Franklin L. Warren

NOTES

ON THE

CHEMICAL LECTURES

FOR

SECOND-YEAR STUDENTS

IN THE

MEDICAL DEPARTMENT OF THE UNIVERSITY OF PENNSYLVANIA.

PUBLISHED BY AUTHORITY OF PROF. THEO. G. WORMLEY.

BY

JOHN MARSHALL.

PHILADELPHIA:
AVIL PRINTING COMPANY.
1894.

THE following notes, by Dr. Marshall, of my Lectures on Chemistry, for students, have been published with my consent and authority.

THEODORE G. WORMLEY.

NOTES

ON

CHEMICAL LECTURES.

ORGANIC CHEMISTRY.

THE production of *organic compounds* was supposed to be due to the influence of a so-called *vital force*. This supposition was shown to be fallacious when *organic compounds* were *produced artificially* (by synthesis).

Liebig defined *organic chemistry* as the chemistry of the compound radicals. This definition is faulty because there are radicals, as NH_4, SO_3, etc., in the domain of inorganic chemistry.

The names *compound radical* and *radical* are synonymous.

A **radical** is a chemical combination of two or more elements capable of playing the part of an elementary form of matter.

Organic chemistry has been defined as the chemistry of the compounds of carbon. It must be remembered that there are two compounds containing carbon in the domain of inorganic chemistry, CO and CO_2.

The **latest definition** of **organic chemistry** is,—the chemistry of the hydrocarbon compounds and their derivatives. This definition applies to all carbon compounds except HCN (hydrocyanic acid) and CN (cyanogen).

The number of elements entering into the composition of organic compounds is comparatively small, but the number of atoms in a molecule of a compound may be very large.

Organic compounds may be composed of simply carbon and hydrogen. Such compounds are termed **hydrocarbons,** as $C_{10}H_{16}$, oil of turpentine, CH_4, methane.

CN, cyanogen is an organic compound composed of two elements.

Other organic compounds may be composed of three elements,—namely, carbon, hydrogen, and oxygen. Compounds of these three elements containing the hydrogen and oxygen atoms in the proportion to form water are called **carbohydrates,** as $C_6H_{10}O_5$, starch, $C_6H_{12}O_6$, glucose, in the latter there are $H_{12}O_6 = 6H_2O$.

There are **three principal groups of carbohydrates:** The

Glucose group.

Saccharose group.

Amylose group.

Organic compounds may be composed of four elements, carbon, hydrogen, oxygen, and nitrogen. Compounds containing nitrogen are termed nitrogenous or azotized, as $CO(NH_2)_2$, urea, $C_5H_4N_4O_3$, uric acid.

Some compounds are composed of only carbon and nitrogen, as CN, cyanogen, and others of carbon, nitrogen, and hydrogen, as HCN, hydrocyanic acid.

HCN, hydrocyanic acid, may be looked upon as CH_4, methane, in which three atoms of H have been replaced by an atom of triad nitrogen, as $C\equiv N$ / H

A few organic compounds contain carbon, hydrogen, oxygen, nitrogen, and sulphur, as $C_{204}H_{322}N_{52}O_{66}S_2$, egg-albumen. Some few contain phosphorus, as $C_{42}H_{84}NPO_9$, lecithin.

A very few organic compounds contain, in addition to carbon, hydrogen, oxygen, nitrogen, and sulphur, a *metal,*—as iron in $C_{636}H_{1025}N_{164}O_{189}FeS_3O_2$, oxyhæmoglobin, the molecular weight of which is 14161.

All organic compounds occurring in nature may be considered as falling under the above classification. By chemical means nearly all of the elements may be introduced into organic chemical compounds.

The number of possible combinations of these elements to produce new organic compounds is almost infinite. Ordinarily, however, only fourteen or fifteen elements are concerned in chemical combinations in organic chemistry.

Q. Disinfectant is an agent that destroys the microorganisms

An Antiseptic is an agent that arrests the growth of a microorganism and stops the multiplication of them

All disinfectants are Antiseptics.

Most powerful disinfectant is $HgCl_2$ It is used 1 in 1000.

Temp of 104°C. destroys all micro orgs. Most spore producing org. are easily destroyed simple boiling will do it. H_2SO_3 gas is a good disinfectant.

Poisons

Sepsine, Cadaverine, Putrescine.

Organic compounds containing mercury, copper, gold, etc., and iodine, bromine, chlorine, etc., may be produced artificially by chemical means. These compounds do not occur already formed in nature.

An **organic body** is an aggregation of distinct organic compounds, called *proximate* constituents. An *organic body* may also contain inorganic compounds. Examples, conium maculatum, opium, etc. The percentage proportion of the proximate constituents contained in an organic body is not definite,—*i. e.*, two or three samples of opium selected at random will not contain, except by chance, the same percentage of morphine.

A **proximate principle** is an organic compound which is contained in an organic body and to which the physiological action of the organic body is due. Examples, coniine, $C_8H_{15}N$, the proximate principle of conium maculatum, morphine, $C_{17}H_{19}NO_3$, codeine, $C_{18}H_{21}NO_3$, etc., proximate principles of opium.

Oxygen is the predominating element in inorganic chemistry, carbon in organic chemistry.

In organic chemistry the same laws of combination apply as in inorganic chemistry.

The belief that a so-called vital force was a necessity in the formation of organic compounds was overthrown in 1828, by Woehler's synthetical production of urea from substances considered inorganic,—namely, ammonia and cyanic acid (the two forming NH_4CNO, ammonium cyanate).

$$NH_3 + HCNO = NH_4CNO$$

By slowly heating NH_4CNO, ammonium cyanate, on a water-bath at a temperature between 50° C. and 70° C. a rearrangement of the atoms occurs with the formation of urea.

$$\underset{\text{Ammonium cyanate.}}{NH_4CNO} = \underset{\text{Urea.}}{CO(NH_2)_2}$$

The next organic compound produced synthetically was CN, cyanogen, by Fownes in 1841. This was produced by passing nitrogen over red-hot charcoal (carbon).

$$C_2 + N_2 = C_2N_2$$

Berthelot followed in 1856 by the synthetical production of $HCHO_2$, formic acid. This was produced by passing CO, carbon monoxide, over heated KOH, potassium hydroxide, forming $KCHO_2$, potassium formate. The latter compound when treated with HCl (hydrochloric acid) breaks up into $HCHO_2$, formic acid, and KCl, potassium chloride.

$$CO + KOH = KCHO_2$$
$$KCHO_2 + HCl = HCHO_2 + KCl$$

Wurtz in 1862 produced C_2H_5OH, ethyl alcohol, synthetically. The alkaloid coniine, $C_8H_{15}N$, has been produced synthetically.

Thousands of organic compounds have been produced synthetically in the past twenty-five years, some of them of the greatest importance; for example, the aniline coloring-matters, indigo, and many of the medicines as antipyrin, phenacetin, etc., now largely used in medical practice. The number of organic compounds produced synthetically is far greater than those existing in nature.

It was urged that the synthesis of urea was only the production of a simpler substance from a more complex one. $K_4Fe(CN)_6$ having been the complex substance used in the production of the NH_4CNO, ammonium cyanate, from which the urea was finally produced. This was met by the synthetical production of a complex substance, $C_{21}H_{18}N_2$, benzaldehyde, from a more simple one, C_7H_6O, benzamide, oil of bitter almonds. This was effected by passing NH_3, ammoniacal gas, into C_7H_6O, oil of bitter almonds.

$$3C_7H_6O + 2NH_3 = C_{21}H_{18}N_2 + 3H_2O$$

A **radical** is a chemical combination of two or more elements capable of playing the part of an elementary form of matter.

Behaving as an element, radicals must have valence and electrical affinities corresponding with elements. They may be monivalent, divalent, etc., and either electro-positive or electro-negative. As they are unsaturated molecules a neutral radical is an impossibility.

In 1872, Scheele recognized the compound $Hg(CN)_2$, mercuric cyanide, and in 1815, Gay-Lussac isolated CN, *it being the first radical isolated.*

CN (or Cy, which is an abbreviation of the name—cyanogen,) may be taken as the type of the *negative* radicals.

Cyanogen, in combining with positive elements or with positive radicals, may be compared with the negative element Cl, chlorine, for example,

KCl, also KCN, a simple salt,—potassium cyanide.
AgCl, also AgCN, a simple salt,—argentic cyanide.
HCl, also HCN, a hydrogen acid,—hydrocyanic acid.
HClO, also HCNO, an oxyacid,—cyanic acid.
KClO, also KCNO, an oxyacid salt,—potassium cyanate.

CN therefore unites with—

1. Hydrogen to form a hydrogen acid.
2. Metals to form simple salts.
3. Hydrogen and oxygen to form an oxyacid.
4. Hydrogen, oxygen, and a metal to form an oxyacid salt.

A radical may contain a metal. When KCN, potassium cyanide, is heated with metallic iron in the presence of air, a radical, called ferrocyanogen, $Fe(CN)_6$, is formed.

$$6KCN + Fe + O = K_4Fe(CN)_6 + K_2O$$

The radical $Fe(CN)_6$ has never been isolated. Sometimes it is expressed $FeCy_6$ or Cfy. It is a tetrad radical. The K in $K_4Fe(CN)_6$ may be replaced by H, forming $H_4Fe(CN)_6$, ferrocyanic acid.

If chlorine be passed through a solution of $K_4Fe(CN)_6$, one atom of K is withdrawn, and $K_6Fe_2(CN)_{12}$, potassium ferricyanide, is formed.

$$2K_4Fe(CN)_6 + Cl_2 = K_6Fe_2(CN)_{12} + 2KCl$$

The negative radical $Fe_2(CN)_{12}$ is a hexad. It has never been isolated. The K in $K_6Fe_2(CN)_{12}$ may be replaced by H, forming $H_6Fe_2(CN)_{12}$, ferricyanic acid.

C_2H_5, ethyl, may be taken as the type of the *positive* organic radicals. Ethyl, in combining with negative elements or with negative radicals, may be compared with the positive element K, potassium, for example,

KCl,	also	C_2H_5Cl,	ethyl chloride.
K_2S,	"	$(C_2H_5)_2S$,	ethyl sulphide.
K_2O,	"	$(C_2H_5)_2O$,	ethyl oxide.
$KHSO_4$,	"	$C_2H_5HSO_4$,	ethyl sulphuric acid.
KCN,	"	C_2H_5CN,	ethyl cyanide.
KCNO,	"	C_2H_5CNO,	ethyl cyanate.

C_2H_5 therefore unites with—

1. Members of the chlorine group to form simple salts.
2. Oxygen to form an oxide.
3. An oxyacid to form an oxyacid salt.

The first positive radical containing a metal isolated was $(CH_3)_2As$, kakodyl (alkarsin, dimethylarsin). It is sometimes represented by the abbreviation Kd. It is a monad radical. It has an intense affinity for oxygen, combining with it to form $((CH_3)_2As)_2O$, kakodyl oxide, sometimes represented by the abbreviation Kd_2O. Kakodyl combines with chlorine to form $(CH_3)_2AsCl$, kakodyl chloride, and CN to form $(CH_3)_2AsCN$, kakodyl cyanide.

Kakodyl alone and in combination (except as kakodylic acid) is very poisonous.

Kakodyl when exposed to the air in the presence of water forms $(CH_3)_2AsOOH$, kakodylic acid, sometimes abbreviated to $HKdO_2$.

Kakodylic acid contains 54 per cent of metallic arsenic, equivalent to 71.4 per cent of As_2O_3, arsenious oxide, but is not poisonous. It is the only kakodyl compound that is not poisonous.

Kakodyl, in combining with negative elements or with positive radicals, may be compared with K, for example,

KCl,	also	$(CH_3)_2AsCl$,	kakodyl chloride.
K_2O,	"	$((CH_3)_2As)_2O$,	kakodyl oxide.
K_2SO_4,	"	$((CH_3)_2As)_2SO_4$,	kakodyl sulphate.

$(CH_3)_2As$, kakodyl, therefore unites with—

1. Members of the chlorine group to form simple salts.
2. Oxygen to form an oxide.
3. An oxyacid to form an oxysalt.

There are similar methyl compounds of antimony and of zinc.

In addition to $(CH_3)_2As$, dimethylarsin, there also exist,

CH_3As, monomethylarsin.
$(CH_3)_3As$, trimethylarsin.

CH_4, methane, may be considered a type of the saturated organic compounds. All of the carbon bonds are satisfied, and it is therefore a saturated molecule. One, two, three, or all four atoms of H in CH_4 may be replaced by certain other elements. The radical remaining after the withdrawal of each atom of hydrogen has a valence corresponding to the number of hydrogen atoms withdrawn.

1. $CH_4 - H = CH_3$ (methyl), univalent.
2. $CH_4 - H_2 = CH_2$ (methene), bivalent.
3. $CH_4 - H_3 = CH$ (formyl), trivalent.
4. $CH_4 - H_4 = C$ (carbon), quadrivalent.

Thus replacing the H by Cl we have—

1. $CH_4 + 2Cl = CH_3Cl + HCl$.
2. $CH_4 + 4Cl = CH_2Cl_2 + 2HCl$.
3. $CH_4 + 6Cl = CHCl_3$ (chloroform) $+ 3HCl$.
4. $CH_4 + 8Cl = CCl_4 + 4HCl$.

Or replacing the H by iodine we have—

1. $CH_4 + 2I = CH_3I + HI$.
2. $CH_4 + 4I = CH_2I_2 + 2HI$.
3. $CH_4 + 6I = CHI_3$ (iodoform) $+ 3HI$.
4. $CH_4 + 8I = CI_4 + 4HI$.

A **type** is a form of chemical combination common to a class of compounds.

NaCl, CH_3Cl, types of simple salts.
K_2SO_4, $(C_2H_5)_2SO_4$, types of oxysalts.
H_2O, $(C_2H_5)_2O$, the water type.
NH_3, the ammonia type.

By **substitution** is meant the replacing of one or more elements or radicals in a compound by one or more elements or radicals without changing the type of the compound.

When chemical combination occurs it does not necessarily follow that substitution has taken place.

Alcohols and ethers may be viewed as substitution compounds.

An alcohol may be considered after the type of water, in which *one* atom of H in H_2O has been replaced by an alcohol radical, as

H OH, water.
C_2H_5OH, ethyl alcohol.

An ether may be considered after the type of water, in which *both* atoms of H in H_2O have been replaced by alcohol radicals, as

H_2O, water.
$(C_2H_5)_2O$, ethyl ether.

EXAMPLES OF SUBSTITUTION.

When $HgCl_2$, *mercuric chloride*, is treated with NH_3, ammoniacal gas, one of the atoms of Cl in the $HgCl_2$ is replaced by the amido-radical NH_2, and $HgNH_2Cl$, *amido-mercuric chloride* (white precipitate), is formed.

$$HgCl_2 + 2NH_3 = HgNH_2Cl + NH_4Cl$$

When Hg_2Cl_2, *mercurous chloride*, is treated with NH_3, ammonia, one of the atoms of Cl in the Hg_2Cl_2 is replaced by the amido-radical NH_2, and Hg_2NH_2Cl, *amido-mercurous chloride* (black precipitate), is formed.

$$Hg_2Cl_2 + 2NH_3 = Hg_2NH_2Cl + NH_4Cl$$

$HgNH_2Cl$ and Hg_2NH_2Cl are inorganic substitution compounds after the type of NH_4Cl, ammonium chloride, in which two hydrogen atoms have been substituted by mercury.

One, two, or all three atoms of H in NH_3 may be replaced by other elements or radicals.

An **amide** may be defined as a substitution compound in which the hydrogen of NH_3 has been partly or wholly replaced by an alcohol radical or a metal.

Amides may unite with an acid without replacing the hydrogen of the acid, as in $N(CH_3)_3HCl$ trimethylamide hydrochloride.

An amide may be a constituent of a double salt, as in $N(CH_3)_4CNAgCN$ tetramethylargentammonium cyanide.

We may have NH_2K, potass-amide, produced by the replacement of one atom of H in NH_3 by K. By the replacement of H in NH_3 by radicals we may have

NH_2CH_3, methylamide.
$NHCH_3C_2H_5$, methyl-ethylamide.
$NCH_3C_2H_5C_3H_7$, methyl-ethyl-propylamide.
$NHCH_3CH_3$, dimethylamide.
$NCH_3CH_3CH_3$, trimethylamide.

All the atoms of hydrogen in a compound are not necessarily replaceable. For example, only one atom of hydrogen in $HC_2H_3O_2$, acetic acid, is replaceable.

When $C_6H_{10}O_5$, *cellulose* (cotton), is treated with nitric acid, three atoms of hydrogen in the cellulose are replaced by the NO_2, nitro radical, forming the explosive compound $C_6H_7(NO_2)_3O_5$, *trinitrocellulose* (gun-cotton).

$$C_6H_{10}O_5 + 3HNO_3 = C_6H_7(NO_2)_3O_5 + 3H_2O$$

When $C_3H_8O_3$, *glycerine*, is treated with nitric acid, three atoms of hydrogen in the glycerine are replaced by the NO_2, nitro radical, forming the explosive compound $C_3H_5(NO_2)_3O_3$, *trinitroglycerine.*

$$C_3H_8O_3 + 3HNO_3 = C_3H_5(NO_2)_3O_3 + 3H_2O$$

The hypothetical H_2CO_3, carbonic acid, is dibasic; has two replaceable atoms of hydrogen.

$$C{=}O{<}\begin{matrix}OH\\OH\end{matrix}$$, carbonic acid.

By replacing one of the OH hydroxyl groups in carbonic acid by NH_2, an amido-acid, carbamic acid is obtained.

$$C{=}O{<}\begin{matrix}OH\\NH_2\end{matrix}$$, carbamic acid.

Carbamic acid is not known in its free state. It is always in combination as a *salt* or an *ether*. The ammonium salt is produced by bringing together dry CO_2, carbon dioxide, and dry NH_3, ammoniacal gas.

$CO_2 + 2NH_3 = CO(ONH_4)(NH_2)$ or $NH_4NH_2CO_2$, ammonium carbamate.

$$C{=}O \begin{cases} \diagup\ ONH_4 \\ \diagdown\ NH_2 \end{cases}$$, ammonium carbamate.

When treated with water it decomposes into ammonium carbonate.

$$\underset{\text{Ammonium carbamate.}}{CO(ONH_4)(NH_2)} + H_2O = \underset{\text{Ammonium carbonate.}}{(NH_4)_2CO_3}$$

The **ethers of carbamic acid** are called **urethans.** They are formed by replacing the atom of hydrogen in the remaining OH hydroxyl group in $CONH_2OH$, carbamic acid, by an alcohol radical.

$$C{=}O \begin{cases} \diagup\ OCH_3 \\ \diagdown\ NH_2 \end{cases}$$, methyl urethan.

$$C{=}O \begin{cases} \diagup\ OC_2H_5 \\ \diagdown\ NH_2 \end{cases}$$, ethyl urethan.

Ethyl urethan under the name of "*urethan*" is used in medicine.

By replacing both of the hydroxyl groups in H_2CO_3, carbonic acid, by NH_2, an amide is formed, $CO(NH_2)_2$, carbamide, urea.

$$C{=}O \begin{cases} \diagup\ NH_2 \\ \diagdown\ NH_2 \end{cases}$$, carbamide (urea).

Antipyrin, phenyldimethyl pyrazolon, $C_{11}H_{12}N_2O$, or $(C_3H(CH_3)_2N_2(C_6H_5)O)$, is a substitution product.

The aniline coloring-matters are also substitution products.

Molecular wt. is the sum of the wts of its molecular constituents.

$$v' = \frac{v \times (B - A)}{760 \times 1 + .00367t}$$

10 mg $C_2H_4O_2$ = 3.72 CC

1 mg H = 11,16 CC

10 = 111,6 ÷ 3,72 = 30

C H_2O = 30

$C_2H_4O_2$ = 60 mol wt of $C_2H_4O_2$

The **composition of organic compounds** may be expressed by—

1. Empirical formula.
2. Molecular formula.
3. Rational formula.
4. Graphic formula.

1. **Empirical formula:** The simplest possible expression by formula of the composition of a compound. It indicates simply the elements that enter into the composition of the compound in their least atomic proportions. Thus CH_2O for acetic acid.

An empirical formula may be deduced from the results of a quantitative analysis of a compound.

2. **Molecular formula:** A formula that expresses a quantity of a compound by weight twice its specific gravity in the gaseous state compared with hydrogen.

Or: A quantity of a compound by weight in the gaseous state twice the volume of the atom of hydrogen. Hence all molecules must be of the same size.

A molecular formula may be determined:

a. By determining the specific gravity of the compound in the gaseous state.

Victor Meyer's method: Depends on the vaporization of a weighed quantity of a compound and measuring, by means of a eudiometer, the volume of air the vapor displaces in the apparatus, correcting for temperature and pressure, and comparing the volume of air displaced with the volume occupied by a quantity of hydrogen equal to the weight of the compound volatilized.

The molecular weight of a compound which undergoes decomposition before its point of vaporization is reached cannot be determined by this method.

Method: A substance having a fixed boiling-point is placed in the outer large glass tube of the apparatus. For compounds having a low vaporizing-point water may be used. Generally $(C_6H_5)_2NH$, diphenylamine, having a boiling-point of 310° C., is employed. Melted lead may be used for compounds having a higher vaporizing-point. The smaller bulbed glass tube having

a curved delivery-tube attached is lowered into the larger glass tube until the bulbed end is a few inches above the surface of the substance having a fixed boiling-point. A small quantity of the compound, of which the molecular weight is to be determined, is weighed off in a very small glass tube, which is closed at one end. The tube containing the compound is placed with the opening upward in a perforation in the cork of the smaller bulbed tube, where it is held in place by a wire thumb-screw contrivance, and the cork thus arranged placed in the smaller bulbed tube. Gentle heat is applied to the outer large tube, and gradually increased until the substance with the fixed boiling-point boils. After boiling a few minutes and the vapor of the boiling material reaches and is condensed to a liquid at a point opposite the bulb of the smaller glass tube, the delivery-tube is adjusted so that the exit is below the surface of water contained in a suitable vessel. When bubbles of air cease to ascend through the water (at the same time the heating of the material having a fixed boiling point is kept up so that it is boiling), a eudiometer filled with water is inverted over the exit of the delivery-tube under water, and by turning the thumb-screw contrivance the tube containing the compound under examination is allowed to drop to the bottom of the inner bulbed tube. (To break the fall of the tube containing the compound it is customary to previously place pieces of broken glass tubing in the bottom of the bulb of the inner glass tube.)

Volatilization of the compound immediately occurs, and a volume of air is displaced and collected in the eudiometer exactly equivalent to the gaseous volume furnished by the weight of the compound volatilized. This final part of the operation requires but a few minutes.

When bubbles of air cease to come over, the cork is taken out of the apparatus and the eudiometer cautiously removed and placed in a vessel containing water. After a time the air in the eudiometer becomes of the same temperature as the water surrounding it. The volume of air is now read off, the temperature of the water surrounding the eudiometer and the barometric pressure are observed, and the necessary calculations performed.

Suppose 0.010 gramme acetic acid were volatilized and the volume of air displaced and collected in the eudiometer, corrected for temperature (0° C.) and barometric pressure (760 mm.), measured 3.72 c.c.

0.010 gramme hydrogen at 0° C. temperature and 760 mm. pressure measure 111.6 c.c.

Then $111.6 \div 3.72 = 30$

showing the vapor of acetic acid to be 30 times more dense, volume for volume, than hydrogen.

	c.c. H.	Grm. H.	c.c. H.	Grm. H.
Or	11160 :	1 ::	3.72 :	0.0013

All molecules, elementary or compound, must be equal in volume to the volume occupied by the molecule of hydrogen, —*i. e.*, twice the atomic volume.

If 30 grammes of acetic acid in the form of vapor will occupy the same volume as the atomic volume of hydrogen,—namely, 11160 c.c.,—then 60 grammes of acetic acid will occupy twice the atomic volume, or the molecular volume of hydrogen,—namely, 22320 c.c.

A formula for acetic acid representing 30 as its molecular weight would be $CH_2O = 30$, really its empirical formula, and expresses a quantity in the gaseous state equal to its specific gravity compared with hydrogen. By multiplying the 30 by $2 = 60$, we have a number representing its molecular weight. A formula for the acid constructed to represent this number would be $C_2H_4O_2 = 60$,—*i. e.*, a formula that expresses a quantity by weight of the compound twice its specific gravity in the gaseous state compared with hydrogen.

b. The molecular weight of an organic compound, especially those compounds which are not vaporizable, may be determined,—

1. If an acid, by combining it with a metal to form a salt and determining the quantity of the metal in combination in a given quantity of the salt.

2. If a basic substance, by combining it with an acid and determining the quantity of acid in combination in a given quantity of the salt.

The molecular formula of many compounds which will not vaporize or form salts is determined by analyzing their substitution products.

The molecular formula of some compounds which will not vaporize, form salts or substitution products, is not definitely known, as cane-sugar, starch.

For example, the molecular formula of acetic acid, which forms a salt with silver, may be determined by estimating the quantity of metallic silver in its silver salt.

Suppose 1.0 gramme of argentic acetate be placed in a crucible and heated until all the organic matter is driven off and nothing remains but metallic silver. After cooling, the silver is weighed.

Quantity of argentic acetate taken	1.0000 grm.
Weight of resulting metallic silver	0.6468 "
Quantity of organic compound C, H, and O, which must have been in combination with the silver	0.3532 "

Then

$$0.6468 : 0.3532 :: \overset{\text{At. wt. of silver.}}{108} : 58.98 = \text{C, H, and O, in combination with one atom of silver.}$$

Acetic acid is a monobasic acid,—*i. e.*, having one replaceable atom of hydrogen. Consequently, as silver is a monad, it would take the place of one atom of hydrogen in the molecule of acid. Add 1 to 58.98 = 59.98, and allowing for error in analysis, say 60.0. A formula constructed to equal that number would be $C_2H_4O_2 = 60$, acetic acid.

The formula of the silver salt is $AgC_2H_3O_2$.

A recent method for determining molecular weight is based upon the observations of Raoult (1883),—namely, that the *lowering of the freezing point* of a solution is proportional to the absolute quantity of substance in solution and *inversely* as its molecular weight. (Law of Raoult.)

Beckmann's method of determining the molecular weight of a compound by the depression of the freezing point of

the solvent employed, according to the Law of Raoult, is as follows:

15 to 20 grammes of the solvent, accurately weighed, are placed in a hard glass tube 2 to 3 centimetres in width, having a tube projecting from the side, and closed with a perforated cork, through which are passed an accurately standardized thermometer (Waldferdin Thermometer) and a stout platinum wire which serves as a stirring rod. This tube is then fixed to the depth of the side-projecting tube in a cork fitted to a larger and wider tube. The latter serves as an air jacket. The entire apparatus consisting of one tube inserted in another is fixed in an aperture in the cover of a large glass vessel which contains a freezing mixture. The congealing point of the solvent is first determined by cooling it 1° to 2° below its freezing point and then by agitation with the platinum wire (after having added platinum clippings) inducing the formation of crystals. During this operation the temperature rises and when the mercury in the thermometer is stationary it indicates the freezing point of the solvent. The mass of crystals is permitted to melt and an accurately weighed quantity of the compound, the molecular weight of which is to be determined, is introduced through the side-projecting tube. When the compound introduced is completely dissolved the freezing point of the solution is determined after the manner just described.

Rule for calculating results in Beckmann's method; Multiply the percentage of compound in solution by the constant T of the solvent employed and divide by the depression of the freezing point.

The solvents usually employed in Beckmann's method are water, benzol and glacial acetic acid.

The constant T of the solvents is as follows:

Water	19.0
Benzol	4.9
Glacial acetic acid .	39.0

Example of determination of molecular weight of a compound by Beckmann's method:

Example: Suppose a definite weight of oxyberberine acetate ($C_{20}H_{17}NO_5HC_2H_3O_2$ molec. wt. 411) is employed and glacial acetic acid is used as the solvent.

1.573 grms. oxyberberine acetate employed.
100.400 grms. glacial acetic acid employed.

Hence 100.4 : 1.573 : : 100 : 1.56 grms. (percentage of oxyberberine acetate in the solvent).

Freezing point of glacial acetic acid . . . . . = 16.262° C.
Freezing point of glacial acetic acid containing the oxyberberine acetate . . . . . = 16.112° C.
Depression of the freezing point . . . . . = 0.150° C.

Therefore,

$$39 \times \frac{1.56}{0.150} = \frac{60.84}{0.150} = 407 \text{ molecular weight}$$

of oxyberberine acetates from which the molecular formula is constructed.

The molecular formula of many substances which before the introduction of Beckmann's method could not be accurately determined, are now determined with accuracy by this method. Example, glucose.

3. A **rational formula** attempts to express the arrangement of the atoms or groups of atoms in the molecule of a compound.

The same compound may be represented by various rational formulas, depending on the views of different chemists,—*e. g.*, over twenty rational formulas have been suggested for acetic acid.

4. A **graphic formula** attempts to express the arrangement of the atoms or groups of atoms in the molecule of a compound by means of lines (pictures).

Acetic acid.

Empirical formula, CH_2O
Molecular " $C_2H_4O_2$
Rational " $HC_2H_3O_2$ or CH_3COOH

Mol wt. Raoult's Method.

Mol. wt = C (constant) × $\frac{\%\ \text{Sub in sol}}{\text{rise in boiling point}}$

Labrais Method.

Mol wt. = C × $\frac{\text{Grams of Sub}}{\text{rise in boiling point}}$

Cane sugar $C_{12}H_{22}O_{11}$ in water in which $C = 5.2$

13.775 % in Sol

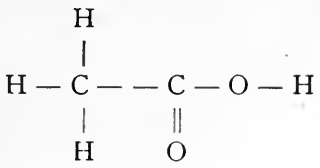
.205 rise in boil. point

Hence $5.2 \times \frac{13.775}{.205} = 349.4$ mol. wt.

342. Theoretical.

The molecular formula of a compound is always twice it vapor density compared with Hydrogen

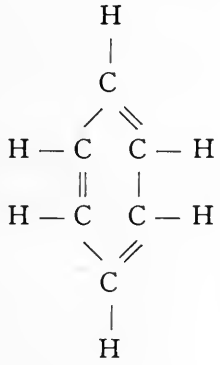
Graphic formula,



Benzol (benzene).

Empirical formula, CH
Molecular " C_6H_6

Graphic formula, (Kekulé's Benzol ring.)



All of the atoms of hydrogen in benzol are replaceable. The hydrogen atoms in benzol may be replaced by single elements, as chlorine, or iodine, or bromine, or by radicals, or all of the hydrogen may be replaced by elements unlike each other or by unlike radicals.

By replacing the hydrogen atoms in benzol with chlorine we may have

C_6H_5Cl, monochlor benzol.
$C_6H_4Cl_2$, dichlor "
$C_6H_3Cl_3$, trichlor "
$C_6H_2Cl_4$, tetrachlor "
C_6HCl_5, pentachlor "
C_6Cl_6, hexa- or perchlor benzol.

Amido-benzol, or *aniline*, $C_6H_5NH_2$, is a compound formed by replacing one of the atoms of hydrogen in benzol by the NH_2, amido group.

There are three distinct isomeric dinitrobenzols, (dinitrobenzenes). They are

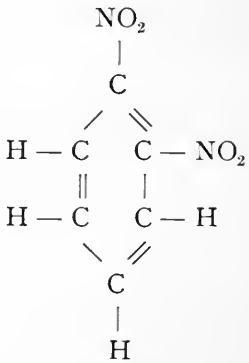
Orthodinitrobenzol.
Metadinitrobenzol
Paradinitrobenzol.

Kekulé's Benzol ring.

When the hydrogen atoms at the positions 1 and 2 have been replaced, the compound is termed an *Ortho* compound.

Example:

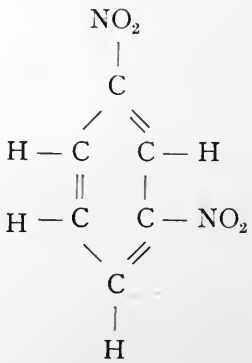
Orthodinitrobenzol,



When the hydrogen atoms at the positions 1 and 3 have been replaced, the compound is termed a *Meta* compound.

Example;

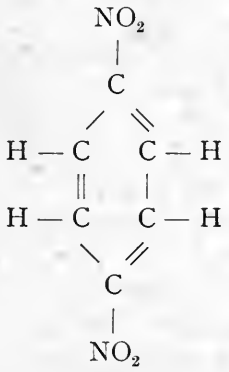
Metadinitrobenzol,



When the hydrogen atoms at the positions 1 and 4 have been replaced, the compound is termed a *Para* compound.

Example;

Paradinitrobenzol,



Phenol, or carbolic acid, C_6H_5OH, may be produced by replacing one of the hydrogen atoms in benzol by the OH, hydroxyl group.

Phenol is eliminated in the urine as $C_6H_5HSO_4$, phenol-sulphuric acid, or in combination as a salt, $C_6H_5KSO_4$, phenol-potassium sulphate. Phenol (carbolic acid) is poisonous, but the salts of phenol-sulphuric acid are not poisonous. Sodium sulphate, or magnesium sulphate is recommended as an antidote in carbolic acid poisoning, converting the acid into the non-poisonous salt phenol-sodium sulphate, or phenol-magnesium sulphate.

Precipitation of barium as barium sulphate does not occur on the addition of barium chloride to a solution of the salts of phenol-sulphuric acid. Thus, from the amount of precipitate of barium sulphate obtained on the addition of barium chloride to the urine, after the ingestion of carbolic acid, it appears that the quantity of sulphuric acid is diminished, whereas it really is unchanged or perhaps increased.

Phenol cannot be detected directly in the urine by any of the tests. It must first be liberated from its combination as phenol-sulphuric acid, distilled, the distillate agitated with ethyl ether, the ethyl ether allowed to separate and then removed from the aqueous solution and allowed to evaporate. The residue is dissolved in a small quantity of water and the tests for phenol applied.

Detection of phenol in the urine.—25 c.c. of sulphuric acid are added to 500 c.c. of the urine, the mixture is distilled until bromine water added to a part of the last portion of the distillate fails to produce a turbidity. The distillate is agitated with ethyl ether, after the ether has separated from the water, it is removed with a pipette, and allowed to evaporate. The residue is dissolved in a small quantity of water and the tests for phenol are applied.

On the addition of a dilute neutral solution of ferric chloride to an aqueous solution of phenol (carbolic acid) an intense purple color is produced.

On the addition of bromine water to an aqueous solution of phenol until a permanent yellowish coloration is produced, a yellowish white, crystalline precipitate of $C_6H_2Br_3OH$, tribromphenol is formed. If the dilution is 1 to 40,000 the turbidity appears immediately, if the dilution is 1 to 50,000 a crystalline precipitate appears only after the solution has stood several hours.

Isomerism is a term applied to bodies containing the same elements united in the same relative proportions by weight, but differing more or less widely in their physical, physiological, and chemical properties.

The first isomeric compounds discovered were

CH_4, methane.
CH_4, attar of roses.

Isomeric compounds are of two classes.—1. Polymeric: Where the *percentage* composition is similar, but the *molecular* composition dissimilar,—*i. e.*, the same empirical but different molecular formula.

The olefines are examples of polymeric compounds, as are also the cyanogen oxyacids.

$HCNO$, cyanic acid, monobasic.
$H_2C_2N_2O_2$, fulminic acid, bibasic.
$H_3C_3N_3O_3$, fulminuric acid, monobasic.
$H_3C_3N_3O_3$, cyanuric acid, tribasic.

2. **Metameric:** Where both the percentage and the molecular compositions are alike.

acidic : acid = methyl formate

Weight the molecular formula $C_6H_{12}O_6$ (grape sugar) by the freezing and boiling point methods of obtaining vapor density. Meyer's method can not be used because the sugar decomposes under the heat

The oils of turpentine, lemons, bergamot, cloves, and pepper are examples of metameric compounds. They all have a similar percentage and molecular composition, $C_{10}H_{16}$.

$C_3H_6O_3$, lactic acid,
$C_3H_6O_3$, paralactic acid,
$C_3H_6O_3$, hydracrylic acid,
} are metameric.

An **homologous series** is a series of chemical compounds made up of the same elements, but having a common difference in their molecular formula.

Hydrocarbons of equal equivalence, as CH_4, may exist separately, whilst hydrocarbons of unequal equivalence, as CH_3, are incapable of existing in the free state, unless, perhaps, as double molecules,—*i. e.*, $\begin{matrix} CH_3 \\ | \\ CH_3 \end{matrix} = C_2H_6$.

As examples of homologous series we have the—

1. Olefines.
2. Alcohol radicals.
3. Paraffins.
4. Alcohols.
5. Aldehydes.
6. Volatile fat acids.
7. Ethers.

In the above homologous series the common difference in the molecular formula is CH_2.

1. **Olefines**: Diatomic, polymeric, hydrocarbon compounds.

		Vapor density compared with H.
1.	CH_2, methene (hypothetical)	7
2.	C_2H_4, ethene	14
3.	C_3H_6, propene	21
4.	C_4H_8, butene	28
5.	C_5H_{10}, pentene	35
6.	C_6H_{12}, hexene	42
7.	C_7H_{14}, heptene	49
8.	C_8H_{16}, octene	56
9.	C_9H_{18}, nonene	63
10.	$C_{10}H_{20}$, decene	70

and continuing to

30. $C_{30}H_{60}$, melene, found in wax.

2. **Alcohol radicals** (may exist only as double molecules): Olefines + one atom of H, hydrides of the olefines, monatomic.

1. CH_3, methyl.
2. C_2H_5, ethyl.
3. C_3H_7, propyl.
4. C_4H_9, butyl.
5. C_5H_{11}, amyl.

Etc.

The alcohol radicals form salts, as CH_3I, CH_3Cl, etc.

3. **Paraffins**: Alcohol radicals + one atom of H, hydrides of the alcohol radicals, saturated hydrocarbons.

1. CH_4, methane.
2. C_2H_6, ethane.
3. C_3H_8, propane.
4. C_4H_{10}, butane or quartane.
5. C_5H_{12}, pentane.
6. C_6H_{14}, hexane.

4. **Alcohols**, monatomic or monohydric, ethylic series: Alcohol radicals + OH (hydroxyl), may be regarded after the type of H_2O, in which one atom of H in H_2O has been replaced by an alcohol radical.

	Vapor density compared with H.	Boiling point.
1. CH_3OH, methylic alcohol (wood spirit) . .	16	150° F.
2. C_2H_5OH, ethylic " (ordinary alcohol)	23	173°
3. C_3H_7OH, propylic "	30	205°
4. C_4H_9OH, butylic "	37	233°
5. $C_5H_{11}OH$, amylic "	44	270°
6. $C_6H_{13}OH$, caprylic "	51	305°

Etc.

5. **Aldehydes** of the acetic series (alcohol dehydrogenatum): Alcohols minus two atoms of H. Under the influence of oxidizing agents alcohols give up two atoms of H, forming aldehydes,—viz., $CH_3OH + O = CH_2O + H_2O$.

Alcohol may be regarded as a substance in which one or more of the hydrogen atoms in a paraffin has been replaced by a hydroxyl

1. CH_2O, methylic or formic aldehyde.
2. C_2H_4O, ethylic or acetic "
3. C_3H_6O, propylic "
4. C_4H_8O, butylic "
5. $C_5H_{10}O$, valeric "

Etc.

6. **Fat acids,** acetic series: Aldehydes + one atom of oxygen, monatomic.

1. CH_2O_2, formic acid, found in red ants.
2. $C_2H_4O_2$, acetic " " " vinegar.
3. $C_3H_6O_2$, propylic " " " oils.
4. $C_4H_8O_2$, butyric " " " rancid butter.
5. $C_5H_{10}O_2$, valeric " " " valerian.
6. $C_6H_{12}O_2$, caproic " " " rancid butter and sweat.

And continuing regularly to

20. $C_{20}H_{40}O_2$, butic acid, found in butter.
30. $C_{30}H_{60}O_2$, melissic " " " beeswax.

7. **Ethers, of monohydric alcohols;** Oxides of the alcohol radicals, after the type of H_2O, in which both atoms of H in the H_2O are replaced by alcohol radicals.

1. $(CH_3)_2O$, methylic ether.
2. $(C_2H_5)_2O$, ethylic "
3. $(C_3H_7)_2O$, propylic "
4. $(C_4H_9)_2O$, butylic "
5. $(C_5H_{11})_2O$, amylic "

Every alcohol has its corresponding aldehyde, acid, and ether.

The **paraffins** are saturated hydrocarbon compounds.

By replacing 3 atoms of H in a paraffin with 3 hydroxyl (OH) groups a **triatomic** (trihydric) **alcohol** is formed. Glycerine ($C_3H_5(OH)_3$) is a triatomic alcohol.

Replacing 3 atoms of H in the paraffin C_3H_8, propane, with

$$\begin{matrix} & & OH \\ & & | \\ C_3H_5 & - & OH \\ & & | \\ & & OH \end{matrix} = C_3H_8O_3,$$

3 hydroxyl groups, a triatomic alcohol, glycerine (propenyl alcohol), is formed.

Glycerine occurs in most animal and vegetable fats in combination with the acids of the acetic and oleic series,—as glycerides. Suet contains stearin, $C_3H_5(C_{18}H_{35}O_2)_3$, a glyceride of stearic acid.

By the action of superheated steam on the stearin in fats glycerine and stearic acid are set free.

$$\underset{\text{Stearin.}}{C_3H_5(C_{18}H_{35}O_2)_3} + \underset{\text{Water.}}{3H_2O} = \underset{\text{Glycerine.}}{C_3H_5(OH)_3} + \underset{\text{Stearic acid.}}{3C_{18}H_{36}O_2}$$

All the monatomic alcohols of the ethylic series (the ordinary alcohols) excepting the first two members of the series have numerous isomeric modifications. They are distinguished especially by their behavior on oxidation. Kolbe gave to methylic alcohol, CH_3OH, the name carbinol, whilst all the succeeding alcohols of the series he termed carbinols, regarding them as derivatives of the first term CH_3OH, methylic alcohol, and formed by the replacement of hydrogen by monad alcohol radicals.

1. **Primary alcohols**: Compounds in which one atom of H of the CH_3 of carbinol (CH_3OH) is replaced by an alcohol radical.

a. $CH_2CH_3OH = C_2H_5OH$, methyl carbinol (ethylic alcohol).

b. $CH_2C_2H_5OH = C_3H_7OH$, ethyl carbinol (propylic alcohol).

Primary alcohols on oxidation yield—

1. An aldehyde.
2. A fat acid.
3. An ethereal salt.

1. $\underset{\text{Ethylic alcohol.}}{C_2H_5OH} + O = \underset{\text{Acetic aldehyde.}}{C_2H_4O} + H_2O$

2. $\underset{\text{Acetic aldehyde.}}{C_2H_4O} + O = \underset{\text{Acetic acid.}}{C_2H_4O_2}$

3. $\underset{\text{Acetic acid.}}{C_2H_4O_2} + \underset{\text{Ethylic alcohol.}}{C_2H_5OH} = \underset{\text{Ethylic acetate (acetic ether).}}{C_2H_5C_2H_3O_2} + H_2O$

2. **Secondary alcohols**: Compounds in which two atoms of H of the CH_3 of carbinol are replaced by alcohol radicals.

$C_6H_{14}O_6$

a. $CHCH_3CH_3OH = C_3H_7OH$, dimethyl carbinol (isomeric with propylic alcohol).

b. $CHCH_3C_2H_5OH = C_4H_9OH$, ethyl methyl carbinol (isomeric with butylic alcohol).

Secondary alcohols on oxidation yield—

1. No aldehyde.
2. A ketone.
3. An acid containing a less number of carbon atoms than the alcohol oxidized,—*i.e.*, an acid of the fat series.

Ketone: An aldehyde in which one atom of hydrogen is replaced by an alcohol radical.

$$\underset{\text{Acetic aldehyde.}}{C_2H_4O} = \underset{\text{Acetic ketone.}}{C_2H_3CH_3O}$$

3. **Tertiary alcohols:** Compounds in which three atoms of H in the CH_3 of carbinol are replaced by alcohol radicals.

a. $CCH_3CH_3CH_3OH = C_4H_9OH$, trimethyl carbinol (isomeric with butylic alcohol), $CH_3{-}\overset{\displaystyle CH_3}{\overset{|}{C}}{-}OH = C_4H_9OH$, trimethyl carbinol (isomeric with butylic alcohol).

b. $CCH_3CH_3C_2H_5OH = C_5H_{11}OH$, ethyl dimethyl carbinol (isomeric with amylic alcohol).

Tertiary alcohols on oxidation yield—

1. No aldehyde.
2. No ketone.
3. One or more acids of the acetic series.

Four primary alcohols;
Three secondary alcohols;
One tertiary alcohol;

are possible, having the same empirical and molecular formula. Six of these are well known, the other two have not yet been discovered.

DECOMPOSITION OF ORGANIC SUBSTANCES.

All organic substances are naturally prone to undergo decomposition. If the organic substance contain nitrogen, the tendency towards decomposition is increased.

DECOMPOSING AGENTS.

1. Oxygen.

1. **Direct combustion.**

2. **Slow combustion** as in the eremacausis (slow oxidation) of oak wood.

$$\underset{\text{Oak wood.}}{C_{18}H_{22}O_{11}} + O_2 = 2H_2O + C_{18}H_{18}O_{11}$$

then

$$C_{18}H_{18}O_{11} = CO_2 + C_{17}H_{18}O_9$$

i. e.,—as soon as two atoms of oxygen have taken away four atoms of hydrogen, one atom of carbon unites with two atoms of oxygen, and so on until finally nothing remains of the oak wood but carbon. This is sometimes used as an illustration of the formation of coal.

3. **When nitrogen** is present in the compound, fermentation or putrefaction may take place. Ammoniacal gas, NH_3, may be given off.

Chlorine, bromine, and iodine may produce decomposition. New compounds are formed.

$$C_6H_6 + Cl_2 = C_6H_5Cl + HCl$$

Continuing the addition of chlorine, all of the hydrogen in C_6H_6 may be replaced, leaving C_6Cl_6, hexachlorbenzol (perchlorbenzol).

2. Heat.

1. Some compounds when heated to a certain temperature volatilize, and when cooled sublime unchanged; examples: strychnine, morphine, benzoic acid.

2. Other compounds when heated directly in the air undergo decomposition (burn). If inorganic matter be absent, no residue remains.

3. When organic compounds are heated in a closed vessel they undergo destructive distillation, as in the destructive distillation of coal in the manufacture of illuminating gas. Pyro-acids may be formed as in the production of pyroligneous acid in the destructive distillation of wood.

3. Acids.

1. **Nitric acid.**

a. If the organic compound be basic, it may combine with it and form a salt, as

$C_{21}H_{22}N_2O_2HNO_3$, strychnine nitrate.

b. It may effect the oxidation of the organic compound, as

$$\underset{\text{Cane sugar.}}{C_{12}H_{22}O_{11}} + 9O_2 = 6\underset{\text{Oxalic acid.}}{H_2C_2O_4} + 5H_2O$$

c. It may form substitution compounds, as

$$\underset{\text{Benzol.}}{C_6H_6} + HNO_3 = H_2O + \underset{\text{Nitrobenzol (oil of mirbane).}}{C_6H_5NO_2}$$

$$\underset{\text{Glycerine.}}{C_3H_8O_3} + 3HNO_3 = \underset{\text{Trinitroglycerine.}}{C_3H_5(NO_2)_3O_3} + 3H_2O$$

$$\underset{\text{Phenol (carbolic acid).}}{C_6H_5OH} + 3HNO_3 = \underset{\text{Trinitrophenol (picric acid).}}{C_6H_2(NO_2)_3OH} + 3H_2O$$

2. **Sulphuric acid.**

a. If the organic compound be basic, it may combine with it and form a salt, as

$(C_{17}H_{19}NO_3)_2H_2SO_4 + 5H_2O$, morphine sulphate.
$(C_{20}H_{24}N_2O_2)_2H_2SO_4 + 8H_2O$, quinine "

b. It may decompose the organic compound, as

$$\underset{\text{Formic acid.}}{CH_2O_2} + H_2SO_4 = \underset{\text{Carbon monoxide.}}{CO} + H_2O + H_2SO_4$$

c. It may abstract the elements of water from the organic compound, as

$$\underset{\text{Oxalic acid.}}{H_2C_2O_4} + H_2SO_4 = CO_2 + CO + H_2O + H_2SO_4$$

d. It may introduce the elements of water into the organic compound (assimilation of the elements of water), as

$$\underset{\text{Starch.}}{C_6H_{10}O_5} + H_2SO_4 + H_2O = \underset{\text{Glucose.}}{C_6H_{12}O_6} + H_2SO_4$$

The property possessed by H_2SO_4 of converting starch into glucose is made use of in determining starch quantitatively, —*i. e.*, converting the starch into glucose and determining the quantity of the latter, and calculating the amount of starch from the amount of glucose obtained.

C_6H_5OH, phenol (carbolic acid), treated with H_2SO_4 forms $C_6H_5HSO_4$, phenol-sulphuric acid.

$$C_6H_5OH + H_2SO_4 = C_6H_5HSO_4 + H_2O$$

This acid ($C_6H_5HSO_4$) is formed when carbolic acid is ingested.

The salts of phenol-sulphuric acid are not poisonous. Antidotes for carbolic acid, sodium sulphate, magnesium sulphate, or any soluble non-poisonous sulphate.

4. Alkalies.

a. If the organic compound be an acid, alkalies will combine with it and form salts, as

$$\underset{\text{Oxalic acid.}}{H_2C_2O_4} + 2NaOH = \underset{\text{Sodium oxalate.}}{Na_2C_2O_4} + 2H_2O$$

b. Alkalies may cause combination of the elements in a compound.

$$KOH + CO = \underset{\text{Potassium formate.}}{KCHO_2}$$

c. If the compound acted upon by the alkali contain nitrogen directly combined, the nascent hydrogen evolved in the decomposition combines with the nitrogen to form NH_3 ammonia.

The alkaline substance usually employed is soda-lime, composed of

One part NaOH (caustic soda).
Two parts CaO (caustic lime).

PUTREFACTION AND FERMENTATION.

Putrefaction; Chemical decomposition of nitrogenous organic compounds, under certain conditions, by bacteria, with the evolution of more or less disagreeable odors.

Professor Sir Henry Roscoe, F. R. S., says, in connection with the causation of the symptoms in infectious diseases, that

Fermentation first pointed out by Schwann in 1837.

"the symptoms of infectious diseases are no more due to the *microbes* which constitute the infection than alcoholic intoxication is produced by the yeast-cell, but these symptoms are *due to the presence of definite chemical compounds*, the result of the life of these microscopic organisms."

Fermentation: Decomposition of certain non-nitrogenous organic compounds in the presence of certain nitrogenized substances known as fermentation fungi.

Ferment: A nitrogenous body capable of inducing fermentation in a non-nitrogenous body. The yeast-cell is an example of a ferment.

Ordinary yeast is composed principally of two varieties of cells,—

Torula cerevisiæ (large round cells).
Penicilium glaucum (small oval cells).

Fermentescible body: A non-nitrogenous body capable of undergoing fermentation. Glucose is an example of a fermentescible body.

Amygdalin, a fermentescible body, is broken up by *emulsin*, a ferment (both being constituents of bitter almonds, peach-kernels, etc.), into

$$\underset{\text{Amygdalin.}}{C_{20}H_{27}NO_{11}} + 2H_2O = \underset{\text{Oil of bitter almonds.}}{C_7H_6O} + \underset{\text{Hydrocyanic acid.}}{HCN} + \underset{\text{Glucose.}}{2C_6H_{12}O_6}$$

Certain conditions are necessary in a fermentation,— viz., the presence of a ferment, a fermentescible body, and moisture, a certain temperature, 20° to 40° C. (70° to 100° F.). Air must be present, at least at the beginning of the fermentation. The presence of a small quantity of salts of the alkaline earths facilitates the progress of fermentation.

Fermentation may be prevented by metallic salts, as $HgCl_2$, $CuSO_4$, etc., a temperature above 100° F. and below 70° F.

There are **five varieties of fermentation,** their distinctive names being derived from the principal product furnished:

1. Alcoholic, or vinous,	in which	alcohol	is	produced.
2. Acetous,	"	"	acetic acid	"	"
3. Lactic,	"	"	lactic acid	"	"
4. Butyric,	"	"	butyric acid	"	"
5. Viscous,	"	"	a gummy matter	"	"

1. **Alcoholic fermentation** is fermentation characterized by the formation of alcohol. It results from the action of yeast on a solution of glucose. The active agent or *ferment* is the *torula cerevisiæ* of the yeast. Only 95 per cent. of the glucose is fermented. The remaining five per cent. is converted into aldehydes, fat acids and perhaps other alcohols.

1 molecule.		2 molecules.		2 molecules.
180.		$2 \times 46 = 92$.		$2 \times 44 = 88$.
$C_6H_{12}O_6$	=	$2C_2H_5OH$	+	$2CO_2$
Glucose.		Ethyl alcohol.		Carbon dioxide.

Advantage is taken of this action to determine the quantity of glucose in urine.

When the alcohol in the solution reaches 20 per cent., fermentation ceases. A solution containing 25 per cent. of glucose will not undergo fermentation.

2. **Acetous fermentation** is fermentation characterized by the formation of acetic acid. It is an advanced stage of the alcoholic fermentation. The active agent or *ferment* is the *mycodermæ aceti*. It appears to act as a carrier of oxygen.

$$\underset{\text{Ethyl alcohol.}}{C_2H_5OH} + O_2 = \underset{\text{Acetic acid.}}{C_2H_4O_2} + H_2O$$

3. **Lactic acid fermentation** is fermentation characterized by the formation of lactic acid. It results from the action of putrefying cheese or milk on glucose or milk sugar. The active agent or *ferment* is the *penicilium glaucum*.

$$\underset{\text{Milk sugar.}}{C_{12}H_{22}O_{11}} + H_2O = \underset{\text{Lactic acid.}}{4C_3H_6O_3}$$

$$\underset{\text{Glucose}}{C_6H_{12}O_6} = \underset{\text{Lactic acid.}}{2C_3H_6O_3}$$

Milk sugar and *lactose* $C_{12}H_{22}O_{11} + H_2O$ are synonymous terms for the same compound. *Galactose*, $C_6H_{12}O_6$, is derived from milk sugar. It results from boiling milk sugar with dilute sulphuric acid.

4. **Butyric acid fermentation** is fermentation characterized by the formation of butyric acid. It is an advanced stage of the lactic acid fermentation. The *ferment* is the *penicilium glaucum*, the same as in lactic acid fermentation.

$$\underset{\text{Lactic acid.}}{2C_3H_6O_3} = \underset{\text{Butyric acid.}}{C_4H_8O_2} + 2CO_2 + 2H_2$$

(2). $C_2H_5OH + O = H_2O + C_2H_4O$ aldehyde

$C_2H_4O + O = C_2H_4O_2$ acetic acid

5. **Viscous fermentation** is a fermentation characterized by the formation of gummy matters. It occurs in the fermentation of the juice of the sugar-beet, and also in sweet white wines, the liquid becoming "ropy." It may be arrested by the addition of a little alum or calcium sulphite. It does not occur in red wines because of the presence of astringent substances. The particular *ferment* causing this fermentation is *unknown*.

ORGANIC ANALYSIS.

PROXIMATE AND ULTIMATE.

Proximate analysis: The separation and determination of the organic compounds contained in an organic body, as the separation of morphine, etc., from opium.

Ultimate analysis: The detection and determination of the ultimate elements entering into the composition of an organic compound, as the quantity of C, H, O, and N in morphine.

That the compound is organic may be shown by heating it on platinum foil; if it chars it is organic. Some compounds volatilize before the temperature of the charring-point is reached, and others undergo decomposition without charring. Such compounds must be heated in a sealed glass tube or with cupric oxide. If CO_2 or H_2O are given off when the compound is heated with cupric oxide it is organic.

Organic compounds may be composed of C and H or C and N; C, H, and O; C, H, N, and O; C, H, N, O, and S; C, H, N, O, S, and P.

Compounds artificially prepared may contain Cl, Br, I, As, Sb, etc.

QUALITATIVE ANALYSIS.

Presence of C and H: Shown by the compound charring when heated alone, or producing CO_2 and H_2O when heated with cupric oxide.

Presence of nitrogen: *a.* Many compounds containing nitrogen, when burned, evolve an odor similar to that of burnt feathers.

b. Many compounds containing nitrogen, when heated with an alkali or with soda-lime, give rise to the formation of ammoniacal gas (NH_3), which may be detected by its odor, and, when in solution, by its forming a precipitate of $(NH_4Cl)_2PtCl_4$ on the addition of platinic chloride.

c. To detect nitrogen in other compounds, they are heated with a small piece of metallic potassium or sodium, thereby forming cyanogen, which combines with the potassium or sodium forming a cyanide, the residue is dissolved in water and the solution tested for a cyanide with $FeSO_4 + Fe_2Cl_6 + HCl$, = formation of prussian blue.

Presence of sulphur: *a.* If the compound be a solid, it is heated with a mixture of solid potassium hydroxide (KOH) (twelve parts) and solid potassium nitrate (KNO_3) (six parts): the sulphur is oxidized to sulphuric acid (which combines with the potassium to form K_2SO_4). The fused mass is dissolved in water and tested with barium chloride for a sulphate.

b. If the compound be a liquid, it is boiled with nitric acid alone or with potassium chlorate: the sulphur is oxidized to sulphuric acid. The liquid is tested with barium chloride for a sulphate.

If the compound be a volatile liquid, it is heated in a sealed glass tube with about twenty to thirty times its volume of nitric acid and the liquid is diluted with water and tested for a sulphate.

c. To determine if the sulphur in the compound is *directly* (unoxidized) or *indirectly* (oxidized) combined with the carbon, the compound is heated with a solution of potassium hydroxide (KOH). If the sulphur is *directly* combined, a sulphide (K_2S) will be formed. The solution is tested for a sulphide with lead acetate, $Pb(C_2H_3O_2)_2$, black lead sulphide (PbS) will be formed, or tested for a sulphide with sodium nitroprusside ($Na_2NOFe(CN)_5$), with which an intense purple-red color is produced. If the sulphur is *indirectly* combined, the solution will not respond to the tests for a sulphide, but in some cases may respond to the tests for a sulphate.

Presence of phosphorus: *a.* The substance is fused with the mixture before stated of KOH and KNO_3, (*a*) or is boiled with nitric acid, as in the case of sulphur, and the aqueous

5 cc ammo 10 c

Iodine Test. — add dilute H_2SO_4 then add alkaline a. nitrate which liberates I add starch chloroform you get a marked coloration due to the $CHCl_3$ taking up the iodine

solution tested with ammonium chloride, ammonium hydroxide, and magnesium sulphate (magnesia mixture) for a phosphate (formation of a crystalline precipitate of $MgNH_4PO_4$).

b. If the organic compound be a volatile liquid, it is heated in a sealed glass tube with about twenty to thirty times its volume of nitric acid and the liquid is diluted with water and tested for a phosphate.

Presence of inorganic matter; *a.* The substance is heated on platinum foil, thereby burning off the organic matter, and leaving the inorganic matter as a fixed residue.

Presence of chlorine, iodine, or bromine: *a.* The organic compound is mixed with caustic lime (CaO), and heated in a combustion-tube. The mixture is suspended in water, slightly acidulated with nitric acid, filtered, and the filtrate tested with argentic nitrate for a chloride, iodide or bromide.

QUANTITATIVE ORGANIC ANALYSIS.

Ultimate or elementary analysis:

Carbon	is determined as	CO_2.	
Nitrogen	"	"	" NH_3 or as N.
Sulphur	"	"	" SO_3, $(\overset{233}{BaSO_4} = \overset{32}{S})$.
Phosphorus	"	"	" P_2O_5, $(\overset{222}{Mg_2P_2O_7} = \overset{62}{P_2})$.
Hydrogen	"	"	" H_2O.

Oxygen is determined by difference,—*i. e.*, after the percentages of the elements in the compound have been determined the percentages are added together, and if the result does not foot up 100, the difference between the footing and 100 is ascribed to oxygen.

Conditions to be observed in ultimate analysis: 1. The compound to be analyzed must be pure and dry. A crystalline substance may be purified by repeated recrystallizations. The compound may be dried by allowing it to remain some time over sulphuric acid or calcium chloride in a desiccator, or it may be dried in a hot-water or air oven. When two weighings agree with each other,—*i. e.*, the compound ceases to lose weight,—it is considered dry.

2. The compound must be completely burned.

3. The products of the combustion must be accurately collected and weighed or measured.

Requisites for an elementary analysis: 1. A combustion-tube of difficultly fusible Bohemian glass, about eighteen inches in length and drawn out at one end in a bayonet-like form and sealed at the drawn out end.

2. A combustion-furnace.

3. An aspirator.

4. A gas-holder containing air or oxygen.

5. Cupric oxide (CuO) or fused granular lead chromate ($PbCrO_4$) to furnish oxygen during the progress of the combustion.

6. A U-shaped tube containing calcium chloride to absorb the H_2O formed in the combustion.

7. Geissler's or Liebig's bulbs containing a strong solution of potassium hydroxide to absorb the CO_2 formed in the combustion.

Method. Determination of carbon and hydrogen: A Bohemian glass combustion-tube is about half filled with freshly heated, perfectly dry, granular cupric oxide (or lead chromate). The accurately weighed organic compound to be analyzed is placed in the tube on top of the cupric oxide, some fine cupric oxide added, and the compound thoroughly mixed with the cupric oxide by means of a copper wire terminating in a spiral. The tube is then filled to within nearly an inch of the end with more granular cupric oxide, a plug of loose asbestos inserted, the tube placed on a combustion-furnace, and a previously weighed tube, containing calcium chloride in small pieces to absorb the H_2O produced in the combustion, is attached to it by means of a closely-fitting perforated rubber stopper. Previously weighed Geissler's or Liebig's bulbs, containing a solution of KOH (specific gravity 1.27) to absorb the CO_2 formed in the combustion, having a tube attached containing small pieces of KOH (previously weighed with the Geissler's bulbs) to absorb the last traces of CO_2, and also to hold any moisture that might be carried over from the KOH solution in the bulbs by the current of gas, are attached to the calcium chloride tube.

The combustion-tube is heated first at the end to which the calcium chloride tube is attached; when the cupric oxide in

this part of the tube is of a dull red heat the heating is commenced at the other end of the tube, and continued until the cupric oxide in that part is also of a dull red heat. The heat is then gradually extended to the middle of the tube until finally the whole tube is heated. The heating must be gradual, so that the combustion is not too rapid, or some of the products may pass through the absorption-bulbs unabsorbed. When the combustion is completed the liquid in the bulb of the Geissler's bulbs nearest the calcium chloride tube will ascend. An aspirator is attached to the Geissler's bulbs, and a rubber tube leading from a drying apparatus is attached to the bayonet-like end of the combustion-tube, the end of the glass tube broken off while in the rubber tube, and the aspirator started. Oxygen or air, free from CO_2 or H_2O, is slowly drawn from the gas-holder through the combustion-tube to burn any of the organic compound which may have escaped decomposition, and also to convey any CO_2 or vapor of H_2O remaining in the combustion-tube into the Geissler's bulbs and calcium chloride tube. The drying apparatus through which the oxygen or air is caused to pass before entering the combustion-tube is composed of a series of three cylinders, the first containing a solution of KOH to absorb CO_2; the second and third containing respectively sulphuric acid and pieces of calcium chloride to absorb H_2O. After drawing oxygen or air through the tube a few minutes the flames are extinguished, the Geissler's bulbs and the calcium chloride tube are detached (the openings stoppered) and allowed to cool. When cool they are unstoppered and weighed. The increase in weight of the Geissler's bulbs containing the KOH will indicate the quantity by weight of CO_2 which resulted from the combustion of the carbon in the organic substance employed.

The increase in weight of the tube containing calcium chloride will indicate the quantity by weight of H_2O which resulted from the combustion of the hydrogen in the organic substance employed.

The quantity of carbon is calculated from the weight of CO_2 obtained, as follows:

$$\overset{44}{CO_2} : \overset{12}{C} :: \text{wt. of } CO_2 \text{ obtained} : X$$

The quantity of hydrogen is calculated from the weight of H_2O obtained, as follows:

$$\overset{18}{H_2O} : \overset{2}{H_2} :: \text{wt. of } H_2O \text{ obtained} : X$$

If the compound for analysis be a liquid, it is placed in a small weighed glass bulb having a drawn-out tube. This is accomplished by rarefying the air in the bulb by heating and holding the drawn-out end in the liquid. On cooling, the liquid will ascend and occupy the space of the expelled air. The tube is then sealed over a Bunsen flame and the bulb weighed. The increase of weight is the weight of the compound contained in the bulb. The bulb containing the liquid is dropped into the combustion-tube containing the cupric oxide, and the method as before described employed.

If the compound to be analyzed contain nitrogen in addition to the carbon and hydrogen or oxygen, oxides of nitrogen may be formed during the combustion, and these oxides being absorbed with the CO_2 by the KOH solution in the Geissler's bulbs, would lead to inaccurate results by apparently increasing the quantity of CO_2. In this case, metallic copper (in the form of turnings or gauze) is heated in a current of hydrogen and then placed in the combustion-tube, at the end at which the calcium chloride tube is attached, in order to decompose the oxides of nitrogen into free oxygen and nitrogen.

If sulphur be present in the compound, SO_3 (sulphuric anhydride) may be formed in the combustion and be absorbed with the CO_2 by the KOH in the Geissler's bulbs. In this case, PbO_2 (lead dioxide) should be placed in the combustion-tube containing the cupric oxide, at the end at which the calcium chloride tube is attached. The SO_3 will combine with, and be held by, the lead as $PbSO_4$. Lead dioxide need not be placed in the tube if lead chromate instead of cupric oxide is used in the combustion.

Compounds containing chlorine, iodine, or bromine when burned with cupric oxide form cupric chloride, iodide, or bromide, which volatilize at a high temperature and condense in the calcium chloride tube, thus causing a fictitious increase in the weight of the H_2O. Compounds containing these

elements must be burned with lead chromate ($PbCrO_4$); non-volatile plumbic chloride, iodide, or bromide will be formed and retained in the combustion-tube.

Compounds difficult of combustion should be burned with lead chromate or with pure oxygen. The latter is employed especially for coals and coke. The substance to be burned is placed in a porcelain boat, and, instead of glass, an iron combustion-tube is often employed.

DETERMINATION OF NITROGEN METHODS:

1. **Dumas' method:** Depends upon the decomposition of the organic substance with the evolution of all of the nitrogen, which is collected and measured in a eudiometer.

The nitrogenous compound is burned in a combustion-tube, sealed at one end, with cupric oxide and copper-wire gauze as in the determination of carbon and hydrogen, except that a layer of about three inches of acid sodium carbonate ($NaHCO_3$) or of magnesite (magnesium carbonate, $MgCO_3$) is placed at the closed end of the tube. When filled the tube contains the following substances in the given order:

1. $NaHCO_3$ or $MgCO_3$ (to the depth of about three inches).
2. Cupric oxide (nearly to the middle of the tube).
3. Mixture of organic substance and cupric oxide.
4. Cupric oxide.
5. Copper gauze to decompose oxides of nitrogen.

A delivery-tube is attached to the open end of the combustion-tube (the exit of the delivery-tube being brought below the surface of mercury contained in a trough), and a glass tube, containing mercury and a solution of potassium or sodium hydroxide, is inverted over the exit of the delivery-tube. The acid sodium carbonate, or the magnesium carbonate is heated first. Decomposition takes place and CO_2 is evolved.

$$2NaHCO_3 = Na_2CO_3 + H_2O + CO_2$$
$$MgCO_3 = MgO + CO_2$$

The heating of the carbonate is continued until all the air is driven out of the combustion-tube by CO_2. This is

accomplished when the bubbles of evolved gas are completely absorbed by the KOH solution.

When all the air is driven out, the heating of the carbonate is discontinued, and the KOH tube is removed. A eudiometer, containing mercury and a layer (about three or four inches in thickness) of solution of KOH, is now inverted over the exit of the delivery-tube. Heat is applied to the end of the tube (No. 5) containing the copper gauze, and also to the part containing cupric oxide (No. 2) next to the carbonate. The heat is gradually extended to the middle of the tube, until the entire tube, except (No. 1) carbonate part, is heated. The heating is discontinued when gas-bubbles cease to come over into the eudiometer. At this time the acid sodium carbonate is again heated, more CO_2 is evolved which drives out the remaining nitrogen. The heating is continued until the gas-bubbles entering the eudiometer are completely absorbed by the KOH.

The CO_2 and H_2O produced in the combustion are absorbed by the solution of KOH. The nitrogen is not absorbed, and collects in the upper part of the eudiometer.

The eudiometer containing the nitrogen is transferred to a vessel of water, the atmospheric pressure is equalized, and the volume of nitrogen read off and corrected for temperature and pressure.

14 grm. nitrogen = 11160 c.c.

11160 : 14 :: 1 c.c. : 0.001256 grm.

1 c.c. of nitrogen at 0° C. and 760 mm. weighs 0.001256 grm.

2. **Will and Varrentrapp's method:** Depends upon the formation of NH_3 (ammoniacal gas) when an organic substance containing nitrogen is heated with soda-lime.

This method is not applicable for the determination of nitrogen in compounds in which the nitrogen is present as a nitro group.

A combustion-tube is nearly half-filled with dry soda-lime. The weighed organic substance is added and thoroughly mixed, by means of a wire stirrer, with the soda-lime. More soda-lime is added until the tube is within about an inch of being filled, and a plug of loose asbestos is placed in the end.

The tube now contains the following substances in the following order:

1. Soda-lime.
2. Mixture of organic substance and soda-lime.
3. Soda-lime.
4. Plug of loose asbestos.

a. **Gravimetric determination of nitrogen.**

The combustion-tube is connected with Will's bulbs, containing dilute hydrochloric acid, and is heated in a combustion-furnace. The heating should be commenced simultaneously at each end of the tube, and gradually extended to the middle.

The NH_3 is absorbed by the HCl in the Will's bulbs, forming NH_4Cl.

The contents of the Will's bulbs are emptied into a dish; excess of platinum chloride, which precipitates the ammonia, is added, and the whole evaporated to dryness on a water-bath. The residue is collected on a weighed filter by means of a mixture of alcohol and ether, and thoroughly washed with a mixture of the same liquid.

When the precipitate, $(NH_4Cl)_2PtCl_4$, is dry it is weighed while on the filter. Every 100 parts of $(NH_4Cl)_2PtCl_4$ contains 6.318 parts of nitrogen.

$$\begin{matrix} (NH_4Cl)_2PtCl_4. & N_2. \\ 443.3 & : 28 :: 100 : 6.318 \end{matrix}$$

or the quantity of nitrogen may be calculated as follows:

$$\begin{matrix} (NH_4Cl)_2PtCl_4. & N_2. \\ 443.3 & : 28 : \text{weight of precipitate} : X \end{matrix}$$

Instead of weighing the precipitate as $(NH_4Cl)_2PtCl_4$, it may be incinerated and weighed as metallic platinum; then

Every 100 parts of platinum are equivalent to 14.410 parts of nitrogen.

$$\begin{matrix} Pt. & N_2. \\ 194.3 & : 28 :: 100 : 14.410 \end{matrix}$$

or the quantity of nitrogen may be calculated as follows:

$$\begin{matrix} Pt. & N_2. \\ 194.3 & : 28 :: \text{weight of metallic platinum} : X \end{matrix}$$

b. **Volumetric determination of nitrogen.**

A measured volume of a normal solution of oxalic acid ($H_2C_2O_4 + 2H_2O$) may be used in the Will's bulbs, instead of dilute HCl, to absorb the NH_3, and the quantity of nitrogen calculated from the number of cubic centimetres of oxalic acid solution, neutralized by the NH_3, as determined by titering with a normal solution of NaOH.

$H_2C_2O_4 + 2H_2O$, a dibasic acid.

$$\frac{126}{2} = 63$$

$$H_2C_2O_4 + 2H_2O + 2NH_3 = (NH_4)_2C_2O_4 + 2H_2O$$

Oxalic acid.		NH_3.
126 grm.	=	34 grm.
63 "	=	17 "

A normal solution of oxalic acid is prepared by dissolving 63 grm. of pure oxalic acid in 1000 c.c. water; then

	Oxalic acid.		NH_3.		N.
1000 c.c.	= 63.0 grm.	=	17.0 grm.	=	14.0 grm.
1 c.c.	= 0.063 "	=	0.017 "	=	0.014 "

10 c.c. of the oxalic acid solution are placed in the Will's bulbs. The combustion of the organic compound is performed and the NH_3 evolved is absorbed by the oxalic acid solution.

When the combustion is completed the oxalic acid solution in the Will's bulbs is emptied into a beaker, the bulbs rinsed with water and the wash water also placed in the beaker, a few drops of litmus added, and the oxalic acid solution titered with a normal solution of NaOH.

1000 c.c. of normal sol. of NaOH contains 40.0 grm. NaOH.
1 c.c. " " " " " 0.040 " "

0.040 NaOH = 0.063 oxalic acid.

Consequently 1 c.c. normal solution of NaOH is equal to (will neutralize) 1 c.c. normal oxalic acid solution, and 10 c.c. normal NaOH solution are equal to 10 c.c. normal oxalic acid solution.

On titering the 10 c.c. oxalic acid solution from the Will's bulbs, it will now be found that less than 10 c.c. of normal

NaOH solution will be required, showing that some of the oxalic acid has been neutralized by the NH_3.

Suppose only 6 c.c. of normal NaOH solution were required to neutralize the 10 c.c. oxalic acid solution from the Will's bulbs, then the difference between 6 and 10 = 4 indicates the number of cubic centimetres of oxalic acid solution neutralized by the NH_3 which resulted from the combustion of the organic compound.

1 c.c. of oxalic acid solution is neutralized by 0.017 NH_3, equivalent to 0.014 N, and as 4 c.c. were neutralized then $4 \times 0.014 = 0.056$ grm. nitrogen present in the weight of organic compound analyzed.

3. **Kjeldahl's method:** Depends upon the conversion of the nitrogen in a compound into ammonia, by boiling it with sulphuric acid. The ammonia combines with the sulphuric acid to form ammonium sulphate. The ammonium sulphate, $(NH_4)_2SO_4$, is decomposed into NH_3 and Na_2SO_4 by boiling it with sodium hydroxide. The evolved ammoniacal gas (NH_3) is collected in dilute hydrochloric acid and precipitated with platinum chloride as $(NH_4Cl)_2PtCl_4$.

The precipitate is collected on a filter, washed with a mixture of alcohol and ether, and treated exactly as in Will and Varrentrapp's method.

The NH_3 may also be collected in a measured volume of normal oxalic acid solution and titered with a normal solution of sodium hydroxide, as in Will and Varrentrapp's method.

DETERMINATION OF SULPHUR.

Sulphur in an organic compound is determined quantitatively by fusing the compound with about 12 parts of potassium hydroxide (KOH) and 6 parts of potassium nitrate (KNO_3), or by boiling the compound with nitric acid, as in the qualitative detection of sulphur. The sulphur is oxidized to sulphuric acid, the latter is then precipitated with barium chloride ($BaCl_2$) as barium sulphate ($BaSO_4$), and the quantity of sulphur is calculated from the amount of $BaSO_4$ obtained.

233 parts of $BaSO_4$ (molec. wt.) = 32 parts of S.

$BaSO_4$. S.

233 : 32 :: wt. of precipitate ($BaSO_4$) : x

DETERMINATION OF PHOSPHORUS.

Phosphorus is determined quantitatively by fusing the compound with 12 parts of KOH and 6 parts of KNO_3, or by boiling it with HNO_3, as in the determination of sulphur. The phosphorus is oxidized to phosphoric acid, which is precipitated by the magnesia mixture ($NH_4Cl + NH_4HO + MgSO_4$), and weighed as magnesium pyrophosphate, $Mg_2P_2O_7$.

222 parts of $Mg_2P_2O_7$ (molec. wt.) = 62 parts of P.

$Mg_2P_2O_7$. P.
222 : 62 :: wt. of magnesium pyrophosphate : x

DETERMINATION OF CHLORINE, BROMINE, AND IODINE.

Chlorine, bromine, and iodine are determined quantitatively by heating the compound in which the element is contained, in a combustion-tube with caustic lime, as in the qualitative detection of these elements, dissolving in water, filtering, neutralizing with HNO_3 and precipitating with argentic nitrate ($AgNO_3$), and weighing as argentic chloride (AgCl), argentic bromide (AgBr), and argentic iodide (AgI).

CALCULATION OF RESULTS AND DEDUCTION OF FORMULAS.

Rule: Determine the percentages of all the elements in the compound. Divide the percentage of each element by its atomic weight. Then divide the quotients obtained by the smallest quotient, and, if necessary, multiply these final quotients by the least number that will make all of them whole or nearly whole numbers. The formula thus obtained is the empirical formula, and at the same time may be the molecular formula. The molecular formula is determined by observing the vapor density of the compound, etc.

Example: A combustion of 0.340 grm. of **oil of turpentine,** a compound containing only carbon and hydrogen, furnished

CO_2 . . . . . 1.100 grm.
H_2O . . . . . 0.360 "

$\overset{CO_2.}{44} : \overset{C.}{12} :: \overset{\text{Wt. of } CO_2.}{1.100} : \overset{C.}{0.300}$ grm.

then, *a.*

$\overset{H_2O.}{18} : \overset{H_2.}{2} :: \overset{\text{Wt. of } H_2O.}{0.360} : \overset{H.}{0.040}$ grm.

Hence 0.340 grm. of the oil contained

0.300 carbon.
0.040 hydrogen.

b. $\overset{Oil.}{0.340} : \overset{C.}{0.300} :: 100 : 88.235$ per cent. carbon.

$\overset{Oil.}{0.340} : \overset{H.}{0.040} :: 100 : 11.765$ per cent. hydrogen.

The percentage composition is,—

Carbon	88.235
Hydrogen	11 765
	100.000

c. $88.235 \div 12 = 7.353 \div 7.353 = 1 \times 5 = 5$ atoms C.
$11.765 \div 1 = 11.765 \div 7.353 = 1.599 \times 5 = 7.995$ atoms H, or, approximately, 8 atoms of H.

Hence the empirical formula of oil of turpentine is C_5H_8. (The molecular formula is twice C_5H_8, or $C_{10}H_{16}$.)

Or, if 0.340 grm. oil of turpentine furnished

CO_2	1.100 grm.
H_2O	0.360 "

then, *a.*

$\overset{Oil.}{0.340} : \overset{CO_2.}{1.100} :: 100 : \overset{CO_2.}{323.53}$ grm.

$\overset{Oil.}{0.340} : \overset{H_2O.}{0.360} :: 100 : \overset{H_2O.}{105.88}$ grm.

If 100 grm. of the oil yields 323.53 grm. CO_2 and 105.88 grm. H_2O, then to obtain the percentage proportions of carbon and hydrogen,—

b. $\overset{CO_2.}{44} : \overset{C.}{12} :: 323.53 : 88.235$ per cent. carbon.

$\overset{H_2O.}{18} : \overset{H_2.}{2} :: 105.88 : 11.765$ per cent. hydrogen.

And from these percentages of carbon and hydrogen we find,—

c. 88.235 ÷ 12 = 7.353 ÷ 7.353 = 1 × 5 = 5 atoms C.
11.765 ÷ 1 = 11.765 ÷ 7.353 = 1.599 × 5 = 7.995 atoms H,

or, approximately, 8 atoms of H.

Hence the empirical formula for the compound is C_5H_8.

Oxalic acid is composed of,—

Carbon	26.66 per cent.
Hydrogen	2.22 "
Oxygen	71.12 "
	100.00

Then,

C 26.66 ÷ 12 = 2.22 ÷ 2.22 = 1 atom carbon.
H 2.22 ÷ 1 = 2.22 ÷ 2.22 = 1 " hydrogen.
O 71.12 ÷ 16 = 4.44 ÷ 2 22 = 2 atoms oxygen.

Hence the empirical formula of oxalic acid is HCO_2. (The molecular formula is twice HCO_2, or $H_2C_2O_4$.)

Composition of cane sugar according to Liebig,—

Carbon	42.30 = 1.001
Hydrogen	6.45 = 2.009
Oxygen	51.25 = 1 000
	100.00

Formula for cane sugar according to different chemists,—

Berzelius	$C_{12}H_{23}O_{11}$
Doebereiner	$C_6H_{12}O_6$
Dumas	$C_5H_{10}O_5$
Prout	$C_8H_{16}O_8$

THE URINE.

The **urine** is the secretion of the kidneys, in which effete nitrogenized products are thrown out from the system.

The color of normal urine is yellow. The intensity of color is proportionate to the specific gravity, except diabetic urine, in which the specific gravity may be high and the color pale yellow.

The **reaction** of normal urine just voided is usually *acid.* The *acid reaction is not due to the presence of free acid* but to *acid salts,* principally to *acid sodium phosphate* (NaH_2PO_4) arising from the sodium phosphate (Na_2HPO_4) of the blood coming in contact with the uric acid of the urine.

$$2Na_2HPO_4 + H_2C_5H_2N_4O_3 = 2NaH_2PO_4 + Na_2C_5H_2N_4O_3$$

Neutral sodium phosphate. Uric acid. Acid sodium phosphate. Neutral sodium urate.

Urine having an alkaline reaction may be voided, especially after a very hearty meal, and also after the ingestion of alkalies and substances, as the acetates, tartrates, and citrates (lemon-juice) of the alkalies, which in the organism are converted into and voided as alkaline carbonates.

On the ingestion of acids, urine having a strong acid reaction may be voided.

Normal urine, on standing some time, may become alkaline in reaction, due to the presence of ammonium carbonate resulting from the action of certain micro-organisms (micrococcus ureæ) on the urea.

$$CO(NH_2)_2 + 2H_2O = (NH_4)_2CO_3$$

Urea. Ammon. carbonate.

Normal urine may have an amphoteric reaction,—*i. e.*, changing blue litmus-paper to red, and red litmus to blue,—due to the simultaneous presence in the urine of acid and neutral phosphates.

In collecting urine for a quantitative determination of its constituents, the total quantity voided in 24 hours should be collected and, that the liquid collected shall be homogeneous in constitution, the separate portions collected during the 24 hours must finally be placed in one bottle and thoroughly mixed.

In collecting the urine passed in 24 hours the collection should be begun at a certain time. For example, at 8 a. m. of one day the bladder should be emptied and this portion of urine discarded. The urine voided from this time until 8 a. m. the next day (at which time the bladder should be finally emptied), should be collected in a bottle,—including that voided in the final emptying of the bladder. During the period of collection the urine voided should be kept in a cold place.

Acidity of the urine: The acidity of the average quantity of urine voided in a day will require from 1.0 to 1.5 grm. NaOH to neutralize it.

The average quantity of sodium acid phosphate (NaH_2PO_4) daily eliminated in the urine is equivalent to about 2.3 grm. of phosphoric anhydride (P_2O_5), and to convert 2.3 grm. P_2O_5 as NaH_2PO_4 into Na_2HPO_4 will require 1.3 grm. NaOH.

The **acidity of the urine** is determined by means of a deci-normal solution of sodium hydroxide (NaOH).

A normal solution of NaOH contains 40.0 grm. of NaOH in 1000 c.c. distilled water. A deci-normal solution contains $\frac{1}{10}$ of 40.0, or 4.0 grm. of NaOH in 1000 c.c. of distilled water.

NaOH.

1000 c.c. = 4.0 grm.

1 c.c. = 0.004 "

Method: To 100 c.c. of urine, two or three drops of rosolic acid, or of phenol-phthalein solution are added, a deci-normal solution of NaOH is run in from a burette until a change takes place in the color of the indicator (rosolic acid, or phenol-phthalein), the number of c.c. NaOH solution required to produce the change in color is read off, and this number is multiplied by the value of 1 c.c. of the deci-normal solution expressed in NaOH units,—namely, 0.004.

Example: 24 hours urine = 1200 c.c.

100 c.c. of the urine required 30 c.c. NaOH solution.

$30 \times 0.004 = 0.120$ grm. NaOH required for 100 c.c. urine.

$12 \times 0.120 = 1.440$ grm. NaOH required to neutralize the acidity of 1200 c.c. urine (quantity of urine voided in 24 hours).

Specific gravity of urine: The specific gravity of normal urine varies from 1015 to 1025. The average is about ~~1017,~~ 1020 water taken as 1000.

The specific gravity may be as low as 1003 or even lower, and as high as 1030 or higher.

The specific gravity may be determined by—

a. Urinometer.

b. Specific gravity bottle (picnometer).

The specific gravity is affected by temperature. It should be taken with the liquid at a temperature of 15° C. or 60° F.

The specific gravity is lowered one unit (the specific gravity of water expressed as 1000) for every increase of 3° C. temperature.

Determination of quantity of solid matter in urine; 1. A ready method is to evaporate 10 c.c. urine, in a weighed dish, to dryness on a water-bath. The residue is further dried in a drying-oven at 100° C., and placed in a dessicator. After a time it is weighed, and the drying operation and weighing are repeated until a constant weight is obtained. The final weight, minus the weight of the dish, is the amount of solid matter in 10 c.c. of the urine.

This method is not very accurate, because during the evaporation some of the urea is decomposed with the evolution of ammoniacal gas (NH_3), thus—

a. $CO(NH_2)_2 + H_2O = 2NH_3 + CO_2$
b. $NH_3 + CO_2 + H_2O = NH_4HCO_3$
c. $NaH_2PO_4 + NH_3 = NaNH_4HPO_4$
d. $NaNH_4HPO_4$ at 100° C. $= NaH_2PO_4 + NH_3$

2. An **accurate method** is to evaporate to dryness 2 c.c. urine in a weighed porcelain boat in Neubauer's special drying apparatus, collecting the NH_3 which results from the decomposition of some of the urea and finally weighing the boat with the dry residue. The NH_3 evolved from the decomposition of the urea in the process of drying is collected in a normal solution of sulphuric acid and the quantity of the latter neutralized by the NH_3 is determined by means of a normal NaOH solution, and from the quantity neutralized the equivalent of urea is deduced and the weight of urea found to have been decomposed in the operation of drying is added to the weight of the dry residue in the porcelain boat.

2 molecules ammonia. 1 molecule urea.
$$2NH_3 = CO(NH_2)_2$$

Or,

34 parts of ammonia = 60 parts of urea.

Example: 2 c.c. urine employed.

Weight of boat plus residue	0.640 grm.
" " minus "	0.600 "
Weight of dry residue	0.040 "

The NH_3 evolved was collected in 10 c.c. normal solution of sulphuric acid. After collecting the NH_3 in the 10 c.c. of normal solution of sulphuric acid, the latter was titered with a normal solution of sodium hydroxide. 8 c.c. of the NaOH solution were required to neutralize the 10 c.c. of sulphuric acid solution. Previous to the collection of the NH_3 in the 10 c.c. normal solution of sulphuric acid, 10 c.c. of normal solution of sodium hydroxide would have been required to neutralize it. Now, however, some of the sulphuric acid has been neutralized by the NH_3 and consequently a less volume of sodium hydroxide solution will be required to neutralize the 10 c.c. of normal sulphuric acid solution. As 8 c.c. of normal sodium hydroxide solution were required it indicates that 2 c.c. of the normal sulphuric acid solution were neutralized by the ammonia evolved.

Thus,

1 c.c. of normal sulphuric acid solution = 0.017 grm. NH_3
2 c.c. = $2 \times 0.017 = 0.034$ grm. NH_3

As 34 parts of NH_3 equal 60 parts of urea, therefore,

$$\overset{NH_3.}{34} : \overset{Urea}{60} :: \overset{NH_3.}{0.034} : x = 0.060 \text{ grm. urea.}$$

Weight of residue in porcelain boat	0.040 grm.
Weight of urea as determined by amount of NH_3 evolved	0.060 "
Weight of solid matter in 2 c.c. of the urine .	0.100 "

$$\overset{c.c.}{2} : \overset{grm.}{0.100} :: \overset{c.c.}{100} : x = 5.0 \text{ grm. solid matter.}$$

3. The solid matter may be approximately determined by multiplying the last two figures of the specific gravity by Haeser and Neubauer's factor, 2.33. Thus if the specific gravity is 1017,

$17 \times 2.33 = 39.61$ grm. solid matter in 1000 c.c. of the urine.

Or more closely, (if the specific gravity be expressed with five figures), by multiplying the last three figures by the factor 0.233. Thus if the specific gravity is 1020.3,

$203 \times 0.233 = 47.299$ grm. solid matter in 1000 c.c. of the urine.

AVERAGE COMPOSITION OF HUMAN URINE.

			Voided per day. Grains.	Grammes.
Water	950.00			
Urea	28.00	Organic matter. 37.60	520.80	35.00
Uric acid	0.60		11.16	0.75
Hippuric acid	0.35		6.51	0.44
Creatinine	0.65		12.09	0.81
Extractives	8.00		148.80	10.00
Sodium chloride	8.00	Inorganic matter. 12.40	148.80	10.00
Phosphoric acid	2.00		37.20	2.50
Sulphuric acid	1.25		23.45	1.56
Lime (CaO)	0.25		4.65	0.31
Magnesia (MgO)	0.30		5.58	0.37
Potash (K_2O) and soda (Na_2O)	0.60		11.16	0.75
	1000.00		930.20	62.49
Water	950.00			
Organic matter	37.60		699.36	47.00
Inorganic matter	12.40		230.64	15.49

Average quantity of urine voided per day, about 40 fluid-ounces, or 1200 c.c.

ANALYSIS OF THE URINE.

The analysis of urine may have for its object—

1. The **qualitative** detection of the normal and abnormal constituents.

2. The **quantitative** determination of the normal and abnormal constituents.

The percentages of solids in urine are expressed as percentages by volume,—*i. e.*, 100 c.c. by volume contain a given weight of a solid.

DETERMINATION OF SODIUM CHLORIDE (NaCl) IN URINE.

The chlorine in urine is in combination as a chloride, chiefly as sodium chloride (NaCl).

It may be detected qualitatively by strongly acidulating the urine with nitric acid and adding a solution of argentic nitrate ($AgNO_3$). If NaCl be present in rather large quantity, a curdy precipitate of argentic chloride (AgCl) will be formed; if the quantity present be small, only a milkiness will be produced. If the urine contain albumen, it must be removed before making this test.

QUANTITATIVE DETERMINATION OF SODIUM CHLORIDE.

1. **Gravimetric method**: Principle: when argentic nitrate ($AgNO_3$) is added to sodium chloride (NaCl) all of the chlorine is precipitated as argentic chloride (AgCl).

$$AgNO_3 + NaCl = \overset{143.5.}{AgCl} + NaNO_3$$

The quantity of sodium chloride, or of chlorine, is calculated from the quantity of AgCl obtained.

Every 143.5 parts of AgCl = 58.5 NaCl or 35.5 Cl.

Hence

$$\overset{AgCl.}{143.5} : \overset{NaCl.}{58.5} :: \text{weight of precipitate of AgCl} : \overset{NaCl.}{X}$$

or,

$$\overset{AgCl.}{143.5} : \overset{Cl.}{35.5} :: \text{weight of precipitate of AgCl} : \overset{Cl.}{X}$$

Method: 10 c.c. of urine, to which is added about 2 grm. potassium nitrate (KNO_3) to destroy the organic matter, are evaporated to dryness in a small porcelain evaporating dish on a water-bath (*absolute* dryness cannot be obtained) or over a flame, care being taken to avoid loss from spurting. (The contents of the dish must not be stirred.) The dish containing the residue is held by means of a crucible tongs over a flame and heated, very gently at first, then with strong heat, until all the organic matter is destroyed as indicated by the disappearance of the charred material. The dish containing the residue while cooling is held at the outer edge by a crucible

tongs and given a rotary motion until the fused material has distributed itself as a thin layer over the dish and finally solidified. By this procedure the cracking of the dish will be prevented. Care must be taken that in this procedure none of the fused material comes in contact with the crucible tongs or is projected from the dish. When the dish is thoroughly cooled the residue is treated with 5 c.c. of water and eight or ten drops of strong nitric acid. The nitric acid is added to neutralize the KOH and K_2CO_3, which are produced in the decomposition of the KNO_3, with the organic matter. The liquid in the dish is warmed to hasten solution. This first portion of 5 c.c. is poured into a beaker (keeping back any enamel that may have separated from the dish), and washing the dish with portions of 5 c.c. of water is repeated (without further addition of nitric acid), and heating, if necessary, until the dish has been thoroughly washed. Each portion of 5 c.c. is poured into the beaker until 30 c.c. have been employed and collected in the beaker. If the liquid is not acid in reaction, it must be acidulated with nitric acid. The solution is warmed and excess of $AgNO_3$ solution is added. The liquid is stirred with a glass rod until the precipitate separates in curd-like masses. The precipitate is collected on a filter, and washed with distilled water until the filtrate shows no trace of the presence of silver. This is determined by collecting in a test tube a portion of the filtrate as it comes from the funnel and adding hydrochloric acid. The precipitate is dried while still on the filter, as much as is possible, is detached from the filter, and placed in a weighed crucible. The folded filter is held by means of a platinum wire over the crucible, ignited, and the ash allowed to fall into the crucible. The ash is moistened with two or three drops of strong nitric acid to dissolve the metallic silver which may have resulted from the reduction of AgCl in burning the filter, and the crucible is warmed very gently. When cool, the ash is moistened with two or three drops of hydrochloric acid to reprecipitate, as chloride, the silver dissolved by the nitric acid. The excess of acid is expelled by gently heating the crucible over a very small flame. The crucible is allowed to cool and is then weighed. The weight of the empty crucible and the filter ash is deducted from the weight

of the crucible, precipitate and ash. The difference in weights is the weight of the argentic chloride.

Example:

Weight of crucible + prec. + filter ash . . .	10.623
" " " — " — " " . . .	10.420
" " prec. + filter ash	0.203
" " filter ash	0.003
" " prec. of AgCl	0.200

Calculation:

$\overset{AgCl.}{143.5} : \overset{NaCl.}{58.5}$ :: weight of precipitate of AgCl : X = quantity of *sodium chloride* in the volume (10 c.c.) of urine employed. Multiply by 10 = percentage of NaCl.

Or,

$\overset{AgCl.}{143.5} : \overset{Cl.}{35.5}$ :: weight of precipitate of AgCl : X = quantity of *chlorine* in the 10 c.c. urine employed. Multiply by 10 = percentage of chlorine.

If 0.200 grm. AgCl obtained, then

$\overset{AgCl.}{143.5} : \overset{NaCl.}{58.5} :: \overset{Wt.\ of\ prec.}{0.200} : \overset{NaCl.}{0.08153} \times 10 = \overset{NaCl.}{0.8153}$ per cent. in a volume of 100 c.c. urine.

In 100 grm. of urine.

Suppose specific gravity of urine was 1020.

Then, $1020 : 1000 :: 0.815 : \overset{NaCl.}{0.799}$ per cent. in 100 grm. of the urine.

2. **Mohr's volumetric method for the determination of sodium chloride:** Depends upon the precipitation of the chlorine of the sodium chloride by means of a standard solution of argentic nitrate and calculating the quantity of sodium chloride present from the quantity of standard solution required for complete precipitation.

The standard solution of argentic nitrate is prepared of convenient strength,—*i. e.*, so that 1 c.c. of the solution shall equal 0.010 of NaCl. 1 c.c. of the solution is also equal to 0.006068 chlorine;—*i. e.*,

$$\overset{NaCl.}{58.5} : \overset{Cl.}{35.5} :: \overset{NaCl.}{0.010} : \overset{Cl.}{0.006068}$$

Preparation of the standard solution of $AgNO_3$:

$$\overset{58.5.}{NaCl} + \overset{170.}{AgNO_3} = AgCl + NaNO_3$$

$$\overset{58.5.}{NaCl} + \overset{\text{At. wt. of Ag, 108.}}{AgNO_3} = AgCl + NaNO_3$$

170 grm. of $AgNO_3$ are equal to 58.5 grm. of NaCl.

170 grm. of $AgNO_3$ (equal to 58.5 grm. NaCl) contain 108 grm. of metallic silver, consequently 108 grm. of silver are also equal to 58.5 grm. of NaCl.

The quantity of $AgNO_3$ necessary to prepare 1000 c.c. of standard solution so that 1 c.c. of it shall equal 0.010 NaCl or 0.006068 Cl is determined by

$$\overset{NaCl.}{58.5} : \overset{AgNO_3.}{170} :: \overset{\text{Grm. NaCl.}}{10} : \overset{AgNO_3.}{29.059 \text{ grm.}}$$

Or of metallic silver to be converted into nitrate, by treating with nitric acid, evaporating excess of acid, and dissolving the residue in water, and diluting to 1000 c.c.

$$\overset{NaCl.}{58.5} : \overset{Ag.}{108} :: \overset{\text{Grm. NaCl.}}{10} : \overset{\text{Metallic silver.}}{18.461 \text{ grm.}}$$

Preparation of standard solution of argentic nitrate: 29.059 grammes pure, dry $AgNO_3$ are dissolved in distilled water and the solution diluted with distilled water to a volume of 1000 c.c.

Or, 18.461 grammes pure metallic silver are dissolved in nitric acid, the excess of acid is expelled by evaporation on a water-bath and the residue (which is $AgNO_3$) is dissolved in distilled water, and the solution diluted with water to 1000 c.c. Then,

$$\begin{aligned} 1000 \text{ c.c.} &= \overset{AgNO_3.}{29.059} \text{ grm.} = \overset{NaCl.}{10.0} \\ 10 \text{ c.c.} &= 0.29059 \text{ “} = 0.100 \\ 1 \text{ c.c.} &= 0.029059 \text{ “} = 0.010 \\ 1 \text{ c.c.} &= 0.029059 \text{ “} = \overset{\text{Chlorine.}}{0.006068} \end{aligned}$$

Sometimes it is necessary to prove the accuracy of the solution by standardizing it with a standard solution of sodium chloride.

Thus:

1.0 grm. NaCl is dissolved in distilled water and the solution diluted to 100 c.c.

	NaCl.	
100 c.c.	= 1.0	grm.
10 c.c.	= 0.100	"
1 c.c.	= 0.010	"

Standardizing the argentic nitrate solution: To 10 c.c. of the NaCl solution two drops of potassium chromate (K_2CrO_4) solution (the indicator), are added, and the $AgNO_3$ solution run into the solution from a burette until the lemon-yellow color of the liquid is changed to a very slight orange-red. The red color is due to the formation of red argentic chromate (Ag_2CrO_4), and indicates that all of the NaCl has been decomposed and a slight excess of the $AgNO_3$ solution has acted on the potassium chromate present.

The 10 c.c. of NaCl solution, which contains 0.100 grm. of NaCl should require exactly 10 c.c. of the $AgNO_3$ solution to completely precipitate the chlorine and act upon the indicator

a. **The standard solution may be too strong,** *i. e.*,—requiring the addition of *less* than 10 c.c. of the $AgNO_3$ to the 10 c.c. of solution containing 0.100 grm. NaCl. It may be corrected by determining and adding the volume of water necessary to dilute it to the proper strength.

Suppose 8 c.c. instead of 10 c.c. of the $AgNO_3$ solution have been required for the 10 c.c. of solution containing 0.100 grm. NaCl. Then to every 8 c.c. of standard solution remaining a volume of distilled water equal to the difference between 8 and 10 c.c., or 2 c.c. is added.

1000 c.c. solution prepared.
8 c.c. used.
8)992 c.c. on hand.
124 × 2 = 248 c.c. water to be added to the 992 c.c. standard solution.

10 c.c. will now equal 0.100 grm. of NaCl.
1 c.c. " " 0.010 " " "

b. **The standard solution may be too weak,** *i. e.*,—requiring the addition of *more* than 10 c.c. of the $AgNO_3$ solution to the

10 c.c. of solution containing 0.100 grm. NaCl. The $AgNO_3$ solution may be corrected by adding more crystallized $AgNO_3$ to the solution, titering with 10 c.c. of standard NaCl solution and then correcting after the manner of the correction of a standard solution that is too strong (see *a* before mentioned).

If the $AgNO_3$ solution is thus standardized and the residue from 10 c.c. urine is dissolved in 30 c.c. water, then, in actual analysis, because of increased dilution, 0.2 c.c. must be deducted (0.1 for every 10 c.c. above a fixed 10 c.c.) from the number of c.c. $AgNO_3$ solution required.

c. **The $AgNO_3$ solution may be standardized so that in actual analysis no correction** (deduction of 0.2 c.c.) *will be necessary.*

10 c.c. standard solution of NaCl (containing 0.100 grm. NaCl) are diluted with 20 c.c. water, so that the volume (30 c.c.) shall equal the volume of the final solution in the actual work with the urine. Two drops of K_2CrO_4 solution are added and the $AgNO_3$ solution from a burette is run into the NaCl solution until the lemon-yellow color of the liquid is changed to a slight orange-red.

If more or less than 10 c.c. of standard $AgNO_3$ solution should be required to give the reaction with the indicator, the $AgNO_3$ solution should be corrected, after the manner of the correction of standard solutions that are too weak or too strong as before described, so that exactly 10 c.c. shall be required for the 10 c.c. of standard solution of NaCl diluted with 20 c.c. of water.

Method: 10 c.c. of urine, to which is added about 2 grm. potassium nitrate (KNO_3) to destroy the organic matter, are evaporated to dryness in a small porcelain evaporating dish on a water-bath (*absolute* dryness cannot be obtained) or over a flame, care being taken to avoid loss from spurting. (The contents of the dish must not be stirred.) The dish containing the residue is held by means of a crucible tongs over a flame and heated, very gently at first, then with strong heat until all of the organic matter is destroyed as indicated by the disappearance of the charred material. The dish containing the residue, while cooling, is held at the outer edge by a crucible tongs and given a rotary motion until the fused material

has distributed itself as a thin layer over the dish and finally solidified. By this procedure the cracking of the dish will be prevented. Care must be taken that in this procedure none of the fused material comes in contact with the crucible tongs or is projected from the dish. When the dish is thoroughly cooled the residue is treated with 5 c.c. of water and eight or ten drops of strong nitric acid. The nitric acid is added to neutralize KOH and K_2CO_3 which are produced in the decomposition of the KNO_3 with the organic matter. The liquid in the dish is warmed to hasten solution. This first portion of 5 c.c. is poured into a beaker (disregarding any enamel which may have separated from the dish), and washing the dish with portions of 5 c.c. of water is repeated (without further addition of nitric acid), heating, if necessary, until the dish has been thoroughly washed. Each portion of 5 c.c. is poured into the beaker until 30 c.c. have been employed and collected in the beaker. If the liquid is not acid in reaction it must be very slightly acidulated with nitric acid. (Up to this point, except disregarding the enamel, the method is practically similar to the one employed in the gravimetric determination of sodium chloride).

The liquid is neutralized by the addition of an excess of precipitated calcium carbonate ($CaCO_3$). The point of neutralization is determined by the cessation of effervescence and the remaining of a portion of undecomposed calcium carbonate at the bottom of the beaker. (Neutralization of the solution is necessary because the nitric acid present would dissolve the orange-red argentic chromate produced, and thus prevent a visible reaction on the indicator).

Two drops of solution of potassium chromate (the indicator) are added and the standard solution of $AgNO_3$ is run from the burette into the 30 c.c. of liquid containing the sodium chloride until the lemon-yellow color of the liquid is changed to a slight orange-red.

The number of cubic centimetres of standard $AgNO_3$ solution required is read off and this number is multiplied by the value of 1 c.c. of the standard $AgNO_3$ solution expressed in terms of NaCl,—namely, 0.010. The result obtained is multiplied by 10 in order to obtain the percentage of NaCl in the urine.

If the standard $AgNO_3$ solution has been standardized with 10 c.c. of standard NaCl solution diluted with 20 c.c. of water making a volume of 30 c.c. no correction is necessary.

Example: Suppose 8.3 c.c. $AgNO_3$ solution have been required. Then $8.3 \times 0.010 = 0.083$ grm. NaCl in the 10 c.c. urine employed. $0.083 \times 10 = 0.83$ per cent. NaCl.

3. **Liebig's method for the determination of sodium chloride:** Depends upon the formation of a soluble salt of mercury (mercuric chloride, $HgCl_2$) on the addition of mercuric nitrate, $Hg(NO_3)_2$, to a solution of sodium chloride.

$$2NaCl + Hg(NO_3)_2 = \overset{117.}{HgCl_2} + 2NaNO_3$$

Liebig's method for the determination of NaCl in the urine is not accurate and is rarely used.

The mercuric nitrate solution is prepared of such strength that 1 c.c. of it shall equal 0.010 grm. NaCl.

117 grm. NaCl require 200 grm. of mercury (once the atomic weight of Hg expressed in grammes).

Hence

$$\overset{2NaCl.}{117} : \overset{Hg.}{200} :: \overset{NaCl.}{10} : \overset{Hg.}{17.094}\text{ grm.}$$

Or, 117 grm. NaCl require 216 grm. mercuric oxide (HgO) (once the molecular weight of HgO expressed in grammes).

$$\overset{2NaCl.}{117} : \overset{HgO.}{216} :: \overset{NaCl.}{10} : \overset{HgO.}{18.461}\text{ grm.}$$

17.094 grm. of metallic mercury, or 18.461 grm. of mercuric oxide, are equal to 10 grm. NaCl.

Preparation of standard solution of mercuric nitrate: 17.094 grm. of mercury, or 18.461 grm. of mercuric oxide, are dissolved in an excess of nitric acid to which is added a small quantity of water. The excess of acid is expelled by evaporation and the residue is dissolved in distilled water and slowly diluted to 1000 c.c. If on dilution with water a canary-yellow precipitate of basic mercuric nitrate should separate, it is allowed to subside, the supernatant liquid is poured off and the precipitate is dissolved in a few drops of strong nitric acid, and the solution returned to the previously poured off supernatant liquid.

$$\begin{aligned} \overset{Hg(NO_3)_2\text{ sol.}}{1000\text{ c.c.}} &= \overset{NaCl.}{10\,0} && \text{grm.} \\ 10\text{ c.c.} &= 0\,100 && \text{“} \\ 1\text{ c.c.} &= 0\,010 && \text{“} \\ & \overset{Chlorine.}{} \\ 1\text{ c.c.} &= 0.006068 && \text{“} \end{aligned}$$

The mercuric nitrate solution may be standardized with a solution of NaCl containing 1.0 grm. NaCl in 100 c.c. water, using a pinch of urea as the indicator.

The phosphates, sulphates, and carbonates in the urine interfere with the application of the method. They are removed by the *baryta mixture*, composed of

2 volumes of a cold saturated solution of barium hydroxide ($Ba(OH)_2$).
1 volume of a cold saturated solution of barium nitrate ($Ba(NO_3)_2$).

The first action of the mercuric solution will be on the NaCl in the urine to form soluble $HgCl_2$; as soon as all the NaCl present has been decomposed, any excess of mercuric nitrate solution added will act upon the urea to form a white insoluble compound of mercuric oxide and urea, $(HgO)_2CO(NH_2)_2$,

$$2Hg(NO_3)_2 + CO(NH_2)_2 + 2H_2O = (HgO)_2CO(NH_2)_2 + 4HNO_3$$

and thus the urea acts as the indicator.

Method: 40 c.c. of urine are mixed with 20 c.c. of baryta mixture and filtered through a filter that has not been previously moistened with water. The filtrate is neutralized with a drop or two of nitric acid. 15 c.c. of the filtrate (representing 10 c.c. of urine and 5 c.c. of baryta mixture) are transferred by means of a pipette to a beaker. The standard mercuric nitrate solution is run from the burette into the liquid until a permanent milky turbidity is produced. (Action on the urea.)

The number of cubic centimetres of standard mercuric nitrate solution required is multiplied by 0.010 and the result multiplied by 10 furnishes the percentage of NaCl in the urine.

UREA (Carbamide).

$CO(NH_2)_2$. *Molec. wt.*, 60.

CON_2H_4, urea. Generally considered to be $CO(NH_2)_2$, carbamide. Gamgee considers that it is not carbamide, because, when urea is heated with potassium permanganate ($K_2Mn_2O_8$) and potassium hydroxide, all of its nitrogen is evolved as free nitrogen, whereas salts of ammonium (NH_4) and amides yield their nitrogen as N_2O_5.

Compounds isomeric with urea:

NH_4CNO, ammonium cyanate.
CON_2H_4, isuretin.

Isuretin, CON_2H_4, is not urea, because it is

$$NH_2OH + HCN = CH\begin{matrix}NH\\OH\end{matrix}$$

Hydroxylamide. Hydrocyanic acid. hydroxyl-methenyldiamide.

Urea was first recognized in the urine, and obtained in an impure state, by Rouelle, in 1773. It was obtained in a purer condition, by Fourcroy and Vauquelin, in 1799. It was prepared artificially, by Woehler, in 1828, it being the first organic compound produced artificially.

Urea is found in the urine of all mammals. The urine of birds and reptiles contains it in small quantity. Thirty per cent. of the solid matter of the vitreous humor of the eye is urea. It is contained in almost all the animal fluids,—blood, lymph, chyle, etc.,—and in the liver and spleen. It is the principal solid constituent of the urine. Ninety per cent. of the nitrogen eliminated by the urine is in the form of urea.

Urea may be prepared artificially by fusing and oxidizing potassium ferrocyanide ($K_4Fe(CN)_6$) with manganese dioxide (MnO_2) or with minium ($Pb_3O_4 = 2PbO + PbO_2$), with the production of potassium cyanate (KCNO), which, when warmed with a solution of ammonium sulphate ($(NH_4)_2SO_4$), forms ammonium cyanate (NH_4CNO), and by continued warming changes into urea ($CO(NH_2)_2$). The solution is evaporated to dryness on a water-bath, and the residue extracted with strong alcohol. The alcohol dissolves only the urea, which may be obtained in crystalline form by allowing the alcohol to evaporate at ordinary temperature.

1. $K_4Fe(CN)_6 + O_9 = 4KCNO + 2CO_2 + FeO + N_2$
2. $2KCNO + (NH_4)_2SO_4 = 2NH_4CNO + K_2SO_4$
3. $NH_4CNO = CO(NH_2)_2$

It may also be prepared by warming plumbic cyanate ($Pb(CNO)_2$) with a solution of ammonium sulphate,

$$Pb(CNO)_2 + (NH_4)_2SO_4 = PbSO_4 + 2NH_4CNO$$

on further warming, the NH_4CNO is converted into urea.

UREA MAY BE OBTAINED FROM THE URINE BY THE FOLLOWING METHODS.

1. As **urea:** Baryta mixture is added to the urine, the liquid is filtered and the filtrate is evaporated to dryness on a water-bath. The residue is treated with strong alcohol and the solution filtered and evaporated to dryness. The residue is dissolved in water and the solution decolorized by being passed through animal charcoal. The decolorized liquid is evaporated to dryness and the residue treated with strong alcohol and the liquid filtered. The filtrate is allowed to evaporate at ordinary temperature, and needle-like crystals of urea will separate

2. As **urea nitrate**: (soluble in 8 parts of water). 250 c.c., or more, of urine are evaporated on a water-bath to about one-sixth its original volume. When cold, nitric acid, of about 1.25 specific gravity, is added to the liquid and the mixture kept cold. Crystals of $CO(NH_2)_2HNO_3$ will separate. The mass of crystals is collected on a moistened piece of muslin and the excess of liquid squeezed out. The mass is scraped off the muslin and dissolved in water. Barium carbonate ($BaCO_3$) is added to the solution to separate the HNO_3 from the $CO(NH_2)_2HNO_3$.

$$2CO(NH_2)_2HNO_3 + BaCO_3 = Ba(NO_3)_2 + H_2O + CO_2 + 2CO(NH_2)_2$$

The solution is decolorized by being passed through animal charcoal. The decolorized liquid is evaporated to dryness on a water-bath, the residue is treated with strong alcohol and the liquid is filtered, and the filtrate allowed to evaporate at ordidinary temperature. Crystals of urea will separate.

3. As **urea oxalate**: (soluble in 25 parts of water). The method is similar to that with nitric acid except that oxalic acid ($H_2C_2O_4$) (strong solution or in powder) is used instead of nitric acid, and calcium carbonate ($CaCO_3$) is used instead of barium carbonate.

$$\underset{\text{Urea oxalate.}}{(CO(NH_2)_2)_2H_2C_2O_4} + CaCO_3 = \underset{\text{Calcium oxalate.}}{CaC_2O_4} + H_2O + CO_2 + 2CO(NH_2)_2$$

Properties of urea: Urea is a white, odorless compound, crystallizing in four-sided prisms. It has a cooling, bitter-like taste, somewhat resembling potassium nitrate. It is very soluble in water, 1 in 1; soluble in alcohol, 1 in 5; insoluble in ether. Melts at a temperature of 130° C.

Urea in solution has no action on blue or red litmus-paper, is neutral, yet it will combine with acids, bases, and salts.

a. $CO(NH_2)_2HNO_3$
b. $(HgO)_2CO(NH_2)_2$
c. $CO(NH_2)_2NaCl$

It unites with acids without displacing the hydrogen in the acid.

a. Urea in solution is decomposed by sodium hypochlorite with the evolution of CO_2 and N.

$$CO(NH_2)_2 + 3NaClO = 3NaCl + 2H_2O + CO_2 + N_2$$

b. Urea in solution is decomposed by nitrous acid (HNO_2) with the evolution of CO_2 and N.

$$CO(NH_2)_2 + 2HNO_2 = 3H_2O + CO_2 + 2N_2$$

c. Urea heated with water in a glass tube sealed at both ends is converted into ammonium carbonate.

$$CO(NH_2)_2 + 2H_2O = (NH_4)_2CO_3$$

A like conversion occurs when urea in solution is exposed for a time to the air, due to the action of micrococcus ureæ.

a. Urea in crystals heated to a temperature of 150°–170° C. fuses and yields ammonia (NH_3) and a compound called biuret ($C_2H_5N_3O_2$).

$$2CO(NH_2)_2 = C_2H_5N_3O_2 + NH_3$$

Biuret in solution produces a violet-red color on the addition of a few drops of very dilute cupric sulphate solution, and afterwards a solution of potassium or sodium hydroxide. (Biuret reaction.)

Peptones and albumoses respond to the same test.

b. Urea heated to a higher temperature (over 170° C.) yields ammonia NH_3, and cyanuric acid ($H_3C_3N_3O_3$).

$$3CO(NH_2)_2 = 3NH_3 + H_3C_3N_3O_3$$

c. Urea heated to a still higher temperature yields ammonia, and melanuric acid, $H_4C_3N_4O_2$.

$$4CO(NH_2)_2 = 4NH_3 + CO_2 + H_4C_3N_4O_2$$

QUANTITATIVE DETERMINATION OF UREA IN URINE.

1. **Davy's method,** introduced in 1854, depends upon the decomposition of the urea in the urine by means of sodium hypochlorite with the evolution of carbon dioxide and nitrogen. The CO_2 is absorbed by the excess of alkali (NaOH) in the NaClO solution, the nitrogen remains unabsorbed, and is collected, and measured in a tube graduated in cubic inches.

Davy's method is inaccurate, is of historical interest only and is not used.

The volume of nitrogen is corrected for temperature (60° F.) and barometric pressure (30 inches), and the quantity of urea calculated from the volume of nitrogen obtained.

$$\overset{60.}{CO(NH_2)_2} + 3NaClO = 3NaCl + 2H_2O + CO_2 + \overset{28.}{N_2}$$

1 molecule (60) of urea contains two atoms ($2 \times 14 = 28$) of nitrogen.

28 grains N = 93.33 cubic inches.

60 grains urea contain 28 grains N = 93.33 cubic inches nitrogen, at temperature of 60° F. and barometric pressure of 30 inches.

Grains urea.		Cubic inches.		Grain urea.		Nitrogen.
60	:	93.33	::	1	:	1.55 cu. in.

Consequently 1.55 cubic inch of nitrogen is evolved from 1 grain of urea, or 1 cubic inch of nitrogen is evolved from 0.645 grain of urea.

Cu. in. N.		Grain urea.		Cu. in N.		Urea.
1.55	:	1	::	1	:	0.645 grain.

Method: A tube graduated in cubic inches is filled with mercury to one third of its capacity. 100 grains of urine are added and the remainder of the tube is rapidly filled with sodium hypochlorite solution. The thumb is quickly placed over the opening of the tube and the latter is inverted in a trough containing mercury. Decomposition of the urea occurs and the evolved nitrogen collects in the upper part of the tube.

After the lapse of about half an hour the opening of the tube is closed with the thumb and the tube transferred to a vessel containing water. The atmospheric pressure is equalized and the number of cubic inches of nitrogen evolved is read off. The number of cubic inches of nitrogen evolved is divided by 1.55 or multiplied by 0.645, and the result will be the percentage of urea in the urine. (100 grains of urine having been employed.)

Suppose 4.65 cubic inches nitrogen evolved.

$$4.65 \div 1.55 = 3 \text{ per cent. urea.}$$

Or, $4.65 \times 0.645 = 2.99$ per cent. (3 per cent.) urea.

Objections to this method: According to Fenton, NaClO in presence of caustic alkalies causes the evolution of only one-half of the nitrogen of urea, the remainder being retained as cyanate, thus:

$$2CO(NH_2)_2 + 3NaClO + 2NaOH = 3\,NaCl + 2NaCNO + 5H_2O + N_2$$

2. **Fowler's modification of Davy's method** for the determination of urea: Depends upon the decomposition of urea in solution by sodium hypochlorite (NaClO), thereby causing a reduction in the density of the solution. Fowler found that a loss of one degree in specific gravity in a mixture of one volume of urine and seven volumes of hypochlorite solution represented the presence (decomposition) of 0.77 per cent. of urea.

Seven volumes of hypochlorite solution of 1035 specific gravity will decompose the urea in one volume of an average urine.

The method is inaccurate and is rarely used.

Method: The specific gravities of the urine and of the hypochlorite solution are determined separately. To one volume of the urine seven volumes of hypochlorite solution are added,—say 10 c.c. urine and 70 c.c. hypochlorite solution.

(The specific gravity of this mixture is determined by calculation.) After allowing the mixture to stand until the decomposition is completed (about half an hour) the specific gravity of the mixture is determined. This latter specific gravity is deducted from the specific gravity (obtained by calculation) of the mixture. The loss of specific gravity multiplied by 0.77 furnishes the percentage of urea in the urine.

Example:

7 volumes of NaClO sol., specific gravity . . .	$1036 \times 7 = 7252$
1 volume of urine, " " . . .	$1025 \times 1 = 1025$
8 volumes	8)8277
Specific gravity of mixture before decomposition . .	1034.6
" " " after " . .	1030.0
	4.6

Hence $4.6 \times 0.77 = 3.524$ per cent. urea.

3. **Hypobromite method** for the quantitative determination of urea: Depends upon the decomposition of the urea in the urine by means of sodium hypobromite (NaBrO) with the production of sodium bromide (NaBr), water, carbon dioxide (CO_2), and the evolution of nitrogen.

This is the most accurate method for the quantitative determination of urea.

The CO_2 is absorbed by the excess of alkali (NaOH) in the NaBrO solution, and the nitrogen remains unabsorbed, and is collected, and measured in a tube graduated in cubic centimetres.

The volume of nitrogen is corrected for temperature (0° C.) and barometric pressure (760 mm.), and the quantity of urea is calculated from the volume of nitrogen obtained.

$$\overset{60.}{CO(NH_2)_2} + 3NaBrO = 3NaBr + 2H_2O + CO_2 + \overset{28.}{N_2}$$

1 molecule urea. 1 molecule (2 atoms).

$$\overset{60.}{CO(NH_2)_2} = \overset{28.}{N_2}$$

60 grammes of urea contain 28 grammes nitrogen.

28 grammes nitrogen = 22.32 litres or 22320 c.c.

60 grammes urea evolve 22.32 " 22320 c.c. nitrogen.

Grms. urea. Grm. urea. Litres. Litre.

60 : 1 :: 22.32 : 0.372 or 372 cubic centimetres.

1 gramme of urea will evolve 372 c.c. of nitrogen (at 0° C. and 760 mm.).

c.c. N Grm. urea. c c. N. Grm. urea.

372 : 1 :: 1 : 0.002688

1 cubic centimetre of nitrogen, measured at 0° C. temperature and 760 mm. pressure, is evolved from 0.002688 grm. urea (2.688 milligrammes urea).

1.0	gramme	urea	evolves	372.0	c.c.	nitrogen.
0.001	"	"	"	0.372	"	"
0.010	"	"	"	3.72	"	"
0.020	"	"	"	7.44	"	"
0.030	"	"	"	11.16	"	"

1 c.c. of a 1 per cent. solution of urea (containing 0.010 urea) will evolve 3.72 c.c. nitrogen.

1 c.c. of a 2 per cent. solution of urea (containing 0.020 urea) will evolve 7.44 c.c. nitrogen.

The **sodium hypobromite solution** is prepared by dissolving 100 grammes sodium hydroxide (NaOH) in 250 c.c. water, cooling the solution, and adding 25 c.c (75 grammes) of bromine.

The reagent ($NaBrO$ solution) should be freshly prepared.

$$\overset{160.}{2Br} + \overset{80.}{2NaOH} = NaBrO + NaBr + H_2O$$

2Br.		2NaOH.		Grm. Br.		NaOH.
160	:	80	::	75	:	37.5 grm.

Hence the 75 grammes of bromine will combine with only 37.5 grammes of the 100 grammes of NaOH employed in the preparation of the solution, leaving the difference between 37.5 and 100, or 62.5 grammes, free NaOH in the solution, to absorb the CO_2 evolved in the application of the method.

There are many forms of apparatus employed in the determination of urea by sodium hypobromite,—

Russell & West's.
Huefner's.
Greene's.
Marshall's.
Etc.

Method, with the use of Marshall's apparatus: The side opening of the bulbed tube is closed with the thumb and the tube is filled with hypobromite solution (which may previously be diluted with an equal volume of distilled water). The upper

opening of the tube is closed with a rubber stopper and the tube is inclined so as to allow any air-bubbles which may be just below the rubber stopper to escape through the side opening. The tube is inverted and the end closed with the rubber stopper is fixed in the saucer-shaped vessel.

1 c.c. of the urine is slowly run from the graduated pipette through the side opening of the tube into the hypobromite solution, and the pipette quickly withdrawn.

1 c.c. of urine, in the great majority of cases, suffices; if, however, the urine contain very little urea, more than 1 c.c. may be employed.

When the decomposition is completed (about twenty minutes) and all the gas-bubbles have collected in the upper part of the tube (may be facilitated by gently tapping the tube with the finger), the atmospheric pressure is equalized by attaching the funnel-tube to the side opening of the hypobromite tube, and pouring hypobromite solution into it until the surfaces of the liquid in both tubes are equal in height. The number of cubic centimetres of nitrogen is read off, the temperature of the air and the barometric pressure are observed and the amount of urea calculated from the volume of nitrogen evolved after having been corrected for temperature and pressure.

Example: Suppose 1 c.c. urine evolved 12.5 c.c. nitrogen, the temperature being 20° C. and barometric pressure 750 mm.

A. **Then, correcting simply for temperature and pressure:**

$$a.\quad 293^{20°} : 273^{0°} :: 12.5^{20°} : 11.64 \text{ c.c. at } 0° \text{ C. temp.}$$

and,

$$b.\quad 760 : 750 :: 11.64 : 11.48 \text{ c.c. at } 0° \text{ C. temp. and } 760 \text{ mm.}$$

Hence

$$11.48 \text{ c.c. N} \times 0.002688 = 0.03085 \text{ urea (in 1 c.c. urine).}$$

$$0.03085 \times 100 = 3.085 \text{ per cent. urea (in 100 c.c. urine).}$$

B. **Correcting for temperature, pressure and tension of aqueous vapor:**

$$a.\quad 293^{20°} : 273^{0°} :: 12.5^{20°} : 11.64 \text{ c.c. at } 0° \text{ C. temp.}$$

b. 750.0 mm. observed barometric pressure,
17.4 correc. in mm. for tension of aqueous vapor at 20° C.
732.6 mm. corrected barometric pressure.

760 : 732.6 :: 11.64 : 11.23 c.c. at 0° C. temp. 760 mm. pressure and corrected for tension of aqueous vapor.

Hence

11.23 c.c. N × 0.002688 = 0.03018 urea (in 1 c.c. urine).
0.03018 × 100 = 3.018 per cent. urea (in 100 c.c. urine).

C. **Correcting for temperature, barometric pressure, and tension of aqueous vapor** using the following formula:

$$V' = \frac{v \times (B-T)}{760 \times (1 + 0.003665\,t)}$$

in which

V′ is the corrected volume of nitrogen in c.c.
v " observed " " "
B " barometric pressure in mm.
T " tension of aqueous vapor for temp. t.
t " observed temperature.

0.003665 is the coefficient of expansion of gases for each degree Centigrade.

Hence, for the above example,

$$\frac{12.5 \times (750 - 17.4)}{760 \times 1.073} = \frac{12.5 \times 732.6}{760 \times 1.073} = \frac{9157.50}{815.48} = 11.23 \text{ c.c. N}$$

11.23 c.c. N × 0.002688 = 0.03018 (in 1 c.c. urine).
0.03018 × 100 = 3.018 per cent. urea (in 100 c.c. urine).

D. To correct for the expansion of mercury at 20° C. in the barometer-tube,

$$0.000171 \times t°$$

thus,
$$0.000171 \times 20 = 0.0034$$
$$0.0034 \times 750 = 2.56 \text{ mm.}$$

so,

Barom. = 750 — 2.56 = 747.44.
750 : 747.44 :: 11.23 : 11.19 c.c. N.
11.19 × 0.002688 = 0.03007 grm. urea.
0.03007 × 100 = 3 007 per cent. urea (in 100 c.c. urine).

Table of Tension of Aqueous Vapor Expressed in Millimetres for Certain Temperatures Centigrade.

Temp.	Tension in mm.	Temp.	Tension in mm.
10° C.	9.139	23° C.	20.857
11° C.	9.767	24° C.	22.152
12° C.	10.432	25° C.	23.517
13° C.	11.137	26° C.	24.955
14° C.	11.883	27° C.	26.470
15° C.	12.673	28° C.	28.065
16° C.	13.510	29° C.	29.743
17° C.	14.395	30° C.	31.509
18° C.	15.330	31° C.	33.366
19° C.	16.318	32° C.	35.318
20° C.	17.363	33° C.	37.368
21° C.	18.465	34° C.	39.522
22° C.	19.629	35° C.	41.784

4. **Liebig's method** for the quantitative determination of urea: Depends upon the production of an insoluble compound of mercuric oxide and urea $(HgO)_2CO(NH_2)_2$ on the addition of mercuric nitrate $Hg(NO_3)_2$ to a solution of urea.

$$2Hg(NO_3)_2 + CO(NH_2)_2 + 2H_2O = (HgO)_2CO(NH_2)_2 + 4HNO_3$$

In the practical application of the method the $Hg(NO_3)_2$ acts first on the NaCl of the urine to form soluble $HgCl_2$. When sufficient $Hg(NO_3)_2$ has been added to combine with all the NaCl present it then acts on the urea.

$$Hg(NO_3)_2 + 2NaCl = HgCl_2 + 2NaNO_3$$

The precipitate formed after the NaCl is satisfied is composed of 2 molecules of HgO in combination with 1 molecule of $CO(NH_2)_2$.

$$\begin{array}{lll} \text{Twice the molecular weight of } HgO & \overset{216.}{} & \text{is } 432 \\ \text{Once } \text{“} \quad \text{“} \quad \text{“} \quad \text{“} & CO(NH_2)_2 & \text{“ } 60 \end{array}$$

In the formation of the compound $(HgO)_2CO(NH_2)_2$, 432 parts by weight of HgO enter into combination with 60 parts by weight of $CO(NH_2)_2$.

Preparation of the standard $Hg(NO_3)_2$ solution: The quantity of HgO necessary to prepare 1000 c.c. of standard $Hg(NO_3)_2$ solution so that 1 c.c. of it shall equal 0.010 urea, is determined by

$$\overset{\text{Urea.}}{60} : \overset{\text{HgO.}}{432} :: \overset{\text{Urea.}}{10} : \overset{\text{HgO.}}{72.0}\text{ grm.} = 66.66\text{ grm. metallic Hg.}$$

In the preparation of the solution 5.2 grm. HgO must be added to the 72.0 grm. to act on the indicator.

$$72.0 + 5.2 = 77.2\text{ grm. HgO} = 71.48\text{ grm. metallic Hg.}$$

Hence

1000 c.c. containing 77.2 grm. HgO = 10.0 grm. urea.
1 c.c. " 0.0772 " " = 0.010 " "

This same solution may be used for the quantitative determination of sodium chloride.

$$\overset{\text{HgO.}}{216} : \overset{\text{2NaCl.}}{117} :: \overset{\text{HgO.}}{77.2} : \overset{\text{NaCl.}}{41.817}\text{ grm.}$$

1000 c.c. containing 77.2 grm. HgO = 41.817 grm.
1 c.c. " 0.0772 " " = 0.041817 "

The insoluble HgO, or the metallic Hg, is converted into soluble $Hg(NO_3)_2$ by dissolving in nitric acid.

77.2 grm. HgO or 71.48 grm. metallic Hg are dissolved in an excess of nitric acid to which is added a small quantity of water. The excess of acid is expelled by evaporation on a sand-bath and the residue is dissolved in distilled water, and slowly diluted to a volume of 900 c.c. If on dilution with water a canary-yellow precipitate of basic mercuric nitrate should separate, it is allowed to subside, the supernatant liquid is poured off and reserved. The canary-yellow precipitate is dissolved in a few drops of strong nitric acid and the solution returned to the previously poured off supernatant liquid. Should there be a recurrence of the precipitation, the precipitate must be allowed to subside, the supernatant liquid poured off, etc., as above described.

The mercuric nitrate solution must be standardized with a standard solution of urea of 2 per cent. strength.

2 or + 16 = 216

216/77.2 (0.3571

648

1240

1280 71.42000

1600

1512

280

If 60 (urea) will prpt 432 (H_2O), 10 will prpt x or 72 gm (H_2O)

Preparation of the standard urea solution: 2.0 grm. dry urea are dissolved in distilled water and the solution is diluted to 100 c.c.

	Urea.	
100 c.c. =	2.0	grm.
10 c.c. =	0.200	"
1 c.c. =	0.020	"

The indicator is a strong solution of sodium carbonate. With mercuric nitrate it produces yellowish-brown basic mercuric oxycarbonate, $HgCO_3(HgO)_3$.

$$4Hg(NO_3)_2 + 4Na_2CO_3 = HgCO_3(HgO)_3 + 8NaNO_3 + 3CO_2$$

Standardizing the mercuric nitrate solution: To standardize the mercuric nitrate solution, 10 c.c. of the standard urea solution (= 0.200 urea) are placed in a beaker, and the mercuric nitrate solution is run into it from a burette, stirring after each addition, until a drop of the liquid in the beaker produces a slight yellow color when brought, by means of a glass rod, in contact with sodium carbonate solution on a porcelain tablet.

If 1 c.c. of the mercuric nitrate solution is equal to 0.010 urea, then 20 c.c. should be required to combine with the urea (0.200) present in the 10 c.c. urea solution and act on the indicator.

1000 c.c. contains 5.2 grm. HgO to act on the indicator.
1 c.c. " 0.0052 " " " " " "

20 c.c. HgO solution having been required for the 10 c.c. urea solution, and as each c.c. HgO solution contains 0.0052 HgO to act on the indicator, 20 × 0.0052 or 0.104 HgO must have been added with the 20 c.c. HgO solution to the 10 c.c. urea solution. Hence, in the combined volumes, 20 c.c. + 10 c.c. = 30 c.c., there were present 0.104 HgO, and in each c.c. 0.104 ÷ 30 = 0.003466 mgrm. HgO to act on the indicator.

If a larger or smaller quantity than 20 c.c. should be required, the mercuric nitrate solution must be corrected after the manner given for solutions too strong or too weak in the correction of the argentic nitrate solution for the determination of sodium chloride.

The phosphates, sulphates, and carbonates in the urine interfere with the application of the method. They are removed by the baryta mixture, composed of

2 volumes of a cold saturated solution of barium hydroxide ($Ba(OH)_2$).

1 volume of a cold saturated solution of barium nitrate ($Ba(NO_3)_2$).

Method: 40 c.c. urine are mixed with 20 c.c. baryta mixture, and filtered through a filter that has not been previously moistened with water. The filtrate is neutralized with a drop or more of nitric acid. 15 c.c. of the filtrate (representing 10 c.c. urine and 5 c.c. baryta mixture) are transferred by means of a pipette to a beaker, and the mercuric nitrate solution is run into it from a burette, stirring after each addition, until a permanent milky turbidity is produced (action on the urea). The quantity of mercuric nitrate solution added up to this point was required by the sodium chloride.

The number of c.c. mercuric nitrate solution added up to this point is noted and the number noted multiplied by 0.0418, will represent the quantity of sodium chloride in 10 c.c. of the urine, and this, multiplied by 10, gives the percentage.

The addition of the mercuric nitrate solution is continued, stirring after each addition, until a drop of the liquid in the beaker produces a slight yellow color when brought, by means of a glass rod, in contact with the indicator (sodium carbonate) on a porcelain tablet.

The total number of c.c. of mercuric nitrate solution required for the sodium chloride and urea is read off. From this number the quantity required for the sodium chloride is deducted and the remainder will be the quantity required for the urea.

If *more than* 30 *c.c.* of mercuric nitrate solution have been required for the urea alone, then *for every* 4 *c.c.* required *over* 30 *c.c. add* 0.1 *c.c.* to the number of c.c. of mercuric nitrate solution required for the urea alone.

If *less than* 30 *c.c.* of mercuric nitrate solution have been required for the urea alone, then *for every* 4 *c.c.* required *less than* 30 *c.c. deduct* 0.1 *c.c.* from the number of c.c. of mercuric nitrate solution required.

The corrected number of c.c. mercuric nitrate solution is multiplied by 0.010, and the result will represent the quantity of urea in 10 c.c. of the urine, and this result multiplied by 10, gives the percentage of urea in the urine.

Example: Suppose a total quantity of 36 c.c. mercuric nitrate solution had been required,

36 c.c.
2 c.c. for NaCl.
34 c.c. for urea.
30
4 (once 4 over 30).

Hence

34.0 c.c. + 0.1 = 34.1 c.c.
34.1 × 0.010 = 0.341 grm.
0.341 × 10 = 3.41 per cent. urea.

These corrections are necessary to make the results correspond to the end reaction obtained in standardizing the mercuric nitrate solution, in which there were just two volumes of the mercuric nitrate solution employed for one volume of the urea solution.

CORRECTIONS FOR VARYING QUANTITIES OF UREA.

a. If the undiluted urine under examination contains *over* 3 per cent. of urea, then on mixing it with the baryta fluid in the proportion of 10 c.c. of the urine with 5 c.c. of the baryta fluid, the resulting *mixture will contain over* 2 per cent. of urea.

Under these circumstances 15 c.c., or say one volume, of the urine mixture will require over 30 c.c., or two volumes, of the mercuric nitrate solution for complete precipitation of the urea.

Consequently, the excess of the mercuric oxide originally present in the mercuric nitrate solution for the purpose of acting upon the indicator (sodium carbonate) *will be under a less degree of dilution* than was present when the mercuric nitrate solution was standardized, and hence a portion of this excess will be consumed in the precipitation of the urea, there still being left in excess a quantity of mercuric oxide equal to

that originally present when the mercuric nitrate was standardized; namely, 3.466 milligrammes in each c.c. of the end mixture.

The quantity of the excess of mercuric oxide thus consumed by urea can be accurately compensated for by adding to the number of cubic centimetres of mercuric nitrate solution employed 0.1 c.c. for every 4 c.c. of the mercuric nitrate solution required above 30 c.c.

Thus if 34 c.c. of the mercuric nitrate solution are required, it should be read 34.1 c.c., which would indicate that the undiluted urine contained 3.41 per cent. of urea.

b. Should the undiluted urine contain *less* than 3 per cent. of urea, then on mixing 10 c.c. of the urine with 5 c.c. of the baryta fluid, the resulting *mixture will contain less* than 2 per cent. of urea, and, consequently, 15 c.c. of the mixture will require less than 30 c.c. of standard mercuric nitrate solution for the complete precipitation of the urea.

Under these conditions the original excess of mercuric oxide, in the mercuric nitrate solution for the purpose of acting upon the indicator, *will be under a greater degree of dilution* than existed when the mercuric nitrate solution was standardized, and, therefore, a portion of the mercuric oxide intended for the precipitation of the urea, will be consumed by acting on the indicator. Hence, when less than 30 c.c. of the mercuric nitrate solution are required for a mixture of 10 c.c. of urine and 5 c.c. of baryta fluid, deduct from the reading 0.1 c.c. for every 4 c.c. of mercuric nitrate solution required less than 30 c.c. Thus, if 26 c.c. of mercuric nitrate solution were required, it should be read 25.9 c.c., which would indicate that the undiluted urine contained 2.59 per cent. of urea.

In other words, these corrections may be stated as follows: For each c.c. of mercuric nitrate solution required above two volumes of the mercuric nitrate solution for one volume of the urine mixture, add 0.025 c.c. to the reading; and for each c.c. of the mercuric nitrate solution required less than two volumes of mercuric nitrate solution for one volume of the urine mixture, deduct 0.025 c.c. from the reading.

Examples: A.—10 c.c. of 2 per cent. *urea solution*, plus 20 c.c. of the HgO solution. Thus, in each c.c. of the HgO

solution there are 5.2 milligrammes of HgO in excess to act on the indicator. In 20 c.c. there are $5.2 \times 20 = 104$ milligrammes excess of HgO in the 30 c.c. of end mixture. Therefore in each c.c. of the 30 c.c. of mixture there are $104 \div 30$ or 3.466 milligrammes of HgO in excess to act on the indicator.

B.—10 c.c. of *urine* containing 3 per cent. of urea, plus 5 c.c. of *baryta mixture*, plus 30 c.c. of HgO solution. In each c.c. of HgO solution there are 5.2 milligrammes excess of HgO. In 30 c.c. there are 30×5.2 milligrammes $= 156$ milligrammes excess in 45 c.c. of end mixture. Therefore in 1 c.c. there are $156 \div 45 = 3.466$ milligrammes.

C.—10 c.c. of *urine* containing 4 per cent. of urea, plus 5 c.c. of *baryta mixture*, plus 40 c.c. of HgO solution. In 40 c.c. of HgO solution there are $40 \times 5.2 = 208$ milligrammes excess of HgO in 55 c.c. of end mixture. Therefore in 1 c.c. there are $208 \div 55 = 3.781$. But adding 5 c.c. of H_2O to the mixture, there are 208 milligrammes of HgO excess in 60 c.c. mixture, and, therefore, in 1 c.c. there are $208 \div 60 = 3.466$.

In the case of a urine containing 4 per cent. of urea, 10 c.c. of the urine would contain 400 milligrammes of urea and, with the dilution of 5 c.c. of baryta mixture, should require 40 c.c. of standard mercuric nitrate solution. As a matter of fact the action on the indicator will occur when 39.76 c.c. of mercuric nitrate solution have been added, for after that quantity has been added, each c.c. of the end mixture will contain 3.460 milligrammes of HgO. Each c.c. of the mercury solution contains 72 mgrms. of HgO * which is designed to combine with 10 mgrms. urea, therefore, 400 mgrms. of urea would require $40 \times 0.072 = 2.880$ grms. HgO. In the 39.76 c.c. mercury solution actually required there are 39.76×0.0772 HgO $= 3.069$ grms. HgO. From this quantity of HgO, which is designed for both urea and indicator, must be deducted the quantity of HgO required for the 400 mgrms. of urea, namely, 2.880 grms. Thus, $3.069 - 2.880 = 0.189472$ mgrms., which is the quantity of HgO distributed in the end mixture to act upon the indicator, consequently in each c.c. of the end mixture (composed of 10 c.c. urine + 5 c.c. baryta mixture + 39.76 c.c. mercury solution = 54.76 c.c.) there are

* Other than the 5.2 mgrms. HgO for the indicator.

0.189472 ÷ 54.76 = 0.00346 mgrms. HgO to act upon the indicator. 39.76 c.c. mercury solution employed — 30 c.c. mercury solution the basis for which no correction is necessary, = 9.76 ÷ 4 = 2.44 the number of tenths which must be added to the number 39.76 to make it correspond to the correct number of cubic centimetres,—namely 40. Therefore, in the correction, each 4 c.c. of mercury solution used above 30 c.c. are equivalent to one-tenth of a cubic centimetre of the standard mercuric nitrate solution.

D.—10 c.c. of *urine* containing 2 per cent. of urea, plus 5 c.c. of *baryta mixture*, plus 20 c.c. of HgO solution. In 20 c.c. of HgO solution there are 5.2 × 20 or 104 milligrammes of HgO excess in 35 c.c. of end mixture. Therefore in each c.c. there are 104 ÷ 35 = 2.971. But 10 c.c. of a 2 per cent. urea solution plus 5 c.c. of baryta mixture will require 20.23 c.c. of HgO. In 20 c.c. of HgO solution there are 20 × 5.2 = 104 milligrammes in excess. Each c.c. of the standard solution of HgO contains 77.2 milligrammes of HgO, therefore 0.23 c.c. will contain 77.2 × 0.23 = 17.75 in excess, plus 104 = 121.75 ÷ 35.23 = 3.466 mgrms.

PHOSPHORIC ACID.

Phosphoric acid occurs in the urine partly in combination with sodium, potassium, and ammonium (alkaline phosphates), and partly with calcium and magnesium (earthy phosphates).

It also occurs in small quantity in the urine as glycerine phosphoric acid ($C_3H_9PO_6$).

$$\begin{matrix} & /OH \\ PO & -OH \\ & \backslash OC_3H_5(OH)_2 \end{matrix} \quad = \text{Glycerine phosphoric acid.}$$

About four-fifths of the phosphoric acid in the urine is in combination as alkaline phosphates, and one-fifth as earthy phosphates.

Phosphoric acid in the urine is determined in terms of P_2O_5 (phosphoric anhydride).

The volumetric method for the quantitative determination of phosphoric acid in the urine depends upon the

$$C_3H_5(HO)_3 + H_3PO_4 = C_3H_5(HO)_2H_2PO_4 + H_2O$$

principle that P_2O_5 in the presence of free acetic acid and an acetate of an alkali, on the application of heat, is precipitated by uranium acetate, or nitrate, as insoluble $(UrO_3)_2P_2O_5$.

It is always necessary to standardize the uranium solution with a standard solution of P_2O_5.

The standard solution of P_2O_5 is prepared so that it shall contain 0.2 per cent. P_2O_5.

1. **Preparation of standard solution of P_2O_5:** Di-sodic hydrogen phosphate ($Na_2HPO_4 + 12H_2O$) is selected for this purpose.

Two molecules of $Na_2HPO_4 + 12H_2O$ contain one molecule of P_2O_5.

$$\begin{array}{rl} 2\ (Na_2HPO_4 + 12H_2O & = 358 \times 2 = 716 \\ P_2O_5 & = 142 \end{array}$$

Hence, to determine the quantity of $Na_2HPO_4 + 12H_2O$ necessary to prepare 1000 c.c. solution, so that it shall contain 2 grm. P_2O_5 (0.2 per cent.),

$$\begin{array}{ccccccc} {\scriptstyle P_2O_5.} & & {\scriptstyle 2Na_2HPO_4+12H_2O.} & & {\scriptstyle P_2O_5.} & & {\scriptstyle Na_2HPO_4+12H_2O.} \\ 142 & : & 716 & :: & 2 & : & 10.085 \text{ grm.} \end{array}$$

or to prepare only 250 c.c. of the standard $Na_2HPO_4 + 12H_2O$ solution,

$$\begin{array}{ccccccc} & & & & & & {\scriptstyle Na_2HPO_4+12H_2O.} \\ 142 & : & 716 & :: & 0.5 & : & 2.521 \text{ grm.} \end{array}$$

10.085 grm. pure non-effloresced *di-sodic hydrogen phosphate* are dissolved in water and the solution diluted to 1000 c.c., or to prepare only 250 c.c. of the solution, 2.521 grm. of the phosphate are dissolved in water and diluted to 250 c.c.

$$\begin{array}{rl} & {\scriptstyle P_2O_5.} \\ 1000 \text{ c.c.} & = 2.000 \text{ grm.} \\ 100 \text{ c.c.} & = 0.200 \text{ "} \\ 50 \text{ c.c.} & = 0.100 \text{ "} \\ 1 \text{ c.c.} & = 0.002 \text{ "} \end{array}$$

2. **Preparation of uranium acetate, or nitrate solution:** The precipitate formed when uranium acetate, or nitrate, is added to a solution containing P_2O_5 is $(UrO_3)_2P_2O_5$,—*i. e.*, two molecules of UrO_3 in combination with one molecule of P_2O_5.

$$\begin{array}{lll} \text{Twice the molecular weight of } & {\scriptstyle 288.} \\ & UrO_3 \text{ is } & 576 \\ \text{Once} \quad \text{"} \quad \text{"} & {\scriptstyle 142.} \\ & P_2O_5 \text{ "} & 142 \end{array}$$

The quantity of uranium oxide (UrO_3) required to prepare 1000 c.c. of standard solution so that 1 c.c. shall equal 0.010 P_2O_5 is determined:

$$\underset{142}{P_2O_5.} : \underset{576}{2UrO_3.} :: \underset{10}{P_2O_5.} : \underset{40.56\text{ grm.}}{UrO_3.} = \text{to } 10\text{ grm. } P_2O_5$$

The quantity of uranium acetate ($UrO_3(C_2H_3O_2)_2 + 2H_2O$) or of the nitrate ($UrO_3N_2O_5 + 6H_2O$) equivalent to 40.56 grm. UrO_3 is determined:

$$\underset{288}{UrO_3.}\ \underset{442}{\text{Uran. acetate.}} :: \underset{40.56}{UrO_3.} : \underset{62.24\text{ grm.}}{\text{Uran. acetate.}}$$

$$\underset{288}{UrO_3.}\ \underset{504}{\text{Uran. nitrate.}} :: \underset{40.56}{UrO_3.} : \underset{70.98\text{ grm.}}{\text{Uran. nitrate}}$$

The quantity of uranium acetate or of nitrate necessary to prepare 1000 c.c. of solution may also be calculated by one proportion:

$$\underset{142}{P_2O_5.} : \underset{884}{\text{2 Uran. acetate.}} :: \underset{10}{P_2O_5.} : \underset{62.24\text{ grm.}}{\text{Uran. acetate.}}$$

$$\underset{142}{P_2O_5.} : \underset{1008}{\text{2 Uran. nitrate.}} :: \underset{10}{P_2O_5.} : \underset{70.98\text{ grm.}}{\text{Uran. nitrate.}}$$

Because of uranium acetate and nitrate being contaminated with oxides of uranium the respective quantities, as indicated by the above proportions, are dissolved in 900 c.c. water instead of 1000 c.c.

Ammonium salts in the urine interfere with uranium nitrate, therefore uranium acetate is preferred in the preparation of the standard solution.

62.24 grm. *uranium acetate* or 70.98 grm. *uranium nitrate* are boiled with about 850 c.c. water, the liquid allowed to cool, the insoluble oxides of uranium are removed by filtration and the solution diluted to 900 c.c.

3. **Preparation of the solution containing an acetate of an alkali and free acetic acid:** 50 grm. *sodium acetate* are dissolved in 450 c.c. water, and *acetic acid* is added until the volume reaches 500 c.c.

4. The *indicator* is a *solution* of *potassium ferrocyanide* of about ten per cent. strength. With a soluble uranium salt it produces a chocolate color (due to formation of uranium ferrocyanide).

$2CrO_3 + MnO_5 + 2Na_2HPO_4 = 2CrO_3 + P_2O_5 + 4NaNO_2 + H_2O$

Standardizing the uranium acetate solution: To standardize the uranium acetate solution, 50 c.c. of the standard phosphoric acid solution (containing 0.100 grm. P_2O_5) are placed in a beaker, 5 c.c. of the acetate of an alkali solution are added and the liquid is heated to the simmering-point. The uranium acetate solution (about one-half c.c. at a time) is run into it from a burette, stirring after each addition, until a drop of the liquid in the beaker produces a *slight* chocolate color when brought, by means of a glass rod, in contact with the potassium ferrocyanide solution on a porcelain tablet.

The number of cubic centimetres of uranium acetate solution required to effect this result is noted.

Example: Suppose 8 c.c. of uranium solution were required. The solution is too strong and must be diluted. For every 8 c.c. of the uranium solution (of the 900 c.c.) remaining, a volume of water equal to the difference between 8 and 10, or 2 c.c. must be added.

900 c.c. original volume of solution.
8 c.c. volume used.
8)892 c.c. volume remaining.
111.5 × 2 = 223 c.c. water to be added to the 892 c.c. uranium solution.

P_2O_5.
10 c.c. will now equal 0.100 grm.
1 c.c. " " " 0.010 "

Method: 50 c.c. urine are placed in a beaker, 5 c.c. acetate of an alkali solution are added and the liquid is heated to the simmering-point. The uranium acetate solution (about one-half c.c. or less, at a time) is slowly run from a burette into the liquid in the beaker, stirring after each addition, until a drop of the liquid in the beaker produces a *slight* chocolate color when brought, by means of a glass rod, in contact with potassium ferrocyanide solution on a porcelain tablet.

The first titration (adding about 0.5 c.c. at a time) usually gives only approximate results, unless performed with great care. A second titration should be made, and the uranium solution slowly run in until within 0.5 c.c. of the quantity required in the first titration. The uranium solution is now added, 0.1 c.c. at a time, and continued, testing with the indicator

after each addition, until a slight chocolate color is obtained in the potassium ferrocyanide solution on the porcelain tablet.

The number of cubic centimetres of uranium acetate solution required is read off the burette and this number is multiplied by 0.010, and the result will represent the quantity of P_2O_5 in 50 c.c. urine. This result multiplied by 2 gives the percentage of P_2O_5 in the urine.

Example: Suppose 8.6 c.c. uranium solution were required. Then

$$8.6 \times 0.010 = 0.086 \times 2 = 0.172 \text{ per cent. } P_2O_5 \text{ (total).}$$

QUANTITATIVE DETERMINATION OF THE EARTHY PHOSPHATES IN URINE.

To 200 c.c. urine excess of ammonium hydroxide (NH_4OH) (about 10 c.c. ordinary strength NH_4OH), is added and the liquid allowed to stand twelve hours. The precipitated earthy phosphates (phosphates of calcium and magnesium) are collected on a filter and washed several times with small quantities of water containing a few drops of NH_4OH. A beaker with a 50 c.c. mark on it is now placed under the funnel. The filter, while in the funnel, is pierced with a glass rod, and the precipitate treated drop by drop with about 3 or 4 c.c. acetic acid. This is for the purpose of dissolving the earthy phosphates. The remainder of the precipitate on the filter is washed with water into the beaker below until 50 c.c. liquid are collected in the beaker. Any insoluble matter in the liquid in the beaker is mostly mucous from the urine. No attention need be paid to this.

5 c.c. of acetate of alkali solution are added and the liquid is heated to the simmering-point.

The uranium acetate solution (less than one-half c.c. at a time) is slowly run from a burette into the liquid in the beaker, stirring after each addition, until a drop of the liquid in the beaker when brought, by means of a glass rod, in contact with potassium ferrocyanide solution on a porcelain tablet, produces a *slight* chocolate color.

The number of cubic centimetres of uranium acetate solution required is read off the burette and this number is multiplied

by 0.010, the result is divided by 2 (because 200 c.c. of urine had been originally employed). The final result will represent the percentage of P_2O_5 in combination with the alkaline earths in the urine.

Example: Suppose 5.4 c.c. uranium acetate solution were required.

$$5.4 \times 0.010 = 0.054 \div 2 = 0.027 \text{ per cent. of } P_2O_5$$

The percentage of earthy phosphates is deducted from the percentage of total phosphates (alkaline + earthy), and the remainder is the percentage of P_2O_5 in combination with the alkalies.

Suppose the percentage of total		P_2O_5	$= 0.172$	per cent.
"	"	earthy	" $= 0.027$	"
"	"	alkaline	" $= 0.145$	"

Phosphoric acid often occurs in urinary sediments as triple phosphate (magnesium ammonium phosphate, $MgNH_4PO_4$).

Phosphoric acid may occur in combination in the form of urinary calculi. Phosphatic calculi do not occur as frequently as uric acid calculi.

PHOSPHATIC CALCULI MAY OCCUR IN THREE FORMS.

1. **Composed of calcium phosphate:** Three varieties,—acid, neutral, and basic.

a. $CaH_4(PO_4)_2$
b. $CaHPO_4$
c. $Ca_3(PO_4)_2$

All are soluble in nitric acid; when their solution is neutralized with NH_4OH they are reprecipitated.

Heat does not affect them.

2. **Composed of magnesium ammonium phosphate** ($MgNH_4PO_4$), (triple phosphate) the most common form.

Soluble in hydrochloric acid. When the hydrochloric acid solution is neutralized with NH_4OH, crystalline triple phosphate appears. When heated, ammoniacal gas is evolved.

3. **Fusible phosphates, usually composed of 1 and 2** (above). Contain more or less organic matter.

URIC ACID, (Lithic acid). $C_5H_4N_4O_3$.

Molecular weight, 168.

A dibasic acid,—*i. e.*, contains two replaceable atoms of hydrogen, $H_2C_5H_2N_4O_3$.

Found in urine chiefly as sodium acid urate, $NaHC_5H_2N_4O_3$.

Present from 0.2 part to 1.0 part in 1000 parts urine.

Average percentage in urine 0.05 per cent.

Recognized as a distinct compound by Scheele in 1776. Studied more fully by Liebig in 1838. Was prepared synthetically in 1882.

Formerly called lithic acid.

It is contained in the solid excrement of birds and serpents, from which source it is most readily obtained by treating the excrement with a 5 per cent. solution of NaOH, filtering, and adding HCl to the filtrate. The uric acid is precipitated in an amorphous state or, on standing, may separate in crystalline form.

It may be deposited in the joints, as in the uric acid diathesis (gout).

To obtain pure and colorless crystals the acid obtained from about 200 c.c. of urine is dissolved in about 75 c.c. water to which about 1 c.c. of a 10 per cent. solution of NaOH has been added. The solution is slightly acidulated with HCl, and allowed to stand twenty-four hours in a cool place. Uric acid will separate. If the crystals are not colorless, they are collected on a filter, redissolved in water with the aid of NaOH, and HCl added as before. The operation is repeated, if necessary, until colorless crystals are obtained.

Uric acid crystallizes in many forms, most often in wedge-shaped crystals.

It is soluble in 15,000 parts of cold and 2000 parts of hot water. Insoluble in alcohol and ether. Soluble in an alkaline solution and in sulphuric acid. Insoluble in hydrochloric acid.

When burned, an odor similar to burnt feathers is produced.

It is dibasic; forms neutral and acid salts.

$H_2C_5H_2N_4O_3$

$K_2C_5H_2N_4O_3$

Neutral salts: *a.* $K_2C_5H_2N_4O_3$, potassium urate, soluble in 44 parts of cold water.

b. $Na_2C_5H_2N_4O_3$, sodium urate, less soluble than the corresponding neutral potassium salt.

c. The neutral ammonium salt is unknown.

Acid salts: *a.* $KHC_5H_2N_4O_3$,, acid potassium urate, soluble in 800 parts of water.

b. $NaHC_5H_2N_4O_3$, acid sodium urate, less soluble than the corresponding potassium salt.

c. $NH_4HC_5H_2N_4O_3$, acid ammonium urate, soluble in 1800 parts of water.

Uric acid treated with *cold strong nitric acid* (specific gravity 1.41) is oxidized, with the formation of *alloxan* ($C_4H_2N_2O_4$). Effervescence occurs, and urea is produced at the same time.

$$\underset{\text{Uric acid.}}{C_5H_4N_4O_3} + H_2O + O = \underset{\text{Alloxan.}}{C_4H_2N_2O_4} + CO(NH_2)_2$$

Uric acid treated with *hot dilute nitric acid* is oxidized, with the formation of *alloxantin* ($C_8H_4N_4O_7$).

QUALITATIVE TESTS FOR URIC ACID.

1. **Murexide test:** The solid uric acid, or its solution evaporated to dryness, is placed in a small porcelain dish and the uric acid covered with strong nitric acid. The mixture is evaporated to dryness on a water-bath, the dish allowed to cool and the residue is moistened with a drop of very dilute ammonium hydroxide. A beautiful red color will appear, due to the formation of murexide (ammonium purpurate), $C_8H_8N_6O_6 = (NH_4C_8H_4N_5O_6)$.

2. **Schiff's test:** The uric acid is dissolved in a solution of sodium carbonate, and a drop of the solution is brought in contact with filter-paper which has been previously saturated with a solution of argentic nitrate. Spots of reduced silver of a yellowish-brown or deep-black color, depending upon the quantity of uric acid in the solution, will appear on the paper.

Hippuric acid ($C_9H_9NO_3$) is contained in very small quantity in human urine.

Benzoic acid ($C_7H_6O_2$) when taken into the human organism is converted into hippuric acid through the agency of glycocol ($C_2H_5NO_2$), a substance formed in the liver, thus:

$$\underset{\text{Benzoic acid.}}{C_7H_6O_2} + \underset{\text{Glycocol.}}{C_2H_5NO_2} = \underset{\text{Hippuric acid.}}{C_9H_9NO_3} + H_2O$$

Quinic acid ($C_7H_{12}O_6$), one of the acids contained in cinchona bark, and also in coffee-beans, is eliminated as hippuric acid. Hippuric acid appears in the urine after eating cranberries.

QUANTITATIVE DETERMINATION OF URIC ACID BY MEANS OF HCl.

1. Heintz's method: If albumen be present it must be removed by coagulation by heat and filtration. To 200 c.c. urine (if not clear, filter,) 5 or 10 c.c. HCl are added and the liquid is allowed to stand about twenty-four hours. Uric acid separates in crystalline form. Some of the crystals float on the surface of the liquid. The crystals separated in this manner are brownish-red in color owing to urine coloring matter which they have taken up in the process of crystallization.

The crystals are collected on a washed, or on an equipoised, or a weighed washed filter. The filtrate is used to wash the crystals out of the beaker. The crystals are washed with small portions of water (5 c.c.) at a time until the last portions of the filtrate coming from the funnel are free from hydrochloric acid (test with $AgNO_3$ solution).

The two equipoised filters are separated, the crystals allowed to dry on the filter, and the two filters are weighed.

The weight obtained is the quantity of uric acid in 200 c.c. urine. This weight divided by 2 furnishes the percentage of uric acid in the urine.

If over 30 c.c. of water are required in the washing of the crystals, then for every cubic centimetre of water employed over 30 c.c. 0.000045 grm. must be added to the weight of uric acid obtained. (1 c.c. water applied in this manner dissolves 0.000045 grm. uric acid).

No correction is necessary when only 30 c.c. are required in the washing.

The uric acid in crystallizing takes up coloring-matter from the urine, but the amount of uric acid dissolved by the 30 c.c. water used in washing is just sufficient to compensate for the increase of weight due to the coloring-matter taken up.

2. **Salkowski-Ludwig method:** Depends upon the principle that when an ammoniacal solution of argentic nitrate is added to a solution of uric acid, to which an ammoniacal mixture of magnesium sulphate and ammonium chloride has been previously added, the uric acid is precipitated as a magnesio-silver salt. This is collected on a filter, washed, and decomposed by means of sodium or potassium sulphide, whereupon the uric acid passes into solution as sodium or potassium urate. On the addition of an excess of hydrochloric acid to this solution the urate is broken up and uric acid separates. The uric acid is collected on a previously weighed or an equipoised filter and weighed.

3. **Haycraft's method:** Depends upon the principle that when uric acid is precipitated by an ammoniacal solution of argentic nitrate, in the presence of an ammoniacal mixture of magnesium sulphate and ammonium chloride, the precipitate is considered to contain one atom of silver to each molecule of uric acid. The precipitate is collected on a filter and dissolved in nitric acid of about 1.12 to 1.18 specific gravity, in which solution the uric acid is determined indirectly by determining the amount of silver by means of a one-fiftieth normal solution of potassium sulphocyanate.

Other methods for the quantitative determination of uric acid are those of Fokker, Camerer, and Czapek.

CREATININE. $C_4H_7N_3O$.

Molecular weight, 113.

First recognized in 1844. Isolated by Liebig in 1847.

Occurs in the urine of man (0.06 per cent.), horse, cow, sheep, and dog.

A substance supposed to be creatinine was found in muscle, but was afterwards shown to be creatine ($C_4H_9N_3O_2$).

Creatinine crystallizes in colorless prisms. Soluble in 12 parts of water and in 100 parts of alcohol.

Its solution is strongly alkaline and will change red litmus to blue, and will change turmeric paper brown. It has a caustic taste, somewhat like dilute ammonium hydroxide. It is the strongest of all bases of animal origin.

It unites with acids, without displacing the hydrogen in the acid, to form salts, as

$$C_4H_7N_3O \cdot HCl \text{ (creatinine hydrochloride).}$$

It combines with one molecule of water to form creatine:

$$\underset{\text{Creatinine.}}{C_4H_7N_3O} + H_2O = \underset{\text{Creatine.}}{C_4H_9N_3O_2}$$

It combines with zinc chloride to form creatinine zinc chloride, $(C_4H_7N_3O)_2ZnCl_2$, which contains 62.432 per cent. of creatinine.

QUALITATIVE TESTS FOR CREATININE.

1. **Picric acid test:** A solution of creatinine treated with a few drops of sodium hydroxide and a little picric acid and then warmed is colored claret-red.

2. **Weyl's test:** A dilute creatinine solution treated with a few drops of *very* dilute sodium nitroprusside solution, and then with a dilute solution of sodium hydroxide, added drop by drop, becomes ruby-red in color, changing in a few moments to an intense straw color, which in turn becomes green when warmed with acetic acid.

Creatinine reduces Fehling's solution changing the blue liquid to yellow. The cuprous oxide does not separate, but remains in solution.

QUANTITATIVE DETERMINATION OF CREATININE IN URINE.

300 c.c. urine are treated with milk of lime until slightly alkaline, and a solution of calcium chloride is added as long as a precipitate forms (may require 5 to 8 c.c. of 5 per cent. $CaCl_2$ solution). The liquid is filtered and the filtrate is evaporated to syrup-like consistence (to about 20 c.c.) on a water-bath and, while warm, about 50 c.c. alcohol (95 per cent.) are added.

The liquid is stirred with a glass rod until a precipitate is formed. (The precipitate may form only after long stirring).

The precipitate is collected on a filter and the dish and the filter are washed with about 10 c.c. alcohol (in 2 or 3 portions), collecting the wash-alcohol with the filtrate. (The precipitate may be thrown away.) The beaker containing the filtrate is covered and allowed to stand twenty-four hours in a cool place.

A precipitate separating after the liquid has stood about twenty-four hours, is collected on a small filter and washed with about 10 c.c. of alcohol (in 2 or 3 portions), and the washings collected with the filtrate.

The filtrate is concentrated by evaporation to about 60 c.c., and, when cold, 1 c.c. of acid free zinc chloride solution of about 1.2 specific gravity is added.

The liquid is stirred with a glass rod until a precipitate (cloudiness) begins to appear. (The precipitate may appear only after long stirring.) The beaker is then covered and allowed to stand two or three days in a cool place to permit crystallization to occur. Creatinine zinc chloride separates in crystalline tufts or rosettes.

The precipitate of creatinine zinc chloride is collected on an equipoised filter (using the filtrate, if necessary, to transfer the precipitate from the beaker).

The precipitate on the filter is washed with alcohol (2 or 3 c.c. at a time) until the filtrate is colorless and free from chlorine (test last portions of the filtrate coming from the funnel with $AgNO_3$). The precipitate is dried on the filter at a temperature of 100° C. and, when dry, weighed.

Every 100 parts $(C_4H_7N_3O)_2ZnCl_2$ contain 62.432 parts creatinine.

Then,

100 : 62.432 :: weight of precipitate : x = quantity of creatinine in the 300 c.c. urine employed.

To obtain the percentage of creatinine divide the result by 3, because 300 c.c. of urine were originally employed.

The creatinine may be obtained from the creatinine zinc chloride by dissolving the latter in hot water and boiling it from one-quarter to one-half hour with freshly precipitated, well-washed plumbic hydroxide (prepared by precipitating

plumbic acetate with ammonium hydroxide) or with basic plumbic carbonate (prepared by precipitating plumbic acetate with sodium carbonate). When cool, the liquid is filtered and the filtrate decolorized by warming it with animal charcoal. The filtrate from the animal charcoal is evaporated to dryness on a water-bath. The residue, in addition to creatinine, contains creatine, which has resulted from the breaking up of some of the creatinine in the boiling with plumbic hydroxide. To separate them the residue is treated with cold concentrated alcohol, which dissolves the creatinine and leaves the creatine undissolved. The liquid is filtered and the filtrate is allowed to evaporate at ordinary temperature. Creatinine will separate from the liquid in crystalline form.

SUGARS.

1. **Glucose group:** $C_6H_{12}O_6$.

   Dextrose (polarizes light to the right).
   Lactose.
   Grape sugar.
   Lævulose (polarizes light to the left).
   Fruit sugar.
   Mannitose.
   Inosite.
   Sorbine.
   Etc.

Glucoses, by the action of a ferment, are converted into alcohol and carbon dioxide.

$$C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2$$

Diastase, a ferment, converts starch ($C_6H_{10}O_5$) into dextrose ($C_6H_{12}O_6$).

Starch warmed with dilute sulphuric acid or dilute hydrochloric acid is converted into dextrose (glucose).

$$3C_6H_{10}O_5 + 3H_2O + (H_2SO_4)_x = 3C_6H_{12}O_6 + (H_2SO_4)_x$$

Dextrine may be formed at the same time.

$$3C_6H_{10}O_5 + H_2O + (H_2SO_4)_x = C_6H_{12}O_6 + \underset{\text{Dextrine.}}{2C_6H_{10}O_5} + (H_2SO_4)_x$$

Dextrine is a gummy substance.

2. **Saccharose group:** $C_{12}H_{22}O_{11}$.

Saccharose (cane sugar).
Milk sugar.
Maltose.
Etc.

Cane sugar, $C_{12}H_{22}O_{11}$, warmed with dilute sulphuric acid is converted into glucose and lævulose, the mixture of the two latter is termed fruit sugar.

$$C_{12}H_{22}O_{11} + H_2O + (H_2SO_4)_x = \underset{\text{Glucose.}}{C_6H_{12}O_6} + \underset{\text{Lævulose.}}{C_6H_{12}O_6}$$

3. **Amylose group:** $C_6H_{10}O_5$.

Starch.
Dextrine.
Cellulose.
Arabinose.
Etc.

According to some investigators, the formula for starch, instead of $C_6H_{10}O_5$, should be $3C_6H_{10}O_5 = C_{18}H_{30}O_{15}$.

Other investigators suggest as the most probable formula $4C_6H_{10}O_5 = C_{24}H_{40}O_{20}$.

Starch is not determined directly, but is converted into dextrose (glucose) by being warmed with dilute sulphuric acid or dilute hydrochloric acid. The quantity of starch is calculated from the quantity of dextrose (glucose) produced.

ABNORMAL CONSTITUENTS OF URINE.

The substances occurring in the urine only pathologically, or only very seldom in soluble form, which need be considered are as follows:

Albumin; globulin; hemialbumose; peptone; mucin; glucose; milk sugar; inosite; dextrin; bile acids; bile coloring-matters; blood coloring-matter; urorubrohæmatin and urorubrofuscin; melanin; leucin; tyrosin; allantoin; fat, lecithin, and cholesterin; acetone and alcohol; Baumstark's substance, $C_3H_8N_2O_8$; urocaninic acid; hydrogen sulphide; diacetic acid; homogentisic acid $C_8H_8O_4$.

The abnormal constituents of the urine occurring most frequently, and of the greatest importance to the physician, are *glucose* and *albumin*.

DIABETIC URINE.

Urine containing glucose is diabetic urine.

Synonyms of glucose: dextrose, grape sugar, diabetic sugar.

Glucose is an abnormal constituent of urine.

It is not contained in normal urine, although Pavy claims that it is present in minute quantity in all urine.

Diabetic urine is generally paler in color than normal urine and has a sweet taste.

The quantity of urine voided in diabetes mellitus is usually greater than in health, and the urinous odor of normal urine is generally absent.

Its specific gravity, as a rule, is higher than that of normal urine (1030–1050, and even 1060).

Glucose may be present, varying from a trace up to 10–12 per cent., and even 14 per cent.

When diabetic urine is exposed to the air for a time, at not too low temperature, the surface often becomes covered with a scum or mould (fungus), due to the multiplication of cells of the torula cerevisiæ, originating from cells derived from the air.

A scum may also form on urine not containing glucose, but the scum in such case is not due to the torula cerevisiæ.

QUALITATIVE TESTS FOR GLUCOSE IN URINE.

1. **Moore's test:** To the urine contained in a test-tube about one-fourth its volume of sodium or potassium hydroxide solution (about 10 per cent. strength) is added and the liquid is boiled. If glucose be present the liquid will become brown or black in color, depending upon the quantity of glucose.

Delicacy of the test, 0.3 per cent. glucose.

The test is unreliable, because normal constituents of the urine, particularly mucin, may give a similar coloration.

2. **Johnson's picric acid test**: Picric acid (carbazotic acid, trinitrophenol), $C_6H_2(NO_2)_3OH$, is a derivative of carbolic acid (phenol), C_6H_5OH.

A few drops of a saturated aqueous solution of picric acid and sufficient sodium hydroxide (NaOH), or KOH solution, to render the urine alkaline are added to the urine and the liquid is warmed.

If glucose be present the liquid will become claret-red in color, owing to the production of picramic acid ($C_6H_2(NO_2)_2NH_2OH$), (resulting from the replacement of one of the NO_2 radicals in picric acid by the NH_2 radical,) which enters into combination with the alkali present to form a salt,—a picraminate of the alkali.

Delicacy of the test, 0.01 per cent. glucose.

This test is unreliable, because creatinine, a normal constituent of the urine, responds in a similar manner,—*i. e.*, gives a claret-red color.

3. **Boettcher's test** (bismuth test): To the urine about one-fourth its volume of sodium or potassium hydroxide solution is added. A portion of bismuth oxynitrate (subnitrate) about the size of a mustard-seed is then added and the liquid is boiled.

If glucose be present the bismuth oxynitrate will be reduced to metallic bismuth, coloring the liquid first gray and then black, or the reduced metallic bismuth may settle in the bottom of the tube.

Delicacy of the test, 0.4 per cent. glucose.

Albumin must be removed before employing this test.

Albumin interferes with this test by producing a similar change of color, due to the formation of black bismuth sulphide (Bi_2S_3). The albumin undergoes decomposition when heated with the caustic alkali, forming sodium sulphide (Na_2S) (the sulphur being derived from the albumin), which acts on the bismuth compound to form bismuth sulphide.

4. **The fermentation test**: Depends upon the breaking up of glucose into alcohol and carbon dioxide by the action of a ferment,—*i. e.*, yeast.

$$C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2$$

A test-tube is filled with the urine, and a drop or two of brewer's yeast or a piece of compressed yeast about the size of a pea is added. The tube is inverted over some of the same urine contained in a dish, and stood aside for six or eight hours in a place where the temperature is between 70° and 100° F.

If sugar be present it will undergo fermentation with the evolution of carbon dioxide, which will collect in the upper part of the inverted tube.

To prove that the gas in the tube is carbon dioxide, a piece of sodium hydroxide is inserted in the tube, the opening of the tube is closed with the thumb, and the tube agitated. The carbon dioxide will be absorbed by the sodium hydroxide, forming sodium carbonate.

As yeast itself may give off gas, a control experiment may be performed by testing the yeast in the same manner, but employing water in the test-tube in the place of urine.

Delicacy of the test, 0.4 per cent. glucose.

The quantity of carbon dioxide evolved from 0.4 per cent. of glucose is just sufficient, at ordinary temperature, to saturate the water in which it is contained and, therefore, will not appear as gas in the upper part of the tube, hence the delicacy of the test, under ordinary conditions, is 0.4 per cent. of glucose.

5. **Trommer's test:** Depends upon the reduction of cupric oxide (CuO) in alkaline solution by glucose to red cuprous oxide (Cu_2O) or yellow cuprous hydroxide ($Cu_2(OH)_2$).

Albumin must be removed from the urine before making Trommer's or Fehling's test.

To about 5 c.c. of the urine about one-fourth its volume of sodium or potassium hydroxide solution is added. Then, *drop by drop*, a solution of cupric sulphate (about 10 per cent. solution) is added and the liquid is *agitated* until the bluish-white precipitate of cupric hydroxide ($Cu(OH)_2$) which first appears ceases to be dissolved and the liquid presents a slightly turbid or opaque appearance. If on the addition of cupric sulphate the bluish-white precipitate of cupric hydroxide should dissolve on agitating the liquid and impart a *purplish* color to the liquid instead of a pure blue, it may be taken as a fair indication of the presence of glucose. The liquid is heated and

if glucose be present the cupric oxide will be reduced to red or brownish-red cuprous oxide or yellow cuprous hydroxide.

Delicacy of the test, 0.01 per cent. of glucose in the urine.

The cupric sulphate solution must never be added to urine containing sodium hydroxide *while hot*, or black cupric oxide will be produced.

Uric acid has the property of reducing cupric oxide in alkaline solution to cuprous oxide.

Creatinine has the property of reducing cupric oxide to cuprous oxide and redissolving the latter.

If on the addition of one drop of cupric sulphate solution to the urine rendered alkaline, and agitating the liquid, the bluish-white precipitate of cupric hydroxide is dissolved, the presence of glucose may be inferred.

If the liquid is heated to the boiling-point, and glucose be present, cuprous oxide should appear. The test, however, may fail to give visible cuprous oxide, or cuprous hydroxide even though glucose be present.

In such a case several drops (3 to 6) of cupric sulphate solution are added to a fresh mixture of urine and sodium hydroxide, and the liquid agitated. If the bluish-white precipitate is dissolved the addition of the cupric sulphate is continued until the liquid presents a slightly turbid or opaque appearance. On heating the liquid, if glucose be present, a precipitate of cuprous oxide or hydroxide should appear.

If the bluish-white precipitate of cupric hydroxide produced on adding a few drops of cupric sulphate solution does not wholly dissolve, and on heating the liquid cuprous oxide or hydroxide fails to appear, it may be concluded that glucose is not present. Its presence, however, is to be suspected when the specific gravity of the urine is high and the cupric hydroxide is dissolved.

If the cupric hydroxide is dissolved, and heating the liquid fails to give cuprous oxide or hydroxide, glucose may still be present, especially if the urine possesses a high specific gravity.

A. If more than 5 to 10 drops cupric sulphate solution are required to produce the turbidity, a fresh portion of the urine is diluted with 4 or 9 volumes of water and Trommer's test (adding sodium hydroxide and 5 to 10 drops of cupric

sulphate) is applied. If the cupric hydroxide wholly dissolves and, on heating, unsatisfactory results are obtained, a portion of the fresh urine is more largely diluted with water, and the test again applied.

B. If the results obtained by the foregoing methods are not satisfactory, as may be the case even when glucose is present in large quantity, a portion of Fehling's solution is diluted with about 4 volumes of water, heated to the boiling-point, and several drops of the suspected urine added to the hot diluted Fehling's solution. This test may fail even when glucose is present, but the results are generally satisfactory.

C. If the results are still unsatisfactory, the urine is passed through animal charcoal and the filtrate tested for glucose.

D. **Bruecke's lead process** for the removal of interfering substances from the urine.

If the results are unsatisfactory, after having followed the foregoing directions, the urine is examined by the lead process.

Lead process: 50 c.c. of the urine are treated with 60 c.c. of about 10 per cent. solution of commercial plumbic acetate, which precipitates the sulphates, phosphates, carbonates, coloring-matter, and some of the uric acid and creatinine. The liquid is filtered and the precipitate is washed once or twice with water. Excess of ammonium hydroxide, which precipitates the glucose in combination with lead as plumbic saccharate $(PbO)_3(C_6H_{12}O_6)_2$, is added to the filtrate.

The precipitate is collected on a filter and washed until free from ammonia. The filter paper is pierced with a glass rod and the precipitate is washed through the aperture into a beaker placed under the funnel. A stream of hydrogen sulphide (H_2S) is passed through the mixture until all of the lead is precipitated as black plumbic sulphide (PbS).

The black plumbic sulphide is filtered off and the precipitate washed once or twice with water. The filtrate, with wash-water, is evaporated to a volume of about 25 c.c., or until free from hydrogen sulphide.

a. Trommer's test is applied to 5 or 6 c.c. of the liquid.

b. Fehling's test is applied to another portion.

After subjecting normal urine to the lead process and testing the final solution, a slight reduction of the cupric oxide may

occur, due to the presence of a small quantity of uric acid which may have escaped removal in the process. This probably is the reason for the statement that glucose is a normal constituent of urine. The final solution may be allowed to stand twenty-four or forty-eight hours and the uric acid will separate in crystals, which may be filtered off and the tests for glucose applied to the filtrate, or the final solution is evaporated to dryness on a water-bath, the glucose is dissolved from the residue with alcohol, the liquid is filtered, the alcohol solution evaporated to dryness on a water-bath, the residue dissolved in about 25 c.c. of water and Trommer's and Fehling's tests applied to portions of the solution.

An inconstant loss of about 50 per cent. of the glucose occurs in the course of the lead process.

6. **Fehling's test:** Depends upon the reduction of cupric oxide (CuO) in alkaline solution by glucose to red cuprous oxide (Cu_2O) or yellow cuprous hydroxide ($Cu_2(OH)_2$).

Fehling's solution must *always* be tested before being used, by *diluting it with about four volumes of water* and heating to the boiling-point. If it has undergone decomposition, a reduction of the cupric oxide with the separation of cuprous oxide or hydroxide will occur on heating the diluted solution.

Fehling's solution which has undergone decomposition is unfit for use.

Application of Fehling's test: About 1 c.c. of Fehling's solution is diluted with about 4 c.c. of water and heated to the boiling-point, and the urine, 2 or 3 drops at a time, is added to the liquid which is heated to the boiling-point after each addition of urine.

If glucose be present reduction will occur, and a precipitate of red cuprous oxide or yellow cuprous hydroxide will be formed.

Delicacy of the test, 0.001 per cent. glucose or one part of glucose in 60,000 parts of dilution.

Often in applying Trommer's test and also Fehling's test to the urine, a brownish-red flocculent precipitate, due to phosphates, is produced. This is often mistaken for cuprous oxide and, in consequence, glucose believed to be present in the

specimen of urine. In differentiating between the precipitate caused by phosphates and the precipitate of cuprous oxide caused by the presence of glucose it may be observed, that the precipitate of phosphates is flocculent, is unevenly distributed throughout the liquid, settles to the bottom slowly and *does not form a compact layer at the bottom of the tube.* The precipitate of cuprous oxide is not flocculent, is evenly distributed throughout the liquid, settles to the bottom rapidly and *forms a compact layer at the bottom of the tube.*

A considerable number of substances occur under normal or pathological conditions in the urine which possess the property of reducing cupric oxide in alkaline solution (Trommer's and Fehling's tests), such as uric acid, creatinine, creatine, allantoin, mucin, milk sugar, pyro-catechin, hydrochinon, glycuronic acid, bile coloring-matters, and homogentisic acid.

Homogentisic acid, $C_8H_8O_4$, may be detected in urine by placing the urine in a test-tube, rendering it alkaline by the addition of sodium hydroxide, closing the tube with the thumb and shaking the tube so that the liquid is brought intimately in contact with the air in the tube. In the presence of homogentisic acid oxidation products will be formed and the liquid will become brown or black in color depending upon the quantity of homogentisic acid present. (Pyrocatechin and hydrochinon respond in a similar manner to the test.)

On the ingestion of certain compounds, such as benzoic acid, salicylic acid, balsam copaiba, oxalic acid, oil of turpentine, glycerine, chloral and chloroform, substances appear in the urine which possess the property of reducing cupric oxide in alkaline solution.

7. **Test with phenylhydrazine hydrochloride** ($C_6H_8N_2HCl$ or $C_6H_5NHNH_2HCl$) (Fischer's test, 1883): Depends upon the formation of a crystalline compound, phenylglukosazon, when phenylhydrazine hydrochloride is brought in contact with glucose. The compound usually separates in rosettes composed of yellow needle-shaped crystals, which melt at a temperature of 204° to 205° C.

Twice as much phenylhydrazine hydrochloride as will cover the end of a penknife-blade is placed in a test-tube and also into the same tube three times as much sodium acetate as will cover the end of a penknife-blade is placed. The test-tube is filled to about one-third its capacity with water, the liquid slightly warmed, and a volume of the urine equal in volume to the liquid in the tube is added.

The tube containing the mixture is placed during fifteen or twenty minutes in boiling hot water, and then in a vessel containing cold water.

If the urine contain a considerable quantity of glucose a yellow crystalline precipitate ($C_6H_{10}O_4(N_2HC_6H_5)_2$ phenylglukosazon) will almost immediately appear. Sometimes the precipitate may appear amorphous macroscopically, and in such cases should be examined microscopically.

If the urine contain a small quantity of glucose, the liquid in the test-tube should be emptied into a conical glass and the sediment examined microscopically for yellow, needle-shaped crystals. The occurrence of rather large yellow plates or brown globules does not indicate the presence of glucose.

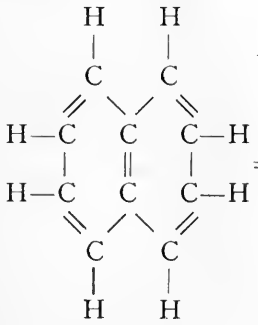
Albumin does not interfere with this test, but if it be present in large quantity it is better to remove the greater part of it by boiling and filtering.

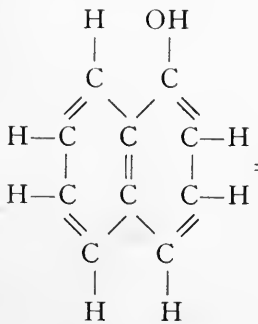
This test is unreliable as Fischer himself has found that other substances in the urine will yield yellow condensation products with phenylhydrazine.

8. **Molisch's tests :**

a. **Test with alpha-naphthol** ($C_{10}H_7OH$).

Alpha-naphthol is a derivative of naphthalene.





The urine is diluted with water (about 100 of water to 1 of urine), and to 1 or 2 c.c. of the dilute urine 2 drops of a 15 or 20 per cent. alcoholic alpha-naphthol solution are added. (The liquid may become turbid owing to the separation of some of the alpha-naphthol.) A quantity of concentrated sulphuric acid equal to

the volume of liquid in the test-tube is added, and if glucose be present a deep violet color, transitory in nature, will be produced. On diluting the liquid with water a bluish-violet precipitate will be formed.

Delicacy of the test, 0.00001 per cent. of glucose (1 part glucose in 10,000,000 parts of water).

The test is not very reliable when applied to the urine.

b. **Test with thymol** ($C_{10}H_{14}O = C_6H_3CH_3C_3H_7OH$, methylpropylphenol).

The test is performed in a manner similar to the alpha-naphthol test.

The urine is considerably diluted with water (1–100), and to 1 or 2 c.c. of the liquid 2 drops of a 15 or 20 per cent. alcoholic thymol solution are added. A quantity of concentrated sulphuric acid equal in volume to the liquid in the test-tube is added and, if glucose be present, a deep cinnabar-red color will be produced, quickly changing to ruby-red, and then to carmine. On diluting with water the carmine color remains.

Delicacy of the test, the same as the alpha-naphthol test.

The test is not very reliable when applied to the urine.

Both of Molisch's tests fail to give a reaction with urea, uric acid, hippuric acid, creatinine, allantoin, pyro-catechin, and indican.

With cane sugar, fruit sugar, and maltose both these tests give reactions similar to those with glucose.

QUANTITATIVE DETERMINATION OF GLUCOSE IN URINE.

1. **Fermentation method by loss in weight**: Depends upon the fermentation of glucose in the urine, thereby causing a loss in the weight of the urine, due to the formation of alcohol and the escape of carbon dioxide.

$$\overset{180.}{C_6H_{12}O_6} = \overset{92.}{2C_2H_5OH} + \overset{88.}{2CO_2}$$

Theoretically, in fermentation, 180 parts glucose evolve 92 parts alcohol and 88 parts carbon dioxide. Hence 1 part by weight of carbon dioxide lost is equivalent to the fermentation of 2.045 parts of glucose.

$$\underset{88}{CO_2.} : \underset{180}{C_6H_{12}O_6.} :: \underset{1}{CO_2.} : \underset{2.045}{C_6H_{12}O_6.}$$

Method: 50 c.c. of urine are placed in a small flask, and a small portion of yeast is added. The flask is closed with a perforated cork to which a small calcium chloride tube is attached (to collect moisture).

The entire apparatus is weighed and stood aside in a warm place that the glucose may ferment.

When the fermentation is completed, the cork is taken out of the bottle, and, by means of a glass tube, the carbon dioxide which may occupy the air-space in the flask, is sucked out. The cork is replaced and the entire apparatus is weighed. Each gramme lost is equivalent to 2.045 grm. glucose. To obtain the percentage of glucose, 50 c.c. of urine having been used, the result must be multiplied by 2.

Water dissolves its own volume of carbon dioxide, therefore the 50 c.c. of liquid holds in solution 50 c.c. of carbon dioxide, which is weighed with the apparatus, and thus causes an error by adding excess of weight equal to the weight of the carbon dioxide retained by the liquid.

22.32 : 44 : : 1 : 1.98

180 : 44.64 : : 1 : 0.248 Co.

To correct this error, the weight of 50 c.c. of carbon dioxide must be added to the loss of weight before multiplying by the factor 2.045.

1 c.c. CO_2 weighs 0.001971 grm.
$50 \times 0.001971 = 0\ 0985$ grm.

Example: Weight of apparatus before fermentation, 67.6 grm.
" " after " 65.6 "
2.0 " loss in weight.
+ weight of 50 c.c. CO_2 = 0 0985
2.0985

$2.0985 \times 2.045 = 4.29$ grm. glucose in 50 c.c. urine; then to obtain the percentage (quantity in 100 c.c. urine),

$2 \times 4.29 = 8.58$ per cent. glucose.

Instead of the foregoing the correction may be made by adding the number 0.4 to the apparent percentage. 0.4 grm. glucose will evolve 0.1955 grm. CO_2, or sufficient CO_2 to saturate 100 c.c. water.

	Glucose.		Carbon dioxide.		Glucose.		Carbon dioxide.
1.	180	:	88	::	0.4	:	0.1955 grm.

	CO_2.		CO_2.		CO_2.		CO_2.
2.	22320 c.c.	:	44 grm.	::	100 c.c.	:	0.1971 grm.

The 50 c.c. liquid absorbed 50 c.c. CO_2, or 50×0.001971 grm. = 0.0985 grm. CO_2, and as the calculation is made on the basis of 100 c.c. of liquid, $2 \times 0.0985 = 0.1971$ grm. CO_2 in 100 c.c. liquid, equivalent to 0.4 grm. glucose.

The first of the foregoing proportions shows that 0.1955 grm. CO_2 is evolved from 0.4 grm. glucose, and as 0.1971 grm. excess of weight of CO_2 is retained by 100 c.c. of liquid, the loss is compensated by adding the fixed number 0.4 to the apparent percentage.

Example: Weight of apparatus before fermentation, 67.6 grm.
" " after " 65.6 "
2.0 " loss in weight.

Then

2 grm. $\times$ 2.045 = 4.090 grm. $\times$ 2 = 8.18
0.4
8.58 per cent. glucose.

To obtain percentage in 100 parts by weight of urine.

Example:

Spec. grav. of urine.		Spec. grav. of water.				
1033	:	1000	::	8.58	:	X

2. **Roberts' differential density method** for the determination of glucose in urine: Depends upon the loss in specific gravity of the urine, due to the fermentation of glucose with the formation of alcohol, and evolution of carbon dioxide.

Each degree in specific gravity lost is equivalent to 1 grain of glucose in 437.5 grains (one imperial fluid ounce) of urine.

Or referred to percentage by volume:

$$437.5 : 1 :: 100 : 0.23 \text{ per cent.}$$

Therefore one degree of specific gravity lost is equivalent to 0.23 per cent. of glucose.

Method: To 60 or 70 c.c. of urine a small quantity of yeast is added and the liquid stood aside in a moderately warm place to ferment. As a control test another portion of 60 or 70 c.c. of the same urine is taken and stood aside without the addition of yeast.

When the fermentation is completed the specific gravities of the fermented and unfermented urines are taken separately. The specific gravity of the fermented urine is deducted from the specific gravity of the unfermented urine, and the result is multiplied by the factor 0.23. The result of the multiplication will be the percentage of glucose.

This method is the one most easily performed by the physician and affords fairly accurate results.

3. **Fehling's method:** Depends upon the reduction of cupric oxide in alkaline solution by glucose to cuprous oxide.

Fehling's solution is prepared so that 1 c.c. of it shall equal 0.005 grm. glucose,—*i. e.*, 0.005 grm. glucose will be required to reduce the cupric oxide in 1 c.c. of the solution to cuprous oxide.

Preparation of the solution: 5 molecules of crystallized cupric sulphate are reduced to cuprous oxide by 1 molecule of glucose.

Then

$C_6H_{12}O_6$.		$5CuSO_4 + 5H_2O$.		Glucose.		Cupric sulphate.
180	:	1247.5	::	5 grm.	:	34.652 grm.

Therefore 34.652 *grm. cupric sulphate* will be reduced by 5 *grm. glucose.*

The cupric sulphate crystals, before being weighed, should be deprived of water, held mechanically, by being crushed and dried between bibulous paper.

Fehling's Solution

1. 34.652 grms $CuSO_4 5H_2O$ in 200 C.C. H_2O
2. 173 grms $KNaC_4H_4O_6$ (Rochelle's salt) in 480 cc NaOH [of 1.14 Sp. G.]

Slowly add 1st to 2nd and dilute to 1000 cc.

Glycerine Fehling's Solution

1. 34.65 grms $CuSO_4 5H_2O$ in 200 c.c. H_2O
   Add Glycerine 11.5 c.c.
2. 130 grms KOH in 650 c.c. "

add 1st to 2nd slowly & dilute to 1000 c.c.

1 c.c. = .005 gr. Glucose

One molecule of glucose is equivalent to 5 molecules of cupric sulphate, $CuSO_4 + 5H_2O$.

One molecule of glucose is equivalent to 5 molecules of cupric oxide, CuO.

A. 34.652 *grm.* pure crystallized *cupric sulphate* are dissolved in about 200 c.c. water.

B. About 173 *grm. sodic potassium tartrate* ($KNaC_4H_4O_6$) (Rochelle salt) are dissolved in about 480 *c.c. of sodium hydroxide solution of* 1.14 *specific gravity.* The cupric sulphate solution is slowly added to the Rochelle salt solution, at the same time the solution is constantly stirred.

The bluish-white precipitate of cupric hydroxide which appears will be completely dissolved by the liquid.

The object of the Rochelle salt is to hold the cupric hydroxide in solution.

The blue liquid is diluted with water to 1000 c.c., then

1000 c.c. = 5.0 grm. glucose.
10 c.c. = 0.050 " "
1 c.c. = 0.005 " "

Fehling's solution is prone to undergo spontaneous decomposition. It may, however, be preserved so as to avoid its undergoing decomposition by keeping the cupric sulphate and Rochelle salt solutions separately and mixing equal volumes of the two solutions when needed, viz.:

34.652 grm. cupric sulphate are dissolved in water and diluted to 500 c.c. The Rochelle salt solution is also diluted with water to 500 c.c. The two solutions must be kept in separate bottles closed with rubber stoppers.

To employ the solutions: 1 volume of the cupric sulphate solution is mixed with an equal volume of Rochelle salt solution.

Example:

5 c.c. cupric sulphate solution.
5 c.c. Rochelle salt "
10 c.c. Fehling's "

The accuracy of the solution may be determined by titering it with a standard solution of glucose, prepared by dissolving 0.5 grm. glucose in 100 c.c. water.

The cupric oxide in 10 c.c. Fehling's solution should be exactly reduced by 10 c.c. of the glucose solution.

In using Fehling's solution the glucose solution must be added to the Fehling's solution, and not the Fehling's to the urine.

If the diabetic urine have a specific gravity of about 1035, it should be diluted with 4 volumes of water,—*i. e.*, 10 c.c. of urine + 40 c.c. of water.

If the diabetic urine have a specific gravity of about 1040, it should be diluted with 9 volumes of water,—*i. e.*, 10 c.c. of urine + 90 c.c. of water.

Albumin, if present in the urine, must previously be removed by coagulation and filtration as it interferes with Fehling's test.

Method: 10 c.c. of Fehling's solution (= 0.050 grm. glucose) are placed in a beaker or dish and diluted with about 40 c.c. of water and the liquid is heated to the boiling-point. If necessary, the urine should be diluted with 4 or 9 volumes of water then placed in a burette. From 0.5 to 1.0 c.c. at a time of the liquid in the burette should be run into the hot diluted Fehling's solution. Heat is applied to the Fehling's solution after each addition of urine to keep it at about the boiling-point. The addition of the urine to the hot Fehling's solution is continued until the reduction of the cupric oxide is completed. This is recognized by the complete disappearance of the blue color of the liquid.

As the point of complete reduction is approached, the precipitate of cuprous oxide will subside more rapidly, thereby allowing the easy observance of the disappearance of the blue color of the liquid.

The first titration usually gives only approximate results unless performed with the greatest care. A second titration should be made, in which the urine (from 0.5 to 1.0 c.c. at a time) is run into the hot Fehling's solution until the quantity added is about 1 c.c. less than that employed in the first titration. The addition of the urine, in small portions, is continued until the blue color of the liquid is completely discharged.

Example: Suppose the urine employed had been diluted in the proportion of 1 volume urine to 9 volumes water, and

1

460) 10.00 (.02
960
40

.23
10
.0230

8 c.c. of this diluted urine had been required to reduce the cupric oxide in 10 c.c. Fehling's solution.

One-tenth of the 8 c.c. of liquid was urine, and must have contained 0.050 grm. glucose (the quantity of glucose required to reduce 10 c.c. Fehling's solution).

	Urine.	Glucose.		Urine.	Glucose.
Then	0.8 c.c.	: 0.050 grm.	::	100 c.c.	: 6.25 per cent.

The process should be performed as rapidly as is consistent with accuracy.

If the urine mixture employed contain more than 0.5 per cent. glucose, it should be still further diluted with a measured volume of water.

If albumin be present, it must be removed before making the determination.

CLINICAL APPLICATION OF FEHLING'S METHOD.

1 c.c. Fehling's solution = 0.005 grm. glucose.

a. 1 c.c. of Fehling's solution is placed in a test-tube and diluted with 4 c.c. water (or 5 c.c. of diluted Fehling's solution, consisting of 1 c.c. Fehling's solution and 4 c.c. water).

The liquid is heated to the boiling-point and 1 c.c. of *undiluted* urine is added and the liquid is again heated.

If the blue color is entirely discharged, 0.5 per cent. or more of glucose is present.

If the blue color is not entirely discharged, less than 0.5 per cent. of glucose is present.

b. To 1 c.c. Fehling's solution + 4 c.c. water heated to the boiling-point 0.1 c.c. of the same urine is added and the liquid heated.

If the blue color is entirely discharged, 5.0 per cent. or more glucose is present.

If the blue color is not entirely discharged, less than 5.0 per cent. glucose is present.

If the color is not discharged, add another 0.1 c.c. urine and again heat the liquid.

If the blue color is entirely discharged, 2.5 per cent. or more glucose is present.

If the blue color is not entirely discharged, less than 2.5 per cent. glucose is present.

The addition of the urine 0.1 c.c. at a time, keeping account of the quantity added, and heating the liquid is continued until the blue color is discharged.

c. 1 c.c. of Fehling's solution is placed in a test-tube and diluted with 4 c.c. of water and the liquid heated to the boiling-point. The urine is added from a pipette, *a drop at a time*, (*the number of drops added is noted,*) and the liquid is heated after the addition of each drop. This addition of a drop at a time of the urine and heating the liquid after the addition of each drop is repeated until finally the blue color of the liquid has disappeared. As two drops of urine are approximately equal to 0.1 c.c., therefore, the number of drops employed divided by 2 will approximately furnish the number of tenths of a cubic centimetre employed. In calculating results the following rule should be used.

Rule: Divide the number 5 by the number of tenths of urine employed converted into whole numbers. The result will be the approximate percentage of glucose.

To obtain more accurate results, the urine should be diluted and titered in the usual manner with 10 c.c. Fehling's solution + 40 c.c. water.

Percentage amount of glucose present in urine, as indicated by the quantity of the urine required to exactly decolorize 1 c.c. of Fehling's standard solution diluted with 4 c.c. of water.

OF UNDILUTED URINE.

c.c. urine.	Glucose, per cent.	c.c. urine.	Glucose, per cent.
0.1 (5 ÷ 1)	5.0	0.4	1.25
0.12	4.2	0.45	1.10
0.14	3.5	0.5	1.0
0.16	3.1	0.6	0.83
0.18	2.7	0.7	0.71
0.2	2.5	0.8	0.62
0.25	2.0	0.9	0·55
0.3	1.66	1.0	0.5
0.35	1.4		

OF DILUTED URINE (1 to 10).

c.c. urine.	Glucose, per cent.	c.c. urine.	Glucose, per cent.
0.4 (50 ÷ 4)	12.5	2.50	2.0
0.5	10.0	2.75	1.8
0.6	8.33	3.00	1.6
0.7	7.14	3.5	1.4
0.8	6.25	4.0	1.25
0.9	5.55	4.5	1.1
1.0	5.0	5.0	1.0
1.2	4.2	6.0	0.83
1.4	3.5	7.0	0.7
1.6	3.1	8.0	0.6
1.8	2.7	9.0	0.55
2.0	2.5	10.0	0.5
2.25	2.2		

4. **Determining glucose quantitatively by means of the saccharimeter:** If light which has undergone double refraction, as in passing through a crystal of Iceland spar, is examined with an analyzer, it is found that both the ordinary and extraordinary rays are completely polarized at right angles to each other. Advantage is taken of this in the construction of the saccharimeter or polariscope.

When 20.51 grm. anhydrous glucose are dissolved in water and diluted to 100 c.c., and an observation-tube 200 mm. in length filled with the solution is placed in Laurent's saccharimeter, the ray of light will be deflected to the right 100 markings or divisions as indicated on the vernier-scale.

Hence

100 divisions on the scale = 20.51 grm. glucose.
1 division " " = 0.2051 " "

Method: 100 c.c. urine are mixed with 10 c.c. basic acetate of lead ($Pb_3O_2(C_2H_3O_2)_2$) solution, and filtered through a filter which has not been previously moistened with water. A 200 mm. (standard) observation-tube is filled with the filtrate.

The field of vision in the saccharimeter must be homogeneous in color, and the zero divisions on the vernier must correspond before the tube is placed in the saccharimeter.

The observation-tube containing the urine is placed in the saccharimeter, and the effect on the field of vision is noted. If the urine contain glucose, one-half of the field of vision will be darker than the other half. The large thumb-screw is then rotated until the field of vision again becomes homogeneous in color.

The reading on the vernier is noted. As the urine was diluted one-tenth by the lead solution, the reading, therefore, is one-tenth too low. This is corrected by adding one-tenth of the reading to the reading. The corrected reading is then multiplied by the value of each division on the vernier,—namely, 0.2051 grm.

Example:

Suppose the reading was	23.1
$\frac{1}{10}$ of the reading added to the reading .	2.31
	25.41

$25.41 \times 0.2051 = 5.211$ per cent. glucose.

If the amount of glucose in the urine be very small, a 400 mm. observation-tube may be used. In such an event the corrected reading must be divided by 2 before multiplying by 0.2051.

RELATION OF SACCHAROSES AND AMYLOSES TO GLUCOSE.

		Mol. wt.	
Glucose,	$C_{12}H_{24}O_{12}(2C_6H_{12}O_6)$,	= 360	= 100 glucose.
Saccharoses,	$C_{12}H_{22}O_{11}$,	= 342	= 95 "
Amyloses,	$C_{12}H_{20}O_{10}(2C_6H_{10}O_5)$,	= 324	= 90 "

SYNOPSIS OF ALBUMINS.

1. **Native albumins,** soluble in water.
   - *a.* Serum albumin, not precipitated by ether.
   - *b.* Egg albumin, precipitated by ether.
   - *c.* Peptones.

Albuminous bodies

1. Serum albumin (+) coag. @ 70°C
2. " Globulin (3) " @ 92°C
3. Hemialbumose
4. Mucin.

Glycocine is acetic acid in which one replaceable H of $C_2H_4O_2$ has been replaced by an amido (NH_2) atom; $-NH_2C_2H_3O_2$

In speaking of albumin we mean serum albumin

2. **Globulins,** insoluble in water, but soluble in 1 per cent. solution of sodium chloride.

a. Fibrinogen.
b. Fibrinoplastin.
c. Myosin.
d. Vitellin.
e. Crystallin.

3. **Derived albumins or albuminates,** insoluble in water or dilute sodium chloride solution, but soluble in acids or gastric juice.

a. Acid albumin (syntonin).
b. Alkali albumin.
c. Casein.
d. Fibrin.
e. Coagulated albumin.
f. Amyloid substance.

ALBUMIN IN URINE.

The albumin in urine in Bright's disease is serum albumin. Serum globulin in small quantity may also be present.

The principal forms of albumin present in urine are serum albumin, globulin, hemialbumose and nucleo-albumin (mucin).

The quantity of albumen varies from a slight trace to 3 per cent.

Albuminous urine is usually pale in color and has a low specific gravity (1006 to 1014).

QUALITATIVE TESTS FOR ALBUMIN.

1. **By heating the urine to the boiling-point (boiling-test).**

a. If a flocculent precipitate appear, it is due either to earthy phosphates or coagulated albumin.

The warm urine in which precipitation has occurred is treated with one or two drops of nitric acid. If the precipitate be dissolved by the acid, it is composed of phosphates; if undissolved, it is albumin.

b. If no precipitate appear on boiling, the warm urine is treated with one or two drops of nitric acid, and if albumin be present it will be precipitated. If the quantity of nitric acid be either too large or too small the precipitation of the albumin may not occur.

Serum albumin and globulin respond to this test. Peptone does not.

If the precipitate separates only after cooling, it is albumose.

2. **Heller's method.**

If not clear, the urine must be filtered before making the test.

A test-tube, containing strong nitric acid to the depth of at least an inch, or better, a conical glass, containing strong nitric acid, is inclined and the urine is slowly poured down the inner side of the vessel so that it shall form a layer above the nitric acid. If albumin be present, a milky zone will be produced at the point of contact of the two liquids.

Delicacy of the test, 0.0025 per cent. albumin.

If the urine contain excess of urates a zone, due to the separation of uric acid, may be formed. This zone is brownish-red in color (the albumin zone is white), and forms, not *at* the point of contact of the urine and acid, but some distance above the point of contact of the urine and acid. The under part of the zone is not so sharply defined as the albumin zone. If in doubt, dilute the urine nearly one-half with water and repeat the test. In the diluted urine uric acid will not interfere.

In the presence of albumin and excess of urates two zones will be produced,—the uric acid zone above the albumin.

On the administration of balsams—such as copaiba—a substance (abietic acid) appears in the urine which, with nitric acid, forms a zone similar to that formed by albumin. This substance also interferes with the first test, by separating as a flocculent precipitate on boiling the urine. The precipitate is soluble in alcohol, albumin is not soluble in alcohol.

If indican be present, the urine will become violet in color at the point of contact with the nitric acid. In presence of biliary matter it will become green, and then change to red-brown.

3. **Picric acid test** ($C_6H_2(NO_2)_3OH$) (carbazotic acid) Gallipe's test).

If the urine is turbid, it must be filtered.

a. Urine to the depth of an inch is placed in a test-tube and a saturated aqueous solution of picric acid is slowly poured down the side of the tube so that it collects as a distinct layer above the urine. If albumin be present, a white zone will be produced at the point of contact of the two liquids.

b. Urine is added, drop by drop, to a saturated solution of picric acid, and, if albumin be present, a sharply defined turbidity will be produced at the point of contact of the drop of urine with the acid.

4. **Potassium ferrocyanide test:** If the urine is turbid, it must be filtered.

The urine is strongly acidulated with acetic acid (to 1 volume of urine add about $\frac{1}{4}$ volume of acid) and, without heating, 3 or 4 drops of potassium ferrocyanide solution are added.

If albumin be present, a turbidity or a flocculent precipitate, depending upon the quantity of albumin, will be produced.

Delicacy of the test, 0.0025 per cent. albumin.

Serum albumin, globulin, and albumose respond to the test, but peptone does not. Nucleo-albumin responds to the test. Nucleo-albumin may be removed by precipitating with plumbic acetate, filtering, removing the lead from the filtrate by means of hydrogen sulphide and applying the ferrocyanide test to the filtrate.

5. **Trichloracetic acid test,** $HCCl_3CO_2$ (Raabe). 5 c.c. of urine filtered through a filter, which has been previously thoroughly washed with water to remove vegetable albumin, are placed in a narrow test-tube and a piece, about the size of a large pea, of trichloracetic acid is placed in the urine. In the presence of albumin a white zone or cloud will be produced at the point where the resulting solution of acid comes in contact with the urine.

Urine containing an excess of uric acid may produce a diffuse turbidity which disappears on heating the liquid. This turbidity, due to excess of uric acid, may not occur if the urine be previously diluted with water. Nucleo-albumin (mucin) responds to the test and as nearly every normal urine

contains more or less nucleo-albumin *the test is not of much value*—unless the nucleo-albumin be removed by precipitation with plumbic acetate, etc., previous to applying the trichlor-acetic acid test.

6. **Biuret test for albumin :** The urine is rendered alkaline with sodium or potassium hydroxide, and a few drops of dilute cupric sulphate solution are added. If albumin be present, the precipitate of cupric hydroxide will dissolve on shaking and a violet-red color will be imparted to the liquid.

Delicacy of test, 0.01 per cent.

Serum albumin, globulin, albumose, and peptone respond to this test.

To distinguish between serum albumin, globulin, hemi-albumose and nucleo-albumin (mucin).

1. The cold urine is treated with an excess of acetic acid.

Serum albumin.	*Globulin.*	*Hemialbumose.*	*Nucleo-albumin. (Mucin.)*
Not precipitated.	Not precipitated.	Not precipitated.	Precipitated.

2. The urine is filtered and solution of potassium ferro-cyanide is added to the cold filtrate.

Serum albumin.	*Globulin.*	*Hemialbumose.*
Precipitated.	Precipitated.	Precipitated.

The liquid is heated to the boiling temperature.

Serum albumin.	*Globulin.*	*Hemialbumose.*
Precipitate remains undissolved.	Precipitate remains undissolved.	Precipitate dissolved.

To separate serum albumin from globulin, a slight excess of ammonium hydroxide is added to the urine to precipitate the phosphates of the alkaline earths. After the liquid has stood several hours the phosphates are filtered off and the filtrate is treated with its own volume of a saturated solution of ammonium sulphate which will precipitate the globulin leaving the serum albumin in solution.

QUANTITATIVE DETERMINATION OF ALBUMIN IN URINE.

1. **By weighing:** If the urine is turbid, it must be filtered. 50 c.c. or 100 c.c. urine, depending upon the quantity of albumin present, are warmed to about blood-heat on a water-bath, and acetic acid added, drop by drop, until the albumin separates in flocculent masses. (The acetic acid keeps the earthy phosphates in solution.) The liquid is heated to the boiling-point, and the coagulated albumin is collected on an equipoised or a weighed washed filter and washed with water (portions of 5 c.c. at a time), until a last portion of filtrate coming from the funnel fails to respond to the test for a chloride with argentic nitrate, or a drop of the filtrate fails to leave a residue when evaporated on platinum foil. The coagulated albumin is washed while on the filter with alcohol, the two (equipoised) filters separated and dried at a temperature not above 100° C., and weighed.

The weight of albumin obtained is the quantity in the volume of urine employed.

2. **Esbach's method:** Depends upon the precipitation of albumin by picric acid.

The quantity of albumin is determined by measuring the height of the precipitate in a specially graduated tube, Esbach's albuminimeter.

The picric acid solution is prepared by dissolving 10 grm. picric acid and 20 grm. citric acid in 900 c.c. hot water, and, after cooling, diluting the solution to 1000 c.c.

The albuminimeter-tube is filled to the mark U with urine, and upon this the picric acid solution is poured until the level of the liquid reaches the mark R. The opening of the tube is closed with the thumb, and the liquids are mixed by inverting the tube several times.

The tube is closed with a rubber stopper and stood aside for twenty-four hours. The depth of the sediment is ascertained by observing where the top of the sediment comes in contact with a line on the scale.

The figures on the scale represent the number of grammes of albumin in 1000 c.c. urine.

The results are not absolutely accurate because of the precipitation of creatinine by picric acid, but sufficiently accurate for clinical purposes.

QUALITATIVE TESTS FOR BILIARY COLORING MATTER IN URINE.

1. Urine containing biliary coloring matter when violently shaken in a test-tube yields a yellow foam; normal urine yields a white foam.

2. The urine is filtered through a white filter paper. The filter paper is taken from the funnel and unfolded and spread on a large porcelain dish or plate. Slightly yellow, strong nitric acid is dropped on the filter. If unaltered biliary coloring matter is present, concentric rings of color will be produced where the drop of nitric acid comes in contact with the filter. The colors from the centre to the periphery are red, violet, blue, and green. Albumin in the urine interferes with this test. It may be removed by boiling and filtering. Small quantities of biliary coloring matter may be precipitated with the albumin but may be recovered by extracting the coagulated albumin with chloroform, evaporating the solution to dryness in a porcelain dish and applying the nitric acid test directly to the residue.

3. The urine is treated with a few drops of sodium hydroxide, to render it slightly alkaline, and calcium chloride is added in excess. The precipitate is collected on a filter. (The precipitate from icteric urine is yellow; from normal urine, white.) When all of the liquid has passed through, the filter is unfolded and spread on a large porcelain dish or plate and slightly yellow, strong nitric acid is dropped on the filter. If unaltered biliary coloring matter is present, concentric rings of color will be produced where the drops of nitric acid come in contact

Blood.

Specimen of H. peroxide used to det. presence of blood add 5 minims of Benzidine to urine. If bloody a pink color appears. $K_2Cr_2O_7$ gives greenish blue with same test

Nitro prussid of Sodium = Na_2NOCy_5Fe

K_2I in urine is det. by addition of HNO_3 & then a little chloroform which will separate as a pinkish ring.
May be det. volumetrically by comparing with standard tubes.

diacetic
$C_4H_6O_3$ is derived from 2 mol. of $C_2H_4O_2$

thus:- $C_2H_4O_2 + C_2H_4O_2 = H_2O + C_4H_6O_3$

acetone
$C_4H_6O_3 = CO_2 + C_3H_6O$

Acetone in urine comes from di-acetic acid or else from alcohol

---

In 1821 Hg & Pb first found in urine.

with the filter. The colors from centre to periphery are red, violet, blue, and green.

Acetone, C_3H_6O. **Detection in urine:** About 600 c.c. of urine are acidulated with acetic acid (about 2 c.c. of acid to every 100 c.c. of urine), and distilled until about 50 c.c. of distillate are collected in the receiver. To this distillate a few drops of a dilute solution of sodium hydroxide are added and then a slight excess of concentrated solution of iodo-potassium iodide (iodine dissolved in potassium iodide solution) is added. In the presence of acetone, the solution, after the lapse of a little time, becomes slightly yellow in color and, after standing some time longer, a yellow, crystalline precipitate of iodoform, which may be recognized by its peculiar odor, appears.

Diacetic acid, $C_4H_6O_3$. **Detection in urine:** Urine containing an appreciable quantity of diacetic acid when treated with a solution of ferric chloride becomes claret red in color. The substances which appear in the urine after the ingestion of antipyrin, kairin, and thallin also produce a claret-red color with ferric chloride.

TOXICOLOGY.

A **poison** is any substance which, when taken into the body and either being *absorbed* or by its *direct chemical action* upon the parts with which in contact, or when applied *externally and entering the circulation*, is capable of producing deleterious effects.

Poisons vary greatly in regard to their toxic action. Thus, *aconitine*, one of the most active poisons, has proved fatal to an adult in the dose of $\frac{1}{16}$ grain. The activity of *aconitine* is about *seven times greater than* that of *strychnine*, and about *forty times greater than* that of *arsenic*. The activity of poisons may be expressed by their fatal toxic action per kilogramme of body weight as follows:

a. **Aconitine,** 0.056 mgrm. per kilo., or about 1 to 18,000,000 parts of body weight.

b. **Strychnine,** 0.400 mgrm. per kilo., or about 1 to 2,500,000 parts of body weight.

c. **Arsenic,** 2.24 mgrm. per kilo., or about 1 to 500,000 parts of body weight.

CAUSES WHICH MODIFY THE EFFECTS OF POISONS.

a. **Idiosyncrasy,** or a peculiarity of constitution, may variously modify the effects of poisons.

b. **Habit** may render certain poisons harmless in doses which to most persons would prove rapidly fatal.

c. **Disease:** In certain diseased conditions of the system there is a diminished susceptibility to the action of certain poisons, whilst in others there is an increased susceptibility, even to the action of the same substance.

d. **Condition of the stomach:** The presence of another substance or poison; sleep.

Sleep:- Symptoms may be delayed by it

CLASSIFICATION OF POISONS.

a. **Irritant poisons,** as a class, produce irritation and inflammation of the stomach and bowels, attended or followed by intense pain in these parts, tenderness of the abdomen, and violent vomiting and purging, the matters evacuated being often tinged with blood.

The irritant poisons may be divided into three sections,—namely, mineral, vegetable, and animal.

b. **Narcotic or cerebral poisons** are such as act principally on the brain and spinal marrow, more especially on the former.

c. **Narcotico-irritants** partake, as indicated by their name, of the action of both the preceding classes.

SOURCES OF EVIDENCE OF POISONING.

1. **Evidence from symptoms.**

a. The symptoms occur suddenly, and soon after the taking of some solid or liquid.

b. The symptoms rapidly run their course.

Adder .0021; Kilo = 1:500,000,000.
Cobra .0000079; " = 1:12,500,000,000.

$HCN + 2H_2O = NH_4CHO_2$ formula of NH_4

Cranberry contains large quantities of Tannic acid and is a good antidote for Strychnine and Morphine

---

Detection of Poisons (metallic) in flour etc. add to flour a little water making a paste. Then a few drops HCl (1/7 vol) heat. Then place a little bright Cu foil in substance. As. Sb. Hg will collect on foil.

Hcy, a very rapid poison, 1-2 Minutes upto 1½ hr
Muscarine acts quickly ~~in~~ in 5 min to 5 Minutes
Aconitine acts in 8 minutes to 4 days.
Strychnine " " 10 " 4 to 9 hrs.
As_2O_3 " " 20 " indefinite

We have no chemical Test for Aconitine we depend on Physiological Tests.
We have no Test for Digitalin
Honey sometimes contains a poison
We have no specific test for Morphine. We have tests common to Morphine & other substances

Mercuric chloride with Nicotine gives a crystalline ppt.

2. **Evidence from post-mortem appearances.**

a. The *irritant* poisons, as a class, usually produce irritation and inflammation of one or more portions of the alimentary canal, the effects being sometimes confined to the stomach, while at other times they extend to a greater or less degree throughout the entire canal.

b. Narcotic poisons, in some instances, produce more or less distension of the veins of the brain, but in others they leave no marked morbid appearances, and in none are the appearances peculiar.

c. Narcotico-irritants partake, in the nature of their effects, of both the preceding classes.

APPEARANCES COMMON TO POISONING AND DISEASE.

a. **Redness** of the stomach and intestines as the effect of poisoning cannot in itself be distinguished from that arising from natural disease.

b. **Softening** of the stomach is another appearance which may give rise to embarrassment. When due to the action of poison, it is usually accompanied by other appearances which readily distinguish it from the effects of ordinary disease or post-mortem changes.

c. **Ulceration** and **perforation** of the stomach are not unfrequently produced by corrosive poisons, but they, especially the latter, are rarely met with as the result of the action of the simple irritants. As the effect of natural disease or post-mortem action they are not uncommon.

d. **Points to be observed in post-mortem examinations:** All investigations of this kind should be made in the presence of the proper law officer; and it is well for the examiner to have the assistance and corroboration of another physician. All appearances observed, whether abnormal or otherwise, should be fully written down at the time of their observance.

All the organs and blood removed for the purpose of examination should be collected in **separate,** *new, clean glass vessels, great care being taken that none of the reserved substances at any time be brought in contact with any substance that afterwards might give rise to suspicion. Before passing out of the sight*

of the examiner, the bottles should be securely sealed and fully labeled. They should then be retained in his sole possession until delivered to the proper legal official.

3. **Evidence from chemical analysis.**

a. In most charges of poisoning the final issue depends upon the results of the chemical analysis. In fact, in many instances in which the evidence from symptoms, post-mortem appearances, and moral circumstances is very equivocal or in part wanting, a chemical examination may at once determine the true cause of death.. It must be remembered, however, a person may die from the effects of poison and not a trace of its presence be discoverable in any part of the body; while, on the other hand, the mere discovery of a poison in the food or drink taken, or in the body after death, is not in itself positive proof that it occasioned death.

b. **Substances requiring analysis:** The substances that may directly become the subject of a chemical analysis in a case of suspected poisoning are: the pure poison in its solid or liquid state; suspected articles of food or medicine; matters ejected from the body by vomiting or purging; the urine; suspected solids found in the stomach or intestines after death; the contents of the stomach or bowels; any of the soft organs of the body, as the liver, spleen, etc., and the blood.

There are some poisons for which no definite chemical test is known.

Some poisons are detected by a combination of tests, and others by a single test or tests.

4. **Limit of tests.**

1 part of arsenic may be detected in 5,000,000 parts water.

Strychnine, $\frac{1}{100000}$ of a grain.

Hydrocyanic acid, $\frac{1}{100000}$ of a grain.

5. **Limit of recovery.**

6. **Failure to detect a poison.**

Numerous instances of poisoning are reported in which persons died from the effects of poison and none was discovered by chemical analysis in the body after death. This result has most frequently been observed in poisoning with organic substances, but it has happened when mineral poisons,

Arsenic may be dispensed throughout the
post mortem
body by imbibition after death.
1

County Commissioner can authorize
a post-mortem examination.

and even those which are most easily detected by chemical tests, had been taken in large quantity.

A failure of this kind may be due to any of the following circumstances: 1. The poison may have been one of the organic poisons, which cannot at present be recognized by chemical tests. 2. The quantity present in the part examined may have been so minute as under the circumstances not to admit of recovery, or at least in a state sufficiently pure to permit its true nature to be established. 3. The poison may have been removed from the stomach and intestines by vomiting and purging or by absorption. 4. The absorbed poison may have been carried out of the system with the excretions. 5. If volatile, like hydrocyanic acid and some few other poisons, it may have been dissipated in the form of vapor. 6. It may have undergone a chemical change in the living body, or, especially if of organic origin, have decomposed in the dead body if far advanced in putrefaction.

7. **Caution regarding the purity of reagents.**

8. **Preservation of chemical results and material.**

9. **Duties and rights of experts.**

An expert cannot be compelled to make a post-mortem examination or chemical analysis.

Having made the examination, the knowledge acquired is common property.

The expert should be cautious in expressing opinions before the case is called for trial.

ARSENICUM.

Atomic weight, 75.

In its pure state arsenicum has a steel-gray color, a bright metallic lustre, and has a crystalline structure. In dry air it remains unchanged, but in the presence of moisture it slowly absorbs oxygen and assumes a dark-gray appearance. It volatilizes at a temperature of 110° C. (230° F.).

Metallic arsenic, when taken into the stomach, is capable of acting as a powerful poison, but perhaps only in so far as the metal becomes oxidized and converted into arsenious acid.

Compounds of arsenicum and oxygen:

As_2O_3, arsenious oxide, molecular weight, 198.
As_2O_5, arsenic " " " 230.

Both form acids with the elements of water:

$As_2O_3 + 3H_2O = 2H_3AsO_3$, arsenious acid.
$As_2O_5 + 3H_2O = 2H_3AsO_4$, arsenic acid.

Arsenious oxide (As_2O_3) is readily obtained by volatilizing metallic arsenicum in a free supply of air.

It is found in commerce either as a white or dull white, opaque powder, or in the form of large, hard masses.

Symptoms of poisoning with As_2O_3: These are subject to great variation. Sooner or later after a large dose of the poison has been swallowed there is usually a sense of heat and constriction in the throat, with thirst, nausea, and burning pain in the stomach. The pain becomes excruciating, and is attended with violent vomiting and retching; the matters vomited present various appearances, being sometimes streaked with blood, and at other times of a bilious character; the pain in the stomach is increased by pressure. As the case progresses the pain extends throughout the abdomen, and there is generally severe purging and tenesmus; the matters passed from the bowels not unfrequently contain blood. The thirst usually becomes very intense; in some instances there is great difficulty in swallowing. The features are collapsed and expressive of great anxiety; the pulse is quick, small, and irregular; the eyes red; the tongue dry and furred; the skin cold and clammy, but sometimes hot; the respiration difficult; and sometimes there are violent cramps of the legs and arms. The urine is frequently diminished in quantity and its passage attended with great pain. Stupor, delirium, paralysis, and convulsions have also been observed. In many cases death takes place calmly, and the intellectual faculties remain clear to the last.

Fatal quantity: 2 grains taken in divided doses during a period of five days have proved fatal. On the other hand, 2 ounces have been ingested and recovery occurred in six hours.

When fatal: Usually in twelve to twenty-four hours, although in three cases death occurred in two hours, and in one case in twenty minutes.

Antidote: Hydrated ferric oxide (ferric hydroxide) is the most important chemical antidote.

The antidotal action of this substance is due to its forming an insoluble compound, ferric arsenate, with the arsenious oxide.

Thus,

$$2Fe_2O_3 3H_2O + 2H_3AsO_3 = Fe_3 2AsO_4 + FeO + 9H_2O$$

The antidote should be given in its *moist* state and administered in large excess. The antidote has no action on arsenious oxide in its solid state, but only when in solution.

Hydrated ferric oxide (ferric hydroxide) may readily be prepared by treating ordinary tincture of ferric chloride with slight excess of ammonia, collecting the precipitate on a muslin strainer, and washing it with water until it no longer emits the odor of ammonia. A tablespoonful or more of the moist magma, mixed with a little water, may be given at a dose. The antidote should always be freshly prepared.

The ordinary magnesia of the shops may be used to add to the tincture of ferric chloride instead of ammonia, and the mixture containing excess of magnesia and hydrated ferric oxide may be administered at once without filtering.

Antiseptic properties of arsenic: The preservative power of arsenic when brought in direct contact with animal textures is well known: and the poison seems to exert a similar action when carried by means of the circulation to the different tissues of the body. The bodies, therefore, of those who have died from the effects of this poison are not unfrequently found in a good state of preservation, even long periods after death.

Solid arsenious oxide. Tests.

a. It is volatile at a temperature of 138° C. (280° F.).

b. When heated on charcoal in the reducing flame it is dissipated in the form of white fumes, and emits a garlic-like odor.

c. When heated in a reduction-tube, arsenious oxide volatilizes without fusing, and recondenses in the cooler portion of the tube in the form of minute, octahedral crystals.

d. When heated in a reduction-tube containing a small piece of ignited charcoal, the arsenious oxide is volatilized and deoxidized in its passage over the ignited charcoal, and deposits in the cooler portion of the tube as a sublimate of metallic arsenic.

A similar reaction occurs when arsenious oxide is heated in a tube with a perfectly dry mixture of powdered charcoal and sodium carbonate.

If the closed end of the tube be removed (by breaking it off) and the metallic sublimate then heated, it is readily volatilized and oxidized into arsenious oxide, which condenses in octahedral crystals.

The metallic sublimate is soluble in a solution of either sodium or calcium hypochlorite.

e. When arsenious oxide is heated in a reduction-tube with a perfectly dry mixture of about equal parts sodium carbonate and potassium cyanide, reduction will take place and a sublimate of metallic arsenic will form in the cooler portion of the tube.

f. Potassium ferrocyanide may be employed as a reducing agent instead of potassium cyanide. The arsenious oxide is mixed with about 6 or 8 times its volume of dry potassium ferrocyanide, and heated in a tube as in the preceding test.

Solutions of arsenious oxide. Tests.

a. **Ammonio-silver nitrate test:** Ammonio-silver nitrate throws down from aqueous solutions of arsenious acid a bright-yellow precipitate of tribasic silver arsenite (Ag_3AsO_3), the reaction being, perhaps,

$$2H_3AsO_3 + 6AgNH_3NO_3 = 2Ag_3AsO_3 + 6NH_4NO_3$$

The precipitate is readily soluble, forming a colorless solution, in ammonium hydroxide and in nitric and acetic acids, sparingly soluble in ammonium nitrate, and insoluble in the fixed caustic alkalies.

b. **Ammonio-copper sulphate test:** Ammonio-copper sulphate produces in solutions of arsenious acid a green,

NaOH will absorb As. from the reagent bottle.

The globules of Hg in Reinsch test are very bright & brilliant

Antimony is a white amorphous deposit

Arsenic gives an octahedral crystalline "

amorphous precipitate of copper arsenite ($CuHAsO_3$), known also as Scheele's green, the reaction being, perhaps,

$$2H_3AsO_3 + 2CuH_2O_2(NH_3)_2, (NH_4)_2SO_4 = 2CuHAsO_3 + 2(NH_4)_2SO_4 + 4NH_4OH$$

The precipitate is nearly insoluble even in large excess of the precipitant, but readily soluble in ammonia and in free acids. From very dilute solutions of the poison the precipitate does not appear of its characteristic color until the mixture has stood some time. The same precipitate is thrown down from solutions of neutral arsenites by copper sulphate alone.

c. **Hydrogen sulphide test:** Hydrogen sulphide throws down from solutions of arsenious acid, previously acidulated with hydrochloric acid, a bright-yellow, amorphous precipitate of arsenious sulphide (As_2S_3), the reaction being

$$2H_3AsO_3 + 3H_2S = As_2S_3 + 6H_2O$$

d. **Reinsch's test:** When a solution of free arsenious acid or an arsenite is strongly acidulated with hydrochloric acid, and the mixture boiled with a slip of bright metallic copper, the latter decomposes the arsenical compound and receives a coating of metallic arsenic, or an alloy of copper and arsenic (As_2Cu_5). The arsenical nature of the deposit may be shown in the following manner: the coated copper, after having been carefully washed with pure water and dried in a water-oven, is heated by means of a spirit lamp or the small flame of a Bunsen burner, in a narrow and perfectly dry and clean reduction-tube, when the arsenic volatilizes, and, becoming oxidized, yields a sublimate of octahedral crystals of arsenious oxide. This sublimate usually forms within from a quarter to half an inch above the point at which the heat is applied. When the sublimate is not exceedingly minute, it presents a well-defined ring of sparkling crystals to the naked eye.

e. **Marsh's test:** When metallic zinc is treated with diluted sulphuric acid, the hydrogen of the latter is displaced by the metal with the formation of zinc sulphate, the hydrogen displaced passing off in its free state:

$$Zn + H_2SO_4 = ZnSO_4 + H_2$$

If, however, arsenious acid or arsenic acid, or any of the soluble compounds of the metal, be present, the nascent hydrogen decomposes the arsenical compound, and, uniting with the metal, forms arsenuretted hydrogen gas (AsH_3), which is evolved in its free state.

The reaction in the case of arsenious acid is as follows:

$$3Zn + 3H_2SO_4 + H_3AsO_3 = 3ZnSO_4 + 4H_2O + AsH_3$$

Metallic zinc is placed in the flask of the apparatus and a quantity of a cooled mixture of 1 volume of pure concentrated sulphuric acid and 4 volumes of distilled water, sufficient to cover the zinc, is poured in through a funnel tube and the decomposition of the acid allowed to proceed. If the zinc should act very slowly upon the acid, as is frequently the case with the pure metal, the action may be hastened by the addition of a few drops of platinic chloride.

Should the zinc or sulphuric acid be contaminated with arsenic this will give rise to arsenuretted hydrogen. Therefore, before applying the test to a suspected solution, the purity of the materials must be fully established. For this purpose, after the apparatus has become completely filled with hydrogen and while the gas is still being evolved, the outer uncontracted portion of the reduction-tube is heated to redness for about fifteen minutes or longer. If this fails to produce a metallic deposit or stain in the contracted part of the tube, in advance of the part heated, the material may be considered free from arsenic. The purity of the materials having been thus established, it may be necessary to wash and renew the zinc, dry the tubes, and add a fresh portion of the diluted acid.

The apparatus being adjusted and completely filled with evolved hydrogen, the jet of gas, as it issues from the drawn-out end of the reduction-tube, is ignited, care being taken not to apply a light until the whole of the atmospheric air is expelled from the apparatus, as otherwise an explosion might occur. A small quantity of the arsenical solution is then introduced into the funnel-tube and washed into the flask by the subsequent addition of a few drops of the diluted sulphuric acid. The decomposition of the arsenical compound, with the evolution of arsenuretted hydrogen, will commence

Or sometimes used as a poison
Many test as follows. Take a fresh amount
of material to be tested. acidulate with HCl &
Take a piece of polished steel. heat from
hot by $Na_2(CO_3)_2$ & heat in material
a reddish or. Iron collects on steel,
This can be tested by HNO_3 & NH_4OH

immediately. The presence of the arsenuretted gas may be established by three different methods,—namely,

a. By the properties of the ignited jet.
b. By decomposing it by heat applied to the reduction-tube.
c. By its action upon a solution of argentic nitrate.

a. **The ignited jet:** As soon as the arsenical solution is introduced into the flask the evolution of gas increases. The flame of the jet will now increase in size, acquire a bluish tint, and, unless only a minute quantity of arsenic is present, evolve white fumes of arsenious oxide; so, also, the flame sometimes emits a peculiar garlic-like odor. If the white fumes be received upon a cold surface they condense to a white powder, which sometimes contains octahedral crystals. This is not a delicate method for detecting the presence of arsenic, and should never be employed to the exclusion of the following modification, viz.:

If the flame be allowed to strike against a cold body, as a piece of white porcelain, it yields a brown to black deposit of metallic arsenic.

Fallacy: Solutions of antimony, under these same conditions undergo decomposition with the production of antimonuretted hydrogen, which, like arsenuretted hydrogen, burns with the evolution of white fumes, and yields metallic deposits upon cold surfaces applied to the flame. The spots produced by arsenic are readily soluble in a solution of either sodium or calcium hypochlorite, whereas those from antimony are insoluble, or dissolve only after prolonged digestion in the hypochlorite solution.

b. **Decomposition of the gas by heat:** When arsenuretted hydrogen, as evolved in Marsh's test, comes in contact with the red-hot portion of the reduction-tube, it is decomposed with the production of a deposit of metallic arsenic in the contracted part of the tube, in advance of the flame.

Fallacy: Antimonuretted hydrogen also is decomposed under the above conditions with the deposition of metallic antimony. Antimony, however, is almost wholly deposited *before* reaching the part of the reduction-tube to which the flame is applied; when it yields deposits on both sides of the

flame, the outer one is quite near the flame. On the other hand, arsenic deposits about one-half to three-quarters of an inch in advance, or on the *outer side* of the flame, and never before reaching the part of the tube to which the heat is directly applied.

c. **Decomposition by argentic nitrate:** If the reduction-tube of the apparatus be substituted by a tube bent at a right angle, and the arsenuretted hydrogen conducted into a solution of argentic nitrate, both the gas and the silver salt undergo decomposition with the production of arsenious acid, which remains in solution, and the separation of metallic silver, which falls as a black precipitate. The reaction is

$$AsH_3 + 6AgNO_3 + 3H_2O = 6Ag + H_3AsO_3 + 6HNO_3$$

The presence of arsenious acid in the solution may be shown by the usual tests for that substance.

SEPARATION OF ARSENIC FROM ORGANIC TISSUES.

Disintegrate the organic matter by means of hydrochloric acid and potassium chlorate, under the action of heat.

The following proportions of tissue, acid, and the chlorate yield very satisfactory results:

Treat 300 grm., or 10 ounces, of the solid tissue, as of the liver, cut into very small pieces and placed in a clean porcelain dish, with a mixture of 60 c.c., or 2 fluid ounces, of strong hydrochloric acid and 240 c.c., or 8 fluid ounces, of water.

Heat the mixture on a sand-bath, and when at about the boiling temperature, add about 1 grm., or 15 grains, of powdered potassium chlorate, and repeat the addition at intervals of a few minutes, with frequent stirring, until about 6 or 7 grm., or 100 grains, have been added and the mass has become homogeneous and of a light-yellow color. During this process water should occasionally be added to replace that lost by evaporation.

The disintegrating action of this mixture is chiefly due to the free chlorine and chlorine peroxide evolved by the mutual decomposition of the chlorate and a portion of the hydrochloric acid.

$$4KClO_3 + 12HCl = 4KCl + 6H_2O + 3ClO_2 + Cl_9$$

Moderately heat the disintegrated mass until the odor of chlorine has entirely disappeared, and then allow to cool. Transfer the cooled mixture to a moistened linen strainer, and, when the liquid has all passed, wash the solids with a little warm water, the washings being collected separately. Concentrate the washings on a water-bath to a small volume, allow to cool, then add the washings to the first strained liquid, and filter the mixed liquid through paper. Any arsenic present will now exist as arsenic acid.

From the filtrate thus obtained free chlorine must be completely expelled, otherwise in the subsequent treatment of the solution with sulphurous acid the latter will be acted upon by the free chlorine resulting in the formation of hydrochloric and sulphuric acids and, consequently, the sulphurous acid would have no action upon the arsenic acid until all the free chlorine was satisfied.

$$SO_2 + Cl_2 + 2H_2O = 2HCl + H_2SO_4$$

To the liquid free from uncombined chlorine add a solution of sulphurous acid until it smells strongly of the gas (sulphurous anhydride). Any arsenic acid present will be reduced to arsenious acid, in which form the metal is more rapidly and more completely precipitated by hydrogen sulphide than when it exists in the form of arsenic acid. The reducing action of the gas may be represented,

$$H_3AsO_4 + SO_2 + H_2O = H_2SO_4 + H_3AsO_3$$

Concentrate the liquid on a water-bath to a volume twice that of the hydrochloric acid employed in preparing the mixture. In this concentrating of the liquid the sulphurous acid must be completely expelled, otherwise in the subsequent addition of hydrogen sulphide decomposition of both compounds occurs with the formation of pentathionic acid and separation of sulphur.

$$5SO_2 + 5H_2S = 4H_2O + H_2S_5O_6 + S_5$$

Allow the concentrated liquid to cool, and then filter.

Pass a slow stream of washed hydrogen sulphide through the filtrate for several hours; then gently warm it, and allow to stand twelve to twenty-four hours. Any arsenic present

will be precipitated as arsenious sulphide (As_2S_3), together with more or less organic matter and free sulphur. Should the liquid contain mercury, antimony, copper, or lead, these metals would also be precipitated as sulphides by the hydrogen sulphide. Liquids prepared as the above may yield with hydrogen sulphide a brownish or yellowish precipitate of organic matter and free sulphur, even in the absence of any metal.

Collect the precipitate on a small filter and wash, at first with water containing a little hydrogen sulphide, until the washings no longer contain chlorine (test with argentic nitrate).

Dissolve the moist precipitate on the filter with dilute ammonium hydroxide (1 to 10), which will dissolve any arsenious sulphide present, with more or less of the organic matter and free sulphur. The sulphides of mercury, antimony, copper, and lead which might be present would remain undissolved, except perhaps a slight trace of the antimony sulphide.

Collect the ammoniacal liquid, usually of a dark-brown color, in a small porcelain dish and evaporate to dryness on a water-bath, treat the residue with a small quantity of strong nitric acid, and again evaporate the liquid to dryness; repeat the operation with nitric acid, if necessary, until the moist residue has a yellow color.

Moisten the residue with a few drops of concentrated solution of sodium hydroxide and evaporate to dryness. Treat the residue with several drops of concentrated sulphuric acid, and heat the mass on a sand-bath until it becomes about dry; again treat the residue with sulphuric acid and heat in the same manner until fumes of the acid are no longer evolved.

Pulverize, if necessary, the carbonaceous residue, and boil it with a small quantity of water containing a drop or two of sulphuric acid, cool the liquid, filter off, and wash the insoluble carbonaceous residue. If in the carbonization the whole of the free sulphuric acid was expelled, the resulting solution (filtrate) will be colorless and entirely free from organic matter. Add 1 or 2 c.c. of solution of SO_2 to reduce As_2O_5 to As_2O_3, and then heat until all the SO_2 has been expelled. Concentrate the solution to a small and definite volume (say 20 c.c.), and divide it into two equal portions. Make the qualitative

Test for blood in urine - 2 samples.

To about 5 c c of urine add a few drops alcoholic solution of guaiacum and shake well mix, then add a few drops of Peroxide of Hydrogen and agitate when a purple or blueish color will be produced.

Strychnine may be removed by alcohol, $CHCl_3$ or ether. Strychnine is taken up in solution and on the membranes or on the Sty. residue is b

tests with one portion and the quantitative determination with the other portion.

With the first portion

a. Apply Reinsch's test.
b. " Marsh's "

With the second portion of 10 c.c. make the quantitative determination.

QUANTITATIVE DETERMINATION.

Acidulate the solution with a few drops of hydrochloric acid, warm to about blood-heat, and pass a stream of hydrogen sulphide through the liquid. The arsenic will be precipitated as arsenious sulphide. Collect the precipitate on an equipoised or on a weighed, washed filter, wash at first with water containing a little hydrogen sulphide, then with pure water until the washings are free from chlorine. Dry the precipitate on the filter at a temperature of about 100° C., and weigh.

The quantity of As_2O_3 corresponding to the weight of As_2S_3 obtained is determined by

As_2S_3. As_2O_3.
246 : 198 :: weight of precipitate : X = one-half, the amount of arsenic as As_2O_3 recovered from the quantity of organic tissue employed.

TESTS FOR SOME OF THE MORE COMMON ORGANIC POISONS.

Opium.

The presence of opium may be inferred by showing the presence of *meconic acid*, an organic acid peculiar to the drug. For this purpose, the aqueous extract of the substance is treated with lead acetate, which will precipitate the acid as meconate of lead. This is collected on a filter and washed, the filtrate being reserved for the examination for *morphine*. The contents of the filter are diffused in a small quantity of water, and the lead precipitated by H_2S. The filtrate from this precipitate is concentrated to a small volume and treated with a drop of *ferric chloride* solution, when, if meconic acid is present, a *deep blood-red coloration* will be produced. Limit of reaction, $\frac{1}{75000}$ solution.

Morphine.

a. **Sulpho-molybdic acid** (Froehde): The reagent is prepared by heating 1 part of molybdic acid with 100 parts of strong H_2SO_4 C. P., until complete solution has taken place. On the addition of a drop of the reagent to morphine or any of its salts in the solid state, on a porcelain test tablet, a *purple or crimson color appears immediately*, passing through various shades and finally to blue, which appears first at the margin of the mixture. Limit, $\frac{1}{100000}$ grain.

b. **Neutral ferric chloride** added to morphine or any of its salts in the solid state, produces a *deep blue color*, which is discharged by acids, alkalies and heat. Limit, $\frac{1}{10000}$ grain.

c. **Iodic acid** in strong solution, added to morphine or any of its salts in the solid state, the acid is decomposed with the liberation of iodine, forming a brown or reddish-brown precipitate. Limit, $\frac{1}{1000}$ grain.

The presence of free iodine may be shown by agitating the mixture with either, carbon disulphide or chloroform, which will dissolve the iodine and assume a purple color.

Strychnine.

a. "**Color-test**;" Strychnine or any of its salts dissolved in a drop of strong, chemically pure H_2SO_4, on a porcelain test tablet, and a minute crystal of potassium dichromate drawn through the solution by means of a glass rod, immediately produces a *characteristic succession of colors* beginning with blue, passing into purple, violet and greenish-yellow. Limit, $\frac{1}{100000}$ grain.

b. **The caustic alkalies** precipitate the free alkaloid from solutions of salts of strychnine in the form of a white powder, which soon assumes the crystalline form. Limit, $\frac{1}{5000}$ grain.

To prove the true nature of the precipitate apply the color test.

c. **Potassium dichromate** produces a bright yellow precipitate of strychnine chromate, which quickly becomes crystalline. Limit, $\frac{1}{10000}$ grain.

This precipitate treated with a drop of strong, chemically pure H_2SO_4 alone gives the color reaction.

Test for blood :- Look for Corpuscles under microscope.
Add Alcohol sol. of Picric acid then add ammonium sulphide sol. of peroxide of hydrogen agitate and if blood is present we get a blueish coloration
Hæmin crystals may be used to detect blood.
Put blood colouring matter with acetate of soda or better NH_4OH & Hæmin acid

Put blood in very small test tube and cover with glacial acetic acid and heat to boiling point then examine with microscope.

Examine morphine and with acetic acid add HNO_3 & get sol. & prop. Etc.

Errata.—Page 24, first line, read *acetic* instead of *sulphuric.*

INDEX.