LABORATORY NOTES

IN

QUALITATIVE ANALYSIS

AND

MEDICAL CHEMISTRY.

BY

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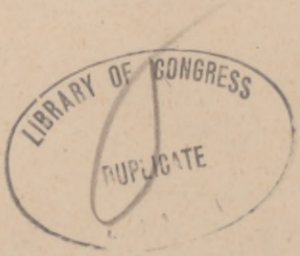

PREFACE.

The writer, in designing this manual, aims to provide the student with an outline sketch of chemical tests which shall be of permanent and practical utility. With this in view all experiments requiring expensive apparatus have been omitted, and those, only, given which would come naturally within the field of the average home or office laboratory. It is intended that the tests shall be criticised, compared, and elaborated, and that additions shall be made under the instructor's advice. By this means it is believed that each student, at the end of his laboratory course, will find himself provided with a handy book of reference. the more valuable because of his own part in its making.

C. P.

CONTENTS.

PART I.

QUALITATIVE ANALYSIS.

TABLE OF PRINCIPAL ELEMENTS.

F. W. CLARKE, 1895 (Cal. to O = 16).

	Symbols.	Atomic Weights.		Symbols.	Atomic Weights.
Aluminum	Al	27	Magnesium	Mg	24.3
Antimony	Sb	120	Manganese	Mn	55
Arsenic	As	75	Mercury	Hg	200
Barium	Ba	137.43	Molybdenum	Mo	96
Bismuth	Bi	208	Nickel	Ni	58.7
Boron	B	11	Nitrogen	N	14.03
Bromine	Br	79.95	Oxygen	O	16
Cadmium	Cd	112	Phosphorus	P	31
Calcium	Ca	40	Platinum	Pt	195
Carbon	C	12	Potassium	K	39.11
Chlorine	Cl	35.45	Silicon	Si	28.4
Chromium	Cr	52.1	Silver	Ag	107.92
Cobalt	Co	59.5	Sodium	Na	23.05
Copper	Cu	63.6	Strontium	Sr	87.66
Fluorine	F	19	Sulphur	S	32.06
Gold	Au	197.3	Tin	Sn	119
Hydrogen	H	1.008	Titanium	Ti	48
Iodine	I	126.85	Tungsten	W	184.9
Iron	Fe	56	Uranium	U	239.6
Lead	Pb	206.95	Vanadium	V	51.4
Lithium	Li	7.02	Zinc	Zn	65.3

THE METALS.

CLASSIFICATION FOR PURPOSES OF ANALYSIS.

THE metals are commonly divided into five groups, according to their behavior with certain general, or *group reagents;* as follows:

GROUP I.—Metals forming *chlorides* insoluble in water, and consequently precipitated from solutions of their salts by Hydrochloric Acid. *Lead*, *Silver*, *Mercury* (Mercurous).

GROUP II.—Metals forming *sulphides* insoluble in water and in dilute acids, precipitated from solutions of their salts by Sulphuretted Hydrogen.

(*a*) The sulphides are soluble in Ammonium Sulphide and in Sodium or Potassium Hydroxides. *Arsenic*, *Antimony*, *Tin*.

(*b*) The sulphides are insoluble in Ammonium Sulphide and in Sodium or Potassium Hydroxides. *Mercury* (Mercuric), *Lead*, *Bismuth*, *Copper*, *Cadmium*.

GROUP III.—Metals forming *sulphides* and *hydroxides*, which are decomposed by acids, but which are insoluble in water, precipitated from neutral solutions by Ammonium Sulphide. *Iron*, *Manganese*, *Aluminum*, *Chromium*, *Cobalt*, *Nickel*, *Zinc*.

GROUP IV.—Metals forming sulphides soluble in water or decomposed by dilute acids, but whose *carbonates* are insoluble, precipitated from solutions of their salts by Ammonium Carbonate. *Barium*, *Strontium*, *Calcium*, *Magnesium*.

GROUP V.—Metals forming chlorides, sulphides, and carbonates soluble in water, and not precipitated by the preceding group reagents. *Potassium*, *Sodium*, *Lithium*. (*Ammonium*, NH_4, is also, commonly included in this group.)

PRELIMINARY TESTS.

GROUP V. The "Alkali Metals."

Potassium, K., Sodium, Na., Lithium, Li., Ammonium, NH_4.

POTASSIUM.

(Use KCl, Solution or Solid.)

1.—Test on clean platinum wire, in Bunsen flame, observe the

violet color developed. In presence of sodium compounds the yellow rays produced thereby may be excluded by use of blue glass.

2.—$PtCl_4$ precipitates from solutions of potassium salts, yellow crystalline, potassium platinic chloride, K_2PtCl_6.

3.—$H_2C_4H_4O_6$ precipitates from concentrated alkaline solutions of potassium salts, white, crystalline, acid potassium tartrate, $KHC_4H_4O_6$.

Sodium.

(Use NaCl, Solution or Solid.)

1.—Test on platinum wire, in Bunsen flame, observe the bright yellow color developed.

2.—Potassium pyroantimoniate, $H_2K_2Sb_2O_7$, precipitates from concentrated solutions of sodium salts, a white, crystalline, sodium pyroantimoniate, $H_2Na_2Sb_2O_7$.

Lithium.

(Use LiCl, Solution.)

1.—Na_2HPO_4 precipitates from hot solutions of lithium salts, lithium phosphate, Li_3PO_4.

2.—Lithium compounds color the non-luminous flame crimson, or carmine red.

Ammonium.

(Use NH_4OH and $(NH_4)_2SO_4$.)

1.—Note the odor of NH_4OH.

2.—Note the action of the fumes on red litmus paper.

3.—Upon one watch glass place a drop of NH_4OH, upon another a drop of HCl. Observe the white fumes of NH_4Cl produced when the glasses are brought together.

4.—Test a solution of $(NH_4)_2SO_4$. Note that no odor is given off. Add a few drops of NaOH (Sol.) and heat. Note the characteristic odor of ammonia.

5.—$PtCl_4$, and $H_2C_4H_4O_6$ produce precipitates with ammonium salts resembling those produced from solutions of potassium compounds.

For the separation of the members of this group, see p. 19.

Group IV. Metals of the "Alkaline Earths."

Barium, Ba., Strontium, Sr., Calcium, Ca., Magnesium, Mg.

Barium.

(Use $BaCl_2$, Solution.)

1.—$(NH_4)_2CO_3$ precipitates white barium carbonate, $BaCO_3$:

$$BaCl_2 + (NH_4)_2CO_3 = BaCO_3 + 2\ NH_4Cl.$$

2.—H_2SO_4 (dil.) precipitates white barium sulphate, $BaSO_4$:

$$BaCl_2 + H_2SO_4 = BaSO_4 + 2\ HCl.$$

3.—Na_2HPO_4 precipitates white barium phosphate, $BaHPO_4$.

4.—K_2CrO_4 precipitates yellow barium chromate, $BaCrO_4$.

5.—Barium compounds impart a green color to the flame.

Strontium.

(Use $Sr(NO_3)_2$, Solution.)

1.—$(NH_4)_2CO_3$ precipitates white strontium carbonate, $SrCO_3$.

2.—H_2SO_4 (dil.) precipitates white strontium sulphate, $SrSO_4$.

3.—K_2CrO_4 precipitates from alkaline solutions yellow strontium chromate, $SrCrO_4$, soluble in $HC_2H_3O_2$.

4.—Strontium compounds impart an intense red or crimson color to the flame.

Calcium.

(Use $CaCl_2$, Solution.)

1.—$(NH_4)_2CO_3$ precipitates white calcium carbonate, $CaCO_3$.

2.—H_2SO_4 precipitates white calcium sulphate, $CaSO_4$, soluble in an excess of water.

3.—Na_2HPO_4 precipitates white calcium phosphate, $CaHPO_4$, soluble in $HC_2H_3O_2$.

4.—$(NH_4)_2C_2O_4$ precipitates white calcium oxalate, CaC_2O_4, soluble in HCl, insoluble in $HC_2H_3O_2$.

5.—Calcium compounds impart a yellowish red color to the flame.

Magnesium.

(Use $MgCl_2$ Solution.)

1.—$(NH_4)_2CO_3$ precipitates white magnesium carbonate, $MgCO_3$.

2.—Add NH_4Cl, then $(NH_4)_2CO_3$. Note that now no precipitate is formed. $MgCO_3$ is soluble in NH_4Cl.

3.—To the solution from the last test, add Na_2HPO_4. Note that a white precipitate of $MgNH_4PO_4$ is formed. This precipitate is aided by the addition of NH_4OH and by agitation.

For the separation of members of this group, see p. 19.

Group III.

Aluminum, Al., Chromium, Cr., Iron, Fe., Nickel, Ni., Cobalt, Co., Manganese, Mn., Zinc, Zn.

Aluminum.

(Use Solution of Potash Alum, or of Al_2Cl_6.)

1.—NH_4OH precipitates white aluminum hydroxide, $Al_2(OH)_6$.

2.—NaOH precipitates white aluminum hydroxide, $Al_2(OH)_6$. Soluble in excess of the reagent.

3.—$(NH_4)_2S$ precipitates white aluminum hydroxide, $Al_2(OH)_6$:

$$Al_2Cl_6 + 3(NH_4)_2S + 6\,H_2O = Al_2(OH)_6 + 6\,NH_4Cl + 3\,H_2S.$$

4.—$(NH_4)_2CO_3$ precipitates white aluminum hydroxide, $Al_2(OH)_6$:

$$Al_2Cl_6 + 3(NH_4)_2CO_3 + 3\,H_2O = Al_2OH_6 + 6\,NH_4Cl + 3\,CO_2.$$

Chromium.

(Use Cr_2Cl_6 Solution.)

1.—NH_4OH precipitates light green chromium hydroxide, $Cr_2(OH)_6$.

2.—NaOH precipitates light green chromium hydroxide, $Cr_2(OH)_6$. Soluble in excess but precipitated again on boiling.

3.—$(NH_4)_2S$ precipitates light green chromium hydroxide, $Cr_2(OH)_6$.

4.—Fused with a mixture of KNO_3 and Na_2CO_3 on platinum foil, yellow sodium and potassium chromates are formed, soluble in water.

5.—Acidify the solution obtained in the last test, with $HC_2H_3O_2$ and then add $Pb(C_2H_3O_2)_2$ (Sol.)—a yellow precipitate of lead chromate, $PbCrO_4$ is formed.

Iron.

Ferrous Compounds. (Use $FeSO_4$ Solution.)

1.—NaOH or NH_4OH precipitates white, or greenish white, $Fe(OH)_2$, turning brown on exposure to the air.

2.—$(NH_4)_2S$ precipitates black ferrous sulphide, FeS.

3.—$K_4Fe(CN)_6$ precipitates bluish white potassium ferrous ferrocyanide, $K_2Fe_2(CN)_6$.

4.—$K_3Fe(CN)_6$ precipitates dark blue ferrous ferricyanide, $Fe_5(CN)_{12}$, known as Turnbull's blue.

5.—K(CN)S gives no reaction.

Ferric Compounds. (Use Fe_2Cl_6 Solution.)

1.—NaOH or NH_4OH precipitates reddish brown ferric hydroxide, $Fe_2(OH)_6$.

2.—$(NH_4)_2S$ precipitates black ferrous sulphide, FeS.

3.—$K_4Fe(CN)_6$ precipitates ferric ferrocyanide, $Fe_7(CN)_{18}$, known as Prussian Blue.

4.—$K_3Fe(CN)_6$ produces no precipitate but imparts a green or brown color to the solution.

5.—K(CN)S produces a blood red color due to the formation of ferric sulphocyanate, $Fe_2(CNS)_6$. The color is destroyed by addition of $HgCl_2$.

Manganese.

(Use $MnSO_4$ Solution.)

1.—NaOH or NH_4OH precipitates whitish manganous hydroxide $Mn(OH)_2$, turning brown and oxidizing, on exposure to the air, to $Mn_2(OH)_6$.

2.—$(NH_4)_2S$ precipitates flesh colored manganous sulphide, MnS.

3.—Fused with a mixture of KNO_3 and Na_2CO_3 on platinum foil, green potassium and sodium manganates are formed.

Zinc.

(Use $ZnSO_4$, or $ZnCl_2$, Solution.)

1.—NaOH added carefully precipitates white zinc hydroxide, $Zn(OH)_2$ easily soluble in excess:

$$ZnCl_2 + 2\ NaOH = Zn(OH)_2 + 2\ NaCl.$$
$$Zn(OH)_2 + 2\ NaOH = Na_2ZnO_2 + 2\ H_2O.$$

2.—NH_4OH precipitates white $Zn(OH)_2$ soluble in excess.

3.—$(NH_4)_2S$ precipitates white zinc sulphide, ZnS.

Nickel and Cobalt.

(Omitted because of relative medicinal unimportance.)

For the separation of members of this group, see p. 18.

Group II.

Arsenic, As., Antimony, Sb., Tin, Sn., Mercury (Mercuric) Hg., Bismuth, Bi., Lead, Pb., Copper, Cu., Cadmium, Cd.

Arsenic.

Arsenous Compounds. (Use As_2O_3, Solution in water.)

1.—H_2S gas precipitates yellow arsenous sulphide, As_2S_3. Insoluble in boiling HCl, soluble in alkalies, and in alkaline sulphides and carbonates.

2.—Ammonio silver nitrate precipitates yellow silver arsenite, Ag_3AsO_3. Soluble in excess of NH_4OH.

3.—Ammonio cupric sulphate precipates green copper arsenite, $CuHAsO_3$. Soluble in excess of NH_4OH.

Arsenic Compounds. (Use NaH_2AsO_4 in Solution.)

1.—H_2S gas precipitates, slowly, arsenous sulphide, As_2S_3, mixed with sulphur.

2.—Ammonio silver nitrate precipitates brown silver arsenate, Ag_3AsO_4. Soluble in excess of NH_4OH.

3.—Ammonio cupric sulphate precipitates bluish green copper arsenate, $CuHAsO_4$.

For Special Tests for Arsenic, see p. 25.

Antimony.

(Use Tartar Emetic in Solution.)

1.—Acidify with HCl and pass H_2S gas, an orange precipitate of Sb_2S_3 is formed. Soluble in alkaline sulphides but insoluble in alkaline carbonates.

2.—NaOH and NH_4OH precipitate antimonous hydroxide, $Sb(OH)_3$. Soluble in excess of the reagent.

3.—In the absence of tartaric or citric acids, *e. g.*, in solutions of the chloride, $SbCl_3$, an excess of water produces a precipitate of a basic salt, the oxychloride, SbOCl, or "Powder of Algaroth."

For Special Tests for Antimony, see p. 26.

Mercury(ic).

(Use $HgCl_2$ Solution.)

1.—H_2S gas precipitates black mercuric sulphide, HgS, insoluble in HNO_3, HCl, or $(NH_4)_2S$.

2.—NaOH precipitates yellow mercuric oxide, HgO.

3.—$SnCl_2$ precipitates white mercurous chloride, Hg_2Cl_2.

$$2\,HgCl_2 + SnCl_2 = Hg_2Cl_2 + SnCl_4.$$

An excess of the reagent precipitates gray metallic mercury.

$$HgCl_2 + SnCl_2 = Hg + SnCl_4.$$

4.—KI precipitates yellow to scarlet mercuric iodide, HgI_2.

For Special Tests for Mercury, see p. 27.

Bismuth.

(Use $Bi(NO_3)_3$ Solution.)

1.—H_2S gas precipitates black bismuth sulphide, Bi_2S_3. Soluble in boiling HNO_3, but insoluble in alkalies and alkaline sulphides.

2.—NaOH and NH_4OH precipitate white bismuth hydroxide, $Bi(OH)_3$.

3.—KI precipitates brown bismuth iodide, BiI_3, soluble in excess of the reagent.

4.—H_2O in excess precipitates basic salts of bismuth, bismuth subnitrate, $BiONO_3$.

Copper.

(Use $CuSO_4$ Solution.)

1.—H_2S gas precipitates black cupric sulphide, CuS, soluble in hot HNO_3, practically insoluble in alkalies and in alkaline sulphides.

2.—NH_4OH precipitates bluish cupric hydroxide, $Cu(OH)_2$, soluble in excess, forming a dark blue solution.

3.—NaOH precipitates blue cupric hydroxide, $Cu(OH)_2$, insoluble in excess, turning black on boiling.

4.—$K_4Fe(CN)_6$ precipitates reddish brown cupric ferrocyanide, $Cu_2Fe(CN)_6$.

Lead, (see Group I.)

Tin and Cadmium.

(Omitted because of relative medicinal unimportance.)

For the separation of the members of this group, see p. 17.

Group I.

Lead, Pb., Silver, Ag., Mercury (Mercurous), Hg_2.

Lead.

(Use $Pb(NO_3)_2$ Solution.)

1.—HCl precipitates white crystalline lead chloride, $PbCl_2$ soluble in hot water.

2.—NaCl precipitates white crystalline lead chloride, $PbCl_2$, soluble in hot water.

3.—H_2SO_4 (dil.) precipitates white lead sulphate, $PbSO_4$.

4.—K_2CrO_4 precipitates yellow lead chromate, $PbCrO_4$, soluble in fixed alkalies.

5.—NH_4OH precipitates white basic lead hydroxide.

6.—H_2S precipitates black lead sulphide, PbS, insoluble in alkalies and alkaline sulphides, but soluble in hot HNO_3.

7.—KI precipitates yellow lead iodide, PbI_2, soluble in hot water.

Silver.

(Use $AgNO_3$, Solution.)

1.—HCl precipitates white silver chloride, AgCl, soluble in NH_4OH, insoluble in HNO_3.

2.—NaCl produces the same precipitate as does HCl.

3.—H_2SO_4 produces no precipitate.

4.—K_2CrO_4 precipitates reddish yellow silver chromate, Ag_2CrO_4.

5.—H_2S precipitates black silver sulphide, Ag_2S.

Mercury(ous).

(Use $Hg_2(NO_3)_2$ Solution.)

1.—HCl precipitates white mercurous chloride, Hg_2Cl_2 which turns black on the addition of NH_4OH, mercurous ammonium chloride, NH_2Hg_2Cl being formed.

2.—H_2SO_4 produces no precipitate.

3.—K_2CrO_4 precipitates orange mercurous chromate, Hg_2CrO_4.

4.—KI precipitates green mercurous iodide, Hg_2I_2.

5.—H_2S precipitates black mercuric sulphide, HgS, mixed with Hg.

For the separation of the members of this group, see p. 17.

ANALYTICAL SCHEME FOR METALS.

Group I.

To the solution, add HCl, drop by drop, until there is no further precipitate. Filter, and wash the precipitate with cold water. (Reserve the filtrate for Group II.). Perforate the filter paper, wash the precipitate into a test tube and heat to boiling with water. Filter rapidly while the liquid is still hot.

The Filtrate contains $PbCl_2$. Test for lead with H_2SO_4 and with K_2CrO_4. **Pb.**	*The Residue* contains AgCl and Hg_2Cl_2. Perforate the filter paper, wash the residue into a test tube, add NH_4OH, warm and filter.

A Black Residue = NH_2Hg_2Cl. **Hg.**	*The Filtrate* contains $(NH_3)_3(AgCl)_2$. Boil off the excess of NH_4OH, add a little HNO_3 to acidify the solution. A white precipitate = AgCl. **Ag.**

Group II.

Pass H_2S gas through the slightly acid filtrate from Group I., as long as a precipitate is formed. Filter and wash the precipitate. (Reserve the filtrate for Group III.). Perforate the filter paper, wash the precipitate into a test tube, add $(NH_4)_2S$, warm gently, filter, and wash.

A. The precipitate contains Hg, Pb, Bi, Cu.

B. The filtrate contains As, Sb.

A. Perforate the filter paper, wash the precipitate into a beaker, add strong HNO_3, boil for several minutes, and filter.

A Black Residue = HgS. **Hg.** Transfer the residue to a porcelain dish, dissolve in HCl + HNO_3. Boil off the excess of acid, dilute with a little water and test for Mercury with $SnCl_2$ or KI.	*The Filtrate* contains Pb, Bi, Cu. Boil off the excess of acid, add a little water, and then a few drops of dilute H_2SO_4. A precipitate = $PbSO_4$. **Pb.** Filter, and add NH_4OH to the filtrate. If there be a precipitate, filter and apply tests for bismuth, **Bi.** If the NH_4OH does not give a precipitate, but forms a dark blue solution, then copper is present. **Cu.**

B. The Filtrate containing As and Sb.

To this solution add HNO_3. The sulphides, if present, are re-precipitated. Filter, and wash the precipitate. Perforate the filter paper and wash the precipitate into a large test tube. Add some fragments of solid $(NH_4)_2CO_3$ and warm for several minutes. Filter and wash.

The Residue contains Sb_2S_3. Dissolve in hot HCl, dilute with water, warm, and pass H_2S gas. An orange red precipitate $= Sb_2S_3$. **Sb.** Apply Special Tests to the original solution.	Acidify the *Filtrate* with HNO_3. A bright yellow precipitate $= As_2S_3$. **As.** Apply Special Tests to the original solution.

GROUP III. (Phosphates, Oxalates, etc., being absent.)

Boil the filtrate from Group II., until all the H_2S is expelled, add a few drops of HNO_3, and boil again. Then add NH_4Cl, and NH_4OH (excess). If there be a precipitate, filter, and wash. (Reserve the filtrate.) Perforate the filter paper, wash the precipitate into a test tube, add NaOH and boil for several minutes. Filter and wash.

The Precipitate contains, $Fe_2(OH)_6$ and $Cr_2(OH)_6$. Test a portion for Chromium, **Cr.**, by Tests 4 and 5 (p. 12). Dissolve the remainder of the precipitate in dilute HCl and test for iron, Fe with KCNS. **Fe.**	*The Filtrate* contains $K_2Al_2O_4$. Acidify slightly with HCl, then add $(NH_4)_2CO_3$. A precipitate $= Al_2(OH)_6$ or— Add an excess of NH_4Cl to the alkaline filtrate, and heat slowly to boiling. A precipitate $= Al_2(OH)_6$. **Al.**

To the filtrate (reserved above) from the ammonia precipitation, add $(NH_4)_2S$. Warm and filter. (Reserve the filtrate for Group IV.) Treat the precipitate with cold dilute HCl, filter and boil the filtrate. Cool, and add a large excess of NaOH. Filter.

The Residue $= Mn(OH)_2$. Dissolve in HCl, and test according to p. 13. **Mn.**	To the *Filtrate* add $(NH_4)_2S$. A white precipitate $= ZnS$. **Zn.**

GROUP III. (Phosphates, etc., present.)

To the filtrate from Group II., add NH_4Cl, NH_4OH, and $(NH_4)_2S$. Filter and reserve the filtrate for Group IV. Wash

the precipitate and dissolve it in cold dilute HCl. Boil, to expel the H_2S, and filter if necessary. Add a few drops of HNO_3 and boil again. Test a small portion of the solution with KCNS for iron, Fe. To the remainder of the solution add Fe_2Cl_6 drop by drop, until all is precipitated, concentrate, neutralize with K_2CO_3, and add an excess of $BaCO_3$. Filter.

Boil the *Precipitate* with NaOH for several minutes.		To the *Filtrate* add HCl, and boil to expel CO_2. Add $(NH_4)_2S$ and NH_4OH. Filter, dissolve the precipitate in HCl, add excess of NaOH. Test the precipitate for Manganese, **Mn.**, and the filtrate for Zinc, **Zn.**
Test the *Solution* for aluminum. **Al.**	Test the *Precipitate* for Chromium. **Cr.**	

Group IV.

To the filtrate from Group III., add HCl, boil, and filter. To the filtrate add NH_4OH and $(NH_4)_2CO_3$. Filter. Test the filtrate for magnesium **Mg.**, with Na_2HPO_4, see p. 12). Dissolve the precipitate in a small amount of HCl, evaporate the solution to dryness, and treat the residue with *absolute* alcohol.

Residue = $BaCl_2$, dissolve in water, and test for barium, **Ba.**	Evaporate the *Solution*, to expel the alcohol, and convert the chlorides to nitrates by repeated evaporations with HNO_3. Evaporate finally to dryness, and treat the residue with absolute alcohol.	
	Residue = $Sr(NO_3)_2$. Dissolve in water and test for strontium. **Sr.**	*Solution* contains $Ca(NO_3)_2$. Test for Calcium, **Ca.** See p. 11.

Group V.

Test the original solution for Ammonium, **NH_4.** (See p. 10.) For Potassium, Sodium, and Lithium, use the flame tests with the original solution, *or*, evaporate the solution containing only members of this group, and heat strongly. Dissolve the residue in a little water, add a few drops of HCl, filter if necessary, and add an excess of $PtCl_4$. A precipitate = K_2PtCl_6, **K.** Add a little water, filter and test the filtrate for sodium by the flame test, and with potassium pyroantimoniate. A precipitate = $H_2Na_2Sb_2O_7$, **Na.** Test another portion of the filtrate for lithium by the flame test, and with sodium phosphate. A precipitate = Li_3PO_4, **Li.**

CHARACTERISTIC TESTS FOR COMMON ACIDS.

Hydrochloric Acid, HCl, and Chlorides.

1.—$AgNO_3$ precipitates curdy white silver chloride, AgCl, soluble in NH_4OH, insoluble in HNO_3.

2.—$Hg_2(NO_3)_2$ precipitates white mercurous chloride, Hg_2Cl_2.

3.—$Pb(C_2H_3O_2)_2$ precipitates white crystalline lead chloride, $PbCl_2$.

4.—H_2SO_4 and MnO_2 warmed with the solution, liberate chlorine gas.

5.—Apply Test 3 under Ammonium (Metals, Group V.).

Sulphuric Acid, H_2SO_4, and Sulphates.

1.—$BaCl_2$ precipitates white barium sulphate, $BaSO_4$, insoluble in HCl.

2.—$Pb(C_2H_3O_2)_2$ precipitates white lead sulphate, $PbSO_4$, soluble only in hot concentrated acids.

Nitric Acid, HNO_3, and Nitrates.

1.—Boiled with copper filings red fumes are produced, the liquid turning green. If the nitric acid be present in combination, add H_2SO_4 to decompose the nitrates.

2.—Add to the solution in a test tube an equal bulk of H_2SO_4. Cool the mixture and float over it a solution of $FeSO_4$. At the contact of the two liquids a brown ring will develop.

3.—A small quantity of the fluid added to a solution of *brucia* in concentrated H_2SO_4, develops a fine red color. (Chloric acid gives the same reaction.)

4.—Nitrates and nitric acid are reduced by a mixture of zinc and H_2SO_4, NH_3 being formed.

Acetic Acid, $HC_2H_3O_2$, and Acetates.

1.—Note the characteristic odor of the acid.

2.—Add Fe_2Cl_6 then NH_4OH to neutralization, the liquid acquires a dark red color. The same is given in solutions of the neutral acetates without the addition of NH_4OH.

3.—Warm the solution with a few drops of H_2SO_4 and the same of C_2H_5OH. The characteristic odor of ethyl acetate, $C_2H_5(C_2H_3O_2)$ is developed.

Oxalic Acid, $H_2C_2O_4$, and Oxalates.

1.—$CaCl_2$ with NH_4OH precipitates white calcium oxalate, CaC_2O_4, soluble in HCl, insoluble in $HC_2H_3O_2$.

2.—$AgNO_3$ precipitates white silver oxalate, $Ag_2C_2O_4$, soluble in hot concentrated HNO_3, and in NH_4OH.

Tartaric Acid, $H_2C_4H_4O_6$, and Tartrates.

1.—$CaCl_2$ precipitates white calcium tartrate, $CaC_4H_4O_6.4H_2O$, soluble in $HC_2H_3O_2$. Soluble also in NaOH, from which solution it is reprecipitated on boiling.

2.—From solutions of normal tartrates, $AgNO_3$ precipitates white silver tartrate, $Ag_2C_4H_4O_6$, which blackens on boiling.

3.—Heated on platinum foil, tartaric acid, or tartrates, fuse, carbonize, and give off the characteristic odor of burnt sugar.

Citric Acid, $H_3(C_6H_5O_7)$, and Citrates.

1.—$CaCl_2$ precipitates white calcium citrate, $Ca_3(C_6H_5O_7)_2$, insoluble in NaOH. The precipitate is most easily obtained from the hot solution. Calcium citrate is soluble in $HC_2H_3O_2$. (Distinction from oxalates.)

2.—$Ca(OH)_2$ in excess precipitates, from the boiling solution only, white calcium citrate, $Ca_3(C_6H_5O_7)_2$.

3.—Heated on platinum foil, citric acid fuses, carbonizes, and gives off pungent acid fumes.

Phosphoric Acid, H_3PO_4, and Orthophosphates.

1.—Fe_2Cl_6 with $NaC_2H_3O_2$ precipitates yellowish-white ferric phosphate, $Fe_2(PO_4)_2$.

2.—$CaCl_2$ with NH_4OH precipitates white calcium hydrogen phosphate, $CaHPO_4$, soluble in $HC_2H_3O_2$.

3.—$AgNO_3$ precipitates yellow silver phosphate, Ag_3PO_4, soluble in HNO_3 and in NH_4OH.

4.—Ammonium molybdate $(NH_4)_2MoO_4$ precipitates yellow ammonium phosphomolybdate, $(NH_4)_3PO_4(MoO_3)_{10}2H_2O$.

5.—"Magnesia Mixture" precipitates white magnesium ammonium phosphate, $Mg(NH_4)PO_4$.

Hypophosphites: 1.—On ignition inflammable PH_3 is given off. 2.—$AgNO_3$ precipitates white silver hypophosphite, turning black on exposure.

Pyrophosphates: 1.—$AgNO_3$ precipitates white silver pyrophosphate. 2.—

$MgSO_4$ precipitates magnesium pyrophosphate, soluble in excess of the reagent. 3.—$(NH_4)_2MoO_4$ reacts very slowly, or not at all.

Metaphosphates: 1.—$AgNO_3$ precipitates white silver metaphosphate. 2.—$(NH_4)_2MoO_4$ causes no precipitate. 3.—Albumen forms a white precipitate.

Chromic Acid, H_2CrO_4, and Chromates.

See p. 12. Chromium.

Permanganic Acid, $H_2Mn_2O_8$, and Permanganates.

See p. 13. Manganese.

Hydrocyanic Acid, HCN, and Cyanides.

1.—Note the characteristic odor.

2.—$AgNO_3$ precipites white silver cyanide, AgCN, soluble in KCN, slightly soluble in NH_4OH, and in boiling HNO_3, but insoluble in cold dilute HNO_3. To obtain the reaction with cyanides, such as KCN, add first a little HNO_3 to decompose the cyanide and then add the $AgNO_3$. The precipitate is distinguished from AgCl by its sparing solubility in NH_4OH, and by the odor of HCN developed on warming with HCl.

3.—Evaporate to dryness at a low temperature with NH_4HS. Dissolve the residue in water and add Fe_2Cl_6. The solution turns blood red in color.

Hydroiodic Acid, HI, and Iodides.

1.—$AgNO_3$ precipitates yellow silver iodide, AgI, insoluble in HNO_3 or in NH_4OH.

2.—$Hg_2(NO_3)_2$ precipitates green mercurous iodide, Hg_2I_2.

3.—$HgCl_2$ precipitates red mercuric iodide, HgI_2.

4.—Add a few drops of "Chlorine water," and then a little starch paste. A blue color is developed which disappears when the solution is heated, but reappears when the solution is cooled. For free iodine the same test is used without the addition of "Chlorine water."

Hydrobromic Acid, HBr, and Bromides.

1.—$AgNO_3$ precipitates yellowish-white silver bromide, AgBr, insoluble in HNO_3, slightly soluble in NH_4OH.

2.—$Hg_2(NO_3)_2$ precipitates yellowish mercurous bromide, Hg_2Br_2.

3.—Add a little carbon disulphide, CS_2, and then a few drops of chlorine water. Mix well by shaking. The CS_2 acquires a reddish-yellow tint. (With iodides by the same test, the CS_2 is colored violet-red.)

4.—With starch paste and chlorine water a yellow color is developed.

Carbonic Acid, H_2CO_3, and Carbonates.

1.—Acids, such as HCl, produce an effervescence of CO_2 gas.

2.—$Ca(OH)_2$ and $Ba(OH)_2$ precipitate white $CaCO_3$, and $BaCO_3$, soluble in acids with effervescence.

ANALYTICAL SCHEME FOR METALS AND ACIDS.

1.—Evaporate some of the solution to dryness. Note the character of the residue. (Presence or absence of organic compounds, etc.). Cool, add a few drops of H_2SO_4, and warm again. Note any effervescence (carbonates), peculiar odors, or fumes (sulphides, sulphites, iodides, bromides), or characteristic acid fumes, (HCl, HNO_3, etc.). See Preliminary Examination of Solids.

2.—Examine the solution for metals, according to the Analytical Scheme, p. 17.

3.—Test the original solution for HCl, HNO_3 and H_2CO_3.

4.—If arsenic or antimony be present, precipitate with H_2S gas. In absence of arsenic and antimony, or after their removal, (boil off the H_2S gas), add concentrated Na_2CO_3 solution and boil. Filter off the precipitate, if any, add a little HNO_3 and boil again to drive off the CO_2. Test the solution for H_2SO_4, $H_2C_2O_4$, H_3PO_4, HI, HBr, HCN, etc.

If H_2SO_4 or sulphates are present, these must be removed before testing for oxalic or phosphoric acids. The following scheme of separation may be used: To the neutral solution add $BaCl_2$ *and* $CaCl_2$. Filter and digest the precipitate with dilute HCl (a residue = $BaSO_4$), filter, add NH_4OH to an alkaline reaction, then add $HC_2H_3O_2$ to an acid reaction. (A residue = CaC_2O_4, shows presence of oxalic acid). Filter and add NH_4OH to an alkaline reaction. (A precipitate = $CaHPO_4$, shows presence of phosphoric acid).

The tests obtained in this solution are to be confirmed by tests with the original solution.

To complete the examination of an unknown liquid, preceding tests being negative, apply the special tests given on pages 25–28. Finally, test for the common alkaloids, p. 28.

EXAMINATION OF SOLIDS.

Preliminary Examination.

1.—Heat some of the substance on a loop of platinum wire, in a flame. The flame is colored *yellow*, by sodium compounds; *crimson*, by strontium; *violet*, by potassium; *yellowish-red*, by calcium; *green*, by barium and copper; *blue*, by arsenic and antimony; *carmine-red*, by lithium.

2.—Heat some of the substance on a piece of platinum foil, or in a small tube. If *combustible*, we have, probably, organic compounds, or C, S, P; if *volatile*, ammonia (note odor), Hg, As, etc., or volatile salts; if *fusible*, salts of alkalies, certain metallic salts, etc.; if *water vapor be evolved*, a crystalline salt containing water of crystallization, hydroxides, etc.

3.—Add a little powdered charcoal to the substance, and heat on platinum foil. If the substance deflagrates, we have probably, nitrates, chlorates, iodates, etc.

Preparation of the Solution for Analysis.

Reduce the substance to a fine powder and dissolve in boiling water, if possible. If all does not go into solution, add a little HCl, and boil again. If some still remains undissolved, try a fresh portion of the powder with HNO_3, and, finally, dissolve any remaining residue in a mixture of HNO_3 (1 pt.) and HCl (3 pts.) If strong acids be required for the solution, it is best to evaporate to dryness and to redissolve in water before proceeding with the analysis.

In treating with the acids, note any peculiar appearances. For example, a sudden effervescence on adding HCl, would indicate the probable presence of carbonic acid, and therefore the salt is probably a carbonate. An odor of HNO_3 would indicate, nitrates, or the odor of H_2S, sulphides, etc.

A residue insoluble in acids may be due to the presence of one of the following substances: Silica (sand), silicates (glass, etc.), $BaSO_4$, $SrSO_4$, $PbSO_4$, AgCl, AgBr, AgI, etc. In such a case try the action of an alkali on a small portion of the powder, and then

fuse the dry powder with Na_2CO_3, or with a mixture of Na_2CO_3 and KNO_3. The fused mass will now be soluble in acidified water. Carbon, sulphur, etc., insoluble in acids, will be recognized by their characteristic appearances in the preliminary tests. CS_2 is the most convenient solvent for sulphur, and may be used to separate it from other substances insoluble in that reagent.

Treat the solution obtained according to the general scheme for analysis.

SPECIAL TESTS.

Arsenic.

1.—*Marsh's Test.*—The apparatus consists of a flask provided with a safety tube, for the introduction of the solution, and a delivery tube for the exit of the gases evolved. The latter pass into a wide tube containing calcium chloride, and thence into a long tube of smaller bore, contracted at intervals and drawn to a fine point at the end. Zinc, water, and sulphuric acid are brought together in the flask, and the solution under analysis added. Hydrogen gas, and, in presence of arsenical compounds, arsenetted hydrogen gas are produced. The inflammable gas issuing at the end of the tube, in presence of arsenetted hydrogen burns with a bluish-white flame, and gives off white fumes which may be collected and examined microscopically for crystals of As_2O_3. If a cold surface, such as a piece of porcelain, be held in the flame, metallic arsenic is deposited in a brilliant steel gray to brown mirror. By heating the long tube near one of its contractions a fine mirror of arsenic is deposited on the glass just in advance of the flame. If the gas be passed into a solution of silver nitrate, metallic silver is deposited in black flakes. After filtering, the clear solution may be examined for As_2O_3. Antimony gives somewhat similar tests, but may easily be distinguished. (See under Antimony.) Organic matter must be absent and the reagents used must be absolutely pure.

2.—*Fleitmann's Test.*—This is similar to the Marsh's Test, depending upon the production of arsenetted hydrogen by the action of nascent hydrogen on a reducible arsenical compound. Potassium hydroxide is used in place of the sulphuric acid, and the test is commonly made in a test tube. A paper moistened with silver nitrate is held at the mouth of the tube; the mixture is boiled,

and in the presence of arsenic the paper is blackened by the reduction of the silver nitrate to metallic silver.

3.—*Reinsch's Test.*—The solution to be tested is acidulated with hydrochloric acid, a strip of pure bright copper foil is introduced and the mixture boiled. In the presence of arsenical compounds, a steel-gray deposit of arsenic forms upon the copper. Antimony, mercury, and even organic matter, may produce a similar appearance, but the arsenic may be identified as follows: The copper slip is removed, washed carefully, and dried between folds of filter paper. A strip is then cut, rolled into a small coil, introduced into a clean reduction-tube and heated. The arsenic volatilizes, and collects in the cooler portions of the tube in white octahedral crystals of As_2O_3. Organic matter is burned away without the formation of a sublimate. (For Antimony and Mercury, see below.)

4.—Heated with charcoal, arsenous and arsenic oxides are volatilized, giving off the characteristic odor of garlic. (Other tests for arsenic, see p. 14.)

Antimony.

1.—*Marsh's Test.*—This test is performed as described under arsenic, similar mirrors of metallic nature being formed. Antimony is distinguished from arsenic as follows: The deposit obtained by holding a cold surface in the flame is insoluble in solutions of sodium or calcium hypochlorite, (arsenic spots—soluble.) If the spot be dissolved in a drop of nitric acid, the solution evaporated to dryness, and the residue moistened with a drop of silver nitrate, no color is developed, (arsenic—a brick red color). The spot dissolved in ammonium sulphide and evaporated to dryness, yields an orange red residue, (arsenic—bright yellow). The antimony mirror obtained by heating the tube is formed immediately above the flame, (with arsenic, in advance of the flame), is darker than the arsenical mirror and less volatile. Antimonetted hydrogen does not precipitate metallic silver from solutions of silver nitrate, but does precipitate black silver antimonide.

2.—*Reinsch's Test.*—Performed as indicated under arsenic. The antimony coating is distinguished from arsenic by the fact that when heated in the reduction-tube, the sublimate produced is either amorphous or composed of fine acicular crystals. (Other tests for antimony, see p. 14).

Mercury.

1.—The solution to be tested is acidulated with hydrochloric acid, and a strip of pure bright copper is introduced. A deposit of metallic mercury (silvery white by gentle friction) is formed *in the cold.* (Compare with Reinsch's Test for Arsenic.) If the copper be dried and heated as in Reinsch's Test, a sublimate of metallic globules of mercury is formed.

To test for corrosive sublimate in calomel, treat a few grains of the calomel with boiling water, filter, and test the filtrate for mercury. Calomel is insoluble in water, corrosive sublimate is soluble. (Other tests for mercury, see pages 15 and 16).

Phenol; (Carbolic Acid,) C_6H_5OH.

1.—Note the characteristic odor, and the greasy stain upon paper.

2.—Heated with a little nitric acid the solution turns yellow, trinitro-phenol (picric acid), $C_6H_2(NO_2)_3OH$, being formed.

3.—A few drops of ferric chloride impart a violet-blue color to the solution.

4.—Add a few drops of the solution to a little hydrochloric acid in a test tube, then add one drop of nitric acid and warm gently. A purple-red color is developed.

5.—Mix the solution with one-quarter volume of ammonia, add a few drops of sodium hypochlorite solution, and warm. A bluish green color is developed, turning to a red on addition of hydrochloric acid.

6.—The addition of bromine water produces a yellowish-white precipitate of tribrom-phenol, $C_6H_2Br_3OH$.

Chloroform, $CHCl_3$.

1.—To some alcoholic potassium hydroxide in a test tube add a few drops of aniline, and one or two drops of chloroform, or of the solution to be tested. Warm gently; the disagreeable odor of benzo-isonitril, C_6H_5NC, is produced.

2.—A strip of paper moistened with chloroform, when ignited, burns with a greenish flame, and gives off fumes of hydrochloric acid.

3.—Heat some of the solution to be tested with Fehling's solution. Red cuprous oxide is precipitated as in the test for glucose (q. v.).

Alcohol, C_2H_5OH.

1.—To a dilute solution of potassium dichromate add a few drops of sulphuric acid, and then a little alcohol, or the solution to be tested. Warm the mixture gently: it turns green, and the characteristic odor of aldehyde is produced.

2.—To the liquid to be tested add a few drops of dilute potassium hydroxide, and a small crystal of iodine, *or*, add to the alkaline solution, a solution of iodine in potassium iodide until the liquid is faintly colored. A precipitate of iodoform will be produced. Note the characteristic odor.

$$C_2H_5OH + 6KOH + I_8 = CHI_3 + CHO_2K + 5KI + 5H_2O.$$

An excess of alcohol holds the iodoform in solution.

3.—Add a little sulphuric acid and some strong solution of sodium acetate. The characteristic odor of acetic ether, (ethyl acetate) $C_2H_5(C_2H_3O_2)$, is developed on warming.

Glycerol, (Glycerin,) $C_3H_5(OH)_3$.

1.—Add sodium hydroxide to a slightly alkaline reaction, and heat, in a non-luminous flame, a borax bead moistened with this solution. Boric acid is produced and the flame is colored green.

2.—Warm the solution with sulphuric acid. The characteristic odor of acrolein, C_3H_4O, is produced.

For medicinal use, the aqueous solution of glycerol should be neutral to litmus paper, no brown color should develop when treated with sulphuric acid, and no red precipitate should be obtained on heating with Fehling's solution.

Ethyl Ether, $(C_2H_5)_2O$.

Ether is best recognized by its odor, volatility and inflammability. It burns with a luminous flame. With sulphuric acid and potassium dichromate a green color is developed, as in the alcohol test (q. v.).

For medicinal use, it should be neutral in reaction, should leave no residue on evaporation, and when shaken with sodium hydroxide, no color should be developed.

SPECIAL TESTS FOR THE ALKALOIDS.

The alkaloids may be defined as organic, nitrogenous substances, basic in character, and capable of combining directly with acids

to form salts. They are commonly divided into two groups: (1) Liquid or Volatile Alkaloids, consisting of Carbon, Hydrogen and Nitrogen. *Nicotine*, *Sparteine*, and *Coniine*. (2) Solid or Non-Volatile Alkaloids, consisting of Carbon, Hydrogen, Nitrogen and Oxygen. *Morphine*, *Quinine*, *Atropine*, *Strychnine*, etc.

General Properties.—Most alkaloids are insoluble, or very slightly soluble in water; more soluble in alcohol, chloroform and benzene. The salts of the alkaloids, on the other hand, are generally soluble in water and in alcohol, but insoluble, or slightly soluble, in chloroform, benzene, and ether. In appearance, they are generally white, with strong taste, and characteristic physiological action. The hydroxides of the alkalies and alkaline earths precipitate alkaloids from aqueous solutions of their salts. Alkali carbonates precipitate most of the alkaloids. Among other precipitants applicable in general to the whole class, we have tannic acid, picric acid, phospho-molybdic acid, mercuric potassium iodide (Mayer's solution), and the chlorides of platinum and gold.

Volatile Alkaloids.

These are volatile liquids, colorless when pure and first separated, but turning brown on exposure to the air. They are characterized by disagreeable penetrating odors.

Nicotine, $C_{10}H_{14}N_2$. 1.—Acrid odor and taste (*a rapidly fatal poison*), soluble in ether, chloroform, turpentine, water and alcohol.

2.—Picric acid, gold chloride, mercuric chloride, and platinic chloride, produce precipitates generally amorphous at first, changing to crystalline.

3.—Hydrochloric acid develops a violet color; nitric acid, an orange color.

4.—If one drop be placed upon a watch glass and covered with a second watch glass carrying a drop of nitric or hydrochloric acid, white fumes are produced.

5.—An ethereal solution of iodine added to a solution of the alkaloid in ether, separates a brownish oil, which gradually becomes crystalline.

Coniine, $C_8H_{17}N$. 1.—Resembles nicotine in physical properties, and in its precipitation by the general alkaloidal reagents.

2.—Evaporated with hydrochloric acid, a greenish-blue crystalline residue is obtained.

3.—Evaporated with sulphuric acid, a red color is developed changing to green.

4.—If a drop be placed upon a watch glass and covered with a second watch glass carrying a drop of hydrochloric acid, dense white fumes are developed, and the drop of coniine assumes a crystalline form.

Sparteine, $C_{15}H_{26}N_2$. 1.—General characters same as above.

2.—An etherial solution of iodine added to a slightly ammoniacal etherial solution of sparteine, separates minute dark greenish-brown crystals.

Non-Volatile or Fixed Alkaloids.

By far the greater number of alkaloids are included here. They are mostly white, odorless solids, fusing at a temperature above 100° C. without change, but decomposed when heated above their fusing points.

Morphine, $C_{17}H_{19}NO_3.H_2O$. 1.—A white crystalline solid, insoluble in ether and chloroform, soluble in boiling alcohol, used generally in form of its salts.

2.—Nitric acid dissolves morphine and its salts with effervescence, producing a red solution gradually changing to yellow.

3.—Sulphuric acid dissolves it, forming a colorless solution, which is turned *green* on addition of a crystal of potassium dichromate, or *pink* on addition of a trace of nitric acid.

4.—Neutral ferric chloride develops a blue color changing to green on addition of an excess of the reagent.

5.—Neutral solutions of morphine are precipitated with gold chloride, platinic chloride, potassium dichromate, and with picric acid, but not with mercuric chloride.

Codeine, $C_{18}H_{21}NO_3H_2O$. 1.—Sparingly soluble in water, easily soluble in alcohol and chloroform.

2.—Cold concentrated sulphuric acid dissolves codeine, forming a colorless solution which turns blue after several days, or when warmed, best after the addition of a trace of ferric chloride.

3.—Nitric acid dissolves codeine, producing a yellow solution.

4.—With chlorine water a colorless solution is obtained, which turns red with ammonia.

Meconic Acid, $C_7H_4O_7.3H_2O$. 1.—Soluble in water, more soluble in alcohol.

2.—To a drop of the solution on a watch glass add a drop of

ferric chloride. A red color appears which is not destroyed by mercuric chloride (difference from ferric sulphocyanate).

3.—Silver nitrate produces a white precipitate which turns red on addition of ferric chloride.

4.—Barium chloride produces a white precipitate.

Quinine, $C_{20}H_{24}N_2O_2.3H_2O$. *Quinine sulphate*, $(C_{20}H_{24}N_2O_2)_2H_2SO_47H_2O$. *Quinine bisulphate*, $C_{20}H_{24}N_2O_2 . H_2SO_4 . 7H_2O$. 1.—A flaky white powder nearly insoluble in water, soluble in dilute acids, in alcohol, chloroform, ether, etc. The sulphate is more soluble in water, and the bisulphate is easily soluble.

2.—Dissolve a few grains of the substance in a little dilute sulphuric acid, and add water. A blue fluorescence indicates quinine.

3.—Test the solution obtained above as follows: *a.*—Add tannic acid, = a white precipitate. *b.*—Add picric acid, = a yellow precipitate. *c.*—Add sodium hydroxide, = a white precipitate.

4.—Add to the solution a few c.c. of bromine water, or of chlorine water, and then add an excess of ammonia. A green color is developed.

5.—Treat the solution with chlorine water and then with a few grains of solid potassium ferrocyanide. The solution turns pink, changing to red, best after the addition of a little ammonia.

Cinchonine, $C_{19}H_{22}N_2O$. 1.—Forms in white crystalline needles almost insoluble in water, slightly soluble in alcohol and chloroform, easily soluble in dilute acids.

2.—Chlorine water forms a yellowish-white precipitate, insoluble in ammonia.

2.—Potassium ferrocyanide forms a white precipitate soluble in excess of the reagent.

3.—Ammonia forms a white precipitate, insoluble in excess.

4.—Solutions of the sulphate are not fluorescent. (Unlike Quinine.)

Caffeine, (*Thein*), $C_8H_{10}N_4O_2.H_2O$. 1.—Long silky needles, soluble in water, more soluble in alcohol. The solutions are neutral in reaction.

2.—Concentrated nitric acid dissolves it, forming a yellow solution, which, on evaporation and warming with ammonia, turns purple.

3.—Sulphuric acid forms a colorless solution.

Strychnine, $C_{21}H_{22}N_2O_2$. 1.—White crystalline powder, with

intensely bitter taste (very fatal action). Sparingly soluble in alcohol, ether, or in water, soluble in chloroform and in dilute acids. The official salt is the sulphate, $(C_{21}H_{22}N_2O_2)_2H_2SO_4.5H_2O$.

2.—Dissolve a minute crystal of strychnine in one or two drops of strong sulphuric acid, and draw through the solution, which should be colorless, a small fragment of potassium dichromate. A blue color is developed, rapidly changing to violet, cherry-red, and finally to yellow. Black oxide of manganese, potassium ferricyanide, or potassium permanganate may be used in place of the dichromate. The permanganate, however, colors the solution, and thus interferes with the delicacy of the test.

3.—Test a solution of strychnine with the following reagents: (*a*) Picric acid produces a yellow crystalline precipitate. (*b*) Tannic acid produces a white precipitate. (*c*) A solution of iodine in potassium iodide produces a brownish precipitate. (*d*) A solution of potassium dichromate gives a yellow crystalline precipitate. (*e*) Mercuric chloride gives a white precipitate.

4.—Dissolved in strong nitric acid, the solution should be colorless or yellow. A red or pink color denotes presence of brucine.

Brucine, $C_{23}H_{26}N_2O_4.4H_2O$. 1.—Soluble in alcohol and chloroform, sparingly soluble in water and in ether. The physiological action is similar to that of strychnine, though not quite so energetic.

2.—Treated with strong nitric acid, brucine is colored red, turning to a yellow on standing or when heated. Stannous chloride changes the red to a violet. (With morphine, there is no change on addition of stannous chloride.)

3.—Chlorine water added slowly to a strong solution of brucine, develops a red color, changed to yellowish-brown by ammonia.

4.—Test the solution with the following: (*a*) Tannic acid gives a white precipitate. (*b*) Picric acid gives a yellow precipitate.

Atropine, $C_{17}H_{23}NO_3$. 1.—White crystalline powder, sparingly soluble in cold water, more soluble in hot water, and easily soluble in alcohol and chloroform. The solutions are alkaline in reaction.

2.—Solutions of atropine are not colored by nitric acid, and are only very slowly colored by potassium dichromate.

3.—Dissolve a fragment of potassium dichromate in sulphuric acid, add a grain of atropine and a few drops of water, and warm

the mixture. A pleasant odor resembling that of orange blossoms is developed.

4.—Moisten the alkaloid with strong nitric acid, dry on the water bath, cool and add a few drops of alcoholic potassium hydroxide. A violet color is developed, changing slowly to red.

5.—Test a solution of atropine as follows: (*a*) Sodium hydroxide forms a white precipitate. (*b*) Gold chloride forms a yellow precipitate. (*c*) Picric acid forms a yellow precipitate. (*d*) Bromine in hydrobromic acid forms a yellow amorphous precipitate which afterwards becomes crystalline.

6.—The physiological test, dilatation of the pupil, is characteristic.

Veratrine, $C_{32}H_{50}NO_9$(?). 1.—White amorphous, occasionally crystalline, powder, bitter taste, insoluble in water, soluble in alcohol, chloroform, ether, etc. When heated it melts and gives off acrid fumes.

2.—Sulphuric acid dissolves it, giving a solution yellow at first, turning to an orange and finally to carmine-red. The solution shows a partial green fluorescence.

3.—Hydrochloric acid dissolves the alkaloid, forming a colorless solution which turns dark red on warming.

4.—Bromine water produces a violet coloration.

5.—Test the solution with the following reagents: (*a*) Gold chloride gives a yellow precipitate. (*b*) Iodine in potassium iodide gives a brownish precipitate. (*c*) Bromine in hydrobromic acid gives a brownish precipitate.

Aconitine, $C_{33}H_{43}NO_{12}$(crystalline variety). 1.—A white powder slightly soluble in cold water, more soluble in hot, soluble in alcohol, ether and chloroform. A rapidly fatal poison.

2.—Concentrated sulphuric acid dissolves aconitine, forming a yellowish-brown solution.

3.—Dissolved in aqueous phosphoric acid and the solution evaporated, a violet color is produced.

4.—Test the solution of aconitine with the following reagents: (*a*) Sodium hydroxide forms a white precipitate. (*b*) Tannic acid forms a precipitate. (*c*) Iodine in potassium iodide forms a precipitate. (*d*) Picric acid gives no precipitate. (*e*) Mercuric chloride gives no precipitate.

5. The taste is characteristically acrid, causing a tingling, benumbing sensation in the mouth. This test must be performed with the greatest precaution.

Physostigmine, $C_{15}H_{21}N_3O_2$. 1.—White amorphous, tasteless powder, sparingly soluble in water, soluble in alcohol, ether and chloroform. The solutions are alkaline.

2.—Sulphuric acid dissolves the alkaloid, forming a yellow solution turning to olive-green.

3.—The sulphuric acid solution, neutralized carefully with ammonia and warmed, is colored first red, then reddish-yellow, green and blue.

4.—Bromine in potassium bromide produces a red color.

Cocaine, $C_{17}H_{21}NO_4$. 1.—A white crystalline powder, fusing at 98° C., sparingly soluble in water, soluble in alcohol, ether and chloroform. The solutions are strongly alkaline. The hydrochlorate, $C_{17}H_{21}NO_4HCl$, is easily soluble in water, the solutions having a slightly bitter taste and producing a tingling sensation, followed by numbness, on the tongue.

2.—The alkaloid should give colorless solutions with sulphuric acid and with nitric acid.

3.—Test a solution of cocaine with the following reagents: (*a*) Gold chloride gives a yellow crystalline precipitate. (*b*) Platinic chloride, a yellowish-white precipitate. (*c*) Picric acid, a yellowish crystalline precipitate. (*d*) Mercuric chloride, a white flocculent precipitate. (*e*) Iodine in potassium iodide gives a rose-colored precipitate in dilute solutions, a brown precipitate in strong solutions.

SEPARATION OF METALS, ALKALOIDS, ETC., FROM ORGANIC MATTER.

The special tests given for the metals and alkaloids are, as a rule, applicable only in absence of organic matter. When, as is often the case, an organ, a tissue or an organic fluid is presented for examination, it becomes necessary to either remove or destroy the organic matter before proceeding with the analysis. Many processes have been proposed, but all, though simple in theory, require expert chemical knowledge for their successful application. The methods given below for metals are particularly adapted for the separation of arsenic, but apply with slight modifications to all of the metallic poisons.

Separation of Metals. *Method of Fresenius and Babo.*—The solid matter is finely divided and treated with an equal weight of pure hydrochloric acid and water. The mixture is then digested

on a water-bath and small quantities of potassium chlorate added from time to time. When the solid matter has been entirely decomposed the clear yellow liquid is evaporated, until the odor of chlorine has disappeared, and then filtered. The solution obtained can be examined by the usual tests for the metals; in the case of *arsenic*, best after the addition of sodium sulphite and subsequent heating to drive off the sulphur dioxide gas.

By Distillation.—In the case of arsenic, and of certain other volatile compounds of metallic poisons, the following method may be used: The finely divided organic matter is dried on the water bath, mixed with its own weight of pure hydrochloric acid and distilled from a glass retort provided with a condenser. The distillate is received in cold water, and may be examined at once for poisons.

By Dialysis.—The finely cut material is digested in cold water—or in dilute acid—for 24 hours, and then placed in a dialyzer. The latter is suspended in a larger vessel containing distilled water, and at the end of 24 hours again, the water is evaporated and the residue examined for poisons. This method is applicable also to the separation of the alkaloids.

Separation of Alkaloids, Ptomaines, etc. The separation of the alkaloids from organic matter is one of the most difficult and, on the whole, one of the most unsatisfactory problems of chemical toxicology. The following outlines will indicate the general nature of the processes used:

Stas-Otto Method.—Treat the finely comminuted mass with twice its weight of pure 90 per cent. alcohol, and with 10 to 30 grains of oxalic acid. Digest at 70° C., and filter. Evaporate the filtrate in vacuo, over sulphuric acid, dissolve the residue in absolute alcohol, filter, and again evaporate at a low temperature. Dissolve in water, add sodium hydrogen carbonate to alkaline reaction, agitate with ether, and separate the etherial layer. Allow the ether to evaporate spontaneously and examine the residue for alkaloids, or, redissolve in water and again extract with ether to further purify the residue before testing.

Dragendorff's Method.—This method is convenient, as affording a partial separation of the alkaloids during their extraction. The finely divided substance is digested for several hours with water acidulated with sulphuric acid. The extract is removed and the process repeated, the temperature being maintained at from 40° C.

to 50° C. The extracts are united, evaporated to a syrup, and digested with 4 volumes of alcohol for 24 hours at 30° C. The alcoholic extract is filtered, the residue washed with 70 per cent. alcohol, and the united extracts freed from alcohol by evaporation. The aqueous residue, diluted if necessary, is filtered, and the acid liquid, containing the sulphates of the alkaloids, treated with the following reagents :

1.—Agitate with petroleum ether, remove etherial layer, repeat extraction, evaporate extracts. Residue consists chiefly of *coloring matters.*

2.—Extract with benzene. Evaporate extract. Residue, if crystalline, may be *cantharidine*, *santonine*, or *digitaline;* if amorphous, *elaterine*, or *colchicine.*

3.—Extract with chloroform. Evaporate extract. Residue may be *cinchonine*, *digitaline*, or *picrotoxine.*

4.—Treat again with petroleum ether, remove etherial layer, render alkaline with ammonia. Treat the alkaline solution with petroleum ether at 40° C., and remove extract while warm. Evaporate extract. Residue may be *strychnine*, *quinine*, *brucine*, or *veratrine.* Extract again with *cold* petroleum ether. Residue may be *coniine* or *nicotine.*

5.—Extract the alkaline solution with benzene. Evaporate extract. Residue may be *strychnine*, *brucine*, *quinine*, *cinchonine*, *atropine*, *hyoscyamine*, *physostigmine*, *aconitine*, *codeine*, *thebaine*, or *narceine.*

6.—Extract with chloroform. Evaporate extract. Residue may be *morphine.*

7.—Extract with amyl alcohol. Evaporate extract. Residue may be *morphine*, *solanine*, or *salicine.*

8.—Evaporate remainder of the solution with powdered glass. Extract with chloroform. Evaporate extract. Residue may be *curarine.*

PART II.

THE CARBOHYDRATES.
THE PROTEIDS.

THE CARBOHYDRATES.

The carbohydrates form an important group of compounds, chiefly of vegetable origin, but occurring also in smaller quantities in the animal tissues and fluids. They may be classified as follows:

Amyloses.	*Glucoses.*	*Saccharoses.*
$(C_6H_{10}O_5)n$	$(C_6H_{12}O_6)n$	$(C_{12}H_{22}O_{11})n$
Cellulose,	Dextrose, (Glucose)	Sucrose, (Cane Sugar).
Starch,	Levulose, (Fruit Sugar)	Lactose, (Milk Sugar).
Dextrin,	Galactose,	Maltose,
Granulose,	Inosite,	Melitose,
Glycogen.	Sorbose.	Mycose.

The carbohydrates are nearly all neutral in reaction; some are crystalloids, others colloids. Of the three series, the first, the amyloses, are the most complex in nature, and most easily broken down into simpler forms. The glucoses are the most permanent, and resist efforts to transform them. They may, of course, be decomposed, but the products of decomposition are not carbohydrates.

Cellulose.

Prepared by reducing vegetable tissue to a pulp and washing out the starches, gums, salts, etc., present.

Chemical filter paper, which is nearly pure cellulose, is suitable for most of the tests.

1.—To some shreds of filter paper in a test-tube add a little sodium hydroxide. Warm the mixture and let it stand. Note that the paper fibres swell slightly and become more or less gelatinous.

2.—To a second sample add strong sulphuric acid and warm gently. Note that the paper turns brown or black, and goes partially or entirely into solution.

3.—Dissolve some pure cellulose in a solution of ammonio-cupric hydroxide. Then add hydrochloric acid carefully until

the blue color of the solution is destroyed, and note that the cellulose is precipitated in a stringy mass.

4.—Cellulose is insoluble in water, alcohol, or in ether.

Starch.

1.—Add a few grains of starch to a little sodium hydroxide in a test-tube. Note that the starch swells and forms a thick paste in the cold.

2.—Prepare some starch paste as follows: Add sufficient ground starch to water in a test-tube to form a milky fluid. Pour this milky fluid into a beaker of *boiling* water. Note that the milky appearance disappears. Dilute some of the "paste" so formed, in another beaker with water. When cool, add a few drops of iodine solution. A blue color is produced. Divide this blue solution into three parts. (*a*) Heat one part carefully until the color disappears. Upon cooling, the color will again develop. If carefully performed this may be repeated several times. (*b*) To the second part, add a few drops of sodium hydroxide. The blue color is destroyed, but may be reproduced by the addition of dilute hydrochloric acid. (*c*) To the third part, add mercuric chloride. The blue color is destroyed.

3.—Starch is insoluble in cold water, or in alcohol. When heated with water it is partially dissolved, the soluble portion being known as *granulose*, the insoluble portion, as *starch-cellulose*. To some of the clear starch solution obtained in (2) add alcohol. The granulose is precipitated.

4.—To some of the solution obtained in (2) add dilute sulphuric acid and heat to boiling. Test a small portion of the solution in another test-tube, with iodine. If the boiling has been sufficient no blue color will appear, showing the conversion of the starch into dextrin and dextrose.

$$2(C_6H_{10}O_5) + H_2O = C_6H_{12}O_6 + C_6H_{10}O_5.$$

The dextrin first produced gives a red or brown coloration with the iodine.

5.—By the action of diastase, starch is converted into maltose and dextrin.

$$3(C_6H_{10}O_5) + H_2O = C_{12}H_{22}O_{11} + C_6H_{10}O_5.$$

By continued action the maltose is converted into dextrose.

6.—Add a few grains of starch to nitric acid in a test-tube, and warm, if necessary, to start the reaction. Note the violent ebullition, red fumes, etc.

Dextrin.

An amorphous substance, readily soluble in water, insoluble in alcohol and in ether.

1.—Drop some of the aqueous solution into alcohol; a white precipitate is formed.

2.—Add ammonia and basic lead acetate; a white precipitate is formed.

3.—With iodine solution a red or brown coloration is obtained, which disappears when the solution is heated.

4.—Boiling with hydrochloric acid converts dextrin into dextrose.

Glycogen.

A white or yellowish-white tasteless amorphous powder, insoluble in alcohol or ether, imperfectly soluble in boiling water.

1.—With iodine in potassium iodide, glycogen gives a deep red color when in solution, a brown color when in form of powder. On heating the solution the color disappears, but reappears on cooling. (Unlike dextrin.)

2.—With sodium hydroxide and one or two drops of copper sulphate a blue coloration is obtained, but there is no precipitate on boiling. (See Trommer's Test, under Dextrose.)

3.—Basic lead acetate, alone, precipitates glycogen from its aqueous solution. (Unlike dextrin.)

4.—Boiling with dilute hydrochloric acid converts glycogen into dextrose.

Dextrose. (Glucose, Grape Sugar, etc.)

A white powder, more or less crystalline, soluble in water, less soluble in alcohol, insoluble in ether. It is sweet to the taste, but less so than cane sugar.

1.—*Moore's Test.*—Add to a dilute solution of glucose, one-half volume of sodium hydroxide, and heat to boiling. A brown coloration is obtained. Add a little nitric acid, the color disappears in part and the characteristic odor of caramel is given off.

2.—*Picric Acid Test.*—Add to the solution a few drops of picric

acid and a little sodium hydroxide. Heat the mixture and a mahogany-brown color is developed.

3.—*Silver Test.*—To some ammoniacal silver nitrate add a few grains of glucose. When dissolved, heat to boiling. The solution turns dark and a metallic mirror of silver is formed at the bottom of the tube. Tartaric acid and aldehyde each give the same test.

4.—*Fermentation Test.*—To the solution in a test-tube add a small piece of dry yeast and invert the tube over mercury. After standing for 24 hours in a warm place, carbonic anhydride gas will be found to have accumulated at the top of the tube. The liquid may be tested for alcohol. It is well to make a control test with yeast and pure water in a second test-tube.

5.—*Böttger's Test.*—To the solution of glucose in a test-tube add sodium hydroxide and then a few grains of bismuth subnitrate. Mix the solution well and heat to boiling. A black precipitate of metallic bismuth is formed. Sodium carbonate may be used in place of sodium hydroxide.

6.—*Trommer's Test.*—To the solution add an excess of sodium hydroxide, and then a solution of copper sulphate drop by drop, until a slight permanent precipitate is formed. In the presence of glucose the bluish white precipitate of cupric hydroxide first formed dissolves on agitation, producing a dark blue solution. Heat the liquid and, in presence of glucose, yellow cuprous hydroxide and red cuprous oxide are precipitated just as the liquid begins to boil. The same precipitation occurs, but much more slowly, in the cold.

7.—*Fehling's Test.*—Heat some diluted Fehling's solution (See Appendix) to boiling in a test-tube, and add, drop by drop, the solution to be tested. A yellowish-red precipitate indicates glucose.

Various modifications of Fehling's Test have been proposed, chief among which are, *Pavy's Ammoniated Cupric Test*, depending upon the decolorization of the solution instead of the production of the red precipitate, *Haines' Test*, *Loewe's Test*, *Schmiedeberg's Test*, etc. Formulæ for Pavy's Solution and for Haines' Solution are given in the Appendix. Haines' Solution is less subject to decomposition than Fehling's *completed* solution, and thus possesses a certain advantage. It is best, however, to preserve Fehling's Solution in two parts and to mix a sufficient amount for each test, at the time of execution.

8.—*Indigo-Carmine Test.*—Add sodium carbonate to the solution,

to render it alkaline, and then add sufficient indigo-carmine solution to impart a blue color. Boil, and the solution turns first violet, then yellow, but the blue color may be restored by agitation with air.

9.—*Phenylhydrazine Test.*—Add to the solution two grammes of phenylhydrazine hydrochloride, and four grammes of sodium acetate. Dissolve the salts by agitation and warm on the water-bath for 45 minutes. If glucose be present, on cooling, if not before, a yellow crystalline precipitate of phenyl glucosazone will separate out.

10.—*Alpha-naphthol Test.*—Add to the liquid a saturated solution of alpha-naphthol and an excess of sulphuric acid. In presence of glucose (and of other sugars) a violet color is developed. The addition of water causes a blue precipitate to form, soluble in alcohol, ether, and sodium hydroxide, with the production of a yellow solution.

For the detection of glucose in the urine, see p. 68, *and for the quantitative estimation of glucose, see p.* 69.

Sucrose. (Cane Sugar.)

A white crystalline solid, easily soluble in water, insoluble in absolute alcohol and in ether.

1.—To a little sugar in a test-tube add sulphuric acid and warm gently. The sugar is charred.

2.—Heat some sugar on a piece of platinum foil. It melts, darkens, chars, and burns, giving off inflammable gases and the characteristic odor of caramel.

3.—To a solution of sugar add a little sodium hydroxide and warm. If the sugar be pure there is no change of color as there is with glucose.

4.—Warmed with nitric acid, sugar is converted into saccharic, tartaric, and, finally, oxalic acid, red fumes of nitrogen oxides being evolved.

5.—Cane sugar does not reduce Fehling's Solution ; with Trommer's Test a blue solution is obtained, but this is not reduced on boiling.

6.—By the action of yeast and, also, by boiling with dilute acids, cane sugar is converted into glucose. To a dilute solution of sugar, add a little sulphuric acid and boil for several minutes. Cool the solution and apply Trommer's Test.

$$C_{12}H_{22}O_{11} + H_2O = C_6H_{12}O_6 + C_6H_{12}O_6.$$
(Cane Sugar.) (Dextrose.) (Levulose.)

Lactose. (Milk Sugar.)

A white crystalline solid, less soluble in water than cane sugar or dextrose, insoluble in alcohol or ether. It possesses only a faint sweet taste.

Lactose responds to the same tests as glucose; it reduces Fehling's Solution, though less strongly. By boiling with acids and under the influence of ferments it is converted into galactose. It differs from glucose in undergoing lactic fermentation, but after conversion to galactose the latter is subject to ordinary alcoholic fermentation. Lactose may be prepared from milk by acidulating with acetic acid, boiling, and filtering off the casein, fat, albumen, etc. On evaporation of the filtrate, crystals of lactose will separate out.

Maltose.

A white, crystalline substance, needle-shaped crystals, soluble in both water and alcohol. Like lactose, it responds to nearly all of the glucose tests. Its reducing power with Fehling's Solution is one-third less than glucose. It readily undergoes alcoholic fermentation, and by prolonged boiling with water, or, more readily, by boiling with dilute acids, it is converted into dextrose.

THE PROTEIDS.

THE proteids occur in both the vegetable and animal kingdoms, most abundantly in the latter. They all contain carbon, hydrogen, oxygen and nitrogen; most of them contain sulphur, and phosphorus is present in a few. Various formulæ have been calculated for the simpler proteids, but little, however, is known regarding their true constitution. They vary in composition as follows:

	C	H	N	S	O
From	51.5	6.9	15.2	0.3	20.9
To	54.5	7.3	17.0	2.0	23.5

Classification:

A. ANIMAL PROTEIDS.

I. *Albumins.* 1. Serum-Albumin. 2. Egg-Albumin. 3. Cell-Albumin. 4. Muscle-Albumin. 5. Lact-Albumin.

II. *Globulins.* 1. Vitellin. 2. Crystallin. 3. Myosinogen. 4. Fibrinogen. 5. Fibrinoplastin or Paraglobulin. 6. Globin.

III. *Albuminates* (Derived Albumins.)
1. Acid Albumins. (*a*) Acid Albumin. (*b*) Syntonin.
2. Alkali Albumins. (*a*) Alkali Albumin. (*b*) Caseinogen.

IV. *Proteoses.* 1. Albumoses. 2. Globuloses, etc.

V. *Peptones.* 1. Hemipeptone. 2. Antipeptone.

VI. *Coagulated Proteids.*

VII., VIII., IX. Fibrin, Lardacein, Meta- and Para-Albumins. See p. —, under Albuminoids.

B. VEGETABLE PROTEIDS.

These resemble the animal proteids in their properties and classification. The *Vegetable Globulins* are the most important and abundant, though under the action of reagents they are often separated from the plant in other, derived, forms.

C. ALBUMINOIDS.—(See p. 51.)

Tests for the Proteids.

All proteids are insoluble in alcohol. Some are soluble in water, others are not. Many not soluble in water are soluble in dilute saline solutions. Some are soluble in concentrated saline solutions, others are insoluble. All are soluble when heated with strong acids, and all are soluble, after change, in the gastric and pancreatic juices. The classification and subdivision of the proteids depend upon their behavior with the above reagents.

"*The Protein Reactions.*" (Applying to all proteids.)

1.—*Xantho-proteic Reaction.*—To a little of the solution in a test-tube, add a few drops of conc. nitric acid and heat to boiling. A yellow color is produced. Cool, and divide the solution into two parts; to one add ammonia, to the other add sodium hydroxide. An orange-yellow color appears in each instance.

2.—*Millon's Reaction.*—To the solution add a few drops of Millon's Reagent (See Appendix) and boil. The white precipitate first formed turns red on heating. The presence of sodium chloride interferes with this reaction.

3.—*Piotrowski's Reaction.*—To the solution add a drop of copper sulphate and then an excess of sodium hydroxide; a violet coloration is obtained which becomes darker on boiling. The same result is obtained with Fehling's Solution.

Removal of Proteids from Solutions.

It is frequently necessary to remove the proteids from a solution preparatory to tests for other substances. In urine analysis, for instance, the albumin must be removed before testing for sugar, or for urea. This removal may be accomplished, in most cases, by boiling the slightly acid solution. Albumins and Globulins are coagulated and may be filtered off. If the solution is not already acid, render so by addition of acetic acid. The same result may be obtained by the addition of an excess of absolute alcohol to the slightly acid solution, all proteids being thereby precipitated. A third method, of wide application, is the following: Add to the solution a few drops of acetic acid, (sufficient to acidulate it) then add an equal volume of a strong solution of ammonium sulphate. Boil for several minutes and filter. Saturation with ammonium sulphate precipitates all proteids except peptones.

ALBUMINS.

1.—*Mercuric chloride* precipitates white albuminate of mercury.

2.—*Copper sulphate* precipitates blue albuminate of copper.

3.—*Lead acetate* precipitates white albuminate of lead.

4.—Add to the solution a few drops of *acetic acid*, and then a drop or so of *potassium ferrocyanide*. A white precipitate is formed.

5.—*Picric Acid Test.*—Add to the solution a few drops of picric acid; a precipitate is formed. This is best performed as a "contact test," floating the solution of picric acid over the solution to be tested. A white zone of precipitated albumin will form between the two liquids.

6.—*Tannic Acid Test.*—Add to the solution a few drops of a solution of tannin. A precipitate is formed.

7.—*Nitric Acid* forms a white precipitate if not added in excess.

8.—*Nitric Acid Contact Test.*—Place about one inch of strong nitric acid in a test-tube, and float over it carefully, so as to avoid admixture, some of the solution to be tested. In the presence of albumin, a white cloudy ring, or zone, will form at the contact of the two liquids.

9.—*Acidulated Brine Test.*—Heat the acidulated brine (See Appendix) in a test-tube to boiling, and float over this the solution to be tested. A white precipitate is formed at the juncture of the two liquids.

10.—*Tanret's Test.*—Heat the Tanret's Solution (See Appendix) in a test-tube, and float over this the solution to be tested. A white precipitate is formed at the juncture of the two liquids.

11.—*Trichloracetic Acid Test.*—Add some of the crystals to the solution to be tested, and allow them to dissolve without agitation at the bottom of the tube. A white precipitate is formed.

12.—*Heat Test.*—Heat some of the aqueous solution of albumin just to boiling. The albumin is coagulated, forming a white precipitate. The solution should be slightly acid. The temperature at which the coagulation takes place averages between 60° C. and 75° C.

13.—Burn a small fragment of solid albumin on a piece of platinum foil. Note the characteristic odor of burnt horn.

Egg- and Serum-Albumin may be readily distinguished by the following tests:

Egg-Albumin.

1.—Rapidly precipitated by alcohol.

2.—Precipitated by ether.

3.—Readily precipitated by HCl, the precipitate not dissolving in excess.

4.—Readily precipitated by HNO_3, the precipitate not dissolving easily in excess.

Serum-Albumin.

1.—Slowly precipitated by alcohol.

2.—Not precipitated by ether.

3.—Not readily precipitated by HCl, the precipitate dissolving easily in excess.

4.—Precipitated by HNO_3, the precipitate dissolving easily in excess.

For the detection of Albumin in the Urine, see p. 65.

Albuminates.

1.—To the solution of an albumin add nitric acid carefully until a precipitate is formed. Then add a slight excess of acid and the precipitate will redissolve. (An acid-albuminate has been formed.)

2.—Boil the solution obtained in the first test and note that the albuminate does not coagulate.

3.—Apply the Xantho-Proteic Reaction to some of the same solution.

4.—Then add to the same solution dilute sodium hydroxide until a precipitate is formed. Add an excess of sodium hydroxide and the precipitate redissolves.

5.—To the solution of an albumin, add a few drops of strong sodium hydroxide, and warm the mixture. (An alkali-albuminate is formed.)

6.—Boil the solution obtained in the last test and note that the albuminate does not coagulate.

7.—Apply Piotrowski's Reaction to the same solution.

8.—Then add to the same solution (5) dilute hydrochloric acid; a precipitate will form, soluble in excess of the acid.

9.—Try solubility of casein in water. (Insoluble.)

10.—Make a solution of casein in water, to which a little sodium hydroxide has been added, and test this solution as follows: (*a*) By heat (No coagulation). (*b*) Neutralize with acid and note the precipitate formed, soluble in excess of acid. (*c*) Apply Piotrowski's Reaction.

Peptones.

The peptones are formed by the action of the gastric and pan-

creatic juices on albuminous bodies, and also, possibly, by the action of dilute acids. They differ from true albumins in containing less carbon, and less nitrogen, also in their power of diffusion. Test a solution of peptone as follows :

1.—Apply the Xantho-Proteic Reaction.

2.—Apply Millon's Reaction.

3.—Add an excess of sodium hydroxide, and a trace of copper sulphate, as in Piotrowski's Reaction. The solution turns rose-red in color. (With ammonia the solution acquires a violet-red tint.) This is known as the Biuret Reaction, because of the similar result obtained with a urea product, biuret, $C_2O_2N_3H_5$.

4.—Mercuric chloride precipitates a white peptonate.

5.—Picric acid forms a yellowish-white precipitate.

6.—Nitric acid, acetic acid and potassium ferrocyanide, and copper sulphate, form no precipitates with peptones.

7.—Boil the solution and note that the peptone does not coagulate.

Coagulated Proteids.

Coagulate some egg-albumin by heat and test the coagulum as follows :

1.—Try the solubility in water. (Insoluble.)

2.—Apply the Xantho-proteic Reaction.

3.—Apply Millon's Reaction.

4.—Apply Piotrowski's Reaction.

5.—Heat in a test-tube with sodium hydroxide. Alkali-albuminate is formed and goes into solution.

Gluten.

Gluten is a vegetable proteid derived from the vegetable myosin and albumose (proteose) of flour. It may be prepared for testing by kneading dough in a stream of water until free from starch.

1.—Apply the Xantho-proteic Reaction.

2.—Apply Millon's Reaction.

3.—Boil with water and note that it does not dissolve.

4.—Add sodium hydroxide to the mixture obtained in the last test, and boil again. Note that the gluten gradually decomposes and goes partially into solution with the formation of an alkali-albuminate.

Tests for Sulphur in Albumin.

1.—Heat a solution of lead acetate in a test-tube and add sodium hydroxide until the white precipitate of lead hydroxide, first formed, is just redissolved. Boil the clear liquid, and while boiling add a little albumin solution. The mixture turns black from the formation of lead sulphide.

2.—Heat a little of the solid albumin in a tube, and hold in the mouth of the tube a piece of paper moistened with lead acetate. The paper is blackened by the fumes evolved.

3.—Boil some albumin solution with a few grains of bismuth subnitrate and an excess of sodium hydroxide. A black precipitate of sulphide of bismuth is formed.

Separation and Identification of the Chief Protein Classes.

I. *If the proteid be solid*, test its solubility. 1.—Soluble in pure water.

(*a*) Coagulated by heat, *Albumins.*

(*b*) Not coagulated by heat, *Peptones.*

2.—Insoluble in pure water, but soluble in one per cent. solutions of sodium chloride, *Globulins.*

(*a*) Precipitated by saturation with sodium chloride, *Fibrinogen*, *Paraglobulin*, *Myosin.*

(*b*) Not precipitated by saturation with sodium chloride, *Vitellin*, *Crystallin.*

3.—Insoluble in pure water or dilute sodium chloride, but soluble in acids and in the gastric juice.

(*a*) Soluble in dilute hydrochloric acid or in dilute alkalies, *Albuminates.*

(*b*) Insoluble in dilute acids and alkalies, but easily soluble when digested with gastric and pancreatic juice, *Coagulated Proteids.*

(*c*) Insoluble in water, sodium chloride, dilute acids, or gastric juice, soluble in the stronger alkalies and in strong hydrochloric acid. *Lardacein* (Amyloid Substance.)

II. *If the proteid be in solution*, (1) Test a portion of the solution for proteids by the Xantho-proteic, Millon's and Piotrowski's Tests.

2.—Test the reaction of the solution.

(*a*) If acid, apply tests for the *acid-albuminates.*

(*b*) If alkaline, test for the *alkali-albuminates.*

3.—Acidify, if necessary, and boil the solution. If there be a coagulation the proteid is either an *albumin*, or a *globulin.* Add magnesium sulphate (saturated solution) to the original sample. A precipitate indicates *Globulin*, if no precipitate be formed, *Albumin* is present.

4.—If the proteid is not coagulated by heat, it is then probably either an albuminate, an albumose, or a peptone.

(*a*) *Albuminates* were tested for under 2, a. b.

(*b*) An *Albumose* would be recognized by the biuret reaction (similar to that given for peptones) and by the characteristic reaction with nitric acid. Albumose is precipitated by nitric acid in the cold, the precipitate dissolves on heating, and reappears when the liquid cools.

(*c*) *Peptones* may be recognized by the biuret reaction, by giving no precipitate with nitric acid, and no precipitate on saturation with ammonium sulphate.

THE ALBUMINOIDS.

The term albuminoid is best restricted to a group of substances which, while similar to the proteids in many particulars, differs from them in certain others.

Among the albuminoids we have the following:

I. *Collagen*, from the white connective tissue.

II. *Ossein*, a collagen from the bones.

III. *Gelatin*, derived from collagen by boiling with water.

IV. *Chondrin*, similar to the above, derived from the cartilages.

V. *Mucin*, from the cement substance or ground mass of epithelium and connective tissue.

VI.–XIV. *Elastin*, *Nuclein*, *Plastin*; *Nucleo-Albumins*, *Spermatin*, *Keratin*, *Metalbumin*, *Paralbumin*, *Lardacein*, etc. The last three are also often classed as proteids.

Gelatin.

When dried and pulverized bones are digested with dilute hydrochloric acid, the mineral salts are dissolved and ossein left behind. This last boiled with water is rapidly converted into the substance gelatin. Test the properties of gelatin as follows:

1.—Try the action of cold water. The gelatin swells without dissolving.

2.—Warm the mixture and it will be found that the gelatin dissolves. Divide this solution into two parts:

(*a*) Allow one part to cool in a test-tube, and note that on cooling the gelatin separates out, or "gelatinizes."

(*b*) Boil the remainder of the solution for several minutes, and then let it cool. Note that now it does not gelatinize.

3.—To the solution obtained in 2, b., or to a fresh solution of gelatin, apply the following tests:

(*a*) Xantho-proteic = lemon-yellow color.

(*b*) Piotrowski's.

(*c*) Mercuric chloride = precipitate.

(*d*) Picric acid = precipitate.

(*e*) Nitric acid = no precipitate.

(*f*) Acetic acid and potassium ferrocyanide = no precipitate.

In these reactions, with the exception of 2, a., gelatin resembles peptone. The following tests serve to distinguish between the two:

Peptones.	*Gelatin.*
1. Feeble osmotic power.	1. No osmotic power.
2. Alcohol precipitates with difficulty.	2. Alcohol precipitates easily.
3. Treatment with hydrochloric acid produces syntonin.	3. Treatment with hydrochlorine acid does not produce syntonin.
4. Solutions do not gelatinize.	4. Solutions gelatinize.

PART III.

CLINICAL.

MILK.

AVERAGE COMPOSITION OF MILK.

	Human Colostrum (Tidy).	Woman's Milk (Leeds).	Cow's Milk (König).	Cow's Milk (Hoppe-Seyler).
Water,	84.077 p. c.	86.732 p. c.	87.17 p. c.	85 to 86 p. c.
Solids,	15.923 "	13.268 "	12.83 "	14 to 15 "
Casein, Albumin,	3.228 "	1.995 "	3.02 " / 0.53 "	3 to 4 " / 0.3 to 0.5 "
Fat,	5.781 "	4.131 "	3.69 "	— 4.0 "
Lactose,	6.513 "	6.936 "	4.88 "	4.5 to 5.0 "
Salts,	0.335 "	0.201 "	0.71 "	———

The salts consist of chlorides, phosphates and sulphates, of calcium, magnesium, sodium, and potassium, with a small amount of iron and a trace of silica. There are, also, certain gases in solution: According to Pflüger, 100 volumes of milk contain 7.60 of carbonic anhydride, 0.10 of oxygen and 0.70 of nitrogen. Pathological alterations may occur after the administration of certain drugs and in morbid conditions. Albumin may increase, pus and blood may make their appearance. In osteomalacia the salts are increased; in acute fevers the amount secreted diminishes while there is a relative increase in the percentage of casein. In chronic diseases the fat and salts increase while the casein is diminished. In syphilis the salts increase while casein and fat are both diminished. The germs of infectious diseases are best recognized by physiological and microscopical tests. Again, cow's milk occasionally exhibits an abnormal appearance due to the presence of chromogenic bacilli, not necessarily, however, pathogenic in character. "Red" and "Blue" milks are examples of this phenomenon.

To Recognize the Constituents of Milk.

1. Dilute some milk in a beaker, with an equal volume of water, then add dilute acetic acid, and note the coagulation of the

casein. This casein coagulum (formed also by rennet) contains considerable butter-fat, which may be extracted with ether, after first treating the coagulum with absolute alcohol. Filter off the coagulum and reserve the filtrate, the *Whey*, for tests 2 and 3. Test the *Casein* as follows: (*a*) Apply the Xantho-proteic Reaction. (*b*) Apply Millon's Reaction. (*c*) Warm with water and add a few drops of sodium hydroxide. The casein goes into solution and may be reprecipitated by addition, to neutralization, of dilute acetic acid.

2.—Test the whey for *Lactose* as follows: (*a*) By Moore's Test. (*b*) By Trommer's Test.

3.—Test the whey for *Inorganic Constituents*. Place the whey in an evaporating dish, add a few grains of sodium or potassium nitrate, and evaporate to dryness with occasional stirring. When the mixture is near dryness heat cautiously until it ignites. Then heat strongly until only a white ash remains. Let it cool, add a little water and a few drops of dilute nitric acid, warm gently, and filter. Divide the filtrate into three parts and test: (*a*) For *Phosphates*, with ammonia and magnesia mixture. (*b*) For *Chlorides*, with nitric acid and silver nitrate. (*c*) For *Sulphates*, with hydrochloric acid and barium chloride.

4.—To some cream in a test-tube, add a little sodium hydroxide and a few drops of alcohol. Warm gently and note the characteristic odor of butyric ether, indicating the presence of *Butter-Fat*.

5.—To separate the *Butter-Fat*, add to the milk one-half its volume of sodium hydroxide, and one-half of ether. Shake well and let it stand in a warm place. The fat dissolves in the ether and floats on the top. The ethereal layer may be removed and the ether evaporated, leaving the fat in a pure state.

Clinical Analysis of Milk.

Quantity. The normal amount secreted by a healthy woman may be placed at from 700 to 1000 c.c. daily. A cow in good condition secretes 6000 to 7000 c.c. daily, or about four times its body weight in the year.

Reaction. Tested with litmus paper, human milk is normally alkaline. The milk of the cow and other herbivora is alkaline or amphoteric in reaction, while that of the carnivora is acid.

Specific Gravity. The specific gravity of milk varies normally

from 1028 to 1034. It is raised by the removal of the cream and lowered by the addition of water. When the milk has suffered both of these operations, therefore, the specific gravity may be normal. *Method.* The specific gravity is usually taken with a hydrometer after a thorough shaking of the sample. When the temperature of the milk departs considerably from the temperature of registration of the hydrometer (usually 60° F.) a correction must be made. Sufficiently accurate results may be obtained by subtracting *one* from the hydrometer reading for each 10° below 60° F., or by adding *one* to the reading for each 10° above 60° F.

A hydrometer with specially constructed scale, known as the *lactometer*, is frequently used in the municipal control of milk. Upon this instrument the 0° mark corresponds to a specific gravity of 1000, that of pure water, while 100° corresponds to a specific gravity of 1029, the minimum acceptable specific gravity for pure milk. The scale is commonly extended to 130°, 120° corresponding to a specific gravity of 1034, the maximum for pure milk.

FAT. (Cream).—(*a*) *By the Creamometer.*—A 100 c.c. glass cylinder graduated from above downward, is filled to the zero mark with the well shaken sample. After standing for 24 hours in a cool place the percentage of separated cream may be read directly from the graduations. This should be between 10 and 20 volumes. Comparing with the specific gravity, less than 10 volumes in a milk of specific gravity above 1033 indicates skimming. Less than 20 volumes in a milk of specific gravity below 1029, indicates the addition of water. There are, however, several possible sources of error in this method. Cream varies in consistency and consequently in bulk, and moreover, the addition of water causes a rapid separation of the cream with an *apparent* increase in quantity.

(*b*) *By Feser's Lactoscope.*—This method depends upon the fact that the relative opacity of milk varies with the number of suspended fat globules. Four c.c. of milk are introduced into the instrument and water added until the black lines upon the inner cylinder are plainly visible. The volume of the mixture indicates, by graduations on the outer tube, the percentage of fat in the sample. Whole milk should show three per cent. or over, by this method.

(*c*) *By Extraction with Ether.*—The milk is rendered alkaline with

potassium hydroxide, and shaken with ether. The etherial solution of fat is separated and the process repeated until all of the fat is removed. The etherial extracts are united and evaporated in a carefully weighed platinum dish. After drying at 110° C., the weight is again taken, the increase representing the fat in the measured volume of milk under analysis.

Proteids. The milk is diluted and treated with acetic acid and carbonic anhydride gas. The precipitated casein is freed from fat by washing with ether, dried, and weighed. The filtrate from the casein is evaporated on the water bath and the albumin precipitated with acetic acid tannin solution. The tannin is removed by washing with dilute alcohol, and the albumin remaining is dried and weighed.

The determination of the proteids in milk offers many difficulties, and is generally omitted in the ordinary clinical examination, as is also the test for sugar which follows.

Lactose. The milk is acidified with hydrochloric acid, boiled and filtered. The filtrate is boiled to convert the lactose into glucose, and the latter is determined by means of Fehling's solution (see p. —) *or*, the lactose may be directly titrated, 10 c.c. of Fehling's being decomposed by 0.0676 grammes of that carbohydrate.

Total Solids. (*a*) *By Calculation.*—An approximation, sufficiently accurate for clinical purposes, particularly in the examination of mother's milk, may be made by means of Hehner and Richmond's formula.

$$T = \frac{F + (0.2186 \times G)}{0.859}$$

T = Total Solids. F = Fat percentage, as determined by Feser's lactoscope, or by extraction with ether. G = Last two figures of the specific gravity; *e. g.*, if the specific gravity is 1030, then G = 30.

If the specific gravity and total solids be known, the fat can be calculated by the same formula transposed as follows:

$$F = 0.859\ T - 0.2186\ G,$$

or, if the milk is poor and has been skimmed,

$$F = 0.859\ T - 0.2186\ G - 0.05\ (\frac{G}{T} - 2.5).$$

(*b*) *By weight.*—Five grammes of milk are accurately weighed in a platinum dish with about 10 grammes of dry sand or powdered gypsum. The milk is then evaporated and the whole carefully dried at 100° C., until a constant weight is obtained. The loss in weight gives the water of the milk, and, by difference, the total solids.

Ash. By incinerating the solids obtained in the last test, the percentage of ash may be determined.

Detection of Adulterants in Milk.

Certain substances are occasionally added to milk for the purpose of preservation, and while not strictly adulterants, in intention, may be injurious to health and should be mentioned here. Probably the most common of these are sodium carbonate, benzoic acid, salicylic acid, borax, boric acid, etc. These will be detected in the course of the clinical analysis by the increase in the ash, and may then be identified by special tests.

Of adulterants proper, *water* is by far the most common and will be detected by variation in the specific gravity, total solids, etc. Though fraudulent, however, the addition of water, provided the latter be pure, has not the same injurious effect as the *skimming* of the milk, an adulteration by subtraction of food value. *Annatto*, added to increase the rich appearance of the milk, is not of itself harmful. Its presence may be detected by rendering the milk alkaline and soaking in it strips of filter paper. These latter will gradually acquire a yellow tint. *Starch* may be tested for by the addition of iodine solution, a blue color being developed. *Cane Sugar* will reveal itself in the taste and in the proportion of total solids, as well as in the percentage of sugar found. *Chalk* will be deposited on standing, and may be tested for in the ash. Other substances, but rarely met with, are glycerine, magnesium carbonate, tragacanth, dextrin, and arrow-root. These will increase the total solids, and may be identified by special tests.

THE URINE.

Constituents of Normal Urine.—Urea and related substances; uric acid, xanthine, creatinine, etc. Fatty and other non-nitrogenous substances; fatty acids, oxalic, lactic acids, minute quantities of carbohydrates. Aromatic substances; etherial sulphates of phenol, cresol, pyrocatechin, indoxyl and skatoxyl, hippuric acid, etc. Pigments and ferments. Inorganic substances; chlorides, sulphates and phosphates of sodium, potassium, calcium and magnesium, ammonium compounds and carbonates. Gases; nitrogen and carbonic anhydride.

Abnormal Constituents.—Serum albumin and other proteids; blood and bile pigments, bile acids, abnormal urinary pigments; glucose, lactose, and glycuronic acid; leucin and tyrosin; fats, lecithin, chloresterin, cystin; blood corpuscles, pus, casts, renal epithelium, etc.

In the urine of the 24 hours, averaging 1500 c.c., with a total of 60 grammes dissolved solids, the proportions are as follows:—(*Hammarsten.*)

Urea,	30.0 grammes.	Sodium Chloride,	15.0 grammes.
Uric Acid,	0.7 "	Sulphuric Acid,	2.5 "
Creatinine,	1.0 "	Phosphorus Pentoxide,	2.5 "
Hippuric Acid,	0.7 "	Potassium Oxide,	3.3 "
Other Organic Constituents,	2.6 "	Ammonia,	0.7 "
		Magnesium Oxide,	0.5 "
Total Organic,	35.0 grammes.	Calcium Oxide,	0.3 "
		Other Inorganic Constituents,	0.2 "
		Total Inorganic,	25.0 grammes.

General Plan of Clinical Urinary Analysis.

I. Ascertain Quantity passed in 24 hours, and obtain an average Sample.

II. Note Color, Appearance, and Odor.
III. If turbid, test Character of Sediment.
IV. Test Reaction with litmus paper.
V. Determine the Specific Gravity.
VI. Calculate the Total Solids.
VII. Set aside a sample for Microscopic Examination; filter the remainder of the urine and use filtered urine for the following tests:
VIII. Test for Mucin.
IX. Test for Albumin, and, if present, determine amount.
X. Test for Sugar in a sample from which the albumin, if originally present, has been removed by boiling and filtering. If sugar be found, determine amount.
XI. Determine the amount of Urea. (In albumin free Urine.)
XII. Determine the approximate amount of Chlorides.
XIII. Determine the approximate amount of Sulphates.
XIV. Determine the approximate amount of Phosphates.
XV. Test for Blood.
XVI. Test for Bile.
XVII. Make Microscopic Examination of the Sediment.

Notes on the Clinical Analysis.

Quantity.

An adult man passes on an average, 1400 to 1600 c.c. of urine in 24 hours. Women secrete less than men; children absolutely less but relatively more, about 60 c.c. for each kilogramme of body weight, (man, about 23 c.c. for each kilo). The urine is *increased* after the ingestion of much liquid, reaching 2000 to 3000 c.c. It is increased, also, in nervous excitement, hysteria, chorea, in forms of diabetes, and in the albuminuria of contracted kidney. It is *diminished* by profuse perspiration, hence in summer, by abstinence from liquid food, by sleep, often in valvular disease, acute inflammations, fever, diarrhœa, enteritis, etc.; often in chronic Bright's disease, and in uræmia.

The sample selected for analysis, owing to variation in the composition of the urine during the day, should be an average of that passed. If the average sample is not obtainable, note the time of passing; night, morning, before or after a meal, etc.

Color, Appearance and Odor.

Normal urine is described as clear, straw-yellow, sherry colored, or amber. It varies normally in shade from nearly colorless to dark amber. In disease there is a similar variation in shade and also frequently a variation in color. It is *light colored* after ingestion of a large amount of water, and often nearly *colorless* in diabetes. It is *dark* after profuse perspiration, muscular activity, etc., and in acute febrile conditions. It may be *red* from presence of blood pigments, or *greenish-yellow*, *brown*, to *black* from presence of bile. "*Blue*" urine is sometimes observed in cholera and in typhus. Again, the color may be due to drugs ingested; phenol and gallic acid, producing a *black* urine; santonin, chrysophanic acid, rhubarb, senna, etc., a *yellow* urine.

In case the color be so pronounced as to interfere with the chemical tests, the urine should be decolorized by shaking with powdered animal charcoal and filtering.

The urine is usually clear when passed, though a faint cloudiness is not uncommon. All urines become turbid on standing. A turbidity may be due to an excess of mucus, to pus, chyle, semen, phosphates, urates, etc. By heating, the turbidity due to *phosphates* is slightly increased, but it disappears at once on the addition of a few drops of nitric acid or of acetic acid. By heating, the turbidity due to *urates* disappears. If due to *pus* or to *mucus*, the turbidity is increased by heat and also by acetic acid.

The *odor* of normal urine is described as *aromatic*. After standing, however, it may become *ammoniacal*. Asparagus, turpentine, cubebs, valerian, and garlic, all impart characteristic odors. The urine of diabetes has a sweetish odor, that of albuminuria, after standing, often a fetid odor.

Reaction.

The reaction of an average sample of normal urine is always acid; the total acidity in terms of oxalic acid being about 2 grammes for the 24 hours. The acidity is *reduced*, or the urine may become *alkaline*, after hearty meals, hot baths, administration of alkaline salts, etc., in starvation, with a strictly vegetable diet, in general debility, chlorosis, or anæmia. The acidity is *increased* with a meat diet, by muscular activity, in fevers, typhus, and often in pneumonia.

Upon standing the urine may at first become more acid, from decomposition of urates, but later an alkaline fermentation sets in, the urea is decomposed and ammonium carbonate formed. At this stage the turbidity is increased by precipitation of phosphates, and an ammoniacal odor is noticeable.

For the *quantitative determination of the acidity*, see under Volumetric Analysis.

Specific Gravity.

The specific gravity of normal adult urine varies generally between 1010 and 1030, with an average of 1020 for 1500 c.c. passed. In children from two to thirteen years of age the average is about 1012.

In order that the determination of the specific gravity shall be of any value, it is necessary to know the amount passed and to use an average sample. When the amount passed varies from the normal (1500 c.c.) the specific gravity of the average sample may be reduced to the normal by the formula:—

$$\frac{A \times G}{1500} + 1000 = D$$

In which A equals the amount passed, G equals the last two figures of the observed specific gravity, and D equals the specific gravity of the urine reduced to the normal quantity. Thus, suppose 3000 c.c. were passed, and the specific gravity of the average sample to be 1015.

$$\frac{3000 \times 15}{1500} + 1000 = 1030$$

Reduced to the normal, then, of 1500 c.c., the specific gravity is 1030, showing that though the specific gravity of the original sample was low, the total solids are in reality high.

Considered with the amount passed in 24 hours, the specific gravity gives the following indications: A *decreased amount* with *increased specific gravity* indicates diminished secretion, loss of water by other excretions, or the presence of some morbid process, fever, etc. An *increased amount* with *decreased specific gravity* indicates abundant ingestion of water, absorption of exudations, or some form of diseased kidney. A *decreased amount* with *decreased specific gravity* indicates, possibly, uræmia, or some forms of Bright's

disease. An *increased amount* with *increased specific gravity* may indicate diabetes mellitus.

The specific gravity is usually determined by means of the urinometer, a small hydrometer with special scale. This scale is adjusted to give accurate readings at a certain temperature (usually 60° F.) marked upon the instrument, and as the temperature of the urine tested is nearly always above this, it is necessary, in accurate determinations, to make a corresponding correction. The temperature of the urine is determined and for each 6° above 60° F., *one* is added to the observed specific gravity. Thus the corrected specific gravity for a urine reading 1020 at 72° F. is 1022. For more accurate determinations it is necessary to use the pycnometer, for which, see works on physics.

Total Solids.

The total solids average in normal urine from 55 to 65 grammes (840 to 1000 grains) in the 24 hours. They may be calculated with sufficient accuracy for clinical purposes by multiplying the last two figures of the specific gravity by Häser's coefficient, 2.33. The product expresses the number of grammes in 1000 c.c. of urine, and from this the number of grammes in the urine passed may be easily calculated. By using 0.233 instead of 2.33, the *percentage* of total solids may be obtained at once. Other factors or coefficients, which have been proposed, are Trapp's = 2, and that of Loebisch = 2.2; Häser's, however, is generally accepted as the most accurate. For the urine of young children, the coefficient 1.80 should be used. In English measure the number of grains of total solids in 24 hours may be roughly calculated by multiplying the last two figures of the specific gravity by the number of fluid ounces passed.

Mucin.

Mucin may be present in normal urines, but it is greatly increased by irritation of the urinary tract. It is precipitated by acetic acid in the cold (unlike albumin) and may best be tested for by floating the clear filtered urine over acetic acid in a test tube. A cloudy coagulum is formed above the surface of contact. Mucin is not precipitated by boiling, but is precipitated by dilute mineral acids, as well as by acetic, citric, and other organic acids. See, also, under Urinary Sediments.

Albumin.

Normal urine is free from proteids, but, on the other hand, the presence of a trace of albumin is not necessarily always pathological. Temporary albuminuria may occur after severe bodily exertion, after the shock of a cold bath, from excess of albuminous foods, from the presence of semen, etc. When more than a trace is present, or when this trace persists for a considerable time, the existence of a serious abnormal condition is indicated. Serum albumin and serum globulin are the forms most frequently met with, while hæmoglobin, fibrinogen, peptones and proteoses, may also appear. In general, the immediate cause of albumin in the urine may be stated as impaired circulation through the glomeruli of the kidney, a result of either venous or arterial disorder, of changes in the blood itself, or of diseased condition of the kidney. The albuminuria of pregnancy, like that due to ovarian or uterine tumors, is a result of disordered venous reflux, and the same may be said of the albuminuria of heart disease. Albumin may occur in gout, from faulty renal metabolism, in scarlet fever, diphtheria, pneumonia, and in poisoning by phosphorus or morphine. It is typical in Bright's disease. In acute Bright's disease there is much albumin and often blood. In large white kidney there is also much albumin, while in granular contracted kidney and in albuminoid kidney, there is an increase in the amount of urine and a decrease in the albumin.

Typical albuminous urine is pale greenish-yellow, of low specific gravity, forms a persistent froth on shaking, and usually deposits a sediment soon after being passed.

Heat Test. A long test tube is three-quarters filled with clear acid urine and the upper half of this is carefully heated to boiling. A cloudiness appearing in the heated portion may be due to albumin or to phosphates. Add a few drops of nitric acid, phosphates will be dissolved and any cloudiness remaining will be due to albumin.

Precautions.—If the urine is not already acid, acidify, before heating, by addition of a few drops of nitric acid. Acetic acid may be used, but has the double disadvantage of being more likely to cause the solution of a trace of albumin than nitric, and also of precipitating mucin. A cloudiness appearing only after some minutes may be due to albumoses. Urates, if abundant,

sometimes separate on cooling, but are not likely to be mistaken for albumin. When adding the nitric acid, after heating, should the first few drops cause no precipitate, continue the addition of acid until one drop has been added for each c.c. of urine.

Nitric Acid Contact Test. Warm some pure nitric acid in a test tube, and then float over it carefully by means of a pipette, an equal volume of the urine to be tested. A white zone or ring at the contact of the two liquids indicates albumin.

Precautions.—A pink, red, or brown color may appear at the contact, due to action of the acid on the coloring matters of the urine. When cold acid is used in the test, crystalline nitrate of urea may separate at the contact, or acid urates may cause a cloudiness just above. Resinous matters, if present, as after ingestion of turpentine, balsams, etc., may cause a yellowish-white zone, soluble, however, in alcohol. (Unlike albumin.)

Ferrocyanide Test. Add a little dilute acetic acid to the urine, filter if mucin be precipitated, and then float the clear acidified urine over a solution of potassium ferrocyanide in a test tube. A white zone forming at the contact of the two liquids indicates albumin. This is a delicate and accurate test, albumoses and nucleo-albumins being the only other substances precipitated.

Picric Acid Test. Warm some picric acid solution in a test tube and add the urine to it, drop by drop. A slight opalescence as each drop of urine enters the acid indicates albumin. This test may also be performed by the contact method, floating the acid over the urine.

Precaution.—If cold acid be used, peptones, mucin, and alkaloids, may also be precipitated. With the warm acid the test is exceedingly delicate.

For other tests, see Albumin, Part II.

Quantitative Estimation. The quantitative estimation of albumin is difficult, and an accurate determination is rarely possible in the ordinary clinical analysis. A rough comparison of the amount of albumin in the urine from day to day may be made from the bulk of coagulum obtained by the heat test, using always the same size tube and the same amount of urine. A more accurate estimation may be made with *Esbach's Albuminometer*. The urine, diluted with a known volume of water, if there be much albumin, is introduced into the tube to the mark U, and Esbach's reagent (see Appendix) added to the mark R. The mixture is

shaken, the tube stoppered and allowed to stand 24 hours. The volume of the precipitate measured by the graduations, gives the percentage of albumin. Each main division equals 0.1 per cent. There is rarely more than 1.0 per cent. of albumin present.

A still more accurate method, is the following: The urine is diluted with 9 volumes of water, and from this diluted urine (one-tenth urine) test solutions are prepared, each containing 10 c.c. of water and a measured volume of the one-tenth urine; *e. g.*, (1) Contains 10 c.c. of water plus 1 c.c. of the one-tenth urine; (2) Contains 10 c.c. of water plus 2 c.c. of the one-tenth urine, etc. Nitric acid contact tests are now made with each test solution until one is found which responds only after standing for 2–3 minutes. The percentage of albumin in the original urine may be calculated from the formula:

$$\frac{10 + V}{30\,V} = P$$

In which V equals the volume of one-tenth urine added to the 10 c.c. of water, and P equals the percentage sought. For instance, if to the test solution, which produces a zone of coagulum only after standing 2–3 minutes, 5 c.c. of the one-tenth urine had been added, then the percentage of albumin originally present is

$$\frac{10 + 5}{30 \times 5} = 0.10 \text{ per cent.}$$

Sugar in Urine.

Transitory glycosuria may occur in cerebro-spinal meningitis, in epilepsy, from brain injuries, under the influence of strong emotion, in pneumonia, ague, cholera, gout, in chloroform narcosis, and after excessive use of saccharin or starchy food. Lactose is frequently present in the urine of mothers during the weaning period. *Permanent glycosuria* is indicative of diabetes. In general, diabetic urine is pale straw-colored with sometimes a greenish tint, it is often turbid and has a characteristic sweetish odor. The specific gravity is high (1030–1050) and the quantity passed is seldom less than 1600 c.c. in 24 hours. It may vary, however, between the extreme limits of 500 c.c. and 8000 c.c. Diabetes mellitus with polyuria, is far more serious than without, and, indeed, glycosuria without polyuria does not seem to be necessarily

fatal. The percentage of sugar varies from 2–3 per cent. to 12 per cent.

Trommer's Test. Add to the urine in a test tube about one-fourth its volume of sodium hydroxide, and then dilute copper sulphate, drop by drop, until a slight permanent precipitate is formed. In the presence of glucose the bluish white precipitate of cupric hydroxide first formed, dissolves on agitation, producing a dark blue solution. Heat the liquid and, in presence of glucose, yellow cuprous hydroxide and red cuprous oxide are precipitated just as the liquid begins to boil. The same precipitation takes place without heating, but much more slowly.

Precautions.—A normal urine will often decolorize the solution, but no red precipitate is formed. The sodium hydroxide causes a precipitation of flocculent phosphates, which, however, bear no resemblance to the granular cuprous precipitate. A precipitate of yellow cuprous hydroxide, which separates on the cooling of the test, is probably not due to sugar. Uric acid, hypoxanthin, mucus, albumin, peptones, pepsin, creatinine, and excessive urinary pigments, all interfere with the delicacy of the test. Glycuronic acid, (see p. 77), is also a frequent cause of error. When there is but a slight reduction, greater delicacy may be attained by clarifying the urine with animal charcoal or lead acetate, filtering, and testing the filtrate.

Fehling's Test, Haines' Test, etc. (See Appendix for the solutions.)

Heat the diluted test solution in a tube to boiling, and add the urine drop by drop until nearly an equal volume has been introduced. A yellowish-red precipitate, as in Trommer's Test, indicates glucose.

Precautions.—The test solution should remain clear when boiled before addition of the urine. The precautions given under Trommer's Test apply here, and should be carefully observed.

Böttger's Bismuth Test. To a few c.c. of the urine in a test tube, add an equal volume of sodium hydroxide and a few grains of bismuth subnitrate. Mix well and boil for several minutes. In presence of glucose, black metallic bismuth will be precipitated. A slightly more delicate reaction is obtained by using the Almen-Böttger Test, (Nylander's). Ten c.c. of urine are boiled with 1 c.c. of Almen's reagent, (see Appendix); black metallic bismuth is separated.

Precautions.—Albumin, if present, will cause the precipitation of black bismuth sulphide. Many normal urines will cause a slight darkening of the bismuth subnitrate, such as might be produced by a trace of sugar.

PHENYL HYDRAZINE TEST. To 50 c.c. of urine add 2 grammes of phenyl hydrazine hydrochloride, and about 4 grammes of sodium acetate. Dissolve the reagents in the urine and heat on the water bath for 45 minutes. In presence of glucose fine yellow crystalline needles of phenyl glucosazone separate out on cooling.

Precautions.—A similar precipitate is given by other carbohydrates. It may be necessary to examine the precipitate microscopically for the characteristic crystals, or even to determine their melting point (204°–205° C.) in order to positively identify them.

FERMENTATION TEST. (See p. 42). This test is useful in identifying glucose in presence of other reducing substances.

INDIGO-CARMINE TEST. (See p. 42). This is an exceedingly delicate test, but gives a faint reaction with nearly all urines.

ALPHA-NAPHTHOL TEST. (See p. 43). Delicate, and often recommended.

QUANTITATIVE DETERMINATION. *By Fehling's Solution.*—Ten c.c. of Fehling's solution (see Appendix) are measured into a porcelain dish with 30 to 40 c.c. of water. The diluted solution is heated to boiling and the urine added from a burette until the blue color of the Fehling's solution has entirely disappeared. Note the number of c.c. of urine required to produce this result, and calculate the amount of glucose present, by the formula $\frac{5}{U} = P$, in which U represents the number of c.c. of urine added to produce complete decomposition, and P equals the percentage of glucose in the sample. From this the amount passed in the 24 hours urine can be calculated. When considerable sugar is present, it is well to dilute the urine with a known volume of water, to determine the glucose in the diluted sample, and then to calculate back to the original.

This method is by far the most satisfactory of any possible to the clinical observer. The fermentation processes, which follow, are not to be recommended unless reducing substances other than glucose are known to be present. Approximate results only are obtained.

Roberts' Fermentation Method.—After a careful determination of

its specific gravity, the urine, together with a small piece of compressed yeast, is placed in a loosely stoppered flask and left for 24 hours. The alcohol and carbonic anhydride produced by the fermentation, reduce the specific gravity of the solution one degree for each grain of sugar per fluid ounce. At the end of the operation the specific gravity is again determined, the loss representing the number of grains of sugar per fluid ounce. This number multiplied by 0.23 gives the *percentage* of glucose in the sample. It is well to make a parallel test on a sample without sugar, observing any variation that may occur in its specific gravity.

Einhorn's Method.—Ten c.c. of the urine are shaken with about 1 gramme of compressed yeast, and the mixture introduced into Einhorn's apparatus, a small graduated tube of special form. The carbonic anhydride gas evolved is measured at the end of 24 hours, its volume expressing directly, by the graduations on the tube, the percentage of glucose present.

Urea.

The urea varies in normal urine from 20 to 40 grammes (309 to 617 grains) in the 24 hours, with an average of about 33 grammes (500 grains). Women secrete less than men, children absolutely less, but relatively, *i. e.*, in proportion to body weight, more. The urea is *increased* by a nitrogenous diet, by mental and, possibly, physical activity, at the beginning of the crisis in fevers, in ague, in diabetes, in pleurisy, and in acute tuberculosis. The urea is *decreased* by profuse perspiration, by diarrhœa, in cholera, in the later stages of chronic Bright's disease, in diabetic coma, in anæmia, and generally, in most chronic debilitating disorders. Expressing, as it does, the progress of nitrogenous metabolism in the body, the determination of the urea passed is important.

Approximate Estimation.—When the chlorides are normal and when sugar and albumin are absent, the urea may be taken as one-half the total solids.

Hypobromite Method.—There are several applications of this method, depending on differences in the form of apparatus used; the principle is the same, however, in all. A solution of sodium hypobromite containing an excess of sodium hydroxide, (see Appendix), is added to the urine; the urea is decomposed, nitrogen gas is set free, and sodium bromide and carbonate are formed.

$$CON_2H_4 + 3NaBrO + 2NaOH = 3NaBr + Na_2CO_3 + N_2 + 3H_2O.$$

The nitrogen evolved is measured and, from its volume, the percentage of urea calculated.

A solution of chlorinated soda with potassium bromide may be used in place of the alkaline hypobromite. It has the advantage of keeping better and of being more easily obtainable. (See Appendix.)

Twenty c.c. of fresh hypobromite solution (40 c.c. of the chlorinated soda) are placed in a bottle, and the measured urine, contained in a small test tube, is introduced in such a manner that the tube will stand in the bottle without spilling. The bottle is then connected by means of a perforated stopper and rubber tube with the top of an inverted burette standing in a jar of water. All connections are carefully made, and the volume of air in the burette read from the graduations. The urine is now slowly mixed with the hypobromite, nitrogen gas is evolved, and a corresponding volume of air is driven from the bottle, displacing the water in the burette. When the evolution of gas has ceased, let the apparatus stand for a few minutes, and then measure again the air in the burette. The increase in volume represents the volume of nitrogen given off. The observed increase, in c.c., multiplied by the factor 0.0028 gives the weight in grammes of urea in the sample taken; *e. g.*, if 4 c.c. of urine were used for the test, and 20 c.c. of nitrogen were evolved, then we have, 0.056 gramme (0.0028×20) of urea in 4 c.c. of urine, or, 1.40 per cent. ($0.056 \div 4 \times 100$).

Theoretically the factor employed should be 0.00268, but as the theoretical volume of nitrogen is not given off, 0.0028 gives more accurate results. In reading the volume of gas in the burette it is of course necessary that the latter shall be raised or lowered so as to bring the water on the same level inside and out.

The ureameter devised by Prof. Doremus, of N. Y., is convenient and with practice yields excellent results. The small quantity of urine (1 c.c.), however, which is used increases the liability of error. With this apparatus, the percentage of urea is read directly from the graduations on the tube.

Uric Acid and Urates.

Uric acid is normally present in the urine in combination, as a

urate. It is *increased* in pneumonia, indigestion, acute rheumatism, and in disorders of the circulation and respiration. It is *decreased* in most chronic diseases, in the later stages of Bright's disease, in diabetes, in gouty affections, and in chronic rheumatism. It is to be remembered that the appearance of a deposit of uric acid, or of urates, does not necessarily point to an excess of these ingredients. High acidity of the urine, and a decrease in mineral salts, tend to produce a separation of uric acid equally with the presence of an increase of that substance. The deposits are easily recognized. (See under Urinary Sediments.)

Uric acid may be separated from the urine as follows: To 200 c.c. of urine add 20 c.c. of strong hydrochloric acid and let the mixture stand 48 hours. Collect the sediment on a previously weighed filter paper, wash with cold water, dry and weigh. The increase in weight represents uric acid.

To detect uric acid or urates apply the *Murexid Test.* Evaporate the sediment with a drop of strong nitric acid. A yellow residue is obtained, which, when moistened with a drop of ammonium hydroxide, turns purple.

Chlorides.

The chlorine of the 24 hours urine varies from 6 to 10 grammes, equivalent to 10 to 16 grammes of sodium chloride. The average for an adult man may be placed at 7 grammes chlorine (11.5 grammes sodium chloride), for women, at 6 grammes chlorine, and for children, about 5 grammes chlorine. The amount varies during the day, being *increased* several hours after a full meal, and by mental or physical labor. It is *diminished*, in most fevers (increasing again after the crisis), in pneumonia, pleurisy, typhoid, cholera, and in many chronic diseases.

Approximate Estimation.—To the urine in a test tube add a few drops of nitric acid and one drop of a concentrated solution of silver nitrate. If the chlorides be normal, a curdy white precipitate is formed; if low, only a milky cloudiness; if absent, there is no precipitate. For the accurate determination of the chlorides, see under Volumetric Analysis.

Sulphates.

Sulphuric acid is found in the urine combined with both organic and inorganic bases, the latter combination being normally in ex-

cess of the former. The sulphates are *increased* by animal food, by physical activity, by ingestion of sulphur compounds, in acute inflammatory diseases, pneumonia, acute rheumatism, delirium, and often in diabetes insipidus. They are *decreased*, with diminished metabolism in chronic affections, in leukæmia, and in diabetes mellitus.

Approximate Estimation.—Add to the urine a few drops of hydrochloric acid and one-fourth volume of barium chloride. An opaque milky cloudiness indicates normal sulphates; an opaque creamy precipitate, increased sulphates; a faint semi-transparent cloudiness, diminished sulphates.

Sulphates of Organic Bases. Sulphates of phenol, cresol, pyrocatechin, indol, skatol, etc. These compounds are derived partially from the food, but are interesting chiefly as putrefaction products absorbed from the intestine. In normal urine they are present in small amount, probably about 10 per cent. of the total sulphates, but in certain stomach troubles, in disordered absorption, and from abnormal fermentative and putrefactive changes, they may be considerably increased.

Determination of Organic Sulphates.—100 c.c. of the urine are mixed with 100 c.c. of Barium Mixture, (2 volumes of saturated solution of barium hydroxide with 1 volume of saturated barium chloride) and the precipitate formed, filtered off. An aliquot part of the filtrate is acidified with hydrochloric acid and boiled. It is then heated to 100° C. for an hour, allowed to stand until completely settled, and finally filtered through an ashless paper. The precipitate is dried, ignited and weighed. The weight of barium sulphate found multiplied by 0.34335 gives its equivalent in terms of sulphuric anhydride.

The Total Sulphates may be determined by acidifying the urine with hydrochloric acid, boiling, and adding barium chloride until all is precipitated. The precipitate is then treated as described above.

Phosphates.

The phosphates of the urine may be divided into two classes, the *alkaline phosphates*, phosphates of sodium and potassium ($\frac{2}{3}$); the *earthy phosphates*, phosphates of calcium and magnesium ($\frac{1}{3}$). The latter are subject to but little variation in disease. The *total phosphates* are *increased*, in acute inflammatory diseases, in the

early stages of acute fevers, in phthisis, leukaemia, osteomalacia, and sometimes in diabetes. They are *decreased*, in many chronic brain troubles, in epilepsy, general paralysis, melancholia, etc., in some forms of Bright's disease and of heart affections, in chlorosis, rickets, gout and chronic rheumatism.

Total Phosphates.—Add to the urine magnesia mixture (see Appendix) and ammonia. The total phosphates are precipitated, and can be compared with a corresponding precipitate from a normal urine.

Earthy Phosphates.—Approximate Estimation.—Place 2 inches of urine in a 6-inch test-tube, add a few drops of sodium hydroxide and heat to boiling. Set aside for 15 minutes to allow the precipitate to settle. If the phosphates be normal the precipitate will occupy about $\frac{1}{2}$ inch at the bottom of the tube; if high, 1 inch or more; if low, less than $\frac{1}{4}$ inch.

Alkaline Phosphates.—Approximate Estimation.—Add ammonia to the urine, filter from the precipitated earthy phosphates, and to the filtrate add $\frac{1}{3}$ volume of magnesia mixture. A milky appearance on shaking indicates normal phosphates; a creamy appearance, increased phosphates; a slight cloudiness, decreased phosphates.

For the accurate determination of Phosphates see under Volumetric Analysis.

Blood.

Blood in the urine may be derived from the kidneys, in cancer, acute nephritis, after powerful diuretics, etc.; from the bladder, in diphtheritic and acute cystitis, calculi, carcinoma, congestion, etc.; from structural disease of the prostate, and from mechanical injury. When uniformly mixed with the urine the blood is probably from the kidneys; when stringy, or in clots, it is more likely to be from the bladder, prostate, or urethra. Unless in considerable quantity, its recognition is best effected by microscopic examination of the sediment, or by spectrum analysis. When present in any considerable amount, the urine will be dark red or brown, often "smoky" in appearance, and the precipitate of earthy phosphates with sodium hydroxide (*Heller's Test for blood*) will be reddish instead of white. Should the urine be alkaline and the phosphates already precipitated, add an equal volume of normal acid urine and a few drops of barium chloride before boil-

ing with sodium hydroxide. Hæmoglobin may be detected by the *Guaiacum Test.* A mixture of freshly prepared tincture of guaiacum with "ozonized ether," or old oil of turpentine, is added to the urine. A blue color indicates the possible presence of hæmoglobin. *Lecanau's Test* may be used for the detection of hæmatin. The urine is acidulated with acetic acid and boiled. The brownish coagulum of albumin and hæmatin is separated by decantation, washed with water, and shaken with alcohol which has been acidified with sulphuric acid. The reddish-brown solution is filtered and the filtrate examined spectroscopically, *or*, it may be evaporated to dryness and the residue tested for iron.

Bile.

Bile constituents may occasionally appear in the urine of healthy persons, particularly during the heat of summer, but as a rule their presence is characteristic of the condition known as jaundice. Bile pigments and bile acids may both be present, but the clinical examination is practically limited to the former. The urine is generally yellowish-brown to green, sometimes almost black, and yields a characteristic yellow froth on shaking. It must be remembered, however, that certain drugs may produce a similar appearance.

Tests for Bile Pigments. *Ultzmann's Test.*—To 10 c.c. of urine add 3 to 4 c.c. of sodium hydroxide and an excess of pure hydrochloric acid. An emerald green color indicates bile pigments.

Gmelin's Test.—The urine is floated over yellow nitric acid in a test tube. A succession of colors, green, blue, violet and red, will appear at the contact of the two liquids.

Fleischl's Test.—The urine mixed with nitric acid, or with sodium nitrate, is floated over strong sulphuric acid in a test tube. The same succession of colors appears, as in the last test.

Test for Bile Acids. *Pettenkofer's Test.*—Evaporate about 200 c.c. of urine to dryness, extract the residue with absolute alcohol, filter, evaporate the alcoholic filtrate, and dissolve the second residue in a little water. Add a few drops of a concentrated solution of cane sugar, and a drop of sulphuric acid, then warm the mixture in a capsule. A purple or cherry-red color indicates the presence of bile acids.

Instead of evaporating the alcoholic filtrate, the bile acids may be precipitated from that solution by an excess of ether, the pre-

cipitate separated, and dissolved in water. The water solution in either case will probably need to be decolorized by animal charcoal, before applying the color test.

Other Pathological Ingredients.

Serum Globulin.—This resembles serum albumin and generally accompanies it in the urine. It is insoluble in water and may be detected by dropping the urine slowly into a beaker of clear water, each drop as it falls producing a slight cloudy appearance. Serum globulin responds to the regular albumin tests.

Albumose.—Tested for, in presence of other proteids, as follows: Saturate the urine with sodium chloride, acidify with acetic acid, boil, and filter while hot. The albumose separates from the filtrate on cooling, dissolve in water and apply the Ferrocyanide Test. (See p. 66.)

Peptones.—Peptones may appear in the urine, in suppurative diseases, croupous pneumonia, typhoid, small-pox, scarlet fever, and in tuberculosis, though it is probable that deutero-albumose is often mistaken for the peptone. In presence of other proteids, acidify with acetic acid, add ammonium sulphate to saturation, filter and examine the filtrate for peptones by the xantho-proteic and biuret tests. Show the absence of other proteids from the filtrate by the nitric acid and ferrocyanide tests. (See p. 48.)

Acetone.—Found in the urine in febrile conditions and in the later stages of diabetes mellitus. Half a litre of urine is distilled with phosphoric acid and the first 100 c.c. of distillate collected and tested. *Le Nobel's or Legal's Test.*—To 25 c.c. of the distillate add a little fresh solution of sodium nitroprusside and a few drops of strong sodium hydroxide. A ruby red color appears, slowly changing to yellow. Add acetic acid and boil; the color is changed to a greenish blue or violet. *Chautard's Test.*—A drop of aqueous solution of magenta, previously decolorized by sulphurous acid, added to the distillate, develops a rich violet color.

Ethyldiacetic Acid.—This is related to acetone in its occurrence and is often confused with it. Diacetic Acid may be detected by boiling a sample of urine and adding thereto a few drops of ferric chloride. A deep red color is developed. If ferric phosphate should be precipitated, add the ferric chloride carefully until the precipitation ceases, filter, and then add a few drops more of the

reagent. Other substances sometimes present give a red color with ferric chloride, but not after boiling.

Glycuronic Acid.—Occurs in normal urine in traces, but is much increased after ingestion of chloral, chloroform, nitrobenzole, camphor, morphine, etc. It is of importance because of its resemblance to glucose, responding to the principal glucose tests. The identification of glycuronic acid is difficult, but it may be distinguished from glucose by the fact that it does not undergo alcoholic fermentation with yeast.

Pus.—May be due to renal abscess, inflammation or cancer of the bladder, suppuration of the prostate or uretha, to leucorrhœa, etc., etc. When the pus originates in the bladder the urine is often alkaline. It is best recognized microscopically, but if in considerable amount, the following tests may be applied. The whitish sediment always present in a urine carrying pus, is not dissolved by heat. It is insoluble in dilute acids, but soluble in sodium hydroxide, forming a gelatinous, ropy mass. Hydrogen peroxide added to pus causes a rapid effervescence.

Fat.—May be due to an excess of fatty food, or it may occur in fatty degeneration of the liver, in phosphorus poisoning, etc., occasionally in diabetes mellitus and in Bright's disease. When the fat is present in considerable amount the urine will be more or less milky in appearance and, on standing, the fat globules will rise to the surface. The fat may be extracted from such a sample by agitation with ether. When present in small amount the fat globules, or fatty casts, will be recognized in the microscopic examination.

URINARY SEDIMENTS.

Urinary sediments may be divided into two groups, organized and unorganized, according to structure and composition. Organized sediment includes mucus and pus cells, blood corpuscles, epithelium, casts, spermatozoa, fungi, etc. The unorganized sediment varies with the reaction of the urine, *acid urine* containing amorphous urates of sodium and potassium, crystalline uric acid, oxalate of lime, cystin, leucin and tyrosin; while *alkaline urine* may contain amorphous phosphate and carbonate of lime, crystalline urate of ammonium, triple phosphates, and phosphates of calcium and magnesium.

The sediment for examination is separated from the urine by

deposition or by use of the centrifugal machine. When the examination is to be delayed, it is necessary to guard against fermentative changes, and for this purpose camphor, salicylic acid, or better, chloroform, may be added.

Organized Sediments.

Mucus Cells.—Often present in normal urine. Round or oval globules with faintly marked margins, averaging about 0.01 mm. in diameter, but sometimes swelling to twice that size, generally with but a single nucleus. (See also, p. 64.)

Pus Cells.—From suppuration in the urinary tract. Similar to mucus cells in appearance, but distinguished by being generally multi-nuclear. The indistinct nuclei are rendered more prominent by addition of acetic acid. The pus cells are distinguished from white blood corpuscles by their somewhat larger size, their granular appearance and their more irregular outlines. The addition of alkalies converts pus into a gelatinous mass. Urine carrying pus yields albumin by chemical tests. (See also, p. 77.)

Blood Corpuscles.—Recognized as more or less yellow biconcave discs with smooth or crenated margins, generally without nuclei. In dilute urine the corpuscle is often swollen, and occasionally is biconvex in form. In concentrated urine shrunken and crenated corpuscles are common. (See also, p. 74.)

Epithelium.—Occurs in rounded, cylindrical, polygonal, or granular cells, often nucleated. These may originate in the bladder, ureters, pelvis of the kidney, kidney, vagina or urethra. The large, flat "squamous" cells from the vagina and bladder are common in the urine of women. Renal epithelial cells are rounded or polygonal, small, and often with a large nucleus. Certain of the smaller round cells may resemble pus, but their single nucleus and greater size will easily distinguish them. Epithelium is much increased by catarrh in the urinary organs.

Casts.—Moulds of the uriniferous tubules of the kidney, pointing to a diseased condition of that organ, inflammation, congestion, etc. Classed, according to appearance, as hyaline and waxy casts, epithelial casts, blood, fatty and granular casts. They are, in general, cylindrical in shape, with rounded ends, often nearly transparent and difficult to identify. They are best detected after staining with iodine or with magenta, and by use of concentrated light. They are quickly destroyed by the fermentation of the

urine. Casts are found in acute and chronic nephritis, in chronic Bright's disease, in jaundice, etc., or they may be due to irritation by renal calculi. Hyaline casts occur in albuminuria, in fever urine, and in many chronic disorders. As the disease or inflammation progresses, granular, epithelial and blood casts become more numerous. Fatty casts indicate a fatty degeneration. The presence of casts together with pus and blood, establishes the renal origin of the latter.

Fungi, Bacteria, etc.—Absent from normal urine when passed, but often developing rapidly on exposure to the air. The identification of bacteria is difficult, requiring preparation of cultures and examination under high powers with appropriate staining agents. Yeast fungi are often abundant in diabetic urine. The micrococcus ureæ, derived from the atmosphere, multiplies rapidly in urine, and is the chief factor in the ammoniacal fermentation.

Spermatozoa are often present, and may occasionally prove to be of medico-legal interest. Their characteristic form is easily recognizable under a high power.

Unorganized Sediments.

Uric Acid.—Common in acute fevers, uric acid diathesis, gravel, etc. Found in acid urine in red or brownish-yellow crystals. The crystals, which are described as whetstone, envelope, spear and fan shaped, are often gathered together in bunches or in rosettes. They are insoluble in acids, but are dissolved by alkalies and by heat. The murexid test (p. 72) may be applied.

Urates.—Associated with uric acid in acid urines and found also as crystalline, or semi-crystalline, ammonium urate in alkaline urine. The amorphous urates are granular, often provided with spicules, and like the prismatic crystalline forms are reddish or yellow in color. The murexid test (p. 72) may be applied.

Oxalates.—Oxalate of lime occurs in acid urine, in small octahedra or in envelope and dumb-bell forms. Its presence is not significant, though it may point to mal-assimilation, particularly from irregularity of diet.

Phosphates.—In alkaline urine we may have amorphous and crystalline phosphates of lime, crystalline phosphates of magnesium, and crystalline triple phosphate. The latter, triple phosphate, or ammonium magnesium phosphate, occurs in large

crystals of various forms, often as triangular prisms with beveled edges, occasionally in stellate feathery forms. When present in freshly voided urine the triple phosphate indicates decomposition of the urine in the bladder. The presence of other crystalline phosphates and of amorphous phosphates is not particularly significant.

Carbonates.—Rare as a sediment. Amorphous carbonate of lime may occur in alkaline urines, occasionally in imperfect dumb-bell forms, but generally as rounded or oval granules with dark contours.

Leucin.—A rare sediment, occasionally appearing in white shiny lamellæ, generally, however, in groups of yellowish striated spherules, somewhat like those of sodium urate, but distinguished from the latter by not dissolving on application of heat. They are distinguished from oil-drops by their insolubility in ether.

Tyrosin.—A rare sediment, occurring with leucin in acute atrophy of the liver, in small-pox and in typhus. It is usually found in the form of yellowish-green globules, but when pure occurs in fine needle-like crystals radiating from a center.

Cystin.—A rare sediment, found in colorless six-sided transparent plates, often in overlapping masses. The crystals are soluble in hydrochloric acid and in ammonia, but are insoluble in water. The chief interest of cystin lies in its tendency to formation of calculi.

URINARY CALCULI.

Calculi may consist of uric acid and urates, of calcium oxalate, ammonium oxalate, and more rarely of phosphates, carbonates, cystin, xanthin, etc. As a rule, each calculus is built up of two or more of the above substances arranged in concentric layers around a central nucleus. These layers may often be separated, and in the analysis should be examined separately.

Uric Acid and Urates form hard calculi, generally smooth, often reddish or yellowish-brown in color, and of variable size. Pure *Phosphatic Calculi* are of rare occurrence, though we frequently find phosphates deposited around a uric acid nucleus. The *Fusible Calculus* consists of a mixture of calcium, magnesium, and ammonium phosphates. It is readily fusible when heated, giving off vapor of water and ammonia. It resembles chalk in appearance and consistency. *Calcium Oxalate* is frequently met with,

forming calculi often of considerable size, brown or olive in color, and with a rugged surface (*mulberry calculi*). When small and hard, the term *hemp-seed calculus* is applied. The rare *Cystin Calculi* are more or less transparent and waxy in appearance, though crystalline in structure. They are commonly tinted yellow, changing to green on exposure. *Xanthin Calculi* are very rare. They are described as yellowish-brown in color, often with scattered white spots. Occasionally altered blood clots will form concretionary masses known as *Fibrinous Calculi.*

The following scheme will serve as an aid in the recognition of the chief varieties of calculi:

Powder the calculus and heat a small portion of the powder on platinum foil in the Bunsen flame.

A. If it chars, burns, and leaves but little residue, it probably consists of either *uric acid*, *urates*, *cystin*, *xanthin*, or *fibrin.* Test a portion of the powder with boiling water; if soluble, we have urates; if insoluble, uric acid. Confirm in either case by the murexid test.

Treat another portion of the powder with hydrochloric acid, and warm ; a residue may be uric acid; cystin and xanthine go into solution. Cystin gives a brown color with the murexid test and yields a residue soluble in ammonia. Xanthine gives a yellow color. Fibrinous calculi will be completely burned when heated, giving off the characteristic odor of burning feathers, and responding to the proteid tests.

B. Should the powder, when heated, char but slightly and leave a considerable residue, or possibly undergo no change at all, we may have *phosphates*, *oxalates* and *carbonates* of calcium and magnesium.

A fresh portion of the powder is treated with dilute hydrochloric acid ; if soluble with effervescence, carbonates are present ; if soluble without effervescence, we have phosphates or oxalates. A residue will probably consist of uric acid. Filter, render alkaline with ammonia, boil, acidify with acetic acid, and again filter. A white residue is calcium oxalate, which, if dried and heated on platinum, is converted into calcium carbonate, soluble in hydrochloric acid with effervescence.

To a portion of the filtrate, add a drop of ferric chloride; a precipitate indicates phosphates. Add to the remainder of the filtrate, oxalate of ammonium and ammonia; a precipitate indicates

calcium. Filter and test the filtrate for magnesium with sodium phosphate.

C. Should the powder when heated melt and give off water vapor with fumes of ammonia, the calculus consists of a mixture of calcium, magnesium, and ammonium phosphates. (The Fusible Calculus).

THE GASTRIC FLUID.

The gastric fluid is a thin, almost colorless liquid, with an acid reaction, and a specific gravity of 1001 to 1010. The following analysis is given by Schmidt:

Water	994.404
Organic Substances, chiefly pepsin . .	3.195
Hydrochloric Acid	0.200
Sodium Chloride	1.465
Potassium Chloride	0.550
Other Inorganic Salts	0.186

The composition varies, however, during the digestive process and in disease. The average "total acidity" is probably between 0.10 and 0.36 per cent. The "free acid" is commonly stated as from 0.20 to 0.30 per cent., but these figures are undoubtedly high and only to be reached on a carbohydrate diet. With ordinary nitrogenous foods the free acid will rarely exceed 0.10 per cent. The hydrochloric acid formed in the early stages of digestion combines rapidly with the proteids, and is not likely to be detected in the fluid until about one hour after the meal. Lactic acid is commonly present in small amount and, under certain conditions, butyric and other organic acids may appear. In fevers, in anæmia, in catarrh of the stomach, etc., pepsin and hydrochloric acid may both be considerably reduced. Hydrochloric acid may be absent in serious disease of the gastric mucous membrane, in atrophy, gastric cancer and chronic catarrh. On the other hand, it is often largely increased in nervous dyspepsia and in gastric ulcers. As a result of excessive fermentative changes, lactic and butyric acids may appear in large amount. In such a case there is always a corresponding increase in gaseous products, the stomach is distended and gaseous eructations occur. Among the abnormal constituents of the vomit, we may find excessive mucus, albumin, blood and bile, while in uraemia, urea and ammonium carbonate are also often present.

The clinical examination of the gastric fluid (obtained best by use of the stomach tube about one hour after the administration of a test breakfast of bread and water) is practically limited to the determination of the total acidity, hydrochloric acid, organic acids, and pepsin strength. The fluid is filtered and the clear filtrate tested, first qualitatively, for the acids.

Free Hydrochloric Acid, Qualitative. *Gunzberg's Test.*—To a few drops of the filtered gastric fluid add an equal quantity of Gunzberg's reagent (see Appendix) and evaporate to dryness at a gentle heat. A bright red ring will form at the margin. It will be found convenient to use a flat porcelain dish for this test, rotating the same over a small flame and avoiding a high temperature. Organic acids do not give the reaction, but the test is said to respond to one part of hydrochloric acid in 10,000 parts of water.

The materials for Gunzberg's reagent being expensive and sometimes difficult to obtain, *Boas' Test* is often substituted. The procedure is the same as with Gunzberg's test, and the results are practically as accurate. For Boas' reagent, see Appendix.

Congo-red Test.—In presence of considerable free hydrochloric acid, a dark blue spot is obtained by touching a piece of Congo-red paper with a drop of the gastric fluid. A light blue or violet spot may be due to organic acids.

Methyl-Violet Test.—To 10 c.c. of water add a few drops of a solution of methyl-violet. Divide the test solution into 2 parts, and to one add an equal volume of filtered gastric fluid. Compare with the remainder of the test solution. A change in color, from violet to blue, indicates hydrochloric acid, but the delicacy of the test is destroyed by pepsin.

Organic Acids, Qualitative. *Uffelmann's Test.*—Treat the gastric fluid filtrate with ether and evaporate the ether extract to dryness. Dissolve the residue in a small quantity of water and add a few drops of Uffelmann's reagent (see Appendix). The amethyst-blue of the reagent is changed to a canary-yellow by lactic acid (1–10,000). Hydrochloric acid in considerable amount decolorizes the reagent, and butyric acid turns it reddish-brown.

Total Acidity, Quantitative.—Dilute 10 c.c. of the filtered gastric fluid with about 40 c.c. of water, add a few drops of alcoholic phenolphthalein solution, and titrate with decinormal sodium hydroxide until the liquid acquires a faint pink color.

The number of c.c. of the decinormal alkali used, multiplied by 0.00364 will give the weight in grammes of hydrochloric acid in the 10 c.c. of gastric fluid. From this the percentage may be calculated. For convenience the total acids are reported, as indicated above, in terms of hydrochloric acid.

The relative amounts of free acid, organic acids, and acid proteids, may be determined by titrating a second, undiluted, sample of the gastric fluid. The decinormal alkali is added until a drop of the fluid under examination gives no reaction with Gunzberg's reagent. The number of c.c. of alkali used serves for the calculation of the free acid. The addition of the decinormal solution is continued until no reaction is obtained with Congo-red paper, and from the number of c.c. used the organic acids are estimated, being calculated in terms of hydrochloric acid. Phenolphthalein may now be added to the fluid, and the titration continued until the pink color is developed. From this last titration the acid proteids are calculated. The total decinormal alkali used indicates the total acidity.

For the principles involved in the above test, and for the preparation of the decinormal alkali solution, see under Volumetric Analysis.

Pepsin.—The determination of the pepsin is of little practical value, it is rarely absent, and the digestion tests used are subject to other factors than the pepsin strength. Coagulated egg albumin is cut in discs 1 mm. thick and 10 mm. in diameter. Two discs are placed in each of 2 test tubes, together with 10 c.c. of the filtered gastric fluid. To one of the tubes add 2 drops of concentrated hydrochloric acid, and then warm both for 1 to 2 hours at 40° C. Complete solution should take place in both samples.

VOLUMETRIC ANALYSIS.

QUANTITATIVE analyses may be conducted by either *gravimetric* or *volumetric* processes. In the former, the constituents are precipitated from solutions by reagents, the precipitates are dried and weighed, and from their weights the composition of the substance is calculated. Volumetric analyses are, as a rule, more quickly performed and require less extensive laboratory appliances. The process, depending on the principle of Definite and Fixed Proportions in chemical combinations, consists in the determination of the amount of a substance in solution by the addition thereto of a reagent of known strength, the *standard solution*. The reagent is added from an accurately graduated glass vessel, known as a *burette*, and the end of the reaction is revealed either by a change in the liquid itself, or by a change in color of a substance added as an *indicator*.

The reaction between sulphuric acid and potassium hydroxide is expressed by the equation:

$$\underbrace{2\ KOH} + \underbrace{H_2SO_4} = K_2SO_4 + 2\ H_2O$$

Molecular Weights, 111.98 97.82

If sulphuric acid be added to a solution of potassium hydroxide, the solution will remain alkaline until sufficient acid has been added to complete the above reaction. In other words, to 111.98 parts of potassium hydroxide we must add 97.82 parts of sulphuric acid, in order that complete neutralization shall take place. If more acid be added, the solution will become acid in reaction. If 97.82 grammes of sulphuric acid exactly neutralize 111.98 grammes of potassium hydroxide, then if we make a solution containing 97.82 grammes of sulphuric acid in 1000 c.c., each c.c. of this solution will neutralize 0.11198 grammes of potassium hydroxide. So, also, in a solution containing 48.91 grammes of the acid to 1000 c.c., each c.c. will neutralize 0.05599 grammes of the alkali. If now to a solution of potassium hydroxide of unknown

strength we add a standard solution of sulphuric acid (one containing a known weight of the acid in each c.c.) until neutralization is effected, we can calculate the amount of alkali from the number of c.c. of acid used.

Again, were we to add a standard solution of sodium chloride to a solution of silver nitrate, the reaction would be expressed by the equation:

$$\underset{\sim\sim}{AgNO_3} + \underset{\sim\sim}{NaCl} = AgCl + NaNO_3$$

Molecular Weights, 169.55 58.37

The number of c.c. of standard sodium chloride necessary to complete the above reaction (to precipitate all of the silver as silver chloride) affords us the means of calculating the amount of silver nitrate in the solution. The same principle may be applied in other ways, as, for instance, in the determination of the amount of iron in a solution. We may first reduce the iron to the ferrous state by appropriate reducing agents, and then by the addition of a solution of known oxidizing power, we may reconvert the iron to the ferric condition. The number of c.c. of oxidizing solution used, multiplied by the oxidizing power of each c.c., affords the data necessary for the calculation of the iron present.

Solutions Used.

In order, then, to make a quantitative determination of any substance by volumetric processes, we must have an appropriate reagent, of known strength, a *standard solution*. The strength of this reagent will be determined by the nature of the analysis, but it is convenient that the weight in grammes, dissolved in each litre, shall bear an intimate relation to the molecular weight, thus simplifying subsequent calculations. The solutions most commonly used are designated as Normal, ($\frac{N}{1}$), Deci-Normal, ($\frac{N}{10}$), and Centi-Normal, ($\frac{N}{100}$).

A Normal Solution of a univalent substance contains, in each litre, its molecular weight expressed in grammes.

A Normal Solution of a bivalent substance contains, in each litre, $\frac{1}{2}$ its molecular weight expressed in grammes.

A Normal Solution of a trivalent substance contains, in each litre, $\frac{1}{3}$ its molecular weight expressed in grammes.

A Deci-Normal Solution is $\frac{1}{10}$th the strength of the corresponding normal solution.

A Centi-Normal Solution is $\frac{1}{100}$th the strength of the corresponding normal solution.

For example, 1 litre of $\frac{N}{1}$ KOH (normal potassium hydroxide) contains 55.99 grammes of KOH. One litre of $\frac{N}{10}$ KOH contains 5.599 grammes of KOH. One litre of $\frac{N}{100}$ KOH contains 0.5599 grammes of KOH. One litre of $\frac{N}{1}$ H_2SO_4 contains 48.91 grammes of H_2SO_4 (Molecular weight of bivalent $H_2SO_4 = 97.82$). One litre of $\frac{N}{10}$ H_2SO_4 contains 4.891 grammes of H_2SO_4, etc.

Indicators.—The success of the volumetric process depends upon the accuracy of our means of recognizing the completion of the chemical reaction which takes place between the reagent and the substance under titration. This is generally accomplished by adding to the solution a substance which will reveal by change of color the slightest excess of the reagent. The substance so used is known as an indicator, and must answer to the following conditions: The completion of the test, the end reaction, must be marked by an indisputable change in color, but little of the indicator should be used, and the color change must not be interfered with by any impurities present, nor by the products of the reaction itself. The following are some of the indicators in common use. For their preparation, see Appendix.

Litmus.—Red with acids, blue with alkalies. Litmus is used chiefly in the titration of the mineral acids and alkalies; it is not reliable as an indicator in presence of carbonates, phosphates or arsenates.

Phenolphthalein.—Colorless with acids, red with alkalies. This indicator is much used and is extremely delicate, but its value is lessened by presence of ammonium salts or of borax.

When it is necessary to use phenolphthalein or litmus in presence of carbonic acid (carbonates), the solution under titration should be boiled.

Methyl Orange.—Red with acids, yellow with alkalies. It is not affected by carbonic anhydride, and hence may be used in presence of carbonates, but it is not satisfactory with organic acids.

Cochineal.—Yellow with acids, violet with alkalies. Used chiefly with ammonia and ammonium compounds.

Other special indicators will be referred to in describing certain of the processes which follow.

Acidimetry.

The estimation of acids by means of standard alkali solutions. Sodium or potassium hydroxides are the alkalies generally used, the standard solutions being prepared as follows:

Normal Potassium Hydroxide.—If pure potassium hydroxide were obtainable it would be only necessary to dissolve 55.99 grammes of that substance in 1 litre of water. (55.99 being the molecular weight of univalent potassium hydroxide.) It can not be obtained pure, however, owing to its tendency to absorb carbonic anhydride and moisture from the air, and the following U. S. P. method is advised: Dissolve 75 grammes of potassium hydroxide in 1050 c.c. of water at 15° C., and fill a burette with this solution. Dissolve 0.63 grammes of pure crystallized oxalic acid in about 10 c.c. of water and add a few drops of phenolphthalein. Now add the potassium hydroxide, from the burette, until the oxalic acid is just neutralized, a faint pink tint being developed in the solution. Note the number of c.c. of alkali used, and then dilute the remainder of the solution until 10 c.c. will exactly neutralize the 0.63 grammes of oxalic acid. The reaction between the alkali and acid is expressed by the equation:

$$\underbrace{2\,KOH}_{112} + \underbrace{H_2C_2O_4.2\,H_2O}_{126} = K_2C_2O_4 + 4\,H_2O$$

From this it can be seen that 126 parts of oxalic acid are neutralized by 112 parts of potassium hydroxide, or 63 parts by 56 of potassium hydroxide. Normal potassium hydroxide contains 56 grammes to the litre, and 10 c.c. of the normal solution contain 0.56 grammes. Hence, in the preparation of the normal alkali as described, when 10 c.c. exactly neutralize 0.63 grammes of oxalic acid, then that 10 c.c. must contain 0.56 grammes of potassium hydroxide, and the solution must be normal.

Deci-Normal Potassium Hydroxide.—Dilute 100 c.c. of the normal solution to 1000 c.c., with pure water.

Normal Sodium Hydroxide.—This contains 39.96 (40) grammes of sodium hydroxide to the litre. The solution is prepared by dissolving 54 grammes of sodium hydroxide in 1050 c.c. of water, proceeding then exactly as described under potassium hydroxide. The reaction in this case is expressed by the equation:

$$\underbrace{2\,NaOH}_{2)\frac{80}{40}} + \underbrace{H_2C_2O_4.2\,H_2O}_{2)\frac{126}{63}} = Na_2C_2O_4 + 4\,H_2O$$

Each Cubic Centimeter of a Normal Alkali Solution is equivalent to:

	Grammes.
Acid, Acetic, $HC_2H_3O_2$	0.05986
Citric, $H_3C_6H_5O_7.H_2O$ . . .	0.06983
Hydrobromic, HBr	0.08076
Hydrochloric, HCl	0.03637
Hydriodic, HI	0.12753
Lactic, $HC_3H_5O_3$	0.08979
Nitric, HNO_3	0.06289
Oxalic, $H_2C_2O_4.2\,H_2O$ . . .	0.06285
Sulphuric, H_2SO_4	0.04891
Tartaric, $H_2C_4H_4O_6$ . . .	0.07482

Method of Analysis.—A weighed quantity of the acid is diluted with a little water, a few drops of phenolphthalein added, and then the standard alkali, from a burette, until a faint pink tint is developed. The number of c.c. of standard solution used, multiplied by the equivalent of each c.c., gives the weight of pure acid in the solution. From this, and the weight of the sample, the percentage may be calculated.

If 5 grammes of hydrochloric acid solution were taken for analysis, and 20 c.c. of normal alkali were required to develop the pink color, then the 5 grammes of acid solution contain, $20 \times 0.03637 = 0.7274$ grammes of pure acid, or, $0.7274 \div 5 \times 100 = 14.55$ per cent.

Remarks.—It is often inconvenient to weigh the sample taken for analysis, and in such a case, a definite *volume* must be used. The titration with the normal solution will give the number of grammes of pure substance in the sample, and for many solutions we can assume the weight of the sample to be equal to as many grammes as there were c.c. taken. (1 c.c. of water weighs 1 gramme). Or, we can determine the specific gravity of the solution by means of a hydrometer, and calculate the weight of the sample by multiplying its volume by its specific gravity.

The pharmacopœial practice is to weigh off such a quantity of the substance, for analysis, that the number of c.c. of standard

solution used will directly express the percentage sought. Thus, 3.64 grammes of U. S. P. acidum hydrochloricum, containing 31.9 per cent. of pure hydrochloric acid, will be exactly neutralized by 31.9 c.c. of normal alkali. 3.64 grammes of the dilute acid of the pharmacopœia, containing 10 per cent. of pure acid, will be exactly neutralized by 10 c.c. of normal alkali.

The method is, in general, the same for any acid, merely substituting in the calculation the proper equivalent for each c.c. of the standard alkali used.

Total Acidity of the Urine.—To 50 c.c. of urine add several drops of phenolphthalein, and titrate with decinormal potassium hydroxide until the pink color appears. Each c.c. of the decinormal alkali is equivalent to 0.006285 grammes of oxalic acid. Assuming that the 50 c.c. of urine weigh 50 grammes, the percentage may be easily calculated. In case the urine is highly colored, it is best to first remove the color by shaking with animal charcoal and filtering. The total acidity of the 24 hours' urine averages about 2 grammes of oxalic acid. It is to be remembered that the acid reaction of the urine is in reality due to the presence of acid salts and not at all to oxalic acid, this last substance being generally adopted, however, because of the greater ease in calculation and as affording a simple means of comparison.

Total Acidity of the Gastric Fluid.—(See p. 84.)

Alkalimetry.

The estimation of alkalies by means of a standard acid solution. Several of the acids are used for this purpose, the more common being oxalic, sulphuric and hydrochloric. Oxalic acid has the distinct advantage over the others of easy preparation, but, as a rule, sulphuric acid will be found to have the widest application and to give the most satisfactory results.

Normal Oxalic Acid.—Dissolve 62.85 grammes of the pure crystalline acid in water and dilute to 1 litre. (62.85 being $\frac{1}{2}$ the molecular weight of the bivalent, crystalline oxalic acid.)

Deci-Normal Oxalic Acid.—Dilute 100 c.c. of the normal acid to 1000 c.c. with water; or, dissolve 6.285 grammes of the acid in water and dilute to 1 litre.

Centi-Normal Oxalic Acid.—Dilute 10 c.c. of the normal acid to 1000 c.c. with water; or, dissolve 0.6285 grammes of the acid in water and dilute to 1 litre.

Normal Sulphuric Acid.—The normal solution of sulphuric acid contains 48.91 grammes in 1 litre. (48.91 being $\frac{1}{2}$ the molecular weight of the bivalent acid.) It is prepared by mixing 30 c.c. of pure concentrated acid, specific gravity 1.835, with enough water to make 1050 c.c. The mixture is cooled and its strength determined by titration with normal potassium hydroxide. It is then diluted with water until 10 c.c. will exactly neutralize 10 c.c. of the normal alkali; in other words, until each c.c. contains 0.04891 grammes of pure sulphuric acid.

Each Cubic Centimeter of a Normal Acid Solution is equivalent to:

	Grammes.
Ammonia gas, NH_3	0.01701
Ammonium hydroxide, NH_4OH	0.03497
Lithium carbonate, Li_2CO_3	0.03693
Potassium bicarbonate, $KHCO_3$	0.09988
Potassium carbonate, K_2CO_3	0.06895
Potassium hydroxide, KOH	0.05599
Sodium bicarbonate, $NaHCO_3$	0.08385
Sodium carbonate, Na_2CO_3	0.05292
Sodium hydroxide, NaOH	0.03996

Method of Analysis.—A weighed quantity of the sample is diluted with water, or, if solid, is dissolved in water, a few drops of phenolphthalein added, and the standard acid run in from a burette until the pink color of the solution is just destroyed. The number of c.c. of standard acid used, multiplied by the equivalent of each c.c., gives the weight of pure alkali in the solution. From this, and the weight of the sample, the percentage can be calculated.

If 5 grammes of sodium hydroxide solution were taken for analysis, and 25 c.c. of normal sulphuric acid were required to effect neutralization, then the 5 grammes of alkali solution contain $25 \times 0.03996 = 0.999$ grammes of pure sodium hydroxide, or, $0.999 \div 5 \times 100 = 19.98$ per cent.

Remarks.—In the titration of carbonates, methyl orange is to be used as the indicator, the standard acid being added until the solution acquires a faint orange-red tint. Otherwise the process is as described. The remarks on p. 90 are also applicable here.

Estimation of Haloid Salts.

Salts of chlorine, bromine and iodine, may be conveniently estimated by precipitation with a standard solution of silver nitrate. The reagent is added until all of the halogen has been precipitated as silver salt, and from the number of c.c. used the original halogen compound may be calculated. The completion of the reaction may be determined by testing small portions of the solution from time to time, filtering off the precipitate and adding a drop of silver nitrate, until no further precipitation occurs. Much more satisfactorily, however, we can add to the solution a few drops of potassium chromate, which, by formation of red silver chromate when all of the halogen has been precipitated, will reveal the slightest excess of the silver nitrate.

Deci-Normal Silver Nitrate.—Dissolve 16.955 grammes of pure silver nitrate in water and dilute to 1 litre, at 15° C. (The molecular weight of silver nitrate being 169.55, the deci-normal solution will contain $\frac{1}{10}$th of the molecular weight, expressed in grammes.) The solution should be kept in the dark.

Should pure silver nitrate not be available, a trial solution stronger than the deci-normal is first prepared, and then 0.1167 grammes of pure sodium chloride is dissolved in water and titrated. Were the silver nitrate deci-normal, 20 c.c. would exactly precipitate all of the chlorine of the salt as silver chloride; but as the solution is stronger than deci-normal, less than 20 c.c. will complete the reaction. Determine the exact strength of the strong silver nitrate and dilute with such a quantity of water as will reduce it to the strength required, *i. e.*, to the deci-normal.

Each Cubic Centimeter of Deci-Normal Silver Nitrate Solution is equivalent to:

	Grammes.
Ammonium Bromide, NH_4Br . . .	0.009777
Lithium Bromide, LiBr	0.008677
Potassium Bromide, KBr	0.011879
Potassium Chloride, KCl	0.007440
Potassium Cyanide, KCN	0.013002
Potassium Iodide, KI	0.016556
Sodium Bromide, NaBr	0.010276
Sodium Chloride, NaCl	0.005837
Zinc Chloride, $ZnCl_2$	0.006792

Method of Analysis.—To a measured volume of the salt solution, or to a weighed quantity of the salt dissolved in water, add a few drops of neutral potassium chromate, and then the deci-normal silver nitrate, from a burette, until the solution acquires a slight but permanent red tinge. The number of c.c. of the deci-normal solution used multiplied by the equivalent of each c.c., gives the weight of the halogen salt in solution. From this and the weight of the sample, the percentage strength can be calculated.

In titrating potassium cyanide, no indicator is used, but the silver nitrate is added until the appearance of the first, slight, permanent precipitate.

Total Chlorides of the Urine.—Dilute 10 c.c. of the urine (previously decolorized with animal charcoal if necessary) to about 50 c.c. with water. Add a few drops of neutral potassium chromate, as an indicator, and titrate with deci-normal silver nitrate until the solution acquires the red tint indicative of the complete precipitation of the chlorides. The number of c.c. used, multiplied by 0.005837, will give the weight of sodium chloride in the 10 c.c. of urine. From this, the percentage, and also the amount passed in the 24 hours, may be calculated.

It is to be remembered that by this method all of the chlorides of the urine are calculated as the sodium salt, though potassium chloride is also present. This error may be avoided by reporting in terms of chlorine, using the factor 0.003537 instead of 0.005837. The average daily amount passed by adult males is from 6 to 10 grammes of chlorine, equivalent to 10 to 16 grammes of sodium chloride.

Total Phosphates in Urine.

Standard Uranium Nitrate Solution.—Dissolve 35.5 grammes of pure uranium nitrate in 1 litre of water. Each c.c. of this solution is equivalent to 0.005 gramme of phosphoric anhydride, P_2O_5.

Acid Solution of Sodium Acetate.—Dissolve 10 grammes of sodium acetate in 90 c.c. of water and add 10 c.c. of glacial acetic acid.

Method of Analysis.—To 50 c.c. of the urine add 5 c.c. of the acid solution of sodium acetate, and heat the mixture to about 80° C. Run in the uranium nitrate while the solution is still hot, and test from time to time until a drop of the mixture de-

velops a brown color when touched with a drop of potassium ferrocyanide. The number of c.c. of uranium nitrate used, multiplied by 0.005 gives the weight in grammes of phosphoric anhydride in the 50 c.c. of urine.

The amount of phosphoric anhydride (commonly known as phosphoric acid) passed in the 24 hours, varies from 2.5 to 3.5 grammes.

APPENDIX.

WEIGHTS AND MEASURES.

MEASURES OF WEIGHT.

1 milligramme	=	0.001 gramme	= 0.01543 grains, Troy.
1 centigramme	=	0.010 "	
1 decigramme	=	0.100 "	
1 gramme	=	1.000 "	= 15.43235 grains, Troy.
1 decagramme	=	10.000 grammes.	
1 hectogramme	=	100.000 "	
1 kilogramme	=	1000.000 "	= 2.6790 pounds, Troy.
1 kilogramme			= 2.2046 pounds, Av.
1 tonneau	=	1000.000 kilogrammes.	

Troy Weight.

Pound.	Ounces.	Pennyweights.	Grains.		Grammes.
1	12	240	5760	=	373.2419
	1	20	480	=	31.1035
		1	24	=	1.5552

Apothecaries' Weight.

Pound.	Ounces.	Drachms.	Scruples.	Grains.		Grammes.
1	12	96	288	5760	=	373.2419
	1	8	24	480	=	31.1035
		1	3	60	=	3.8879
			1	20	=	1.2959
				1	=	0.0648

Avoirdupois Weight.

Pound.	Ounces.	Drachms.	Grains.		Grammes.
1	16	256	7000	=	453.5926
	1	16	437.5	=	28.3495
		1	27.343	=	1.7718

MEASURES OF CAPACITY.

1 millilitre = 1 cubic centimetre	= 0.061027	cubic inch.
	= 0.033816	U. S. fluid ounce.
	= 16.2310	U. S. minims.
1 litre = 1000 cubic centimetres	= 33.816	U. S. fluid ounces.
	= 35.219	Imperial "
	= 1.0567	U. S. quart.
1 kilolitre = 1000 litres	= 264.18	U. S. gallons.

1 U. S. minim	=	0.06 c.c.
1 U. S. fluid ounce	=	29.57 c.c.
1 Imperial fluid ounce	=	28.39 c.c.
1 U. S. gallon	=	3785.43 c.c.
1 Imperial gallon	=	4543.46 c.c.

MEASURES OF LENGTH.

1 millimetre	= 0.001 metre	= 0.03937 inch.
1 centimetre	= 0.010 "	
1 decimetre	= 0.100 "	
1 metre	= 1.000 "	= 3.28089 feet.
1 decametre	= 10.000 metres.	
1 hectometre	= 100.000 "	
1 kilometre	= 1000.000 "	= 0.62138 mile.

1 inch = 2.53995 centimetres.
1 foot = 0.30479 metre.
1 yard = 0.91438 metre.
1 mile = 1.60931 kilometres.

MEASURES OF TEMPERATURE.

	Water boils.	Water freezes.
Centigrade	100°	0°
Fahrenheit	212°	32°
Reaumur	80°	0°

To convert °C to °F, multiply by 9, divide by 5, then add 32.

To convert °F to °C, subtract 32, then multiply by 5, and divide by 9.

LIST OF REAGENTS.

Acid, Acetic, $HC_2H_3O_2$. Sp. gr. 1.04. 30 per cent.
" Hydrochloric, HCl. Sp. gr. 1.16. 32 per cent.
" Nitric, HNO_3. Sp. gr. 1.24. 32 per cent.
" Nitro-hydrochloric, $NOCl + Cl_2$. About 1 part conc. HNO_3 to 3 parts HCl.
" Oxalic, $H_2C_2O_4$. 1 part crystals in 10 parts water (1–10).
" Phosphoric, H_3PO_4. Sp. gr. about 1.7. 85 per cent.
" Picric, $C_6H_2(NO_2)_3OH$. Aqueous solution.
" Salicylic, $HC_7H_5O_3$. Solid, or in aqueous solution.
" Sulphuric, H_2SO_4. Concentrated, sp. gr. 1.84.
" Tannic, $HC_{14}H_9O_9$. Aqueous solution.
" Tartaric, $H_2C_4H_4O_6$. (1–3.)
" Trichloracetic, $HC_2Cl_3O_2$. Solid.

Acidulated Brine. 500 c. c. of saturated NaCl solution, 30 c.c. HCl.

Alcohol, C_2H_5OH. *Absolute.* Not less than 99 per cent. by weight.

Alcohol, C_2H_5OH. *Ordinary.* About 91 per cent. by weight.

Almen's Reagent. $BiONO_3$, 2 grammes; $NaKC_4H_4O_6$, 4 grammes; NaOH, 8 grammes; water, 100 c.c.

Ammonium Carbonate, $(NH_4)_2CO_3$. (1–4.)
" Chloride, NH_4Cl. (1–8.)
" Hydroxide, NH_4OH. 10 per cent. NH_3.
" Molybdate, $(NH_4)_2MoO_4$. Solution in nitric acid.
" Oxalate, $(NH_4)_2C_2O_4$. (1–24.)
" Sulphate, $(NH_4)_2SO_4$. Saturated aqueous solution.
" Sulphide, $(NH_4)_2S$. By passing H_2S through NH_4OH.

Barium Carbonate, $BaCO_3$. Solid, or sat. solution.
" Chloride, $BaCl_2$. (1–10.)
" Hydroxide, $Ba(OH)_2$. Sat. aqueous solution.
" Nitrate, $Ba(NO_3)_2$. (1–10.)

Bismuth Subnitrate, $BiONO_3$ (?). Solid.

Bleaching Powder, $CaOCl_2$.

Boas' Reagent. Pure resorcin, 5 grammes; White sugar, 3 grammes; dilute alcohol, 100 c.c.

Bromine Water, Br. Aqueous solution of bromine.

Calcium Chloride, $CaCl_2$. (1–8.)

" Hydroxide, $Ca(OH)_2$. Saturated aqueous solution.

Carbon Disulphide, CS_2. Pure.

Carbonic Anhydride, CO_2. By action of HCl on $CaCO_3$.

Chlorine Water, Cl. Aqueous solution of chlorine.

Chlorinated Soda, for Urea Test. 25 c.c. solution of chlorinated soda; 5 c.c. KBr (20 per cent.); 5 c.c. NaOH.

Chloroform, $CHCl_3$. Sp. gr. about 1.49 at 15° C. Boils at 60° C.

Cochineal, Indicator. Macerate 1 gramme for several days with 20 c.c. alcohol and 60 c.c. water. Filter.

Congo-red Paper. Prepared by soaking unsized paper in 1 per cent. aqueous solution of Congo-red.

Copper Sulphate, $CuSO_4$. (1–8.)

Cupric Ammonium Hydroxide. Solution of $Cu(OH)_2$ in ammonia.

Cupric Ammonium Sulphate. To a solution of $CuSO_4$ add ammonia until the precipitate first formed just redissolves.

Esbach's Reagent. Picric Acid, 10 grammes; Citric Acid, 20 grammes; Water to 1000 c.c.

Ether, $(C_2H_5)_2O$. Sp. gr. about 0.727 at 15° C. Boils at 37° C.

Fehling's Solution. Prepared in 2 parts. I. 34.639 grammes of pure crystallized $CuSO_4$, dissolved in water and diluted to 500 c.c. II. 173 grammes Rochelle salts and 60 grammes NaOH, dissolved in water and diluted to 500 c.c. For use mix equal volumes of I. and II. Ten c.c. of the mixed solution = 0.05 gramme glucose.

Ferric Chloride, Fe_2Cl_6. (1–15.)

Ferrous Sulphate, $FeSO_4$. (1–10.)

Gold Chloride, $AuCl_3$. (1–30.)

Gunzberg's Reagent. Phloroglucin, 2 pts.; Vanillin, 1 pt.; Absolute Alcohol, 30 pts., by weight.

Haines' Solution. Dissolve 2 grammes pure $CuSO_4$ (crys.) in 15 c.c. of water, add 15 c.c. of pure glycerin and then 150 c.c. of 5 per cent. KOH solution. A clear dark blue liquid should result.

Indigo-Carmine Solution. 1 gramme commercial indigo-carmine in 150 c.c. of water.

Iodine Test Solution. 1 gramme iodine, 3 grammes potassium iodide, in 50 c.c. water.

Lead Acetate, $Pb(C_2H_3O_2)_2$. (1–10.)

Litmus Test Solution. Exhaust powdered litmus with boiling alcohol. Digest residue in cold water, filter, and extract residue with boiling water. Filter and preserve the filtrate as a test solution. *Litmus paper* is prepared by impregnating unsized paper with the above solution.

Magnesium Chloride, $MgCl_2$. (1–10.)

" Mixture, 1 pt. cryst. $MgCl_2$; 2.5 pts. NH_4Cl; 5 pts. NH_4OH; and 10 pts. water. Let stand, then filter.

" Sulphate, $MgSO_4$. Saturated aqueous solution.

Mayer's Solution. 13.546 grammes $HgCl_2$ dissolved in 600 c.c. of water. 49.8 grammes KI dissolved in 100 c.c. of water. Mix and dilute to 1000 c.c.

Mercuric Chloride, $HgCl_2$. (1–20.)

Mercurous Nitrate, $Hg_2(NO_3)_2$. (1–20.) Acidulate with nitric acid.

Methyl Orange. (Tropæolin D.) 1 gramme in 1000 c.c. of water. Add dilute H_2SO_4 drop by drop, until liquid just turns red. Filter.

Millon's Reagent. 1 pt. mercury treated with 2 pts. HNO_3 in the cold. Then heat on water bath, dilute with 2 pts. water, and after several hours, decant the clear liquid.

Pavy's Ammoniated Test Solution. $CuSO_4$, 4.158 grammes; Rochelle salts, 20.4 grammes; KOH, 20.4 grammes; Strong ammonia, 300 c.c.; Water to 1000 c.c.

Phenolphthalein. (Indicator.) 1 gramme in 100 c.c. dil. alcohol.

Platinic Chloride, $PtCl_4$. (1–10.)

Potassium Carbonate, K_2CO_3. (1–20.)

" Chlorate, $KClO_3$. Solid.

" Chromate, K_2CrO_4. (1–10.)

" Dichromate, $K_2Cr_2O_7$. (1–10.)

" Ferricyanide, $K_3Fe(CN)_6$. (1–12.)

" Ferrocyanide, $K_4Fe(CN)_6$. (1–12.)

" Hydroxide, KOH. (1–9.)

" Iodide, KI. (1–20.)

" Nitrate, KNO_3. Solid.

" Sulphate, K_2SO_4. (1–12.)

" Sulphocyanide, KCNS. (1–12.)

Silver Nitrate, $AgNO_3$. (1–20.)

" Ammonium Nitrate. To solution of $AgNO_3$ add NH_4OH until the precipitate first formed is just redissolved.

Sodium Acetate, $NaC_2H_3O_2$. (1–5.)

" Carbonate, Na_2CO_3. (1–5.)

" Chloride, NaCl. Solid, or saturated solution.

" Hydroxide, NaOH. (1–9.)

" " Alcoholic. Sol. of NaOH in alcohol.

" Hypobromite, NaBrO, for Urea Test. 100 grammes of NaOH in 250 c.c. water. 25 c.c. Bromine added.

" Phosphate, Na_2HPO_4. (1–10.)

" Sulphate, Na_2SO_4. Saturated solution.

" Sulphite, Na_2SO_3. (1–5.)

Stannous Chloride, $SnCl_2$. (1–6.) Acidified with HCl.

Strontium Nitrate, $Sr(NO_3)_2$. Aqueous solution.

" Sulphate, $SrSO_4$. Saturated solution.

Sulphuretted Hydrogen, H_2S. Prepared by action of FeS on HCl.

Tanret's Solution, $HgCl_2$, 1.35 grammes; KI, 3.32 grammes; Acetic acid, 20 c.c. Distilled water to 100 c.c.

Uffelmann's Reagent. 10 c.c. of 4 per cent. Phenol; 1 drop of dil. Fe_2Cl_6; 20 c.c. water.

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