Laboratory manual of general agricultural chemistry. 1920

Madison, Wisconsin: University of Wisconsin, College of Agriculture, 1920

https://digital.library.wisc.edu/1711.dl/A5XSNXDQNHCH58A

LABORATORY MANUAL

OF

General Agricultural Chemistry

THE UNIVERSITY OF WISCONSIN
COLLEGE OF AGRICULTURE
1920

LABORATORY MANUAL

OF

General Agricultural Chemistry

THE UNIVERSITY OF WISCONSIN
COLLEGE OF AGRICULTURE
1920

LABORATORY MANUAL

OF

General Agricultural Chemistry

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. So far as practicable, important reactions have been expressed by equations. These should be completed by the student where only partially given. The notes for quantitative analysis have been adapted for the most part from the *Methods of Analysis of the Association of Official Agricultural Chemists.* Bul. 107. Bur. of Chem. U. S. Dept. Agric.

REFERENCE BOOKS ON AGRICULTURAL ANALYSIS

Prescott and Johnson, or any other good book on qualitative analysis.
Wiley, Agricultural Analysis, 3 vols. (revised edition).
Sherman, Organic Analysis (revised edition).
Hawk, Physiological Chemistry (revised edition).
Treadwell and Hall, or any other good book on quantitative analysis.

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, potas-

sium, 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 either 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 carbohydrates 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 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, and

plant decay, 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 one per cent solution of aspartic acid, and solutions of mineral acids, such as N/200 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. It should be understood, however, that any such arbitrary test is indicative only, and that true availability can be determined only by the growth of plants upon the soil in question.

Potential supply of plant food is an expression intended to cover 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 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 per cent 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.

2. Preliminary Tests

These tests should be conducted by the use of table reagents unless other strengths are specified. The table reagent consists of one volume of the concentrated reagent diluted with four volumes of water. Express the reactions of these tests by equations, so far as possible, reserving the left-hand pages of the notebook for this purpose.

Action of alkalies upon insoluble silicates. Weigh two portions of Kaolinite of 100 mgm. each. Mix one portion with about 2 gm. of a mixture of K_2CO_3 with Na_2CO_3 in equal amounts by weight. Fuse thoroughly in a small iron crucible. Extract the fused mass and the

unfused Kaolinite with about 10 c. c. hot water each and filter. Add excess of concentrated HCl to the filtrates. Explain the results.

$Al_2O_3 2SiO_2 2H_2O + 5Na_2CO_3 = Na_2AlO_3 +$

$Na_2SiO_3 + HCl =$

Separation of silica from solutions of silicates. To about 5 c. c. of HCl add a nearly equal amount of waterglass. Break up the precipitate with a rod, filter and wash. Compare small portions of it with quartz sand for solubility in hot conc. HCl and 10 per cent NaOH. For this purpose, filter, add excess of HCl, evaporate to dryness and digest the residue with dilute HCl. What is the difference in chemical composition between the precipitate and the quartz sand?

Dry a little of the precipitate thoroughly by heating gradually in a porcelain crucible over a low flame. Now test its solubilities in the reagents previously used:

How would you quantitatively remove silica from acid extracts of soil?

$Na_2SiO_3.XH_2O + HCl =$

$H_2SiO_3 + NaOH =$

$H_2SiO_3 + heat =$

Precipitation of phosphoric acid by metals of soil. To 1 c. c. of 10 per cent sodium hydrogen phosphate solution slightly acidified with HCl add 5 c. c. of a 10 per cent solution of aluminum chloride. Add NH_4OH drop by drop until the precipitate formed requires several seconds to dissolve. Heat nearly to the boiling point, add ammonia in excess and boil for a few seconds. Filter, wash with water and test the filtrate and washings for P_2O_5 by making slightly acid with HNO_3, adding 10 c. c. of clear molybdic reagent and heating until perceptibly hot to the touch. Dissolve a little of the precipitate in nitric acid and test for phosphoric acid with molybdate solution as before. $AlCl_3 + H_3PO_4 + NH_4OH = AlPO_4 +$

$H_3PO_4 + 12(NH_4)_2 MoO_4 + 21HNO_3 = (NH_4)_3PO_4.12MoO_3 +$

$21NH_4NO_3 + 12H_2O.$

$MoO_4 + 21HNO_3 = (NH_4)_3PO_4.12MoO_3 + 21\ NH_4NO_3 + 12\ H_2O.$

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

Acid-soluble soil minerals. Shake about 10 gm. of loam soil in a small flask with about 30 c. c. of dil. HCl warming a little. Filter and make alkaline with NH_4OH as in the previous test. Now filter again and add to the filtrate a few drops of saturated $(NH_4)_2C_2O_4$ solution. What precipitate, if any, forms? What compound readily soluble in acids and giving this test is likely to be commonly found in soils?

After standing warm a few minutes, filter off the precipitate, add a few drops of sodium phosphate solution and make slightly alkaline with NH_4OH. What precipitate appears on standing?

$CaCO_3 + HCl =$

$CaCl_2 + (NH_4)_2C_2O_4 =$

$MgCl_2 + NH_4OH + NaH_2PO_4 =$

Nitrates in soil. Shake up about 10 gm. of a rich garden soil with 25 or 30 c. c. of 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 deliver about 2 c. c. of strongest H_2SO_4 from a pipette 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. Explain the formation of calcium nitrate from farm manure and limestone tilled into the soil.

Why do not nitrates accumulate in soils of humid regions?

$Ca(NO_3)_2 + H_2SO_4 =$

$6FeSO_4 + 2HNO_3 + H_2SO_4 = 2NO +$

Organic matter in soil. Heat a thin layer of fertile soil in a covered crucible over a low flame. Charring indicates the presence of organic matter or humus. Which of the chemical elements of the humus produces the char? What are the sources of the organic matter of the soil? By what chemical processes is the organic matter of cultivated soils exhausted? How does tillage affect the humus content of soil?

$2C_{44}H_{90}O_8NP$ (lecithin) $+ O = 2NH_3 +$

Nitrogen in humus. Thoroughly mix in a mortar 5 gm. 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 distal end may be readily immersed in about 5 c. c. of distilled water in another test tube. Test the water with red litmus paper. Now heat the soil mixture gradually from top to bottom. 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 reaction is the source of this water? Heat for some time and then remove the receiving t. t. before allowing the residue to cool. What is the reaction to litmus of the distillate? What compound has been produced? How was it formed?

CH_3CONH_2 (acetamide) $+ NaOH =$

CH_2NH_2COOH (glycocoll) $+ O =$

METHODS FOR THE ANALYSIS OF SOILS

1. Directions for Taking Samples

Remove surface accumulations of decaying leaves, and debris, and take samples with a soil tube or spade 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 ins., or to the change between the surface soil and the subsoil, in case such change occurs between the depth of 6 and 12 ins. Mix the samples of each depth thoroughly and take subsamples of 2 to 4 lbs., drying the latter in a well-aired, cool place.

The depth to which the sample of subsoil should be taken will depend on circumstances, but in ordinary cases 10 or 12 ins. 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. 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 mm. diameter and thoroughly mixing, samples should be drawn and placed in tightly stoppered jars and carefully labelled.

Moisture.

Weigh out 5 gm. of the air-dried soil into a suitable weighed dish, dry at 100° C. for 5 hrs., cool in a desiccator 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.

Acid Insoluble Residue.

Weigh out duplicate 10 gm. portions of air dry soil on a watch glass and by means of glazed or smooth paper transfer to 200 c. c. Erlenmeyer flasks. To each flask add 100 c. c. of HCl of specific gravity 1.115. This is made approximately by mixing 275 c. c. of concentrated acid (sp. gr. 1.20) with 200 c. c. of distilled water. Add more water or acid as required until the hydrometer shows a specific gravity of 1.115. Into each flask place a rubber stopper carrying a glass tube about 5 mm. in diameter and 50 cm. long, to serve as a reflux condensor. Set on the steam bath and digest for 10 hrs., shaking two or three times during this digestion. Remove, let settle and decant the solution into a hard glass beaker. Small quantities of insoluble material passing over will do no harm. Wash the remaining insoluble residue onto a 15 cm. filter with hot water,

letting the filtrate run into the beaker containing the decanted solution. Continue washing the residue until free from chlorides, saving the filter and its contents. To the solution add 5 c. c. conc. HNO_3 and evaporate to dryness on the steam bath. If the residue appears black repeat the evaporation with HCl and HNO_3, until all the organic matter has been oxidized and the residue looks yellow or orange in color. When the final evaporation is complete, add just sufficient conc. HCl to saturate the residue, add 10 to 20 c. c. water, warm on the bath to dissolve all soluble material, filter out the insoluble silica, wash free from chlorides and save the filter and its contents. Evaporate this filtrate to render insoluble any dissolved silica, take up with acid, filter and wash free from chlorides, preserving the filter as before.

The filtrate constitutes the acid extract of the soil, and may be used to determine the so called "potential supply of plant food." For methods of analysis see Official Methods, Bulletin 107, revised, p. 15. Dry the filter papers with contents. Transfer the latter to smooth paper and burn the filter papers in separate porcelain dishes, previously ignited and weighed. Add the silica and the main residue and ignite to constant weight. Do not allow a partial vacuum to form by cooling this large mass in a too tightly closed desiccator. Weigh as the insoluble residue and report as per cent of the soil.

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?

$K_2O.\ Al_2O_3.\ 6SiO_2 + 2HCl + 5H_2O = Al_2O_3\ .2SiO_2\ .2H_2O +$

$Ca_3(PO_4)_2 + HCl =$

Protein $+ H_2O + HCl =$

Polysaccharide $+ H_2O + HCl =$

Waxes and resins $+ H_2O + HCl =$ no action

$C_6H_{12}O_6 + 8HNO_3 = 8NO +$

Total Nitrogen.

Place 10 gm. of the soil (3 gm. in case of a peaty soil) in a 300 c. c. Kjeldahl digesting flask with 30 c. c. of strongest sulfuric acid, 10 gm. of sodium sulphate and 0.7 gm. of copper sulphate and boil gently for 2 hours after the liquid becomes transparent. Oxidize the residue by adding potassium permanganate in small amounts 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 for nitrogen in fertilizers and report the *per cent of nitrogen on the dry soil.*

Total Phosphorus Determination.

SODIUM PEROXID FUSION METHOD

Weigh 5 gm. of soil into a large iron crucible and mix to a thin paste with water. Using an iron rod, stir in 10 gm. of Na_2O_2, adding the latter in several portions and keeping just enough water present to prevent violent oxidation. Place the lid on the crucible so as to leave a narrow opening. Heat the mixture carefully by applying the flame of a Bunsen burner directly to the side of the crucible until the action starts. Keep the crucible covered until reaction is over and keep at a low red heat for 15 minutes. When cool, add hot water until the crucible is about half full, cover and leave on the steam bath for some time. By means of a spatula and hot water remove the fused mass from the crucible and transfer it to a 600 c. c. beaker. Make slightly acid with conc. hydrochloric acid, heat to boiling and test again with litmus. When slightly acid after boiling add 15 c. c. of concentrated hydrochloric acid and boil for 10 min. If the action has taken place properly no particles of undecomposed soil will remain in the bottom of the beaker, the suspended silicic acid will be white and the solution will appear yellowish-green in color. Transfer to a 500 c. c. volumetric flask and make up to the mark when cold.

Allow the silicic acid to settle and draw off 200 c. c. of the clear solution for analysis. In cases of soils abnormally rich in phosphorus, aluminum or ferric chloride should be added at this point, as directed by the instructor. To this solution add ammonium hydroxide drop by drop until nearly neutral. Then heat almost to boiling and add ammonium hydroxide in slight excess. Boil for about one minute and if no ammonia is then given off, as tested by litmus paper, add more until it can be detected. Filter, wash several times with hot water, pierce the point of the filter and return the precipitate to the original beaker by means of a stream of hot water. Digest any precipitate remaining upon the filter with hot, dilute hydrochloric acid, letting the solution run into the beaker containing the precipitate. Add more acid, and evaporate to complete dryness on the steam bath. Digest with dilute hydrochloric acid, filter out the silica and wash. If much silica is present, the evaporation and removal of silica should be repeated. Evaporate the filtrate to about 25 c. c., neutralize with ammonium hydroxide, clear up with a few drops of nitric acid, and then add 10 gm. of ammonium nitrate. To the hot solution add molybdate solution as directed (50 c. c. for every decigram of phosphoric acid (P_2O_5) and digest at 65° C. for 1 hr. Filter and wash with cold dil. HNO_3 (5 c. c. conc. HNO_3 per 100 c. c.) Test the filtrate for phosphoric acid by renewed digestion and addition of 5 to 10 c. c. molybdate solution. *Deposit all molybdic waste of filtrates in jars provided for the same.*

Dissolve the precipitate on the filter with three or four applications of conc. ammonium hydroxid followed by hot water and wash into a beaker to a bulk of not more than 100 c. c. Nearly neutralize with

hydrochloric acid, cool, and stir with 15 c. c. magnesia mixture added slowly from a burette. After fifteen minutes add 12 c. c. of conc. NH_4OH (and cover with watch glass and specific gravity 0.90), let stand for at least two hours. Filter on ashless paper, wash with dil. NH_4OH (9 c. c. conc. NH_4OH per 100 c. c.) until practically free from chlorides. Dry and ignite until white to gray and weigh as $Mg_2P_2O_7$. Calculate and report the per cent of P_2O_5.

Note. The yellow precipitate of this determination 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 c. c. of conc. NH_4OH followed immediately bp 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 at least one hour before filtering, to throw out phosphoric acid which may have redissolved in the weakened alkaline liquor.

$Al_2O_3 . 2SiO_2 . 2H_2O + 5Na_2O_2 =$

$C_6H_{18}P_6O_{24}$ (phytic acid) $+ Na_2O_2 = Na_4P_2O_7 +$

$Na_2AlO_3 + HCl =$

$Na_4P_2O_7 + 4HCl + H_2O = H_3PO_4 +$

$(NH_4)_3PO_4 . 12MoO_3 + 24NH_4OH =$

$(NH_4)_3PO_4 + MgCl_2 + NH_4OH =$

$2MgNH_4PO_4 +$ heat $=$

Total Sulfur.

Ten grams of soil are placed in a 100 c. c. nickel crucible, moistened with water, about ten gm. of a weighed 20 gm. 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. Why is a gas flame objectionable here?

The crucible is removed, cooled, placed in a 600 c. c. casserole, hot water added and the fused mass removed. It is neutralized with hydrochloric acid and then further acidified with 10 c. c. of hydrochloric acid. The volume is made up to about 450 c. c. and boiled for 15 min., 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 c. c. are evaporated below that volume, refiltered and the volume made up to 500 c. c. Aliquots of 250 c. c. each are heated to boiling, barium chloride added, boiled for 5 min. and set aside on a steam bath for 24 hrs. The volume should not be al-

lowed 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 per cent total SO_3.

$(C_2H_5)_2S + 20\ Na_2O_2 = Na_2SO_4 +$

Total Potassium.

REAGENTS AND APPARATUS

Decomposition of the soil is effected by heating it with its own weight of ammonium chloride and eight times as much precipitated calcium carbonate. The former salt must be pure. Correction for alkalies in the carbonate must be made on the basis of control analyses of this reagent.

The ignition may be made in a covered platinum crucible of ordinary shape and of about 20 to 30 c. c. capacity heated to full redness for not more than two-fifths of its height. The elongated form of crucible with cap originately advocated by Smith is very much to be preferred, however, since it permits, when set at an angle through an opening in the side of a fire clay cylinder, the application of the full heat of the two burners; and perfect decomposition invariably results.

Weigh out ½ gm. of fine soil. In a large agate mortar mix with an equal weight of sublimed ammonium chloride, and grind the two thoroughly together. Then add nearly the whole of a 4-gm. portion of calcium carbonate and continue grinding till thorough mixing has resulted.

The contents of the mortar are transferred to the long crucible, the rest of the carbonate being used for rinsing off mortar and pestle. The crucible is then capped and placed in an inclined position in a clay cylinder or through a hole in a piece of stout asbestos board clamped in a vertical position, and heated for about 10 min. by a low flame placed at considerable distance beneath. As soon as the odor of ammonia is no longer perceptible the nearly full flame of two Bunsen burners is substituted, and continued for 40 to 50 minutes. The sintered cake detaches readily from the crucible as a rule; if not, it is softened up in a few minutes by hot water and digested in a dish until thoroughly disintegrated. It is first washed by decantation, breaking any lumps by means of a pestle, and then thrown on the filter and well washed with hot water. The residue should dissolve completely in hydrochloric acid without showing the least trace of unattacked mineral, not even quartz, though sometimes a few black particles of iron ores will dissolve but slowly.

Separation of Calcium and Sulphuric Acid

All but a trifling amount of the calcium is separated at boiling heat in a large platinum dish by double precipitation by ammonia and ammonium carbonate. The combined filtrates are evaporated to dry-

ness and the ammonium salts are carefully driven off by heating over the Bunsen burner.

Take up the residue with a little water and test for SO_4. If an appreciable amount is present add sufficient $BaCl_2$ to precipitate it. Filter and remove the excess of barium by precipitation with $(NH_4)_2CO_3$. If the sulphur is not thus removed there is danger, if not certainty, of the potassium chloro-platinate carrying sodium sulphate. From the acqueous solution of the residue, but a few c. c. in volume, the rest of the calcium is thrown out by ammonia and ammonium oxalate. The filtrate, caught in an untared platinum crucible or small dish, is evaporated to dryness and gently ignited; the residue is moistened with hydrochloric acid to decompose any alkali carbonate that may have been formed, again evaporated, ignited, and weighed. On solution in water a few tenths of a milligram of residue is invariably left, which should be collected, ignited, and weighed in the same crucible or dish in order to arrive at the weight of the chlorides.

Precipitation of Potassium

To the solution of the chlorides in a small porcelain dish an excess of chlorplatinic acid solution is added. The dilution should be such that when heated on the water bath any precipitate that may form wholly redissolves. Evaporation is then carried on till the residue solidifies on cooling. It is then drenched with alcohol of 80 per cent strength, filtered by decantation through a very small filter and washed by decantation with alcohol of the same strength. Avoid transferring the precipitate to the filter so far as possible. Dish and filter are now dried for a few minutes to remove adhering alcohol, the contents of the former are transferred to a weighed platinum crucible or very small dish and what still adheres to the porcelain is washed through the filter with hot water into the weighed receptacle. This is now placed on the steam bath and afterwards heated for one-half hour to 135° in an air oven. It is very important to cover the dish at first in the air bath, for decrepitation with resultant loss sometimes takes place if this is not done. From the weight of K_2PtCl_6 compute the per cent of K_2O.

Humus Determination.

Place 10 gm. of the air dry soil (5 gm. 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 the **washing should be done chiefly by decantation** from a cylinder or tall beaker. This avoids clogging of the filter. With the aid of a wide-stemmed funnel, wash the contents of the crucible (including the asbestos filter), into a narrow **glass-stoppered** bottle, with 500 c. c. of 4 per cent NH_4OH. (Mix 300 c. c. of water with 200 c. c. cone NH_4OH and add more water or ammonia until the hydrometer reads .9604 which indicates a 20 per cent solution of

NH_4OH. Dilute this to 4 per cent with water.) Allow to remain, with occasional shaking, for **24 hrs.** During this time the bottle 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 hrs.** to allow the sediment to settle.

For the convenience of students this time period may be altered, e. g. changed from 12 to 16 hours which can be included from 4 p. m. to 8 a. m. In any case, however, *the arbitrary time periods specified must be followed closely.*

Draw off 300 c. c. of the supernatant liquid with a pipette, without stirring up the sediment, place in a stoppered 500 c. c. flask and let stand for 48 hrs. Carefully pipette off 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 2 hrs. Dissolve the humus out with 200 c. c. of 4 per cent NH_4OH and filter through paper to separate flocculated clay. Evaporate 50 c. c. aliquots, dry at 100°C., and weigh. Then ignite the residue mildly, to oxidize organic matter, 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.

$Ca(C_{17}H_{33}(OH)_2COO)_2$ (Salt of di-hydroxy stearic acid) + HCl =

$C_{17}H_{33}(OH)_2COOH + NH_4OH =$

$Al_2O_3.2SiO_2.2H_2O$ + heat =

$C_{17}H_{33}(OH)_2COOH + O =$

Total Carbon.

Six grams of sodium peroxide are placed in an iron crucible of 40 to 50 c. c. capacity and the soil added. The amount of sample to be taken will depend upon the amount of carbon present. For ordinary soils 1 to 2 gm. should be used, but for peat soils or other organic materials with a carbon content greater than 40 per cent the amount of sample should be reduced to .10 to .20 gm. Addition of .10 gm. of magnesium should be made for the purpose of accelerating the reaction. The charge is now well mixed with a stirring rod, covered with a tight cover, set in another iron or nickle crucible of about 120 c. c. capacity and this outer crucible also covered. This is done for the purpose of preventing absorption of gases from the flame of the burner during the fusion. The further precaution of projecting the botton of the crucible through a hole in an asbestos board should be followed. The mixture is next ignited by applying the flame and regulating the heat so as to avoid too violent a reaction. This can generally be done by withdrawing the burner at the first sign of the beginning of the reaction. If the reaction proceeds with too great violence losses may occur and less sample should be taken. After the reaction has subsided the crucible should be heated 3 to 5 min. longer. The inner crucible and charge are

then placed in a desiccator and allowed to cool. When cool the crucible is put in a 4-in. funnel and the contents transferred to a 250 c. c. flask with carbon-dioxide-free water, keeping the volume down to 150 c. c. (carbon-dioxide-free water should be prepared by boiling one liter of distilled water for 10 to 20 min.) After the first addition of water to the crucible the funnel should be quickly covered with a watch glass and later washed off. If the fusion has proceeded properly there will be no residue of unburned carbon. The flask is next attached to the condenser head, the solution made acid with 25 per cent sulphuric acid and the carbon dioxide distilled into a tower of beads to which has been added 10 c. c. of 30 to 35 per cent NaOH with a bulbed connector between condenser and bead tower.

After distilling over about 60 c. c. the apparatus is detached and the contents of the receiver transferred to a 250 c. c. flask by successive washings with small portions of carbon-dioxide-free water. This is easily accomplished by inclining the end of the pipette-like connector into the flask and pouring the wash water over the beads. The washing should be complete as indicated by phenolphthalein. It is always possible to completely wash the tower and keep within the 250 c. c. of volume. Phenolphthalein is next added to the solution and 25 per cent sulphuric acid run in until the color begins to change. **Care should be taken not to over-step the end point or low results may follow.** The solution is now made up to 250 c. c. and aliquots of 50 c. c. taken for titration. A few drops of phenolpthalein are added and the titration continued with N/10 sulphuric acid to a neutral reaction. At this point when the carbon exists as acid carbonate, methyl orange is added and the titration continued until a change of color occurs. Use only 1 to 2 drops of a dilute solution of methyl orange. The number of c. c. of N/10 acid used during the titration with methyl orange as an indicator, minus the blank, multiplied by the carbon factor .0012 gives the weight of carbon in 50 c. c. of the solution. A correction blank is run in the same manner as above described to determine the carbon in the peroxide and the reagents used. Report the carbon as per cent of the soil.

Carbon Dioxide.

Place 10 gm. of the air-dry soil (5 gm. 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 calcium chloride tube, a soda-lime tube, and an aspirator bottle arranged in successive order from the condenser. The potash bulb and calcium chloride tube are weighed at the beginning of the experiment.

Having the apparatus carefully joined together, test it for leaks by letting the water flow from the aspirator bottle. If it is tight the

bubbling of gas through the various bulbs will cease. Now add drop by drop 20 c. c. of 20 per cent hydrochloric acid to the soil from the funnel, regulating the addition of acid by the rate at which the carbon dioxide comes off—the bubbles should not pass through the absorption bulbs faster than about 2 per second. After all the acid has been added close the glass stop-cock, and **heat the flask gently** to the boiling point of the solution. Boil for two or three minutes, and then before removing the flame **cautiously** open the stop-cock and admit air into the apparatus. Care must be observed lest a sudden rush of air enters the apparatus. Remove the flame and draw air through the apparatus for 5—10 minutes. A soda-lime tube should be fitted into the funnel containing the hydrochloric acid so as to remove the carbon dioxide contained in the air. Remove the bulbs **previously weighed** and **weigh** again. Report the increase in weight, as per cent of carbon dioxide.

What compound in the soil is the chief source of the evolved carbon dioxide? What function does it serve in the soil? Why is not NaOH used to absorb the CO_2?

$CaCO_3 + HCl =$

$KOH + CO_2 =$

Nitrification Experiment.

Wash 500 to 600 gm. of medium fine sand with distilled water and spread it on paper to dry. Mix with the dry sand about 0.3 gm. K_2HPO_4, 0.5 gram $CaCO_3$ and 2.0 gm. fresh garden soil. Place in a large petri dish or percolator, cover with a mulch of cotton, and determine the weight of the system. Now add 50 c. c. of N/20 $(NH_4)_2SO_4$ and enough distilled water to increase the moisture content to 50 per cent of the water-holding capacity of the sand, by weight, as directed. Maintain the above moisture content. After about 3 weeks leach out the sand with distilled water to a volume of 500 c. c., filtering through paper.

Evaporate 10 c. c. of standard nitrate solution to dryness in a porcelain dish on a water or steam bath and treat the same as the percolate below, finally diluting the solution to 100 c.c. This standard colorimetric solution has the strength of 1 part of NO_3 per million, or 0.001 mgm. NO_3 per c. c.

Evaporate 10 c. c. of the extract to dryness in a porcelain dish on a water bath, removing the dish as soon as it is completely dry. Add 1 c. c. of phenoldisulphonic acid reagent 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 min. At the end of this time the acid is diluted with about 15 c. c. of water and made alkaline with ammonium hydroxide, a yellow color being developed when the solution becomes alkaline. This is then diluted to 100 c. c. and compared with the standard colorimetric solution by means of a colorimeter. With diameter of plungers and divisions of scales

equal, the depths of liquid giving equal intensity of color in this instrument vary inversely as the concentrations of NO_3. If the color of the unknown is too intense for direct comparison with the standard, an aliquot portion may be diluted to definite volume and the strength of this determined. Calculate the milligrams of nitrogen as nitrate recovered from the sand and express as per cent of nitrification, as follows:

Milligrams N as NH_4 in Nutrient Solution=

Milligrams N as NO_3 in 10 c. c. percolate=

Milligrams N in total percolate=

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.

$(NH_4)_2\,SO_4 + O = HNO_3 +$

$CaCO_3 + HNO_3 =$

$CaCO_3 + H_2SO_4 =$

$H_2SO_4 + Ca(NO_3)_2 = 2HNO_3 + CaSO_4 +$

$C_6H_3(OH).(SO_3H)_2$ (Phenol disulphonic acid) $+ HNO_3 =$

$C_6H_2(OH)\,(SO_3H)_2\,(NO_2)$ (Nitro phenol disulphonic acid $+ H_2O$

$C_6H_2(OH)\,(SO_3H)_2 + 3NH_4OH = C_6H_2(ONH_4)\,(SO_2NH_4)_2NO$ (Yellow ammonium nitro-phenol disulphonate) $+ 3H_2O$

II. FERTILIZERS AND MANURES

Fertilizers and 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. When furnishing food in themselves these substances 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** and **Phosphorus. Sulphur** is also very low in amount and freely used by plants, but has not been given commercial value in fertilizers. On the other hand, **potassium** is very abundant in most soils, but a very large part of it is in unavailable forms. It is in common use as an ingredient of fertilizers and is given commercial value. Consequently, our commercial fertilizers contain compounds of **N, P** and **K** either singly or in mixtures, a mixture of all three being termed a **"complete fertilizer."**

Farm yard 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 brines and salt deposits; and phosphorus in the form of apatite from Canada

or the rock and pebble ("river") phosphates from southeastern United 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 per cent of nitrogen in organic forms Hair, horn, and hoof, which are sometimes worked into fertilizers, contain much nitrogen in the crude state but decompose so slowly as to be of slight value as available forms of that element unless they have been treated with sulfuric acid. Bones contain phosphorus equivalent to from 20 to 25 per cent 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 ("Thomas Basic Slag") contains as high as 18 per cent 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 to 15 per cent K_2CO_3 were the main source of potash. 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 containing about 20 per cent and 10 per cent of nitrogen respectively.

1. 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 avaiable 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.
		Reverted	Insol. in water. Sol. in dil. acids, and salt solutions.
	Unavailable P_2O_5	**Insoluble**	Sol. only in the strongest acids.

In the fertilizer industry, enough sulfuric acid is used to insure thorough conversion of the insoluble phosphate to soluble forms. More insoluble phosphate is finally added, to remove free phosphoric acid which may have been formed. This causes the soluble phos-

phates to become insoluble again, or revert by conversion to 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:

$$Ca_3(PO_4)_2 + 3H_2SO_4 = 2H_3PO_4 + 3CaSO_4$$

$$2H_3PO_4 + Ca_3(PO_4)_2 = Ca_2H_2(PO_4)_2 + CaH_4(PO_4)_2$$

Insol. $Ca_3(PO_4)_2 + 2H_2SO_4 =$ **Sol.** $CaH_4(PO_4)_2 + 2CaSO_4$

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

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 stanard acid. The "moist combustion" process which Kjeldahl developed is the most rapid and convenient for determining nitrogen. 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 modification was that which insured the fixation of NO_3 by salicylic acid and its subsequent reduction 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 an alkali and distilled into standard acid solution. Since mercury and ammonia form double compounds not readily decomposed by potassium or sodium hydroxide, it is necessary when the former is employed, to precipitate 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 last preliminary test 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 me-

dium 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 unless formed under carefully controlled conditions, but a volumetric method for estimating phosphorus has been based on its reaction with alkalies. The more 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 salts with ammonium chloride and sodium salts with 80 per cent 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

Volatility of Ammonium Salts.

Place about 0.1 gm. each of ammonium carbonate and ammonium sulphate in separate test tubes. Apply gentle heat to each and test the vapors with red litmus paper. Notice the liquid which collects on the walls of the tube. Dissolve a little of the residue, if any, in water and test with blue litmus. Explain the results.

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 evolve heat. How can loss of nitrogen by volatilization be prevented?

$(NH_4)_2SO_4$ + heat =

$(NH_4)HCO_3$ + heat =

$(NH_4)HCO_3 + CaSO_4$ =

Fermentation of Manure.

Fill two deep 2 gallon jars with fresh horse manure. Fill one jar rather loosely but thoroughly compact and wet the contents of the other. Plunge a thermometer well into each lot of manure, and cover loosely with a sack. Read the temperatures at the beginning and daily, for three or four days. When actively fermenting examine moistened litmus paper placed over the manures for a few minutes. What methods does this experiment suggest by which to prevent the loss of nitrogen from manure heaps by bacterial action?

Solubility of Phosphoric Acid in Rock Phosphate.

Transfer 0.5 gm. rock phosphate to a test tube with 10 c. c. H_2O and stir occasionally for 5 minutes. Filter, heat to boiling and add 5 c. c. molybdic solution to the filtrate. Observe the results on standing and preserve. Repeat the test substituting dilute HNO_3 for the H_2O. What do you conclude from the results?

$Ca_3(PO_4)_2 + HNO_3 =$

Preparation of Acid-phosphate.

Place 5 gm. of rock phosphate in a 2-inch porcelain dish. Add slowly, and with constant stirring, 3.3 gm. (1.8 c. c.) of strongest sulphuric acid, using a heavy glass rod for the purpose. The mixing should be done at the hood. Transfer the mixture to a small strip of board and set aside for a few days. Test 0.5 gm. of the preparation for water-soluble phosphates as in the case of rock phosphate. Compare the amount of precipitate with that obtained from the latter, saving both. Describe your observations fully. What is the irritating gas liberated in this experiment? See equations under paragraph on availability and in third test on soil.

Solubility of Di-Calcium Phosphate.

Test 0.5 gm. of di-calcium phosphate for solubility in water as in the case of acid-phosphate and compare the result with the corresponding extracts from rock phosphate and acid-phosphate. Compare the readiness with which 0.1 gm. of powdered dicalcium phosphate is dissolved in 5 c. c. water and in 5 c. c. neutral ammonium-citrate solution (sp. gr. 1.09) when the solvents are just hot to the touch, shaking a few times to aid solution. The digestions should be filtered, and that with citrate solvent evaporated, ignited and digested with dilute HNO_3. Bring both solutions to a volume of about 5 c. c., make weakly acid with HNO_3 and warm with an equal volume of molybdic reagent.

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

Availability of Nitrogen in Organic Forms.

Prepare a pepsin solution by dissolving 0.2 gm. of commercial pepsin in 100 c. c. of water and adding 0.1 c. c. of conc. HCl. Place 0.5 gm. samples each of ground leather and dried blood in separate 200 c. c. Erlenmeyer flasks. (Horn or hoof may be substituted for leather.) Add 50 c. c. of pepsin solution to each flask and incubate at 40° C for about 24 hours with occasional stirring. At the end of this time, observe the comparative depth of color of the solutions. Filter, evaporate, and compare the amount of dissolved matter.

How is solubility in this test related to the value of these substances as fertilizers?

Protein + H_2O + pepsin. HCl =

Water Soluble Matter in a Commercial Fertilizer.

Place two grams of a dry sample of commercial fertilizer in a filter paper previously fitted to a funnel. Pass 250 c. c. of distilled water through the sample in small portions at a time, saving the entire filtrate. Evaporate a 50 c. c. aliquot of 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. Quantitative Analysis of Fertilizers.

Moisture.

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

Neutralizing Value of Various Forms of Limestone, Burnt Lime, etc.

The value of commercial limes depends on their water content and neutralizing power. How would the neutralizing value of a pound of ground limestone, $CaCO_3$, compare with that of a pound of freshly burnt lime, CaO?

Weigh 1 gm. of the material into a 300 c. c. Erlenmeyer flask, add 50 c. c. of normal hydrochloric acid, heat below active ebullition under reflux condenser for 2 hrs. The condenser should consist of a glass tube of $\frac{1}{4}$-in. bore, 2 ft. long and fitted into a cork stopper. Allow the flask to cool, wash out the condenser into the flask, titrate with half normal alkali, using methyl orange. For pure limestone approximately 80 c. c. of alkali are needed to titrate the excess of acid, but titration of aliquots is not satisfactory. The experiment should be conducted in duplicate. Each c. c. of normal acid used equals .05 gm. of calcium carbonate. Calculate the per cent of calcium carbonate in the sample, and also the per cent of calcium oxide.

Total Phosphoric Acid.

Digest 2 gm. of fertilizer in 30 c. c. of concentrated nitric acid and a small quantity of hydrochloric acid until organic matter is de-

stroyed. After solution, cool, dilute to 250 c. c., mix and pour on a dry filter. Ascertain the approximate per cent of P_2O_5 in your sample. Take an aliquot portion of the solution corresponding to 0.4 gm. of fertilizer, neutralize with ammonium hydroxid, and clear with a few drops of nitric acid. Add about 15 gm of dry ammonium nitrate. To the hot solution add 50 c. c. of molybdate solution for every decigram of P_2O_5 present. Digest at about 65° C. for an hour, filter, and wash with cold water or, preferably, with a solution containing 5 c. c. strongest nitric acid in 100 c. c. 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 c. c. Nearly neutralize with hydrochloric acid, cool, and add 15 c. c. magnesia mixture slowly from a burette, stirring vigorously. Proceed from this point as with the determination of phosphorus in soils.

Water Soluble Phosphoric Acid.

Place 2 gm. 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 c. c. Save the filter paper and residue for the determination of citrate-insoluble phosphoric acid. If the filtrate is 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 250 c. c. mix well, take an aliquot, and proceed as under total phosphoric acid.

Citrate-insoluble Phosphoric Acid.

Place 100 c. c. of strictly neutral ammonium citrate solution of 1.09 specific gravity in a 200 c. c. Erlenmeyer flask and heat to 65° C in a 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, support the bottom of the flask with a towel, and shake vigorously 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 30 min.** 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 15cm. 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 c. c. of strong hydrochloric acid, and digest on the steam bath for 20 minutes. Transfer to 250 c. c. volumetric flask and when cold make up to the mark. Mix well, filter

through a dry filter, take a definite portion of the filtrate and proceed as directed below.

Volumetric Method.

For P_2O_5 percentages of 5 or below use an aliquot corresponding to 0.4 gm. of fertilizer for percentages between 5 and 20 use an aliquot corresponding to 0.2 gm. and for percentages above 20 use an aliquot corresponding to 0.1 gm. Neutralize with ammonia, add 2 c. c. of nitric acid and 5 gm. of ammonium nitrate, dilute to 100 c. c., heat in water bath to 65°, and for P_2O_5 percentages below 5 add from 20 to 25 c. c. of freshly filtered molybdate solution. For percentages between 5 and 20 add from 30 to 35 c. c. molybdate solution. Stir, let stand 15 min. and filter **at once.** Wash once or twice with water by decantation, using from 25 to 30 c. c. each time agitating the precipitate thoroughly and allowing to settle. Transfer to filter and wash with cold water until two filterfuls of washings are turned pink with phenolphthalein by one drop of the standard alkali to be used in this determination. Transfer the precipitate and filter to a beaker, dissolve in a measured moderate excess of standard alkali. Dilute with water if necessary, add a few drops of phenolphthalein solution, and titrate with standard acid. From the cu. cm. of standard alkali neutralized by the precipitate calculate the per cent of **total** P_2O_5.

$Ca_3(PO_4)_2 + HCl =$

$H_3PO_4 + (NH_4)_2\,MoO_4 + HNO_3 =$

$2(NH_4)_3PO_4\ 12\ MoO_3 + 46KOH = 2(NH_4)_2HPO_4 + (NH_4)_2MoO_4 + 23K_2MoO_4 + 22H_2O.$

Citrate Soluble Phosphoric Acid.

As indicated in the preliminary notes; this is usually determined indirectly.

The sum of the water-soluble and citrate-insoluble subtracted from the total gives the citrate-soluble P_2O_5. The latter is added to the water-soluble and the same reported as per cent of **available** P_2O_5. Knowing both the total and citrate-insoluble P_2O_5, subtraction of the latter from the former will also give the available P_2O_5.

Total Nitrogen

Testing for the Presence of Nitrates

Mix 2 gm. of the fertilizer with 25 c. c. of hot water and filter. In 5 c. c. of the filtrate dissolve a small crystal of $FeSO_4$ and to it add 2 or 3 c. c. of conc. H_2SO_4, releasing the latter beneath the solution from a pipette, so that the fluids do not mix. If nitrates are present the junction shows at first a purple, afterwards a brown color.

Modified Kjeldahl Method

This method is to be used when nitrates are present.

Place 2 gm. of the fertilizer in a Kjeldahl flask, add 30 c. c. of sulphuric acid containing 1 gm. of salicylic acid, and shake until

thoroughly mixed, then add 5 gm. of crystallized sodium thiosulphate. Finally place the flask under the hood 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 gently and the boiling continued about 5 or 10 min. Add approximately 0.5 gm. of copper sulphate and continue the boiling until the liquid in the flask has been transparent 1 hr. In case the contents of the flask are likely to become solid before this point is rached, add 10 c. c. more of sulphuric acid. Carefully avoid proximity to fumes of NH_3 in this work.

Prepare a receiving flask at the still, containing 100 c. c. N/10 H_2SO_4 and marked at a volume of 300 c. c. After the digested mixture is cool, transfer to an 800 or 1000 c. c. distilling flask with 200 c. c. of distilled water. The latter should be used in portions, with cooling of the first fraction, to prevent violent action with the alkali to be added. If a glass distilling flask is used add a few pieces of granulated zinc, to keep the contents of the flask from bumping during distillation.

Next add 100 c. c. of conc. NaOH (about 40 per cent conc.) 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,** heat gradually to boiling and distill until all ammonia has passed over into the standard acid. The first 200 c. c. of distillate will contain all the ammonia. The distillate is then titrated with N/10 NaOH, using cochineal extract liberally as indicator. 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.**

Note. In applying this method to fertilizers rich in organic nitrogen such as meat scraps and cottonseed meal, it is usually necessary to digest at least 4 hours in all, and in a larger flask. Troublesome frothing at the start may be somewhat remedied by adding a small lump of paraffin. 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 very low there is danger of losing ammonium sulfate by volatilization. **Do not** add water to the acid residue from digestion until it is quite cool. The receiving flask containing the standard acid should be made ready at the still before alkali is added to the distilling flask to liberate ammonia. If distilling from a glass flask the change from green of cupric sulfate to deep blue of cupric hydroxide will serve as an indicator of alkalinity. Do not allow distilling flask to cool during distillation, because the resultant contraction of volume may draw the distillate back into the distilling flask. **Always** remove the **distillate** at the end of the process before turning off the gas flame.

Fixation: $NaNO_3 + H_2SO_4 =$
$C_6H_4OHCOOH$ (salicylic acid) $+ HNO_3 =$
$C_6H_3(NO_2)(OH)COOH$ (nitro-salicylic acid) $+ H_2O$

Reduction: $2Na_2S_2O_3 + 2H_2SO_4 = 2Na_2SO_4 + H_2S_4O_6 + H_2$
$C_6H_3(NO_2)(OH)COOH + 3H_2 =$
$C_6H_3NH_2)(OH)COOH$ (amino salicylic acid) $+ 2H_2O$

Oxidation: H_2SO_4 + heat =
$2CuSO_4$ + reducing substance (organic matter) =
$Cu_2SO_4 + SO_2 + O_2$ (consumed)
$Cu_2SO_4 + 2SO_3 = 2CuSO_4 + SO_2$
CH_2NH_2COOH (glycocoll) $+ 3SO_3 = NH_3 + 3SO_2 + 2CO_2 + H_2O.$
$C_6H_3(NH_2)(OH)COOH + SO_3 =$
$NH_3 + H_2SO_4 =$

Distillation: $(NH_4)_2SO_4 + NaOH =$
$NH_3 + H_2SO_4 =$

Gunning Method

This method to be used when nitrates are absent.

Place two grams of the fertilizer in a digestion flask and add 10 grams of crystalline sodium sulphate, 0.5 gm. copper sulphate crystals, and 25 c. c. of sulphuric acid. Conduct the digestion as in the Kjeldahl process. Digest for one hour after the mixture is transparent or nearly so. Dilute, neutralize, distill, and titrate as in the Kjeldahl method.

Note. 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 gm. 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. The value of the blank may be supplied by the instructor.

Nitrate and Ammoniacal Nitrogen: Ulsch-Street Method

Place 1 gm. of fertilizer (see below for salt) in a 500 c. c. flask. Add about 30 c. c. of water and 2 to 3 gm. of reduced iron, and after standing sufficiently long to insure solution of the nitrates and ammonium salts add 10 c. c. 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 min.. and cool. Transfer to a Kjeldahl flask with about 150 c. c. of water, add a little paraffin and an excess of strong NaOH. Connect with a condenser, and distil the mixture nearly to dryness. Receive 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 2½ grams of the salt are dissolved in water, made to 250 c. c., and 25 c. c. of the nitrate solution, equivalent to 0.25 gm. of sample, are employed with 5 gm. of reduced iron. After boiling add 75 c. c. of water and an excess of sodium hydrate and complete the determination as above.

Note. Zinc and iron in strong alkaline solution reduces 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 Street method.

$NaNO_3 + H_2SO_4 =$

$H_2SO_4 + Fe =$

$HNO_3 + H = NH_3 +$

$NH_3 + H_2SO_4 =$

Synchronous reduction, distillation:

$2NaOH + Zn = Na_2ZnO_2 + H_2$

$NO_3 + H =$

$NH_3 + NaOH =$

Ammoniacal Nitrogen

Place 3 grams of the fertilizer (see below for the salt) in a Kjeldahl distilling flask add 210 c. c. of water and 5 gm. of magnesium oxide free of carbon dioxide. Distil 200 c. c. into standard acid and titrate the residual acid as in the Kjeldahl method. Report as **per cent of nitrogen in ammonium compound.**

In dealing with ammonium salts or substances rich in them, treat 2.5 gm. of the sample with water and make to a volume of 250 c. c. Take 50 c. c. of the solution in a distilling flask, add 160 c. c. distilled water and proceed as in the case of the mixed fertilizer.

$(NH_4)_2SO_4 + MgO =$

Potash

LINDO-GLADDING METHOD

Methods of Making Solution

(a) With potash salts and mixed fertilizers.—Boil 5 gm. of the sample with 300 c. c. 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 (about 1 gm.) to precipitate all the lime present Cool, dilute to 500 c. c., mix and pass through a dry filter.

(b) With organic compounds.—When it is desired to determine the total amount of potash in organic substances, such as cottonseed meal

and farm manure, thoroughly char 5 gm. with strong sulphuric acid, incorporated gradually, and ignite in a muffle or hood at 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, neutralize, precipitate calcium as in (a), make to a volume of 500 c. c., and pass through a dry filter.

Determination

Obtain from your instructor the approximate per cent of K_2O in the fertilizer and evaporate an aliquot of the solution containing about 0.02 gm. K_2O nearly to dryness. Add about 3 c. c. of dilute sulphuric acid, 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 until the residue is quite white. Dissolve the residue in about 25 c. c. of hot water. Filter from any insoluble residue, and wash. Add a few drops of hydrochloric acid, and chloro-platinic acid (1 c. c.) solution in excess. *Avoiding the absorption of ammonia* evaporate on a water bath to a thick paste or just to dryness and treat the residue with 80 per cent alcohol, sp. gr. 0.8645. The alcohol dissolves the excess of platinic acid and the sodium platinic chloride, Na_2PtCl_6, leaving the potassium as potassium platinic chloride,, K_2PtCl_6. Wash the precipitate thoroughly with 80 per cent alcohol both by decantation and on the filter, continuing the washing after the filtrate is colorless. Wash finally with 10 c. c. of the ammonium chlorid solution to remove magnesium chloride and other impurities from the precipitate and repeat this washing five or six times. Wash again thoroughly with about 100 c. c. of 80 per cent alcohol. Dissolve the precipitate into a weighed evaporating dish with boiling hot water, evaporate and dry to constant weight at 100° C. The precipitate should be perfectly soluble in water. Calculate and report as per cent of K_2O.

Note. 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. **Deposit all platinum precipitates and first alcohol washings** in jars provided for the purpose.

$KHC_4H_4O_6$ (potassium acid tartrate) + H_2SO_4 =

$KCl + H_2SO_4 =$

$CaCl_2 + (NH_4)_2C_2O_4 =$

$K_2SO_4 + HCl + H_2PtCl_6 =$

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 composition. 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 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 readily isolated. 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, as in the humus extraction of 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 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, 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 immature grasses and root crops may be derived largely from amino acids and amides, while the nitrogen of the mature seed of cereal plants exists almost entirely in the form of protein compounds. It is apparent then that the crude protein determination in some cases may yield results considerably above the true protein percentage. The proteins may be isolated however, by precipitation with certain reagents and determined from the nitrogen in the precipitate.

Ether extract is a term including all substances soluble in 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 and pentosans, together 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 current 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 volat-

ilize 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 hydro-carbon oil closly related to gasoline) and carbon-tetra-chlorid, have been used. The extraction should be conducted with heat from other sources 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 hemi-celluloses 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.

Direct determination of components 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 different reducing capacities which are jointly expressed by the analyst in terms of glucose. Starch is only approximately measured by the acid hydrolysis of the residue insoluble in water, since pentosans, galactans and other hydrolyzable carbohydrates may be included in this determination Starch may be determined accurately by digesting this residue of the feeding stuff with diastase of malt extract or saliva, washing out the resultant maltose and dextrins and hydrolyzing them to glucose 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 constituents. Burn 2 or 3 gm. of alfalfa or clover hay to a gray ash in a porcelain dish. Add 30 c. c. of dil. HNO_3 and transfer to a beaker. Bring to boiling, filter, and divide the filtrate into three portions.

To one portion add NH_4OH in slight excess, make distinctly acid with acetic acid and then add a few drops of saturated solution of ammonium oxalate. Why is acetic acid used? To a second portion of the filtrate add 2 or 3 c. c. of $BaCl_2$ solution.

To a third portion of the ash extract add a few drops of $AgNO_3$ solution.

Repeat the above experiment using corn meal and adding 0.2 or 0.3 gm. Na_2CO_3 before burning to prevent loss of volatile acidic elements. How does the strength of each test compare with the corresponding test of the previous experiment?

Char 2 or 3 gm. of crushed wheat in a porcelain dish or crucible, transfer to a beaker, add about 30 c c. dil. HNO_3 and boil for a few minutes, breaking up charred particles with a stirring rod. Add water to restore volume, filter and to 10 c. c. add 5 c. c. of molybdate solution. Heat till hot to the touch and let stand a few minutes. The test is due to phytic acid, $C_6H_{18}P_6O_{24}$, present in the grain as a salt of calcium and other metals. Is it abundant in this grain?

Name organic compounds found in plants which contain Ca, Mg, and P. Which of the above feeding stuffs are supplementary in providing for bone formation?

$-(NO_3)_2 + (NH_4)_2C_2O_4 =$

$K_2- + BaCl_2 =$

$Na- + AgNO_3 =$

$C_3H_7PO_2 + O =$

$H_3- + (NH_4)_2MoO_4 + HNO_3 =$

Fats. 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, or acrylic aldehyde, formed by dehydration of the glycerine in the fat. Glycerine is an alcohol or organic base.

$C_3H_5(OH)_3$ (glycerine) + heat =

C_2H_3CHO (acrylic aldehyde) + $2H_2O$

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 warm water. The product should dissolve. Acidify with sulphuric acid. The fat like substance which now separates should be washed by stirring in a few portions of hot water, allowing to cool and pouring off the fatty liquid from the wash water. Apply the acrolein test. What is the general composition of fats and what is the action of alkalis upon them?

$C_3H_5(C_{17}H_{33}COO)_3$(olein) + 3NaOH =
$C_3H_5(OH)_3$(glycerine) + $3C_{17}H_{33}COO$ Na (soap)
$2C_{17}H_{33}COO$ Na + $H_2SO_4 = Na_2SO_4$ +
$2C_{17}H_{33}COOH$ (oleic acid)

Carbohydrates. *Glucose.* To 5 c. c. of the copper sulphate part of Fehlings solution add 5 c. c. of the alkaline tartrate part and 20 c. c. of distilled water. Heat 5 c. c. of this solution to boiling in a test tube and add a few drops of 0.5 per cent solution of glucose. Boil for a few seconds and if no change takes place add a few drops more of the sugar solution. Observe the color changes, and the precipitate formed.

$CuSO_4$ + 2NaOH + $NaKC_4H_4O_6 = Cu(OH)_2 + Na_2SO_4 + NaKC_4H_4O_6$
$Cu(OH)_2 + NaKC_4H_4O_6 = CuNaKC_4H_2O_6 + 2H_2O$ (Fehling's solution)
$2Cu(OH)_2$ + R. CHO (aldose sugar) = $Cu_2O + 2H_2O$ + R. COOH (organic acids)

What is the function of the tartrate in Fehling's solution? What reciprocal changes occur to the glucose and the copper?

Sucrose. Test a 1 per cent solution of sucrose in the manner described for glucose.

To 5 c. c. of the sucrose solution add an equal volume of dil. HCl and heat to boiling for a minute or two. Neutralize with sodium hydroxide and test with Fehling's solution. Explain the change which sucrose has undergone. What are the reducing sugars formed and to what chemical groupings are their reducing properties due?

$C_{12}H_{22}O_{11}$(sucrose) + H_2O + HCl =
HCl + $C_5H_{11}O_5CHO$ (glucose) + $C_5H_{12}O_5CO$ (fructose)

Starch. Add 0.2 gm. of starch to 20 c. c. of water, boil for a short time and cool. Add a drop of iodine solution to a drop of the starch preparation on a porcelain surface. Test another portion of the starch paste with Fehling's solution, using the proportion of solution to reagent which obtained in the test with glucose.

To about 10 c. c. of the remaining starch paste add 10 c. c. dil. 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. Explain the changes to which the results are due.

$(C_6H_{10}O_5)x$(starch) + xH_2O + HCl = HCl +
x $C_6H_{12}O_6$ (glucose)

Add 10 c. c. concentrated H_2SO_4 to 10 c. c. of water in a small flask and cool. Immerse about 0.5 gm. of cotton and heat at about 80° C. for ½ hr.

Neutralize half of this solution with NaOH using first concentrated then dil. solution of the latter. Test for reducing sugars with Fehling's solution. Save the test.

Boil the remaining acid solution of cellulose for 15 minutes, placing a funnel in the neck of the flask to reduce evaporation. Neutralize and test with Fehling's solution as before. Compare with the previous test.

$(C_6H_{10}O_5)x$ (cellulose) + xH_2SO_4 = cellulose sulfates
Cellulose sulfates + $H_2O = C_6H_{12}O_6$ (glucose) + H_2SO_4

Pentosan. Place about 2 gm. of wheat bran in a 200 c. c. Erlenmeyer flask. Add 30 c. c. water and 10 c. c. conc. HCl 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. Note the time required for the color to appear.

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 than 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.

$(C_5H_8O_4)x$ (pentosan) + concentrated HCl = $xC_4H_3O.CHO$ (furfuraldehyde) + $2H_2O$

anilin + furfuraldehyde = addition product of pink color.

$(C_5H_8O_4)x$ + dilute HCl + H_2O = $xC_5H_{10}O_5$ (pentose sugar)

Proteins. Place about 10 gm. of wheat flour in a porcelain dish, add water gradually 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 conc. HNO_3 and heat. Observe any change of color, cool, add excess of conc. NH_4OH 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. The test is given by compounds containing the phenyl (C_6H_5) group.

To another portion of gluten in 5 c. c. water add an equal volume conc. NaOH solution and boil for a minute or two. Cool and 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 gradually through the clear solution. This is the *biuret reaction* of proteins. It derives its name from the compound biuret $NH_2CONH_2:CONH_2$ obtained by heating urea. The protein molecule contains the double NH_2 linking present in this compound.

To a small portion of gluten add 5 c. c. of Millon's Reagent and heat *just hot to the touch*. The brick red color is due to the presence in the protein of tyrosin an amino acid. Any compound containing the oxy-phenyl (C_6H_4OH—) group will give this test.

Dissolve about 0.1 gm. each of egg albumen and asparagin in separate small portions of water. The former should be finely powdered, shaken well with H_2O, let stand a few minutes and filtered. Albumen is a protein, while asparagin is a comparatively simple derivative of proteins, and belongs to a class of compounds called amides, involving both the amide and amino acid groupings. To separate portions of the two solutions add a few drops of a saturated solution of mercuric chloride. Repeat with a solution of copper sulphate. Let stand some

time and record your observations. How would you separate proteins from amides and amino acids where they 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. Quantitative Analysis of Feeding Stuffs

Dry 3 gm. of the substance in the steam oven for 5 hrs. Cool and weigh as usual. Dry again for 3 hrs. and weigh.

Repeat, if necessary, until constant weight has been attained, saving the dried material.

Ether Extract

Transfer the dried sample from the previous determination to a fat-free paper capsule by means of a piece of glazed paper and plug in loosely with a wad of fat-free absorbent cotton. Carefully clean a 50 c. c.Erlenmeyer flask, dry in the oven for ½ hr., cool in desiccator and weigh. Now drop the capsule of dried feeding stuff in an extraction tube, connect the weighed flask, pour 20 c. c. anhydrous alcohol free 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 hrs., remove the flask, evaporate remaining ether, clean the outer surface and dry at 100° C for 2 hrs. or to a minimum weight. Cool and weigh as before. Deduct the weight of flask and report as per cent of ether extract on the dry sample.

Note. Carefully avoid vicinity of flames in this determination. The thimble and residue should be dried in the steam oven for a few minutes, and the contents used for the determination of crude fiber, unless the material is of a sticky nature and can not be easily removed. Do not destroy the thimble, but return it to the store room.

Crude Fiber.

The residue from the ether extraction is used or if this cannot be removed without contamination by the cellulose of the capsule, a separate sample should be weighed into a linen filter wrapped to a cylindrical shape, dried, and extracted with ether for 2 or 3 hrs. only, using an unweighed flask. To this residue of feeding stuff, in a 500 c. c. Erlenmeyer flask, add 200 c. c. of boiling 1.25 per cent sulphuric acid. Connect the flask with an inverted condenser (an inverted 50 c. c. pipette may be used) the tube of which passes only a short distance below the rubber stopper into the flask. Boil at once and continue the boiling for 30 min. A cold, wet cloth may be placed upon the flask to reduce frothing of the liquid. Filter on a linen cloth and wash with boiling water till the washings are no longer acid. Transfer the substance back into the same flask with

200 c. c. of a boiling 1.25 per cent solution of sodium hydroxid, boil at once, and continue the boiling for 30 min., as directed above for the treatment with acid. Filter on a Gooch crucible and wash with boiling water till the washings are neutral. Dry in the oven for 5 hrs., cool and weigh. Incinerate completely and weigh again. Report loss of weight as *per cent crude fiber.*

Note. 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 but filter paper and paper capsules are to be avoided because of the cellulose which they may so readily yield to the sample. The digesting liquor should be boiled gently.

$(C_6H_{10}O_5)x$ (starch) $+HCl + H_2O =$

$(C_5H_8O_4)$ x (pentosan) $+ HCl + H_2O =$

Protein $+$ NaOH $=$ soluble complex.

Starch

If starch is gelatinized by boiling with water and then treated with malt diastase or saliva, it can be converted into maltose and dextrin. These can be separated by filtration from the insoluble residue containing the pentosans and other substances which yield reducing sugars by acid hydrolysis.

Determination. Extract 3 gm. of dry sample with five successive 10 c. c. portions of ether, decanting the washings through a hardened filter. Wash with 100 c. c. of 10 per cent alcohol to remove sugars and dextrins. Transfer the residue to a beaker with 100 c. c. of water and heat gradually to boiling, stirring constantly to prevent bumping or the formation of lumps. Cool to 55° for diastase, or to 38° for salivia and add 10 c. c. of the solution containing the enzyme Keep the mixture stirred and within 2° of the stated temperature for 15 to 20 min. Now heat the solution again to boiling, in order to gelatinize any starch granules which may remain and test by bringing a trace of the digesting mixture in contact with iodine solution on a white porcelain surface, using the tips of stirring rods for securing the test liquids. If starch is still present cool to the proper temperature add 2 to 5 c. c. more of the enzyme solution and digest as before. Continue this treatment until the solution gives no starch reaction after boiling. Dilute to 250 c. c., mix thoroughly and pour on a dry fluted filter. Transfer 150 c. c. of the filtrate to a 250 c. c. flask and add 9 c. c. of conc HCl attach the flask to a reflux condenser and boil gently on a sand bath for 45 min. Cool and neutralize carefully with concentrated, followed by diluted, NaOH. If turbid, clarify by shaking with alumina cream (suspension of $Al(OH)_3$). Make to 500 c. c., mix thoroughly, filter if turbid, and determine the reducing power by the Defren-O'Sullivan method. From the weight of CuO derive the equivalent glucose from the table at the end of the manual. Convert to starch by the proper factor and report as per cent of the feeding stuff.

The Defren-O'Sullivan Method. Mix 15 c. c. of Fehling's copper solution, A, with 15 c. c. of the tartrate solution, B, in a 200 c. c. Erlenmeyer flask, and add 50 c. c. of distilled water. Place the flask and its contents in a boiling water bath and allow them to remain 5 min. Then run rapidly from a pipette into the hot liquor in the flask 25 c. c. of the sugar solution to be tested (which should contain not more than ½ per cent of reducing sugar). Allow the flask to remain in the boiling water bath just 15 min. after the addition of the sugar solution, remove, and with the aid of a suction flask 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 should have been previously ignited, cooled, and weighed. The cuprous oxide precipitate is thoroughly washed with boiling distilled water till the washings are neutral.

Dry the Gooch with its contents in the oven, and finally heat to dull redness, for 15 min., 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 desiccator, cooled and weighed.

Note. The determination should be carried through without interruption. If this is impossible, care must be taken to avoid alcoholic or lactic fermentation. After the digestion with the enzyme is finished, but not before about 0.5 gm. of *salicylic acid* may be added as a preservative. A trace of sodium fluoride may be added at the start to retard lactic fermentation while the digestion with the enzyme is taking place.

When only a few determinations are to be made, freshly collected saliva can conveniently be used, as this is free from carbohydrate. If commercial diastase or an infusion of malt is used, the amount added must be noted and a correction applied for the carbohydrate thus introduced. This is found by heating a quantity of the diastase or infusion with acid and determining the resulting glucose as in the starch determination.

Ash.

Char 2 gm. 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 desiccator and weigh. Report as *per cent of ash* on the *dry* feeding stuff.

Crude Protein.

Determine nitrogen in a 1 gm. sample by the Gunning method as given for fertilizers and multiply the result by 6.25.

True Protein.

Place 1 gm. of the substance in a beaker, add 100 c. c. of water and heat to boiling, or, in the case of substances rich in starch, heat on the water both 10 min. Add a quantity of cupric hydrate suspension (Stutzer's reagent) containing about 0.5 gm. of the hydrate and stir thoroughly. Filter when cold, wash with cold water, and, without removing the precipitate from the filter, determine nitrogen by the Gunning method given for the determination of nitrogen in fertilizers.

Note. 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 is not done, cupric phosphate and free alkali may be formed and the protein-copper precipitate may be partially dissolved in the alkaline liquid, causing low results.

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 anlysis 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. Dissolve about 1 gm. $CuSO_4$ in 10 c. c. hot water and cool the solution. To about 5 c. c. add a few drops of dil. NH_4OH. Now add 5 c. c. NH_4OH and mix. Explain the result.

$CuSO_4 + 2NH_4OH = Cu(OH)_2 + (NH_4)_2SO_4$

$CuSO_4 + 4NH_4OH = Cu(OH)_2 . 2NH_4OH . (NH_4)_2SO_4$

Dilute 2 or 3 drops of the $CuSO_4$ solution with about 500 c. c. of water. To about 5 c. c. of this solution add 2 or 3 drops of dilute $K_4Fe(CN)_6$ solution. Potassium ferro-cyanide detects 1 part Cu in 200,000 parts of solution. Add 2 or 3 c. c. of $K_4Fe(CN)_6$ solution to 5 c. c. of the conc. $CuSO_4$ solution. 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?

$2CuSO_4 + K_4Fe(CN)_6 = Cu_2FeCN)_6 + 2K_2SO_4$

Arsenic. Dissolve about 0.5 gm. each of sodium arsenate and sodium arsenite in separate 10 c. c. volumes of water. Stir 2 or 3 c. c. magnesia mixture into each solution. Explain your results. How could you detect *arsenic* when present in an insecticide as *an arsenite*?

$Na_3AsO_3 + O = Na_3AsO_4$

$Na_3AsO_4 + MgCl_2 + NH_4OH = Mg\ NH_4AsO_4 + 2NaCl + NaOH$

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. This is 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. The precipitate is called Schweinfurth's green or paris green. This is the true paris green.

$CuSO_4 + Na_3AsO_3 + H_2O = Na_2SO_4 + CuHAsO_3 + NaOH$

$4Cu(C_2H_3O_2)_2 + 2\ Na_3AsO_3 =$

$Cu_3(AsO_3)_2 . Cu(C_2H_3O_2)_2 + 6NaC_2H_3O_2$

Acetic acid. Dissolve about 0.5 gm. $NaC_2H_3O_2$ in 5 c. c. water. Add 2 or 3 c. c. conc. H_2SO_4 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. Test a paris green sample in the same way.

$NaC_2H_3O_2 + H_2SO_4 =$

$C_2H_4O_2 + C_2H_5OH =$

Purity of Paris Green. Compare a pure sample of paris green with samples of unknown purity by the following methods:

(a) To about 1 gm. 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 gm. 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.

METHODS FOR THE ANALYSIS OF INSECTICIDES

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

Moisture.

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

Paris Green.

(a) *Insoluble matter.*

Weigh out 1 gm., 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.*

$Cu_3(AsO_2)_2$. $Cu(C_2H_3O_2) + NH_4OH$ (excess) =

(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 hr. 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.*

$(NH_4)_3AsO_3 + HCl$ (excess) =

$Cu(OH)_2$. $2NH_4OH(NH_4)_2SO_4 + HCl$ (excess) =

$CuCl_2 + (NH_4)_2S$ =

(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 to 100 c c. add 10 c. c. dil. H_2SO_4 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) *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 hr., filter (rejecting filtrate), and dissolve the ppt. in KOH. Filter to remove the free sulphur, and pass chlorine gas into the solution for 1 hr. (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 hrs., 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.*

$H_3AsO_3 + H_2S =$

$As_2S_3 + KOH =$

$K_3AsO_3 + H_2O + Cl =$

$H_3AsO_4 + MgCl_2 + NH_4OH =$

$MgNH_4AsO_4 + \text{heat} =$

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

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

This 50 c. c portion is concentrated by boiling in a 250 c. c. 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. The well mixed solution is 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 thiosulphate added, drop by drop, until the solution becomes exactly colorless. This end point is easily 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 c. c. of iodin solution

used and the weight of arsenious oxid taken, the value of each c. c. of iodin in terms of arsenious oxid is determined.

$As_2O_3 + NaOH =$

$KI + HCl =$

$2H_3AsO_4 + 4HI = 5H_2O + 2I_2 +$

$2Na_2S_2O_3 + I_2 = Na_2S_4O_6 +$

$Na_3AsO_3 + HCl =$

$H_3AsO_3 + I + H_2O =$

Determination. Two gm. of paris green are weighed out, transferred to a 250 c. c. flask and about 100 c. c. of water and 2 gm. of sodium hydrate added. This mixture is boiled for 5 to 10 min., 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 c. c. The well-shaken liquid is filtered through a dry filter and 50 c. c. taken for analysis. The analysis is carried out from this point forward the same as when standardizing the iodin solution.

(e) *Soluble Arsenious Oxid.*

METHOD 1

Digest 1 gm. of paris green for about 5 min. with 25 c. c. of hot 50 per cent (by volume) solution of sodium acetate. The solution is then cooled and made up to 100 c. c. Fifty c. c. are filtered off and titrated with standard iodin as in determination (d). Report as *per cent of sodium-acetate-soluble As.*

METHOD II

Method. One gm. of paris green is treated with 1,000 c. c. of previously boiled (CO_2 free) water in a large flask. The flask is stoppered and shaken 8 to 10 times each day for 10 days. At the end of this time the solution is filtered through a dry filter paper. Two hundred c. c. of the filtrate are treated with sodium bicarbonate and titrated with iodin as in determination (d). Report as *per cent of water-soluble As.*

Lime-sulphur.

Determination of Total Sulphur

Place about 10 c. c. of the clear sample in a weighed 100 c. c. volumetric flask, weigh again and fill to the mark. Mix well and analyze 10 c. c. aliquots of this solution by treating with 3 c. c. of conc. KOH solution, followed by 50 c. c. of H_2O_2 solution free from sulphates. Heat on the steam bath for exactly ½ hr. and then acidify with HCl. Heat to boiling and add 10 per cent barium chlorid solution slowly, with stirring until precipitation is complete. Digest on the steam bath until clear, filter, wash free from chloride, ignite and weigh. Before igniting completely, the precipitate should be treated with one or two

drops of concentrated HNO_3 to reoxidize any sulfur which may have been reduced by the burning filter paper. Report the *per cent of total S* in the dip or wash.

$CaS_5 + KOH = K_2S +$

$K_2S + H_2O_2 =$

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 the cells of the udder. This change involves extended 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 or 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:

$$C_3H_5\begin{cases} C_3H_7COO \text{ (Butyric acid radicle)} \\ C_{11}H_{23}COO \text{ (Lauric acid radicle)} \\ C_{17}H_{33}COO \text{ (Oleic acid radicle)} \end{cases}$$

About 90 per cent of these fats are the glycerides of insoluble and non-volatile acids

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

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, 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 tests of feeding stuffs.) To what class of compounds does casein belong?

Albumin. Neutralize the filtrate saved from the previous test, using dilute NaOH. Concentrate 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 ziuret and xantho-proteic tests. What is the nature of albumin?

Ash constituents. Bring the volume of filtrate saved in the previous test to about 50 c. c. Make 10 c. c. distinctly acid with acetic acid and add a few drops $(NH_4)_2\ C_2O_4$ 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?

Lactose. Dissolve about 0.5 gm. powdered 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 per cent strength. Can lactose be determined in the presence of these preservatives? What is the chemical relation between formalin (formaldehyde) and some of the reducing sugars?

METHODS FOR MILK ANALYSIS

Total Solids

Babcock asbestos method.—Obtain a hollow cylinder of perforated sheeet metal, 60 mm. long and 20 mm. in diameter closed 5 mm. from one end by a disk of the same material. The preforations should be about 0.7 mm. in diameter and about 0.7 mm. apart. Fill loosely with from 1.5 to 2.5 gm. 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 gm.) and dry at 100° to constant weight for the determination of total solids.

Note. 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. In general, milk can be conveniently weighed by difference from a small flask, stoppered to

prevent vaporation. 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.

Fat

Extract the residue from the determination of water by the Babcock asbestos method with anhydrous ether for 10 hrs. or more, using weighed extraction flasks.. Evaporate the ether, dry the fat at 100° and weigh.

Nitrogen Compounds

Determination of total nitrogen compounds. Place in a Kjeldahl digestion flask a known weight (about 5 gm.) of milk, and proceed, without evaporation, as described for the Gunning method under nitrogen in fertilizers. Multiply the percentage of nitrogen by 6.38 for nitrogen compounds.

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 24 hrs., add 1 part of formaldehyde to 2,500 parts of milk, and keep in a cool place. Place about 10 gm. of milk in a beaker with about 90 c. c. of water and add at once 1.5 c. c. of a solution containing 10 per cent of acetic acid by weight. Stir well and let stand from 3 to 5 min. 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 is 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 Gunning 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.

Note. 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.

Determination of albumin in milk. The filtrate obtained in the preceding operation is neutralized with caustic alkali, 3/10 c. c. of a ten per cent solution of acetic acid added and the mixture heated to the temperature of boiling water for from 10 to 15 min. The precipitate is collected on a filter, washed, and the nitrogen therein determined. Nitrogen multiplied by 6.38 equals albumin.

Note. These moist protein precipitates readily *decompose on standing.* They should therefore be *immediately treated with the* H_2SO_4 for

the succeeding Gunning digestion, after which they may be allowed to stand for a time *if kept from ammonia fumes.*

Ash

Put about 20 gm. of milk into a tared, ignited porcelain dish, add 6 c. c. of conc. nitric acid, evaporate to dryness, and burn at a low red heat until the ash is free from carbon.

Milk-Sugar *Volumetric Method*

Twenty-five grams of the milk (24.2 c. c.) are transferred to a 250-c. c. flask, 0.5 c. c. of a 30 per cent solution of acetic acid are added and the contents well shaken. After standing for a few minutes, about 100 c. c. of boiling water are run in, the contents again shaken, 25 c. c. 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 c. c. The filtrate must be perfectly clear. The milk sugar in a solution thus precipitated would ordinarily not exceed 0.5 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 c. c. each of copper and alkali solution in 50 c. c. of water, till sufficient has been introduced to completely reduce the copper. This is determined by placing a drop of 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 min. each time, until the ferrocyanide test fails to appear.

As 0.067 gm. milk sugar will reduce 10 c. c. 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 gm. of milk sugar, from which the percentage is readily computed. Thus if 16 c. c. of the milk-sugar solution are necessary to reduce the copper, then 16 c. c. contain 0.067 gm. milk-sugar.

250 c. c. of solution contain 25 gm. milk
1 c. c. of solution contain 0.1 gm. milk
16 c. c. of solution contain 1.6 gm. milk

and 1.6 gm. milk contain 0.067 gm. milk-sugar. Therefore the sample contains .067x100/1.6==4.19 per cent lactose.

Babcock Method for Fat.

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 a min., 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 c. c.

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

DETERMINATION

Pipette off 17.6 c. c. of the carefully mixed sample into a test bottle and add 17.5 c. c. 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 4 min. at the required speed for the machine used. Add boiling hot water, filling to the neck of the bottles, and whirl for 1 min.; again add boiling water so as to bring the fat within the scale on the neck of the bottles, and after whirling for 1 min. 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."

Hart Method for Casein.

REAGENTS

(*a*) *Chloroform.* A high grade of chloroform should be used. When not in use the chloroform bottle should be kept in a cool, dark place.

(*b*) *Acetic Acid.* A 10 per cent solution is made by diluting 10 c. c. glacial acetic acid (99½ per cent pure) to 100 c. c. Then 25 c. c. of the 10 per cent acid are diluted to 1,000 c. c. with water. This gives a 0.25 per cent solution, the correct strength for the casein test.

TEMPERATURE

The testing should be done in a room where the *temperature is 60° to 70° 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° F.* It may be necessary to warm or cool the samples before testing, depending on the season. The acetic acid should be as near 70° F. as possible, although a few degrees variation (not over 5°) either way will not cause any serious error.

It is aboslutely essential that these precautions as to temperature be strictly observed:

DETERMINATION

The test bottles should be placed in a suitable rack in an upright position. Measure 2 c. c. 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 c. c. A pipette should not be used to measure chloroform. Next add with a pipette 20 c. c. of the 0.25 per cent acetic acid to each bottle. Then put 5 c. c. 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 from *15 to 20 secs.* 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 min. at 2,000 revolutions a minute. To secure this speed the handle of the tester must be turned 56 times a minute. The speed can best be regulated by using a metronome, set to beat audibly 56 times a minute.

After whirling, the test bottles should be taken from the machine, replaced in the rack, and allowed to stand 10 min. 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 min. (up to 24 hrs.) before reading without affecting the result, but should *never* be read in *less* than 10 min.

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 also cause ragged edges.

ATOMIC WEIGHTS IN COMMON USE
(International Atomic Weights, 1918)

Aluminum	Al.	27.10	Manganese	Mn.	54.93
Arsenic	As.	74.96	Mercury	Hg.	200.60
Barium	Ba.	137.37	Molybdenum	Mo.	96.00
Calcium	Ca.	40.07	Nitrogen	N.	14.01
Carbon	C.	12.00	Oxygen	O.	16.00
Chlorine	Cl.	35.46	Phosphorus	P.	31.04
Copper	Cu.	63.57	Platinum	Pt.	195.20
Hydrogen	H.	1.008	Potassium	K.	39.10
Iodine	I.	126.92	Silicon	Si.	28.30
Iron	Fe.	55.84	Silver	Ag.	107.88
Lead	Pb.	207.20	Sodium	Na.	23.00
Magnesium	Mg.	24.32	Sulphur	S.	32.06

DEFREN'S TABLE FOR DEXTROSE, MALTOSE, AND LACTOSE

Cupric Oxide mgms.	Glucose mg.	Maltose mg.	Lactose mg.	Cupric Oxide mg.	Glucose mg.	Maltose mg.	Lactose mg.
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	58.8	44.1	220	98.6	161.5	140.6
80	35.4	50.1	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	192.3
160	71.3	116.9	101.7	310	139.9	229.6	199.8
170	75.8	124 4	108.2	320	144.5	237.2	205.9

PREPARATION OF REAGENTS

The following are reagents whose composition is not stated in the text. Values in brackets are approximate requirements of 100 students.

Alkali for nitrogen distillation. Approximately 50 per cent solution of commercial NaOH.

Alumina cream. Add excess of NH_4OH to an alum solution of 10 to 15 per cent strength. Filter, wash free from ammonium hydroxid and suspend to the desired consistency in distilled water (2 liters).

Ammonium chloride, for K_2PtCl_6 wash. Dissolve 100 gm. of NH_4Cl in 500 c. c. water, add 5 gm. pulverized K_2PtCl_6 and shake at intervals for 8 hrs. Let stand over night and filter (15 liters).

Ammonium citrate solution. Dissolve 370 gm. of commercial citric acid in 1,500 c. c. 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°. (15 liters).

Ammonium oxalate. Saturated solution (4 liters).

Anilin acetate. Equal volumes of anilin and 50 per cent acetic acid, mixed. (Table indicator bottles).

Asbestos. Boil long-fibered asbestos for ½ hr. with 20 per cent HNO_3 and wash with hot water. Repeat the boiling with 20 per cent NaOH and wash free from alkali with hot water. Suspend in distilled water in wide-necked bottles.

Barium chloride. Solution of 10 per cent concentration. (4 liters).

Chloro-platinic acid. The red-brown crystals have the formula $H_2PtCl_6 6H_2O$. The solution should be made to contain 1.0 gram Pt. in 10 c. c.

Cochineal indicator. Digest and frequently agitate 3 gm. of pulverized cochineal in a mixture of 50 c. c. strong alcohol and 200 c. c. of distilled water for a day or two at ordinary temperatures and filter. (1 liter.)

Cupric hydroxide suspension (Stutzer's reagent). Dissolve 100 gm. of pure cupric sulphate in 5 liters of water, add 25 c. c. of glycerol, and then a dilute solution of sodium hydrate until the liquid is alkaline. Filter and rub the precipitate up with water containing 5 c. c. of glycerol per liter. 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 suspension. (2 liters.)

Fehling's Solution.

A. Fehling's copper solution. 34.639 gm. of carefully selected crystals of pure copper sulphate dissolved in water and diluted to exactly 500 c. c.

B. Fehling's alkaline tartrate solution. 178 gm. sodium potassium tartrate and 50 gm. sodium hydroxide dissolved in water and diluted to exactly 500 c. c.

A and B are added when used. (15 liters of each part.)

Glycerol-soda solution. Prepare a solution of NaOH by dissolving 100 gm. of the solid in 100 c. c. of water. Add the cool solution to pure glycerol in the ratio of 1 volume of the former to 9 volumes of the latter. (2 liters).

Magnesia mixture. Dissolve 110 grams crystallized $MgCl_2 6H_2O$ in about 1500 c. c. of water and add 280 gm. of NH_4Cl. Mix well, add 260 c. c. concentrated NH_4OH, and dilute to 2 liters. (12 liters).

Methyl orange indicator. A 0.1 per cent aqueous solution.

Millon's reagent. Warm 1 part of Hg with 2 parts of concentrated HNO_3 until the Hg is dissolved. *Avoid inhaling fumes.* (Table reagent.)

Molybdate solution. Dissolve 100 gm. of molybdic acid in 144 c. c. of ammonium hydroxid, specific gravity 0.90, and 271 c. c. of water; slowly and with constant stirring, pour the solution thus obtained into 489 c. c. of nitric acid (specific gravity 1.42), and 1,148 c. c. 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. (15 liters).

Phenol di-sulfonic acid. (Chamot. Jr. A. Chem. Soc. 33:382). To 25 grams of white phenol add 150 c. c. concentrated H_2SO_4 and 75 c. c. fuming H_2SO_4 (13 per cent SO_3) and heat 2 hrs. at 100° C. (500 c. c.)

Phenolphthalein indicator. One gm. of phenolpthalein per 100 c. c. 95 per cent alcohol.

Silver nitrate. One per cent solution (Table reagent).

Standard Acid Solutions

For nitrogen determination. 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 this solution be used in tenth normal strength, but it may be employed with use of an N/10 factor (50 liters).

For the crude fiber determination. This should be standardized to 1.25 per cent strength by titration against standard alkali. (50 liters).

For volumetric P_2O_5 determination.

Solution of any mineral acid standardized against the corresponding alkali. It should be one half the normality of the latter. (10 liters).

Standard alkali solutions.

(For nitrogen determination). The strength of this solution relative to the acid used must be accurately determined. An approximately N/10 solution of NaOH is recommended. (40 liters).

(For crude fiber determination.) This should be made as directed for the corresponding acid. (50 liters).

(For volumetric P_2O_5 determination). Solution of 0.324 normal NaOH free from carbonates. One c. c. is equivalent to 1 mg. of P_2O_5. (6 liters).

Standard nitrate solution. Dissolve 0.1631 gm. of pure, dry potassium nitrate in water and make up to 1 liter. Of this stronger solution 100 c. c. are diluted in 1 liter. This constitutes the standard nitrate solution and contains 0.01 of a mg. NO_3 in each c. c., or 1 part NO_3 per 100,000 of solution. (2 liters).

Starch indicator.—One per cent suspension prepared by short boiling.