place 1 gram of the substance in a beaker, add 100 cc of water, heat to boiling, or, in the case of substances rich in starch, heat on the water bath 10 minutes; add a quantity (see instructor) of cupric hydrate mixture containing about 0.5 gram of the hydrate, stir thoroughly, filter when cold, wash with cold water, and, without removing the precipitate from the filter, determine nitrogen according to one of the unmodified methods given for the determination of nitrogen in fertilizers. The filter papers used must be practically free from nitrogen. If the substance examined consists of seed of any kind, or residues of seeds, such as oil cake or anything else rich in alkaline phosphates, add a few cubic centimeters of a concentrated solution of alum just before adding the cupric hydrate, and mix well by stirring. This serves to decompose the alkaline phosphates. If this be not done, cupric phosphate and free alkali may be formed, and the protein-copper precipitate may be partially dissolved in the alkaline liquid.

7. Determination of Crude Fiber and Carbohydrates.

Weigh 2 grams of the substance into a linen filter, fold and tie up, dry in the oven and extract with ordinary ether as in the determination of the ether extract but for 2 or 3 hours only, using an unweighed flask. To this residue of feeding stuff, in a 500 cc flask, add 200 cc of boiling 1.25 per cent sulphuric acid; connect the flask with an inverted condenser, the tube of which passes only a short distance beyond the rubber stopper into the flask. Boil at once, and continue the boiling for thirty minutes. A blast of air conducted into the flask may serve to reduce the frothing of the liquid. Filter; wash with boiling water till the washings are no longer acid; rinse the substance back into the same flask with 200 cc of a boiling 1.25 per cent solution of sodium hydroxid, free, or nearly so, from sodium carbonate; boil at once, and continue the boiling for thirty minutes, in the same manner as directed above for the treatment with acid. Filter on a gooch, and wash with boiling water till the washings are neutral; dry in the oven for 5 hours, cool and weigh. Incinerate completely and weigh again. Report loss of weight as *per cent crude fiber.*

The filter used for the first filtration may be linen, one of the forms of glass wool or asbestos filters, or any other form that secures clear and reasonably rapid filtration. The solutions of sulphuric acid and sodium hydroxid are to be made up of the specified strength, *determined accurately by titration* and not merely from specific gravity. The digesting liquor should be boiled gently. A simple glass bulb tube will then prove an efficient condenser. Filter paper and paper capsules are to be avoided because of the cellulose which they may so readily yield to the sample.

8. Determination of Reducing Carbohydrates (Estimated as Dextrose).

Frequently stir 3 grams of the sample in a beaker with 50 c. c. water for an hour, warming on the steam bath. Filter into a 250 c. c. flask,

wash and make up to the mark. If the solution be difficult to filter 2 c. c. of alumina cream $Al(OH)_3$ should be added and the whole shaken vigorously.

Determine reducing power as follows: The Defren-O'Sullivan Method. Mix 15 cc of Fehling's copper solution, A, with 15 cc of the tartrate solution, B, in a quarter-liter Erlenmeyer flask, and add 50 cc of distilled water. Place the flask and its contents in a boiling water bath and allow them to remain five minutes. Then run rapidly from a burette into the hot liquor in the flask 25 cc of the sugar solution to be tested (which should contain not more than one-half per cent of reducing sugar). (See instructor.) Allow the flask to remain in the boiling water bath just fifteen minutes after the addition of the sugar solution, remove, and with the aid of a vacuum filter the contents rapidly in a porcelain Gooch crucible containing a layer of prepared asbestos fiber about 1 cm thick, the Gooch with the asbestos having been previously ignited, cooled, and weighed. The cuprous oxide precipitate is thoroughly washed with boiling distilled water till the water ceases to be alkaline.

The asbestos used should be of the long-fibered variety, and should be specially prepared as follows: Boil first with nitric acid (specific gravity 1.05 to 1.10), washing out the acid with hot water, then boil with a 25 per cent solution of sodium hydroxide, and finally wash out the alkali with hot water. Keep the asbestos in a wide-mouthed flask or bottle and transfer it to the Gooch by shaking it up in the water and pouring it quickly into the crucible while under suction.

Dry the Gooch with its contents in the oven, and finally heat to dull redness for fifteen minutes, during which the red cuprous oxide is converted into the black cupric oxide. Considerable care must be taken to avoid cracking the crucible, the heat being increased cautiously and the operation preferably conducted in a muffle furnace. After oxidation as above, the crucible is transferred to a dessicator, cooled, and quickly weighed. From the milligrams of cupric oxide, calculate the milligrams of dextrose from the table at the close of the manual.

Fehling's solution is made up in two separate parts as follows:

A. Fehling's Copper Solution.—34.639 grams of carefully selected crystals of pure copper sulphate dissolved in water and diluted to exactly 500 cc.

B. Fehling's Alkaline Tartrate Solution.—178 grams Rochelle salts and 50 grams sodium hydroxide are dissolved in water and diluted to exactly 500 cc.

Alumina Cream.—Add excess of NH_4OH to an alum solution of 10 to 20 per cent strength. Filter, wash free from ammonium hydroxid and suspend to the desired consistency in distilled water.

NOTE.—Crystalline substances should be pulverized before attempting to dissolve in quantity. Solution will be thus accomplished much more readily. This applies especially to Rochelle salts.

9. Determination of Starch (Direct Acid Hydrolysis).

Dry a 3-gram sample for 1 to 2 hours in the oven, transfer to a smooth filter and wash with 4 or 5 successive portions of gasoline, *avoiding the*

vicinity of flames. Remove the gasoline by drying a few minutes, transfer to a flask with 200 c. c. H_2O and 20 c. c. HCl (sp. gr. 1.125), equip with reflux condenser and heat in boiling water for 2½ hours. Cool, and neutralize carefully with dilute NaOH solution. Clarify if necessary with alumina cream. Mix well, make to 500 c. c., filter and determine the reducing power of duplicate 25 c. c. portions of the solution by the Defren-O'Sullivan method. Calculate as dextrose and convert to starch value by the factor 0.9. Report as *per cent of starch* in the *dry feeding stuff.*

IV. INSECTICIDES.

Economic Entomology has brought into use various preparations for the control or eradication of insect pests. Such materials, known as insecticides, may be divided into internal (or stomachic) poisons and external poisons. Paris Green and London Purple are examples of the former class, kerosene emulsion and lime-sulfur mixture of the latter class of poisons. The horticulturist employs similar preparations for the control of fungus pests. Bordeaux mixture is an example of these.

Contact poisons and fungicides are generally freshly prepared by the sprayer, and their purity and composition are under his control. Stomachic poisons on the other hand are usually bought ready prepared in the open market and are consequently subject to variation in composition and to adulteration. Therefore an analysis of this latter class of insecticides is essential to the welfare of the purchaser.

Chemical examination of insecticides is rendered fairly simple by the fact that arsenic is the chief active ingredient and one easily isolated. Free arsenious acid in solution is destructive to foliage, and as a consequence a determination of this ingredient is of prime importance. Most of the states have passed laws limiting the amount of this ingredient allowed in insecticides. California has set the mark at 4 per cent, Idaho at 6 per cent.

PRELIMINARY TESTS.

Copper. 1. Dissolve about 1 gram $CuSO_4$ in 10 c. c hot water and cool the solution. To about 5 c. c. add a few drops of NH_4OH. What is the ppt.? Now add 5 c. c. NH_4OH and mix. Explain the result.

2. Add 2 or 3 drops of the $CuSO_4$ solution prepared in Exp. 1 to about 500 c. c. water and mix well. To about 5 c. c. of this solution add 2 or 3 drops of dilute $K_4Fe(CN)_6$ solution. What is the result? (Potassium ferro-cyanide detects 1 part Cu in 200,000 parts of solution). Add 2 or 3 c. c. of the $K_4Fe(CN)_6$ solution to 5 c. c. of the $CuSO_4$ solution prepared in Exp. 1. What is the ppt.? Filter, wash a little, and test solubility of small portions in HCl and in strong NaOH solution. What must be the reaction of the medium in which ferrocyanide is used as a test for copper?

Arsenic. 3. Dissolve about 0.5 gram each of sodium arsenate and sodium arsenite in separate 10 c. c. volumes of water. Add 2 or 3 c. c. magnesia mixture with stirring. Explain your results. How could you detect *arsenic* when present in an insecticide as *arsenious acid?*

4. Prepare a solution of sodium arsenite as in the last experiment. To a part of the solution add a little strong $CuSO_4$ solution. Filter and test the solubility of the precipitate in NH_4OH (Ppt. = $CuHAsO_3$, Scheele's green, also often called Paris green).

To the remaining arsenite solution add a solution of copper acetate and boil. Filter and test, solubility in NH_4OH. (Ppt. = copper-aceto-arsenite $(CuOAs_2O_3)_3$ Cu $(C_2H_3O_2)_2$, called Schweinfurth's green or Paris green. This is the true Paris green.)

Acetic acid. 5. Dissolve about 1 gram $NaC_2H_3O_2$ in 10 c. c. water. To 5 c. c. add 2 or 3 c. c. conc. H_2SO_4, mix and heat. What odor do you detect? To the remaining acetate solution add H_2SO_4 as before and also 3 or 4 c. c. strong alcohol. Heat carefully with *t. t. mouth away from the flame.* The fragrant odor evolved is that of ethyl-acetate. How would you test a Paris green sample for the presence of acetic acid?

Tests for Purity of Paris Green. 6. Compare a pure sample of Paris green with impure samples by the following methods:

(a) To about 1 gram of each sample in separate test tubes add 5 c. c. of NH_4OH. A pure Paris green will dissolve and leave no appreciable residue. Impure samples may, however, also be quite readily soluble in NH_4OH.

(b) Place about 0.5 gram of the samples on separate small glass plates of clear glass and oblong in shape. Elevate the end of the plate and tap sharply with a glass rod. Compare the color of the "streaks" or paths of the samples. A pure "green" will show uniform color.

(c) Examine streaks of the different samples under the microscope. A pure green will appear in the form of green, spherical particles, and these are all that should be seen.

The methods for the quantitative analysis of insecticides chiefly involve determinations already familiar to the student of quantitative inorganic analysis.

METHODS FOR THE ANALYSIS OF INSECTICIDES.

1. Moisture.

Dry 2 grams of sample for 10 hours at 110° C. and calculate the loss as moisture.

2. Paris Green.

(a) *Insoluble matter.*

Weigh out 1 gram, add an excess of NH_4OH and stir until all the green particles have dissolved. If there is a residue, filter through one of two counterpoised filters. Dry at 100° C. and report the *per cent of insoluble matter.*

(b_1) *Total copper—Gravimetric method.*

To the filtrate from (a) add HCl till nearly neutral, then yellow ammonium sulphide and digest at moderate heat for ½ hour. Decant the supernatant liquid on a filter and save the filtrate. Digest the residue with more ammonium sulphide, filter and wash with water containing a little ammonium sulphide. Dry, ignite and weigh as $CuOCu_2S$. Assuming equal molecular weights for these compounds, report the *per cent of Cu.*

(b_2) *Total copper—Electrolytic method.*

Pour the cuprous oxid obtained by boiling Paris green with sodium hydroxid (see Volumetric Method for Total Arsenious Oxid) on a filter and wash well with hot water. Dissolve in hot dilute nitric acid and make to a volume of 250 c. c., first filtering out any insoluble residue. To 50 or 100 c. c. add 10 c. c. dilute sulphuric acid (200 c. c. strongest H_2SO_4 per liter) and evaporate on steam bath until the Cu salt has largely crystallized. Heat carefully on a hot plate or asbestos board until evolution of white fumes shows that excess HNO_3 is removed. Add 8 to 10 drops strongest HNO_3 and rinse into a beaker of 100 to 150 c. c. capacity. Precipitate the copper by electrolysis, depositing the metal on a weighed platinum cylinder. Wash thoroughly with water before breaking the current, remove cylinder from the circuit, wash with alcohol and ether successively, dry at 50° C. and weigh. The increase of weight is due to Cu.

(c_1) *Total Arsenic—Gravimetric Method.*

To the filtrate from (b_1) cautiously add HCl to acid reaction. Warm, pass H_2S gas through the solution for ½ hour, filter (rejecting filtrate), and dissolve the ppt. in KOH. Filter to remove the free sulphur, and pass chlorine gas into the solution for one hour (keeping the solution alkaline). Acidulate with HCl (the solution must remain clear), add NH_4OH in excess and then magnesia mixture in excess, let it stand 12 hours, filter through tared filters and wash with a mixture of 1 part NH_4OH and 3 parts water. Ppt. is $MgNH_4AsO_4.6H_2O$. Dry thoroughly and convert by gentle ignition into magnesium pyroarsenate, $Mg_2As_2O_7$. The filter is burned alone, having first been moistened with ammonium nitrate solution. Report the *per cent of As.*

(c_2) *Total Arsenious Oxid—Volumetric Method.*

1. SOLUTIONS REQUIRED.

(a) *Starch solution.*—A starch solution is used which is prepared by boiling 2 grams of starch with 200 cc. of water for about five minutes.

(b) *Standard iodine solution.*—A standard iodine solution is prepared in the following manner: Dissolve 12.7 grams of powdered iodin in about 250 cc. of water to which has been added 25 grams of c. p. potassium iodid, and make the whole up to a volume of 2 liters. To standardize this solution, weigh out 1 gram of dry c. p. arsenious oxid, tranfer to a 250 cc. flask by means of about 100 cc. of a solution containing 2 grams of sodium hydrate in each 100 cc. and boil until all arsenious oxid goes in solution. Make to a volume of 250 cc. and use 50 cc. for analysis.

This 50 cc. portion is concentrated by boiling in a 250 cc. flask to half its volume and allowed to cool to 80° C. An equal volume of concentrated hydrochloric acid is now added, accompanied by 3 grams of potassium iodid, mixed, and the whole allowed to stand for ten minutes (to reduce the arsenic oxid, formed on boiling the alkaline arsenite, to arsenious oxid). The brown solution is then diluted with cold water and an approximately N/10 solution of sodium thiosuphate added, drop

by drop, until the solution becomes exactly colorless. (This end point is easy to read without the aid of starch.) The solution is then made slightly alkaline with dry sodium carbonate (using a drop of methyl orange to read the change), then made slightly acid with hydrochloric acid, taking care that all lumps of sodium carbonate are acted on by the hydrochloric acid. Sodium bicarbonate is now added in excess and the solution of iodin run in, drop by drop, using starch solution to read the end reaction. (Sometimes the solution gets dark toward the end of the titration. This must not be confused with the final dark-blue color given by the iodin and starch.)

From the number of cubic centimeters of iodin solution used and the weight of arsenious oxid taken, the value of each cubic centimeter of iodin in terms of arsenious oxid is determined.

2. *Determination.*—Two grams of paris green are weighed out, transferred to a 250 cc. flask and about 100 cc. of water and 2 grams of sodium hydrate added. This mixture is boiled for five to ten minutes, or until all of the green particles have changed to red cuprous oxid. It is then cooled to room temperature and the volume made to 250 cc. The well-shaken liquid is filtered through a dry filter and 50 cc. taken for analysis. The analysis is carried out from this point forward the same as when standardizing the iodin solution.

(c_3) *Soluble Arsenious Oxid.*

METHOD I.

Solutions required.—A solution of starch and standard iodin prepared as for (c_2) and a solution of sodium acetate containing 12.5 grams of the crystallized salt in each 25 cc.

Method.—Digest over the flame 1 gram of paris green for about five minutes with 25 cc. of the solution of sodium acetate. The solution is then cooled, made up to 100 cc. and 50 cc. filtered off and titrated with standard iodin in the usual way. Report as *per cent of sodium-acetate-soluble As.*

METHOD II.

Solutions required.—A starch and standard iodin solution prepared as for (c_2).

Method.—One gram of paris green is treated with 1,000 cc. of water (previously boiled to expel CO_2 and again cooled to room temperature) in a large flask. The flask is stoppered and shaken 8 to 10 times each day for ten days. At the end of this time the solution is filtered off through a dry filter. Two hundred cubic centimeters of this are treated with sodium bicarbonate and titrated with iodin. Report as *per cent of water-soluble As.*

3. Lime Sulphur Dips and Lime-Sulphur-Salt Mixture.

Determination of Total Sulphur.

(1) SOLUTION REQUIRED.

(*a*) *Alkali solution.*—Use a saturated KOH solution.

(*b*) *Barium chlorid.*—A 10 per cent solution.

(*c*) *Hydrogen perovid.*—An approximately 3 per cent solution, free from sulphates. If the solution contains sulphates add freshly precipitated barium carbonate and shake occasionally for several hours, then filter and use the clear solution.

(2) DETERMINATION.

Measure 10 cc of the clear sample in a 100 cc measuring flask and fill to the mark. Mix well and analyze 10 cc aliquots of this solution by treating with 3 cc of the caustic potash solution, followed by 50 cc of hydrogen peroxid free from sulphates. Heat on the steam bath for one-half hour exactly and then acidify with hydrochloric acid, precipitate with barium chlorid in the usual way in boiling solution, and finally weigh as barium sulphate. Weigh 10 c. c. of the sample in a small corked flask and from the weight of $BaSO_4$ obtained, report the *per cent of total S* in the wash or dip.

V. MILK.

Milk is a biological fluid of complex nature. It is closely related to blood, being produced from the latter by the action of cells of the udder. This change involves intricate reconstruction of the elements of the blood.

Milk serum is a solution of organic and inorganic compounds. In this solution the fat is suspended as minute globules forming an *emulsion. Casein and albumin* are in *colloidal* solution in the serum and are accompanied by at least two other proteins, namely: lactoglobulin and fibrin. Lactose (milk sugar), citric acid (an organic acid) and inorganic salts, chiefly phosphates of calcium and potassium and chlorides of potassium and sodium, form a *true, dilute solution* with the water of the milk.

Lactose is a reducing sugar of the disaccharide group, and upon hydrolysis by acids yields one molecule each of dextrose and galactose. As a result of hydrolysis induced by certain bacteria, lactose produces lactic acid and other products. It is to the lactic acid so produced that the precipitation of casein, commonly known as curdling, is due. *The fats* of milk are not simple, but the same molecule of fat may contain two or even three different fatty acids as indicated:

Glycerin { Butyric acid. / Lauric acid. / Oleic acid.

The complexity of milk is illustrated by the following scheme according to Babcock.

COMPOSITION OF COW'S MILK.

Milk:

- Butter-fat = 3.6..
  - Olein, Palmitin, Stearin, Myristin, Laurin — Glycerides of insoluble and non-volatile acids..... 3.3
  - Butyrin, Caproin, Caprylin (trace), Caprin (trace) — Glycerides of soluble and volatile acids..... 0.3
  - Fat.... 3.6
- Milk serum 96.4..
  - Casein 3.00
  - Albumin 0.60
  - Globulin.....—
  - Enzymes..... 0.20
  - Fibrin (trace)—
  - 3.80 — Containing Nitrogen.. 3.8
  - Milk sugar..... 4.5
  - Citric acid 0.1
  - Potassium oxide 0.175
  - Sodium " 0.070
  - Calcium " 0.140
  - Magnesium " 0.017
  - Iron " 0.001 — 0.7
  - Sulphur trioxide 0.027
  - Phosphorus pentoxide 0.170
  - Chlorine 0.100
  - 0.700 — Ash 9.1
  - Solids not fat 9.1
  - Water..... 87.3

Total solids. 12.7

100.0

Official examination of milk for purity and conformity to standards, and the ordinary requirements of the dairyman or consumer do not require chemical analysis of milk in detail, but rather involve proximate analysis. The following determinations commonly made are listed in their order of practical importance:

Total solids.
Fat.
Protein.
Sugar.
Ash.

PRELIMINARY TESTS.

1. Casein.—Precipitate the casein in 100 c. c. fresh skimmed milk as follows:

Dilute with distilled water to about 500 c. c. and add dilute acetic acid (10%), a little at a time and mixing well, until the curd flocculates sharply. Shake vigorously to gather the finer curd particles and let stand to settle. If the serum is not clear, add a little more acid as before. Filter, and save the filtrate. Wash the casein thoroughly.

Now oxidize some of the casein by boiling with strong HNO_3 until it yields a clear solution. Cool, dilute, nearly neutralize with NH_4OH and test with molybdate solution. What constituent have you detected?

Try the biuret and xantho-proteic tests upon small portions of casein. (See preliminary test No. 11, feeding stuffs). To what class of compounds does casein belong?

2. Albumin.—Neutralize the filtrate saved from the previous test, using dilute NaOH. Boil to a volume of 100 c. c. or less and filter, washing a little and saving the filtrate. Test the residue on the filter by the biuret and xantho proteic tests. What is the nature of albumin?

3. Ash constituents.—Bring the volume of filtrate saved in the previous test to from 50 to 100 c. c. Make 10 c. c. distinctly acid with acetic acid and add a few drops $(NH_4)_2C_2O_4$ solution. To a similar portion apply the test with molybdate solution.

To a third portion add a little HNO_3 and a few drops $AgNO_3$ solution. What elements have you detected? In what inorganic compounds of milk does each occur?

4. Lactose.—Dissolve about 0.5 gram lactose in about 50 c. c. water. Boil about one half the solution and add 10 c. c. Fehling's solution with continued boiling. Is lactose a reducing sugar?

In the place of the sugar solution test corresponding volumes of formalin and $HgCl_2$ solutions of about 1% strength. Can lactose be determined in the presence of these preservatives? What is the chemical relation betwen formalin (formaldehyde) and some of the reducing sugars?

METHODS FOR MILK ANALYSIS.

(*a*) *Determination of Water.*

Heat to constant weight at the temperature of boiling water from 1 to 2 grams of milk in a tared flat dish of not less than 5 cm. diameter.

If desired, from 15 to 20 grams of pure dry sand may be previously placed in the dish. Cool in a desiccator, and weigh rapidly to avoid absorption of hygroscopic moisture.

Babcock asbestos method.—Provide a hollow cylinder of perforated sheet metal, 60 mm. long and 20 mm. in diameter, closed 5 mm. from one end by a disk of the same material. The perforations should be about 0.7 mm. in diameter and about 0.7 mm. apart. Fill loosely with from 1.5 to 2.5 grams of freshly ignited, woolly asbestos, free from fine and brittle material, cool in a desiccator, and weigh. Introduce a weighed quantity of milk (between 3 and 5 grams) and dry at 100° to constant weight for the determination of total solids.

In general, milk can be conveniently weighed by difference from a small flask, stoppered to prevent evaporation. Where the sample is to be added to a weighed dish, the required weights may be put upon the balance and the milk run in rapidly from a pipette just in excess. The weight can then be quickly found on the balance arm to within a few milligrams.

In sampling milk for analysis it should be mixed by pouring gradually from one vessel to another. *Vigorous shaking is not permissible* because it incorporates air with the liquid.

(*b*) *Determination of Fat.*

(1) Paper-coil method.

Coils made of thick filter paper, cut into strips 6.25 by 62.5 cm., are thoroughly extracted with ether and alcohol. From a weighing bottle about 5 grams of milk are transferred to the coil by a pipette, care being taken to keep the end of the coil held in the fingers dry. The coil, dry end down, on a piece of glass, is dried at the temperature of boiling water for one hour, or, better, dried in hydrogen at the temperature of boiling water, transferred to an extraction apparatus, and extracted with absolute ether or petroleum ether boiling at about 45°. The extracted fat is dried to minimum weight.

(2) Babcock asbestos method.

Extract the residue from the determination of water by the Babcock asbestos method with anhydrous ether for 10 hours or more, evaporate the ether, dry the fat at 100°, and weigh.

(*c*) *Determination of Nitrogen Compounds.*

1. *Determination of Total Nitrogen Compounds.*—Place in a Kjeldahl digestion flask a known weight (about 5 grams) of milk, and proceed, without evaporation, exactly as described for one of the methods under nitrogen in fertilizers. Multiply the percentage of nitrogen by 6.38 for nitrogen compounds.

2. *Determination of casein in cow's milk.*—The determination of casein in milk should be made when the milk is fresh, or nearly so. When it is not practicable to make this determination within twenty-four hours, add 1 part of formaldehyd to 2,500 parts of milk, and keep

in a cool place. Place about 10 grams of milk in a beaker with about 90 cc. of water at 40°-42°, and add at once 1.5 cc. of a solution containing 10% of acetic acid by weight. Stir well and let stand from three to five minutes longer. Then decant on filter, wash two or three times with cold water by decantation, and transfer precipitate completely to filter. Wash once or twice on filter. The filtrate should be clear, or very nearly so, and should be saved with the washings. If it be not clear when it first runs through, it can generally be made so by two or three repeated filtrations, after which the washing of the precipitate can be completed. The washed precipitate and filter paper are digested as in the regular Kjeldahl method for the determination of nitrogen, and the process is completed as usual. To calculate the nitrogen into an equivalent amount of casein, multiply by 6.38.

In working with milk which has been kept with preservatives, the acetic acid should be added in small proportions, a few drops at a time, stirring, and the addition continued until the liquid above the precipitate becomes clear, or very nearly so.

3. *Determination of albumin in milk.*—The filtrate obtained in the preceding operation is neutralized with caustic alkali, three-tenths cc. of a ten per cent solution of acetic acid added and the mixture heated to the temperature of boiling water for from ten to fifteen minutes. The precipitate is collected on a filter, washed, and the nitrogen therein determined. Nitrogen multiplied by 6.38 equals albumin.

After adding the concentrated acids to these separated protein bodies they may be left safely for a time, if kept free from ammonia. The moist precipitates readily decompose on standing. They should be immediately treated with the H_2SO_4 for the succeeding digestion.

(*d*) *Determination of Ash.*

In a weighed dish put about 20 grams of milk, add 6 cc. of nitric acid, evaporate to dryness, and burn at a low red heat until the ash is free from carbon.

(*e*) *Determination of Milk-Sugar by Volumetric Method.*

Twenty-five grams of the milk (24.2 cc) are transferred to a 250-cc. flask, 0.5 cc. of a 30% solution of acetic acid are added and the contents well shaken. After standing for a few minutes, about 100 cc. of boiling water are run in, the contents again shaken, 25 cc. of alumina cream are next added, the flask shaken once more, and set aside for at least ten minutes. The supernatant liquid is then poured upon a previously wetted ribbed filter, and finally the whole contents of the flask are brought thereon, and the filtrate and washings made up to 250 cc. The filtrate must be perfectly clear. The milk sugar in a solution thus precipitated would ordinarily not exceed ½ of 1 per cent.

From a burette containing the clear milk-sugar solution above prepared, run a measured volume into the boiling Fehling liquor containing 5 cc. each of copper and alkali solution till sufficient has been introduced to competely reduce the copper. This is determined by placing a

drop of the clear supernatant liquid of the Fehling's test on a glazed porcelain plate and bringing it in contact with a drop of dilute potassium ferro-cyanide solution strongly acidified with acetic acid. The test drop must be free from Cu_2O and, if necessary, may be so obtained by filtering a few drops of the supernatant through a very small filter. Sufficient acid must be present to be still in excess when the drops are mixed. If unreduced copper is present this test produces a brownish coloration. It is not necessary to apply it until the color of the Fehling's solution is seen to be faint. Continue adding the sugar solution in small portions, and boil for 2 or 3 minutes each time, until the ferro-cyanide test fails to appear.

As 0.067 gram milk sugar will reduce 10 cc. of Fehling solution, it follows that the number of cubic centimeters of sugar-containing solution required for the test (using preferably the average of several determinations) will contain 0.067 gram of milk sugar, from which the percentage is readily computed. Thus if 16 cc. of the milk-sugar solution are necessary to reduce the copper, then 16 cc. contain 0.067 gram milk-sugar.

250 cc. of solution contain 25 grams milk,
1 cc. " " " 0.1 " "
16 cc. " " " 1.6 " "

and 1.6 grams milk contain 0.067 grams milk-sugar. Therefore the sample contains $.067 \times 100/1.6 = 4.19\%$ lactose.

(*f*) *Centrifugal Volumetric Methods.*

1. Babcock Method for Fat.

(1) APPARATUS.

(*a*) *Babcock milk-test bottles*, graduated to 10 per cent.

(*b*) *A centrifuge* with sockets for from 2 to 32 bottles, according to the number of tests to be made, and capable of being run at a speed of from 600 to 1,200 revolutions per minute, according to the diameter of the machine. If many tests are made steam turbine testers or electrical testers will be found convenient.

(*c*) *Pipettes*, 17.6 cc.

(*d*) *Graduates*, 17.5 cc, or a Swedish acid bottle delivering that amount, for measuring sulphuric acid.

(2) DETERMINATION.

Pipette off 17.6 cc of the carefully mixed sample into a test bottle and add 17.5 cc of commercial sulphuric acid (specific gravity, 1.82-1.83). Mix, and when the curd is dissolved whirl the test bottles in the centrifuge for four minutes at the required speed for the machine used. Add boiling hot water, filling to the neck of the bottles, and whirl for one minute; again add boiling water so as to bring the fat within the scale on the neck of the bottles, and after whirling for one minute more read the length of the fat column, care being taken to make the readings at a temperature between 130° and 150° F. when the fat is wholly liquid. The readings give the per cent of fat in the milk direct.

For details as to the manipulation of the Babcock test and its application in the analysis of dairy products other than milk the following books may be consulted: Farrington and Woll, "Testing Milk and Its Products," and Van Slyke, "Modern Methods of Milk Testing."

2. Hart Method for Casein.

1. REAGENTS.

(*a*) *Chloroform.* A high grade of chloroform should be used. This can be obtained at local drug stores at about 50 cents per pound, depending upon the quantity purchased. It is better not to buy over 2 pounds at a time. When not in use the chloroform bottle should be kept in a cool, dark place.

(*b*) *Acetic Acid.* A 10 per cent acetic acid solution is usually furnished by the supply houses at 25 cents per quart. If the glacial acetic acid (99½ per cent pure) is purchased, a 10 per cent solution is made by diluting 10 cc. of the strong acid to 100 cc. with clean rain water or condensed steam. Then 25 cc. of the 10 per cent acid are diluted to 1,000 cc. with water. This gives a 0.25 per cent solution, the correct strength for the casein test. The acid bottles should be plainly labeled, in order that acid of wrong strength may not be used.

2. TEMPERATURE.

The testing should be done in a room where the temperature is 60 to 70 degrees F. The curing room of a cheese factory is a good place for the tester; the cheese making room is likely to be too hot in summer or too cold in winter.

The milk samples should be at a temperature of 65 to 75 degrees F. It may be necessary to warm or cool the samples before testing, depending on the season. If they have been properly cared for but little change will be needed. The acetic acid should be as near 70 degrees F. as possible, although a few degrees variation (not over 5 degrees) either way will not cause any serious error.

It is absolutely essential that these precautions as to temperature be strictly observed.

3. MAKING THE TEST.

The test bottles should be placed in a rack in an upright position. Such a rack can easily be made by boring holes in a board and nailing a block under each end. Measure 2 cc. of chloroform into each bottle. If a burette is not available, pour the chloroform into the test bottle up to the 4 per cent mark; it then contains 2 cc. A pipette should not be used to measure chloroform. Next add with a pipette 20 cc. of the 0.25 per cent acetic acid to each bottle. Then put 5 cc. of the milk well mixed and at the proper temperature, into each bottle with a pipette. Care should be taken that this is accurately measured. It should be allowed to run directly into the acetic acid, and not along the sides of the test bottle. As many bottles as the machine will hold should be prepared with the chloroform and acetic acid before putting the milk into any of the bottles.

The test bottles must then be inverted, first closing them by placing the thumb over the neck, and shaken vigorously for *15 to 20 seconds.* A thorough shaking is necessary to mix the chloroform with the precipitated casein, and thus dissolve out the fat. Otherwise too high tests will result. As soon as shaken, place the bottles in the tester, being careful to have them rest firmly on the cork cushion at the bottom of each pocket.

Then whirl the bottles for 7½ to 8 minutes at 2,000 revolutions per minute. To secure this speed the handle of the tester must be turned 56 times per minute. The speed can best be regulated by using a metronome, set to beat audibly 56 times per minute.

After whirling, the test bottles should be taken from the machine, replaced in the rack, and allowed to stand 10 minutes, after which the per cent of casein can be read directly from the scale on the test bottle. The tests may be allowed to stand longer than 10 minutes (up to 24 hours) before reading without affecting the result, but should *never* be read in *less* than 10 minutes.

If the edges of the casein pellet are not sharp and clear-cut, it is probably due to too long or too vigorous shaking. A very high or low temperature may cause ragged edges. The acid also must be of correct strength.

ATOMIC WEIGHTS IN COMMON USE

(International Atomic Weights 1910)

Aluminum	Al.	27.1	Manganese	Mn.	54.93
Arsenic	As.	74.96	Mercury	Hg.	200.0
Barium	Ba	137.37	Molybdenum	Mo.	96.0
Calcium	Ca.	40.09	Nitrogen	N.	14.01
Carbon	C.	12.00	Oxygen	O.	16.00
Chlorin	Cl.	35.46	Phosphorus	P.	31.00
Copper	Cu.	63.57	Platinum	Pt.	195.0
Hydrogen	H.	1.008	Potassium	K.	39.10
Iodine	I.	126.92	Silicon	Si.	28.3
Iron	Fe.	55.85	Silver	Ag.	107.88
Lead	Pb.	207.10	Sodium	Na.	23.00
Magnesium	Mg.	24.32	Sulphur	S.	32.07

DEFREN'S TABLE FOR DEXTROSE, MALTOSE, AND LACTOSE

Cupric Oxide mgms	Dextrose mgms.	Maltose mgms.	Lactose mgms.	Cupric Oxide mgms.	Dextrose mgms.	Maltose mgms.	Lactose. mgms.
30	13.2	21.7	18.8	180	80.4	131.8	114.6
40	17.6	29.0	25.2	190	84.9	139.1	121.0
50	22 1	36.2	31.5	200	89.5	146.6	127.5
60	26.5	43.5	37.8	210	94.0	154.1	134.1
70	30.9	50.8	44.1	220	98.6	161.5	140.6
80	35.4	58.1	50.5	230	103.1	169.1	147.0
90	39.9	65.5	56.8	240	107.7	176.6	153.5
100	44.4	72.8	63.2	250	112.3	184.1	160.0
110	48.9	80.1	69.5	260	116.9	191.6	166.5
120	53.3	87.4	75.9	270	121.4	199.2	173.0
130	57.8	94.8	82.4	280	126.1	206.8	179.6
140	62.2	102.1	88.7	290	130.7	214.3	186.2
150	66.8	109.5	95.2	300	135.3	221.9	192.8
160	71.3	116.9	101.7	310	139.9	229.6	199.3
170	75.8	124.4	108.2	320	144.5	237.2	205.9