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CHEMISTRY

FOR

DENTAL STUDENTS

VOLUME II

ORGANIC AND PHYSIOLOGICAL DENTAL CHEMISTRY

BY

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FOURTH EDITION, REVISED AND ENLARGED

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PREFACE TO VOL. II, FOURTH EDITION.

The increasing tendency to place Dentistry on higher professional planes necessitates a deeper study of the causes of observed conditions, or, in other words, of the relationships between systemic and oral disease. In a consideration of systemic conditions in relation to oral disease, the majority of acute diseases which constitute so large a part of the physician's study may be disregarded. On the other hand, many of the habits of life, including diet, which lead to chronic conditions, healthful or otherwise, form an important part of Physiological Chemistry from the dental standpoint. All of this means that the student of Dentistry must make a more comprehensive study of *Chemistry*.

It is the desire of the authors in this Second Volume to give to the student of Dentistry and to the dental practitioner the elements of Organic and Physiological Chemistry which are recognized to-day as essential to the scientific dentist, and which enable him at least to consider the many systemic factors which may influence oral conditions. The authors hope that this volume will not only succeed in answering questions, but will also raise many questions in the minds of its readers and suggest new possibilities of dental research.

The text contains frequent references to some of the more complete works listed below. The student is expected to have easy access to these, as well as to current dental literature, particularly to Folin's newer micro-methods for blood and urine analysis (*Journal of Biological Chemistry*). The student is urged to make use of these references in order that he may learn how to study — a more important object in any course than the mere familiarity with present-day facts and theories.

Organic Chemistry.....Norris
Organic Chemistry.....Holleman-Walker
Physiological Chemistry .....Hawk, Eighth Edition
Physiological Chemistry.....Mathews
Metabolism.....Tibbles or Taylor

H. C. S.
R. M. S.

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DENTAL CHEMISTRY

PART I.

ORGANIC CHEMISTRY.

CHAPTER I.

THE HYDROCARBONS AND SUBSTITUTION PRODUCTS.

In the present volume we are to consider briefly the organic compounds which will serve as a basis for the intelligent study of physiological chemistry, and also some which are of peculiar interest in dentistry.

We shall touch but lightly on some of the subdivisions of the subject and take up a little organic chemistry proper, a little physiological chemistry, a little pathological chemistry, and from it all pick out such facts as may help us to a better understanding of the problems of dentistry.

As in many other departments of science, absolute rules for classification are impracticable; yet we may consider in a general way that the organic compounds are those containing carbon as a molecular constituent. The old conception that the organic compound must have been produced by a vital process of some sort (animal or vegetable) is of little value unless we confine our thought to substances found in nature only.

The compounds of carbon are practically innumerable and very widely distributed, constituting the great bulk (aside from water) of all vegetable or animal substances.

The carbon compounds contain the elements carbon and hydrogen, and when these two only are present the compounds are *hydrocarbons*. Compounds containing carbon, hydrogen, and oxygen, are of more frequent occurrence, and when the

hydrogen and oxygen are present in the proportions in which they occur in water, the compound is a *carbohydrate* (with exceptions).

In the chemistry of the animal body the majority of substances which we meet contain carbon, hydrogen, oxygen, and nitrogen, and often in addition sulphur or phosphorus. Many other elements, notably the halogens, and often the metals, may be found in organic compounds.

In consideration of an organic substance, therefore, the question of its composition is the first one presenting itself.

The analysis of organic bodies may be made from two distinct standpoints: first, to determine the various substances which may be separated from a given organized body, as from some part of a plant; second, to determine the constituent elements of one of the substances so separated.

As an example of the first sort of analysis, we may find in a potato a certain basic principle (alkaloid), more or less water, and considerable starch. These may be called proximate principles, and the separation of them would be proximate analysis, while the second sort of analysis determines the composition of the starch molecule and is known as ultimate analysis.

Qualitative Tests.

Carbon. — The presence of this element may be shown by the "carbonization," or blackening, of the compound when heat is applied.

Carbon may also be demonstrated by oxidizing the compound and producing carbon dioxide, as follows:

Mix thoroughly a few grains of benzoic acid with about four times as much powdered copper oxide. Put the mixture into a glass tube about 6 inches in length and closed at one end, and shake well into the closed end of the tube. Then introduce three or four times as much coarsely powdered copper oxide and place the tube in a nearly horizontal position, tapping it to distribute powder through about half its length. Now, by means of a short rubber tube, connect a short delivery tube of glass which

dips into about 5 c.c. of lime-water contained in a test-tube. With the Bunsen burner in the hand, heat *first* the unmixed copper oxide, then gradually work toward the closed end of the tube until the whole is hot and the gas given off has produced a precipitate in the lime-water. What reactions have taken place?

Hydrogen shows itself by the production of moisture in these same tests.

Nitrogen may or may not be indicated by a preliminary test. It may be detected with certainty by either of the following methods:

(*a*) Conversion into a cyanogen compound.

By means of a burette clamp, support a test-tube in a vertical position; drop into it a piece of clean, dry sodium, and with burner in the hand melt the sodium and continue heating until vapors begin to rise. Add a very small quantity of powdered albumin and continue to heat for a minute or two. Break lower portion of tube into mortar containing 2 c.c. of alcohol. Watch for the evolution of gas. When no more gas is given off, add a little sodium hydrate solution, and filter. To the clear filtrate add 2 or 3 c.c. of yellow ammonium sulphide, and evaporate the whole to dryness over a water-bath. Dissolve residue in water which has been made slightly acid with hydrochloric acid, filter, and to the clear filtrate add a drop of ferric chloride.

Explain steps in conversion of nitrogen into ferric thiocyanate.

(*b*) Conversion into free ammonia.

Almost any nitrogenous substance may be made to evolve ammonia-gas by simply heating in a test-tube with several times its bulk of soda-lime. Test for ammonia by moistened red litmus paper or by odor. (This test is known as that of Wöhler, also of Will and Varrentrap.)

The Kjeldahl, or moist combustion, process is much employed as a quantitative method but may be used qualitatively as follows: The substance is heated in an ignition-tube with concentrated sulphuric acid till a clear (not necessarily colorless) solution is obtained. The mixture is cooled, diluted with water, an excess of caustic soda added, and heat applied. Am-

monia is then evolved, and may be detected by litmus paper or by odor.

Sulphur and phosphorus are first completely oxidized, either by fusion of the substance with alkali nitrate and carbonate, or by treatment in the wet way with fuming nitric acid or mixture of potassium chlorate and hydrochloric acid. The resulting sulphate or phosphate is detected by the usual qualitative methods.*

A sulphur test may also be made by heating the substance with a little concentrated sodium hydroxide in the test-tube. A little sodium *sulphide*, which may be detected by dropping upon a bright silver coin or by testing with lead acetate solution, will thus be formed.

Halogens. — Chlorine, bromine, and iodine cannot be detected in organic combinations by the ordinary qualitative test with silver nitrate and dilute nitric acid, but must first be converted into corresponding inorganic haloid salts. This may be done by heating the organic substance strongly with pure lime, and thus forming calcium chloride, bromide, etc., which may be dissolved in water and tested in the usual way. (See Vol. I.)

A test for chlorine or iodine may also be made by heating with copper oxide on a platinum wire in the Bunsen flame, chlorine giving first a blue and then a green color to the flame. Iodine gives green only (Beilstein).

Test for presence of C, H, N and S in dried albumin.

Test for P in casein precipitated from milk.

Test a few drops of chloroform for the presence of chlorine.

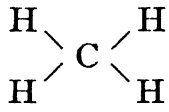
The Hydrocarbons.

The hydrocarbons are organic compounds of carbon and hydrogen only. The simplest of these is marsh gas, or methane (CH_4). The molecule of this substance consists of a single

* Qualitative test for sulphates is the production of a white precipitate, $BaSO_4$, insoluble in hydrochloric acid, upon the addition of $BaCl_2$ to the unknown.

Qualitative test for phosphates is the formation of the yellow ammonium phosphomolybdate when ammonium molybdate in nitric acid is added to the unknown.

carbon atom with each of its four points of atomic attraction (valence) satisfied by an atom of hydrogen.



If one of these four atoms of hydrogen is replaced by a chlorine atom, for instance, we have a *substitution product.* Its formula will be CH_3Cl, its name monochlor-methane or *methyl* chloride. If two molecules of methyl chloride are brought together and the chlorine removed by metallic sodium, the residual molecules (methyl radicals) will unite, forming a new hydrocarbon, as follows:

$$2\ CH_3Cl + Na_2 = 2\ NaCl + C_2H_6 \text{ (ethane).}$$

By a similar reaction we may form the third member of the series, propane, C_3H_8, from ethyl chloride, C_2H_5Cl, and sodium; the fourth member, butane, C_4H_{10}, from propyl chloride, etc. A tabulated list of the first five compounds of this series will plainly show their chemical relationship.

CH_4, methane or methyl hydride (CH_3H).
C_2H_6, ethane or ethyl hydride (C_2H_5H).
C_3H_8, propane or propyl hydride (C_3H_7H).
C_4H_{10}, butane or butyl hydride (C_4H_9H).
C_5H_{12}, pentane or amyl hydride ($C_5H_{11}H$).

Note that the various members of this series differ from one another by CH_2; that is, each higher compound contains one carbon atom and two hydrogen atoms more than its predecessor. This holds true through the series, and the compounds of this or any such series are termed homologues and the series homologous series. Note further that any member of this series (which is known as the paraffin series) may be represented by the general formula C_nH_{2n+2}. This likewise holds true throughout the series, and a compound having sixty carbon atoms will have a formula of $C_{60}H_{122}$. The first four hydrocarbons of this series are gaseous at ordinary temperatures; from C_5H_{12} to

about $C_{16}H_{34}$, the hydrocarbons are liquid; from $C_{16}H_{34}$ (melting at about 18°) up, they are solids.

The hydrocarbons of the paraffin series are known as *straight-chain* or *aliphatic hydrocarbons*, their graphic formulae consisting of " chains " of carbon atoms, as butane, $-\overset{|}{\underset{|}{C}}-\overset{|}{\underset{|}{C}}-\overset{|}{\underset{|}{C}}-\overset{|}{\underset{|}{C}}-$, as distinguished from the closed-chain or cyclic compounds as represented by the "benzene-ring " (page 60), a carbon nucleus with the carbon atoms united in a continuous *closed* chain or " cycle."

The paraffins are called saturated hydrocarbons because they are incapable of forming *addition* products by absorption of chlorine, for instance, without first giving off an equivalent number of atoms of hydrogen. This is because of the complete " saturation " or union of every carbon " bond " with some other atom.* Paraffin wax and mineral oil are mixtures of saturated hydrocarbons and resist chemical action even of strong nitric acid or sulphuric acid.

The name paraffin is derived from the two Latin words *parvus*, little, and *affinitas*, affinity.

The natural sources of hydrocarbons of the paraffin series are natural gas and crude petroleum, or rock oil. Many of these hydrocarbons exist as such in the petroleum, and some undoubtedly are produced by the heat used to effect a separation of the various compounds. This separation may be effected by distilling the oil in an apparatus similar to that pictured in Fig. 1, and is known as fractional distillation, the different hydrocarbons passing over at different temperatures. Separation by this method, however, is by no means complete, and the resulting products are themselves mixtures of hydrocarbons, and are distinguished by physical properties rather than by chemical composition.

When crude petroleum is thus distilled, the following products are obtained: first, rhigoline, which comes over at a temperature

* Notice that while addition products of saturated hydrocarbons cannot be formed, substitution products are easily possible. See page 5.

of 20° to 22° C.; then petroleum ether, or benzine, at from 50° to 60° C.; then gasoline, or naphtha, at about 75° C.; then one or two unimportant commercial products; and then kerosene, or burning oil which is obtained at 150° to 250° C. Above this, we may obtain paraffin oil or light lubricating oils; then the heavy lubricating or cylinder oils; and from the residue we obtain the solid substances known as vaseline, or petroleum jelly, and paraffin of various degrees of hardness.

The first five hydrocarbons of this series we shall consider somewhat in detail, not only because they are important and comparatively common, but also because they serve as types of all other compounds of the series, and reactions which we study with these compounds are, as a rule, general typical reactions which may be produced with other members of the series.

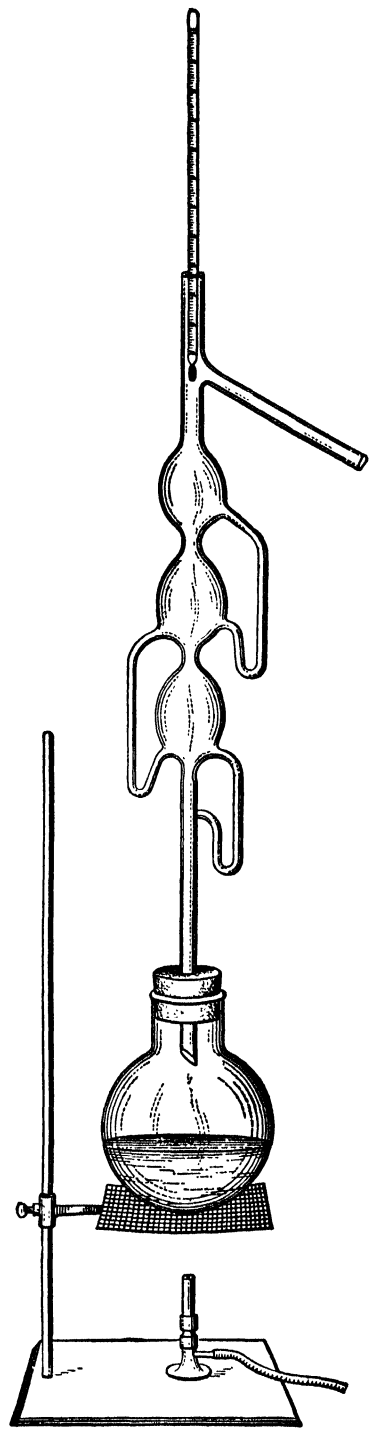


FIG 1.

Methane, CH_4, occurs as marsh gas in stagnant ponds or pools and is a constituent of "fire damp" in coal mines. It is a colorless gas, odorless when pure, and very slightly soluble in water. Methane burns in the air with the production of carbon dioxide and water

$$CH_4 + 2\,O_2 = CO_2 + 2\,H_2O.$$

It may be prepared synthetically by the direct combination of hydrogen and carbon. This reaction is usually brought about by allow-

ing hydrogen to pass over heated nickel mixed with finely divided carbon. The nickel acts as a catalyzer; without it the combination of hydrogen and carbon takes place with difficulty and only at a temperature exceeding 1100°.

Other methods of preparation are:

(a) Anhydrous sodium acetate and sodium hydroxide (marsh gas mixture*) heated together will react according to the following equation:

$$NaC_2H_3O_2 + NaOH \rightarrow CH_4 + Na_2CO_3$$

This method is based on the fact that carbon dioxide can be liberated from acetic acid by means of a strong base, and it is the method recommended for general laboratory use.

(b) Zinc methyl, a very loose chemical compound of two methyl groups and one atom of zinc, may be decomposed by the action of water, giving methane and zinc hydroxide.

$$Zn(CH_3)_2 + H_2O \rightarrow CH_4 + Zn(OH)_2$$

This method and the previous one are of particular importance, as they are general methods adapted for the preparation of any hydrocarbon of the series.

(c) Aluminium carbide treated with water reacts to yield methane and aluminium hydroxide.

$$Al_4C_3 + 12\,H_2O \rightarrow 4\,Al(OH)_3 + 3\,CH_4$$

(d) Methyl chloride, a substitution product of methane, may be reduced with nascent hydrogen.

$$CH_3Cl + H_2 \rightarrow CH_4 + HCl.$$

Ethane, C_2H_6, the second member of the series, occurs naturally in a solution in crude petroleum, and can be artificially prepared by the electrolytic decomposition of a saturated solution of potassium acetate, as follows:

$$2\,CH_3COOK = C_2H_6 + 2\,CO_2 + K_2.$$

* Marsh gas mixture also contains considerable calcium oxide to prevent too rapid action. See Appendix.

The free potassium, of course, decomposes water, liberating hydrogen gas which collects at the negative pole, and, if the solution contains sufficient potassium hydroxide, the carbon dioxide will be dissolved, allowing ethane to collect at the positive pole.

Ethane may also be made from a haloid derivative of marsh gas by the action of metallic sodium; that is, in methane we may replace one of the hydrogen atoms with iodine, forming CH_3I, methyl iodide; then, by treatment with metallic sodium, the following reaction will take place:

$$2\ CH_3I + 2\ Na = C_2H_6 + 2\ NaI.$$

This is the general method employed for building up all the higher hydrocarbons from the lower members of the series. By selecting the correct alkyl* halide, we may make any hydrocarbon from any other hydrocarbon that is a lower member of the series, by treatment with metallic sodium.

Ethane, as indicated under preparation of methane, may also be made by either method (a) substituting sodium propionate for sodium acetate or (b) substituting zinc ethyl for zinc methyl.

Ethane is slightly more soluble in water than methane. It may be condensed to a liquid at a pressure of forty-six atmospheres.

Propane, C_3H_8, also occurs in petroleum, and can be made by treating a mixture of ethyl iodide and methyl iodide with metallic sodium:

$$C_2H_5I + CH_3I + 2\ Na = C_3H_8 + 2\ NaI$$

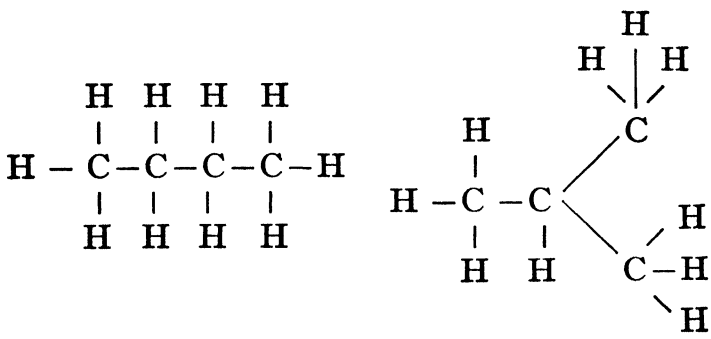
or by any of the synthetic methods before mentioned.

Butane, C_4H_{10}, is the first of the series capable of existing in two forms, isomers. The structural formulae of these two compounds are shown in the illustration of the term isomer on page 10. This compound and many of its higher homologues are of importance only in relation to some of their derivatives which will be subsequently studied.

* Alkyl — a term used to denote any hydrocarbon radical as CH_3-, C_2H_5-, C_3H_7-, etc.

Isomers. — When two or more compounds are of exactly the same molecular composition, or when two compounds have the same percentage composition, the one being a multiple of the other, the compounds are said to be isomers or isomeric compounds.

The isomerism of the first class is said to be metameric. It exists when the atoms of the several compounds are not only the same in kind, but also the same in the number of each kind. For example, $C_{12}H_{22}O_{11}$ is the formula for cane sugar; $C_{12}H_{22}O_{11}$ is also the formula for milk-sugar, and these two compounds have decidedly different properties, the difference being dependent upon the arrangement or relationship of the atoms in the molecule. Another example illustrating this difference may be found in the graphic formula for normal and isobutane, given below.



Note that each molecule has an empirical formula of C_4H_{10}; the normal compound may be represented as $CH_3.(CH_2)_2.CH_3$, the iso-compound as $CH_3.CH.(CH_3)_2$. These will be found to have quite different physical and chemical properties.

The isomerism of the second class is called polymeric; one substance is the polymer of another when the molecules are of the same percentage composition but of different molecular weights. For example, CH_2O is gaseous formaldehyde; $(CH_2O)_3$ is its polymer or polymeric form, known as paraform, a white crystalline solid.

Unsaturated Hydrocarbons.

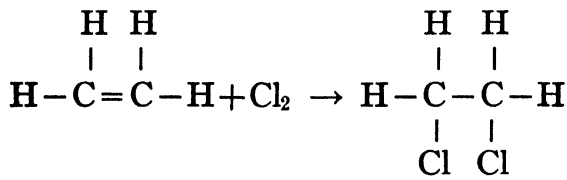
DOUBLE-BONDED HYDROCARBONS.

When a mixture of alcohol and strong sulphuric acid is heated, with the acid in considerable excess, water is withdrawn from the molecule of alcohol, and a gas, found to have the formula C_2H_4, is produced. (See Exp. 7.) The name of this gas is ethylene; it occurs in coal gas and in traces in solution in crude petroleum. It is the first of a series of hydrocarbons which contain double-bonded carbon atoms. The double bond is assumed because it is found to be impossible to produce a lower compound of this series, such as CH_2, which might be called methylene, but which would necessitate a bivalent carbon atom; also because the hydrocarbons of this series are capable of formation of addition products as well as of substitution products.

Note that the formula of ethylene does not conform to the general formula of the paraffins (C_nH_{2n+2}), but is the first member of the new series of " unsaturated " hydrocarbons; the olefin or ethylene series with a general formula of C_nH_{2n}.

Addition Products. — The hydrocarbons of this series are known as unsaturated hydrocarbons, because of the fact that by treatment with certain elements or compounds the double bond existing between two of the carbon atoms may easily be broken, thus giving an additional free bond to two carbon atoms.

For example: when ethylene is treated with nascent chlorine, the double bond is broken and two chlorine atoms add themselves to the molecule, forming a saturated compound, ethylene chloride.



Such a product is called an addition product, and the power of forming addition products is characteristic of all unsaturated compounds. Some of the addition reactions of ethylene, given below, may be considered as typical of the way in which addition products are formed.

Ethylene can be converted into ethane by treatment with nascent hydrogen. Two atoms of hydrogen add themselves to the molecule, forming a saturated compound. Ethylene with any of the halogens produces a reaction similar to the one given above for chlorine. With the halogen acids, except hydrochloric, the acid molecule splits into the positive and negative ions, the positive hydrogen attaching itself to one carbon atom and the negative halogen ion going to the other carbon. The compound produced is $CH_3.CH_2Br$, or ethyl bromide. Sulphuric acid acts similarly, the acid ionizing into H^+ and HSO_4^- and forming, with ethylene, ethyl-hydrogen-sulphate, $CH_3CH_2HSO_4$.

The hydrocarbons of this series take their names from corresponding members of the paraffin series, with " ene " as a distinguishing termination — ethylene, C_2H_4, propylene, C_3H_6, butylene, C_4H_8, etc. They are unimportant in dental and physiological chemistry. Some of the higher oxygenated compounds of this class are, however, of great importance, as olein, which is a constituent of vegetable and animal fats and oils.

TRIPLE-BONDED HYDROCARBONS.

A third series of the straight-chain hydrocarbons is the acetylene series; these are triple bonded, and of course unsaturated, with a general formula of C_nH_{2n-2}.

The only members of this series of special interest are, first, *acetylene*, $H-C\equiv C-H$, (C_2H_2), made from calcium carbide and water (see Exp. 10, page 249). It is poisonous, combining directly with the hemoglobin of the blood, has a disagreeable odor, and is inflammable. Second, *allylene*, C_3H_4, derivatives of which occur in onions, garlic, mustard-oil, etc.

Haloid Derivatives of the Paraffins.

Methane furnishes three chlorine substitution products which are more or less in common use: first, the monochlor-methane, or methyl chloride; second, the trichlor-methane, $CHCl_3$, or chloroform; and third, the tetrachloride of carbon, CCl_4.

Methyl chloride, CH_3Cl, may be made from methyl alcohol, zinc chloride, and hydrochloric acid. It is a colorless gas, con-

densing to a liquid at − 23° C.; it is used as a spray in producing local anesthesia (page 86), also as a constituent of anesthetics, such as anesthol, somnoform, etc.

Dichlor-methane, CH_2Cl_2, also known as methylene chloride, has been used as a general anesthetic, usually mixed in more or less chloroform and alcohol. Its use in this way is open to criticism because of its poisonous action upon the heart.

Chloroform, $CHCl_3$, trichlor-methane, is a general anesthetic prepared by distilling a mixture of chlorinated lime and acetone. Alcohol and water were formerly used in place of acetone (see Exp. 13, page 250). While it is not regarded as inflammable, its heated vapor can be made to burn with a greenish flame. The reaction with alcohol is probably as follows: $4\ C_2H_5OH + 8\ Ca(ClO)_2 = 2\ CHCl_3 + 3\ Ca(CHO_2)_2 + 5\ CaCl_2 + 8\ H_2O$.

A delicate test for chloroform is made by treating a few drops with a mixture of aniline and alcoholic potassium hydroxide. Isobenzonitril, or phenyl-carbylamine, which has a very characteristic and intensely obnoxious odor, is thus produced.

$$CHCl_3 + 3\ KOH + C_6H_5NH_2 \rightarrow C_6H_5NC + 3\ KCl + 3\ H_2O$$

Methyl chloroform, CH_3CCl_3, formed by replacing the hydrogen atom of chloroform by a methyl group, CH_3, has been used as an anesthetic.

Tetrachloride of carbon is a colorless liquid used quite largely as a solvent. It also has anesthetic properties but, like dichlor-methane, is dangerous because of its action on the heart.

Methyl bromide, or monobrom-methane, is used to some extent as a constituent of anesthetics.

Bromoform, $CHBr_3$, tribrom-methane, is prepared from bromine and a solution of alcoholic potash. Its properties are similar to those of chloroform, but it is more poisonous.

Methyl iodide, CH_3I, is a heavy liquid, with pleasant odor, boiling-point 43° C.; it has been used somewhat as a vesicant.

Iodoform, CHI_3, tri-iodomethane, is a much-used and very valuable antiseptic. It is a light yellow, crystalline powder with characteristic persistent odor (Plate III, Fig. 6, page 11).

Iodoform may be made by heating in a retort two parts of

potassium carbonate, two of iodine, one of strong alcohol, and five of water, till the mixture is colorless,

$$C_2H_5OH + 4\,I_2 + 3\,K_2CO_3 = CHI_3 + KCHO_2 + 5\,KI + 2\,H_2O + 3\,CO_2.$$

Iodoform is also produced from action of the above reagents with acetone in place of alcohol. This test is a very delicate one and advantage is taken of it in testing for acetone in saliva, see page 182.

Cacodyl is an example of the arsenic derivatives of the hydrocarbons. It is one of several products which result from the distillation of a mixture of potassium acetate and white arsenic. Its composition is that of dimethyl arsine, $(CH_3)_2As$.

Ethyl chloride, C_2H_5Cl, chlorethyl, may be made by distillation of a mixture of alcohol and hydrochloric acid and purification of the distillate. It is extremely inflammable, boils at 12° C., and is used as a local anesthetic in a similar manner to methyl chloride. Its higher boiling-point makes it the more convenient of the two preparations (see page 82).

Ethyl bromide, C_2H_5Br, is prepared from alcohol, sulphuric acid, and potassium bromide. It is a heavy, colorless liquid, does not burn, and has been used to a considerable extent as a general anesthetic.

CHAPTER II.

ALCOHOLS.

If we substitute, for one of the hydrogen atoms of methane, a hydroxyl group (OH), we shall produce the first of a series of alcohols, several of which will claim our attention.

The alcohols may be considered as hydroxides of alkyl radicals, CH_3OH being methyl alcohol, C_2H_5OH being ethyl or ordinary alcohol; C_3H_7OH being propyl alcohol, and $C_5H_{11}OH$, amyl alcohol or fusel oil.

The alcohols as a class may be prepared by the action of moist silver oxide on the corresponding halogen compounds; e.g.,

$$CH_3Br + AgOH = CH_3OH + AgBr.$$

In many instances, the alkaline hydroxides will act in the same way.

$$CH_3Br + KOH = CH_3OH + KBr.$$

Alcohols treated with metallic sodium or potassium liberate hydrogen gas, forming compounds known as alcoholates; e.g.,

$$CH_3OH + K = CH_3OK + H;$$

or

$$C_2H_5OH + K = C_2H_5OK + H.$$

While these compounds are, as just stated, called alcoholates, they may be distinguished, one from another, by using the name of the alkyl radical involved; CH_3OK will be potassium methylate, while C_2H_5OK will be potassium ethylate.

Alcohols may contain more than one hydroxyl group, and, according to the number of the OH groups, are termed mono-, di-, tri-atomic, etc. Thus, ordinary alcohol, C_2H_5OH, is monatomic; glycol, $C_2H_4(OH)_2$, is diatomic; glycerol, $C_3H_5(OH)_3$, is triatomic, while mannite, $C_6H_8(OH)_6$, is a hexatomic alcohol.

Alcohols may also be classified according to the relative position of the hydroxyl group. By this classification, we may have

primary alcohols with OH replacing a hydrogen of the $-CH_3$ group; secondary alcohols with OH replacing the hydrogen of a $-CH_2$ group; and tertiary alcohols with OH replacing the hydrogen of a $-CH$ group. This may be illustrated by the formula of an alcohol of each class. $CH_3-CH_2-CH_3$ being the hydrocarbon, a primary alcohol will have the formula $CH_3.CH_2.CH_2OH$, and $-CH_2OH$ may be considered the distinctive grouping of the primary alcohols. Again, from the same hydrocarbon, if OH is substituted for an H of CH_2, then the secondary alcohol will be $CH_3-CHOH-CH_3$ and $-CHOH$ may be regarded as a distinctive group of this class.

The tertiary alcohols, however, must be produced from compounds having at least four carbon atoms, as a CH group is only possible when there are sufficient carbon atoms to produce a forked chain; that is, in a compound with three carbon atoms, one must of necessity be placed between the other two, while with four carbon atoms, the carbons may be attached in a straight chain, such as C – C – C – C, or they may be arranged as a forked chain $C-C\begin{matrix}/C\\ \backslash C\end{matrix}$, and by supplying the hydrogen atoms necessary to satisfy the valence of each carbon, in this latter chain we find a CH group. OH introduced in place of the hydrogen of this group gives us the tertiary alcohol,

$$CH_3-COH\begin{matrix}/CH_3\\ \backslash CH_3\end{matrix}$$

Methyl alcohol, CH_3OH, $(H-CH_2OH)$,* wood spirit, carbinol, is a product of the destructive distillation of wood, and can be made synthetically from methane. It is a colorless, inflammable liquid, with a gravity of 0.802 at 15° C., with solvent properties similar to ordinary alcohol. It boils at 66°.

It is customary to test for methyl alcohol by oxidizing it to formaldehyde and then obtaining a color reaction due to the formation of furfural. The details of the test are given in Exp.

* Note that $-CH_2OH$ is the "alcohol group" peculiar to this class of alcohols.

24, page 253, a positive test being the production of a rose-colored ring.* In a mixture of alcohols the test should be applied to the first portion of distillate, as the boiling point of methyl alcohol is lower than that of any of the other common alcohols.

Ethyl alcohol, C_2H_5OH, (CH_3-CH_2OH), methyl carbinol, grain alcohol, or ordinary alcohol, may be made by the action of silver hydrate† on ethyl iodide or bromide, as suggested on page 15. It is made commercially by fermentation of various carbohydrates, and purified by distillation. Carbon dioxide is evolved as follows:

$$C_6H_{12}O_6 = 2\ C_2H_5OH + 2\ CO_2.$$

Ninety-five per cent alcohol has a specific gravity of 0.8164, boils at about 78° C., dissolves many inorganic salts, vegetable waxes, resins (not gums), oils, etc., and is miscible with water, ether, or chloroform.

Propyl alcohol, normal, $CH_3.CH_2.CH_2OH$, occurs with amyl alcohol as a constituent of fusel oil, or may be prepared by general method with moist silver oxide. It is a colorless liquid, and boils at 97° C. The iso-compound,†† $CH_3.CHOH.CH_3$, may be made by reducing acetone with nascent hydrogen; nascent hydrogen may be produced by sodium amalgam.

Butyl alcohol, C_4H_9OH, occurs in four isomeric forms. The normal alcohol is $CH_3.(CH_2)_2.CH_2OH$. It is produced by the fermentation of glycerol. It boils at 117° C. The isobutyl alcohol, $(CH_3)_2CH.CH_2OH$, obtained from fusel oil, boils at 107° C.

Amyl alcohol, $C_5H_{11}OH$, ($C_4H_9-CH_2OH$), consists of about 87 per cent of isobutyl carbinol and about 13 per cent of an isomer known as active amyl alcohol. It is a colorless, oily liquid with a specific gravity of 0.818. It boils at about 130° C., and burns with a bluish flame.

Fusel oil, or potato spirit, consists of amyl alcohol carrying traces of various other alcohols as impurities.

* All the higher alcohols give a brown ring in this test.

† Moist silver oxide.

†† See note on page 22.

Amyl alcohol is a valuable solvent and is largely used in the manufacture of artificial fruit flavors, banana essence, and the like.

Oxidation of the Alcohols.

Aldehydes.

The first step in the oxidation of a primary alcohol consists in the withdrawal of hydrogen; thus the oxidation of methyl alcohol produces formaldehyde (CH_2O) and water,

$$CH_3OH + O = CH_2O + H_2O,$$

and the oxidation of ethyl alcohol produces acetaldehyde and water.

$$C_2H_5OH + O = CH_3CHO + H_2O.$$

The removal of the two hydrogen atoms may be considered as a result of the addition of oxygen to the molecule, as this gives a condition in which two hydroxyl groups are attached to one carbon atom. This condition is an impossible one, and water immediately breaks off, removing two hydrogen atoms and one oxygen atom, and leaving the characteristic aldehyde grouping $-C\begin{matrix}H\\ \diagdown\!\!\!\!\diagup\\ O\end{matrix}$

$$\begin{matrix}H\\ |\\ H-C-OH\\ |\\ OH\end{matrix}$$

With the exception of formaldehyde, all aldehydes may be considered compounds containing an alkyl radical and this distinctive aldehyde group. Acetaldehyde, produced by the first oxidation of ethyl alcohol, as shown above, consists of the methyl group and the aldehyde group, CH_3CHO; propionic aldehyde, of the ethyl group and the aldehyde group, C_2H_5CHO. In the case of formaldehyde, as with methyl alcohol, a hydrogen atom takes the place of the alkyl radical.

Aldehyde reactions. — The aldehyde structure and the consequent reactions to which aldehydes respond are of fundamental importance in the chemistry of carbohydrates. These reactions may be grouped as

(1) Reduction of copper solutions and ammoniacal silver solutions.

The products of the reduction of the copper solutions depend on the preparation of the reagent; but, in general, if much reducing agent is present, cuprous oxide is precipitated. The reduction of various copper solutions by carbohydrates has been used in part as a proof of the presence of an aldehyde structure in some carbohydrate substances and is used extensively as a test for carbohydrates (Fehling's Test, Benedict's Test, etc., page 271.)

The reduction of an ammoniacal silver solution is one of the best known tests for aldehydes, Tollen's Test, Exp. 30, page 254, and is used in Howe's method for the sterilization of root canals, page 84. The aldehyde group is oxidized to form an acid, and metallic silver is precipitated.

(2) *Addition reactions.* — Aldehydes combine directly with ammonia, forming aldehyde ammonias — white, crystalline compounds.

$$CH_3CHO + NH_3 = \begin{matrix} & CH_3 & \\ & | & \\ H & C & OH \\ & & NH_2 \end{matrix}$$

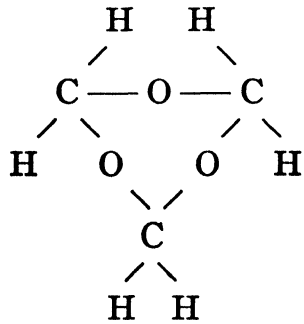
Hydrocyanic acid forms, with aldehydes, compounds known as oxynitrils or cyanides of oxy-organic acids.

$$CH_3CHO + HCN = \begin{matrix} & CH_3 & \\ & | & \\ H & C & OH \\ & & CN \end{matrix}$$

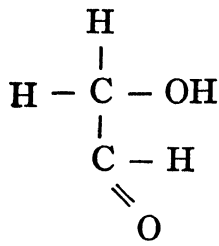
It will be noted, in each case, that the molecule which adds itself to the aldehyde splits; the hydrogen goes to the oxygen of the aldehyde group, making a hydroxyl group and leaving a free bond which holds the rest of the new molecule.

Aldehydes react similarly with phenyl-hydrazine, an aromatic compound, $C_6H_5NH.NH_2$, forming phenyl-hydrozones or osazones, which are important in the detection of various sugars. (See page 99.)

(3) *Condensation of aldehydes.* — Two or more molecules of an aldehyde possess the property of uniting and thus forming new compounds. This union may take place either between the oxygen atoms, forming *polymers*, or between the carbon atoms, forming *condensation products.* Paraform, a polymer of formaldehyde, illustrates the way in which the molecules combine through oxygen, while aldol is an example of a condensation product.



Paraform



Aldol

It is of importance to note that when polymerization takes place the characteristic aldehyde grouping is lost and the new molecule does not possess aldehydic properties. When condensation takes place, however, the aldehyde grouping is always left intact, allowing condensation products to react similarly to aldehydes.

Formaldehyde, H.CHO, is a gas with a decidedly irritating odor, freely soluble in water. The commercial preparation, usually called formalin, is a 40 per cent solution. Formaldehyde is prepared by the oxidation of methyl alcohol. It is readily oxidized to formic acid and carbon dioxide, and possesses the reducing properties common to all the aldehydes. With ammonia its action is unique (page 194).

Formaldehyde forms polymers, of which paraform, a combination of three formaldehyde molecules, is the most important. This compound is known as tri-oxymethylene. Formose, a substance allied to glucose, has been considered as a higher polymer, but in a stricter sense it is a condensation product, as the union of the molecules is probably effected through the carbon atoms.

Because formaldehyde coagulates albumin, hardens gelatin and renders proteins in general tougher and less digestible, it is utilized to a large extent in the preparation and preservation of anatomical specimens. It has been used quite generally as a food and milk preservative and is a valuable disinfectant. In dentistry its use is in connection with formo-cresol and will be studied under dental preparations (page 83).

Acetaldehyde, aldehyde, CH_3-CHO or C_2H_4O, the aldehyde from ethyl alcohol, may be made by addition of H_2SO_4 to a mixture of alcohol and bichromate of potassium. It is a colorless, inflammable liquid with pungent ethereal odor, and boils at 22° C. It responds to the general aldehyde reactions.

Paraldehyde, $(C_2H_4O)_3$, a polymer of acetaldehyde, is a "colorless liquid with a strong pungent odor, soluble in 8.5 parts of water at 15° C., miscible in all proportions with alcohol, ether, and fixed or volatile oils." (U.S.P.) It is a valuable hypnotic.

Chloral, CCl_3CHO, trichlor-aldehyde, is an oily liquid formed by action of dry chlorine gas on pure alcohol; it is soluble in ether and chloroform, boiling at from 94° C. to 98° C., and forming, with a molecule of water, *chloral hydrate,* $CCl_3CHO.H_2O$, a crystalline solid, the chloralum hydratum of the pharmacopœia (see page 81).

Chloral hydrate is decomposed by sodium or potassium hydrate with liberation of chloroform (see Exp. 32, page 254): $CCl_3-CHO + KOH = CHCl_3 + KCOOH$ (potassium formate).

When a drop or two of aniline oil is warmed in an excess of alcoholic potash, chloral hydrate forms, first, chloroform, then phenyl-isocyanide, C_6H_5NC, the persistent disagreeable odor of which furnishes a delicate test for chloroform or chloral (see Exp. 33, page 255). If $CHCl_3$ is used as the reagent in place of the aniline, the same reaction becomes a test for aniline or organic compounds, from which aniline may be produced by heating with alcoholic potash as acetanilide.

Other aldehydes from hexatomic alcohols are dextrose (glucose) and galactose. They are represented by the formula $CH_2OH-(CHOH)_4-CHO$, and will be considered more fully in a subsequent chapter.

KETONES.

The oxidation of *secondary* alcohols (page 16) will not yield aldehydes, but a class of substances known as *ketones*:

$$CH_3-CHOH-CH_3 + O = CH_3-CO-CH_3 + H_2O.$$

Secondary propyl alcohol. Dimethyl ketone.

The converse of this reaction is possible, and, by reduction of a ketone with nascent hydrogen (sodium amalgam), the secondary alcohol will be formed:

$$CH_3-CO-CH_3 + H_2 = CH_3-CHOH-CH_3.$$

Acetone. Isopropyl alcohol.*

Likewise primary alcohols may be produced by the reduction of aldehydes:

$$CH_3-CHO + H_2 = CH_3-CH_2OH.$$

Acetaldehyde. Ethyl alcohol.

It will be noted that the characteristic grouping of the ketone is the $\begin{matrix}|\\ C=O\\ |\end{matrix}$ or *carbonyl group*. Ketones may be remembered as alkyl radicals joined by the carbonyl group. Thus, acetone, the first ketone, formed as is shown above by the oxidation of secondary propyl alcohol, is dimethyl ketone, $CH_3.CO.CH_3$. Another example is methyl ethyl ketone, $CH_3.CO.C_2H_5$. Note that whereas the aldehyde group is necessarily at the end of the molecule, the ketone grouping always falls between two carbon atoms.

Although the two possess some properties in common, ketones differ from aldehydes in their inability to polymerize, their greater resistance to oxidation, and their lessened reducing properties. Ketones will not respond to Tollen's Test for aldehydes, and in general they do not reduce ammoniacal silver solutions. When, adjoining the carbonyl group of a ketone, there is either a primary or a secondary alcohol group, the reducing properties of the ketone seem to be increased; a ketone

* While the prefix "iso" generally means a forked chain compound, its use here indicates a secondary alcohol.

with this structure reacts positively in the reduction of copper solutions. Fructose, a keto-sugar, is an example of this type of ketone and will be studied later in connection with sugars.

When oxidation of a ketone is brought about, the molecule splits at the carbonyl group, forming two acids, the identification of which may sometimes serve as a means of determining the structure of the ketone. If ethyl methyl ketone is oxidized we may think of the melocule as probably splitting as indicated below

$$C_2H_5 / CO.CH_3$$

(the smaller radical going with the carbonyl group), oxidation then taking place by the addition of a hydroxyl group to each half of the molecule. Acetic acid and ethyl alcohol, which readily oxidizes to acetic acid, are then formed.

Acetone, or dimethyl ketone, $CH_3-CO-CH_3$, a colorless liquid of peculiar odor, boils at 56° C. and is made commercially by the dry distillation of acetate of lime.

$$\begin{matrix} CH_3COO \\ \\ CH_3COO \end{matrix} \!\!\diagdown\!\!\diagup Ca \rightarrow CaCO_3 + CH_3COCH_3$$

Ketones in general may be prepared by heating the calcium salts of the organic acids containing the required alkyl groups. For example, if we wished to make propyl ethyl ketone we should distil dry a mixture of calcium propionate and calcium butyrate

$$\begin{matrix} C_2H_5COO \\ \\ C_2H_5COO \end{matrix} \!\!\diagdown\!\!\diagup Ca + \begin{matrix} C_3H_7COO \\ \\ C_3H_7COO \end{matrix} \!\!\diagdown\!\!\diagup Ca \rightarrow 2CaCO_3 + 2C_2H_5.CO.C_3H_7$$

Acetone may be a toxic substance when produced in the body under pathological conditions, and is therefore of clinical importance. From this standpoint it will be discussed in Chapter XX.

Oxidation of Tertiary Alcohols.

While the oxidation of a primary alcohol will produce an aldehyde and the oxidation of a secondary alcohol will produce

a ketone, the tertiary alcohol, by action of a strong oxidizing agent, forms no new class of substances but is split into several simpler compounds.

If we take as an example of a tertiary alcohol $(CH_3)_2COH.C_2H_5$, dimethyl ethyl tertiary alcohol, the oxidation may be illustrated as follows: one bond is broken first and then oxidation takes place, as with the ketone, by the addition of a hydroxyl group to each part of the molecule. Thinking of the molecule as $\begin{matrix} CH_3 \\ CH_3 \\ C_2H_5 \end{matrix} \!\!\!\

converted into the carboxyl group, giving oxalic acid as a final product of oxidation.

CH_2OH	CH_2OH	CH_2OH	CHO	$COOH$
|	|	|	|	|
CH_2OH	CHO	$COOH$	$COOH$	$COOH$
glycol	glycollic aldehyde	glycollic acid	glyoxylic acid	oxalic acid

Glycerol, or ordinary glycerine, is the first of the trihydric alcohols and the only one which we shall consider. It is a substitution product of propane and is assigned the structural formula $CH_2OH.CHOH.CH_2OH$, or $C_3H_5(OH)_3$. It is the base of all common animal fat and vegetable oils and is produced when fat is saponified (page 107). Its dental use and detection are discussed in Chapter X.

The sweet taste noticeable in glycerine seems to increase upon the addition of hydroxyl groups, the hexatomic alcohols — those containing six hydroxyl groups — being directly related to the sugars (see Chapter XII).

CHAPTER III.

ETHERS.

Ethers may be regarded as oxides of the hydrocarbon radicals, as $\begin{matrix} C_2H_5 \diagdown \\ \\ C_2H_5 \diagup \end{matrix}$ O, or as anhydrides of the monatomic alcohols, water having been removed from two molecules of the alcohol:

$$2\ C_2H_5OH - H_2O = (C_2H_5)_2O.$$

Ethers may be simple or mixed. The simple ether is illustrated above by the formula for ordinary or ethyl ether, where two radicals of the *same* kind are united by an atom of oxygen.

In a mixed ether, these radicals will be of different kinds; as, for example, $CH_3-O-C_2H_5$, methyl-ethyl ether.

A general method for the preparation of simple and mixed ethers is that of distillation of the corresponding alcohols with sulphuric acid, as illustrated by Experiment No. 39, page 255. They may also be produced by the action of silver oxide on the corresponding alkyl iodides:

$$2\ C_2H_5I + Ag_2O = (C_2H_5)_2O + 2\ AgI,$$

also, by treating the sodium alcoholate with an alkyl iodide,

$$C_2H_5ONa + C_2H_5I = (C_2H_5)_2O + NaI$$

or

$$CH_3ONa + C_2H_5I = \begin{matrix} CH_3 \diagdown \\ \\ C_2H_5 \diagup \end{matrix} O + NaI.$$

Methyl Ether. — Methyl oxide, $(CH_3)_2O$, also known as formic ether, is isomeric with ordinary alcohol, and may be made in a manner similar to that used in the production of ethyl ether (*q.v.*). At ordinary temperature it is a gas, but it liquefies at $-20°$ C. (Bernthsen). It has been used as a general anesthetic, and the resulting anesthesia is said to be profound and quickly produced (U. S. D. from A. J. P., Sept., 1870).

Methyl-ethyl Ether. — This name, besides indicating a definite compound, as referred to in the preceding paragraph, has been applied to a mixture of methyl ether and ethyl ether, used for purposes of general anesthesia.

Methylene Ether. — A name applied to a mixture of methylene dichloride and ethyl ether. It has been used as an anesthetic, but has been found unsafe (U. S. D.).

Ethyl Ether. — Ethyl oxide, $(C_2H_5)_2O$. The ether used for general anesthesia should contain not less than $95\frac{1}{2}$ per cent or more than $97\frac{1}{2}$ per cent of ethyl oxide, the remainder consisting of alcohol with a little water (U. S. P.). It is a light, colorless liquid with a specific gravity of 0.715 at 25° C., and a boiling-point of about 35° C. It may be made by the action of sulphuric acid on ethyl alcohol, and from this fact has been known as sulphuric ether, but this name is, of course, incorrectly used.

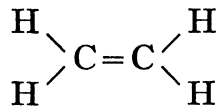
In the preparation of ether, sulphuric acid may be mixed with rather more than its own bulk of alcohol, the mixture heated to a temperature of from 130° to 138° C. in a suitable retort or still, the distillate (ether) being collected in a *cold* receiver.

The reaction takes place in two steps, as follows: one molecule of acid and one of alcohol react to form ethyl sulphuric acid (ethyl acid sulphate) and H_2O, $H_2SO_4 + C_2H_5OH = C_2H_5HSO_4 + H_2O$. Then the ethyl sulphuric acid reacts with a second molecule of alcohol to form ether and sulphuric acid, $C_2H_5HSO_4 + C_2H_5OH = (C_2H_5)_2O + H_2SO_4$. Thus the sulphuric acid, from two molecules of alcohol, has produced one molecule of ether and is in condition to repeat the process, having been changed only to the extent of adulteration with one molecule of water. In accordance with this theoretic formation of ether by simple dehydration of alcohol by sulphuric acid, provision is made for a continuous process, by the introduction of a constant supply of fresh alcohol into the retort during the distillation, and so regulated that the total bulk of liquid is neither increased nor diminished. The product is then purified, and freed from water and traces of acid by redistillation over a mixture of lime and calcium chloride.

Ether, according to the U. S. P. requirements, is "a trans-

parent, colorless, mobile liquid with characteristic odor and a burning and sweetish taste."

It is soluble in about twelve times its volume of water and in all proportions in alcohol, chloroform, petroleum ether, benzene, and oils. It is readily inflammable, and this fact, together with its easy volatility, makes it necessary to use considerable care when handling it.

The action of sulphuric acid upon alcohol needs careful regulation; because there may be produced three other products in addition to the ethyl oxide already considered. These are, first, ethyl sulphuric acid, $C_2H_5HSO_4$; second, ethyl sulphate $(C_2H_5)_2SO_4$, these being respectively the acid and neutral ethyl esters of H_2SO_4; third, the hydrocarbon *ethylene*, C_2H_4. This latter compound is the first of the ethylene series of hydrocarbons with the general formula $C_n H_{2n}$ and containing " double-bonded " carbon atoms,  or $CH.CH_3$.

These are unsaturated hydrocarbons (see page 11). Ethylene is produced by the action of an excess of concentrated sulphuric acid, which abstracts water from each molecule of alcohol ($C_2H_5OH - H_2O = C_2H_4$), whereas in the preparation of ether the more dilute acid abstracts water from *two* C_2H_5OH

CHAPTER IV.

ORGANIC ACIDS AND ESTERS.

If the oxidation of an alcohol is carried beyond the formation of aldehyde or ketone, i.e., if the aldehyde or ketone be oxidized, an organic acid results. The first atom of oxygen involved in this process does not become a constituent part of the new molecule, but simply withdraws hydrogen from the old (the alcohol), as shown in the formation of aldehydes on page 18. The second atom of oxygen, however, attaches itself to the molecule and does become a part of the new substance (the acid):

$$\underset{\text{Alcohol.}}{CH_2OH} + O = \underset{\text{Aldehyde.}}{CHO} + H_2O \qquad \underset{\text{Aldehyde.}}{CHO} + O = \underset{\text{Acid.}}{COOH}$$

The group $-COOH$ is known as carboxyl and is the characteristic group of the acids. The hydrogen of the carboxyl differs from the other atoms of hydrogen in the molecule in that it is united to oxygen rather than to carbon, and constitutes the basic or replaceable hydrogen of the acid; hence, acetic acid is monobasic, and the only possible salt of potassium, for instance, is CH_3-COOK.

The basicity of the acid depends on the number of *carboxyl* groups it contains.

Among the monobasic acids of the fatty or paraffin series which we shall study are the following:

Representative Fatty Acids.

$H.COOH$ = formic acid or hydrogen formate;
$CH_3.COOH$ = acetic acid or hydrogen acetate;
$C_2H_5.COOH$ = propionic acid or hydrogen propionate;
C_3H_7COOH = butyric acid or hydrogen butyrate;
C_4H_9COOH = valeric acid or hydrogen valerate;

$C_{15}H_{31}COOH$ = palmitic acid or hydrogen palmitate;
$C_{17}H_{35}COOH$ = stearic acid or hydrogen stearate.

The acids of these series are represented by the general formula $C_n H_{2n} O_2$. They all are monobasic; i.e., they contain only one atom of replaceable hydrogen.

All of the organic acids of this series may be prepared either by oxidation of the alcohol as suggested above or by treatment of the nitril with water, nitril being the term applied to an organic cyanide.

$$CH_3CN + H_2O \rightarrow CH_3COOH + NH_3$$

More frequently potassium hydroxide is used in place of the water, the potassium salt of the acid then being formed.

Formic Acid, (H.COOH), originally distilled from the bodies of ants (the name being derived from the Latin, *formica*, an ant,) is a colorless, easily volatilized liquid. It may be prepared in the laboratory by heating oxalic acid with glycerol, the oxalic acid breaking up into formic acid and CO_2.

$$C_2H_2O_4 = CO_2 + HCOOH.$$

Carbon monoxide, passed over hot potassium hydroxide, results in the formation of potassium formate,

$$CO + KOH = HCOOK.$$

Also, by treatment of ammonium carbonate with nascent hydrogen (sodium amalgam),

$$(NH_4)_2CO_3 + 2\,H = HCOO(NH_4) + H_2O + NH_3$$

and

$$HCOO(NH_4) + NaOH = HCOONa + NH_3 + H_2O.$$

Formic acid, according to the above reaction, is apparently carbonic acid less one atom of oxygen, and the fact that formic acid acts easily as a reducing agent, taking away oxygen from other bodies and becoming H_2CO_3, is further proof of this relationship.

Acetic acid, CH_3COOH, is obtained commercially by the oxidation of ethyl alcohol. It is the acid of vinegar, which,

according to Massachusetts law, should contain 4 per cent of acid. Glacial acetic acid is a commercial name of the acid containing 1 per cent or less of water; it is a colorless solid at a temperature below 15° C. The U. S. P. acetic contains only 36 per cent (by weight) of the pure acid.

Either one, two, or all three of the hydrogen atoms of the CH_3 group may be replaced by chlorine, forming respectively the mono-, di-, and tri-chloracetic acids, the tri-chloracetic acid being used to a considerable extent in dentistry (page 91).

Acetic acid, by the abstraction of water, forms an anhydride, $C_4H_6O_3$:

$$2\,HC_2H_3O_2 = (C_2H_3O)_2\,O + H_2O.$$

This substance is of considerable importance in organic reactions. It is a colorless liquid with a boiling-point of 138° C., and, with the halogens, forms compounds such as acetyl chloride, C_2H_3OCl, the radical C_2H_3O being known as the acetyl radical.

Propionic acid, $CH_3.CH_2.COOH$, is a colorless liquid, boiling at 140° C. According to Witthaus, it is best prepared by heating ethyl cyanide with caustic potash until the odor of the ester has disappeared:

$$C_2H_5CN + KOH + H_2O = C_2H_5COOK + NH_3.$$

Then, by treatment with H_2SO_4, the propionic acid is liberated, and may be separated by distillation.

Butyric acid, C_3H_7COOH, occurs as a product of fermentation of butter, or other animal fat containing butyrin; also from the decomposition of lactic acid, two molecules of lactic acid furnishing one of butyric acid, two of carbon dioxide and two of hydrogen (H_2). It is an occasional constituent of the gastric contents, and may be detected by formation of the ethyl ester (page 43). The pure acid is a heavy, colorless liquid with characteristic odor, soluble in water in any proportion. See page 43 for the glyceryl ester of butyric acid (butyrin); also for stearic and palmitic acids.

Valeric acid, C_4H_9COOH, may be made by the oxidation of amyl alcohol ($C_5H_{11}OH$). It is an oily liquid, boiling at 174° C.

It occurs as a constituent of valerian, and in consequence has been called valeric acid. Its salts are used in medicine as sedatives.

The valeriate of amyl has an odor resembling that of apples, and is used in alcoholic solutions as apple essence.

Palmitic acid, $C_{15}H_{31}COOH$, a solid "fat acid," occurs as a glyceryl ester in butter (to a very slight extent), in olive oil, palm oil, and bayberry wax. Combined with certain alcohols it occurs in white and yellow wax; also in spermaceti.

Palmitin, $C_3H_5(C_{16}H_{31}O_2)_3$, occurs in all animal fat and in large quantities in human fat.

Stearic acid, $C_{17}H_{35}COOH$, as glyceryl stearate or stearin, occurs in vegetable and animal fats, particularly in tallow. Stearic acid is only slightly soluble in alcohol or in ether. Its melting-point is 69.3° C.

Acrylic Acid Series.

If a hydrogen atom of a double-bonded hydrocarbon be replaced by hydroxyl, a double-bonded alcohol is produced, which, like the saturated alcohol, may be oxidized to form first an aldehyde and then an acid.

From propylene, the second double-bonded hydrocarbon, CH_2CHCH_3, we obtain acrylic aldehyde, $CH_2{:}CHCHO$. This compound, known as acrolein, is a colorless liquid, boiling at 52°C. Its vapor has an irritating, pungent odor, sufficiently characteristic to be used as a qualitative test for glycerol, from which it is obtained by heating with $KHSO_4$.

By oxidation acrylic aldehyde gives *acrylic acid*, which is a type of the double-bonded acids — the *acrylic acid series*. It is a liquid with boiling-point at 140° C. Nascent hydrogen breaks the double bond, forming propionic acid, $CH_3.CH_2.COOH$. Hydriodic acid will also break the double bond by direct union of its constituents, forming CH_2I-CH_2-COOH, β-iodo propionic acid.

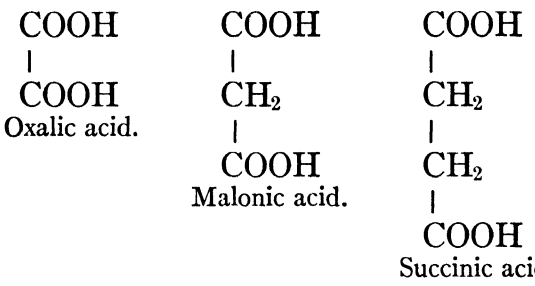
The only other acid of particular interest in this series is oleic acid, $C_{17}H_{33}COOH$. It is an important constituent of oils, both animal and vegetable.

Its glyceryl ester, $C_3H_5(C_{17}H_{33}CO_2)_3$, forms a large part of lard oil, cotton-seed oil, or any oil (of glyceryl base) obtained by cold expression.

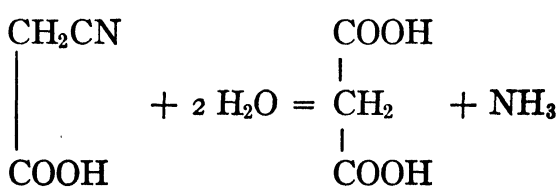
Because of its double bond, it has the property of forming addition products with the halogens. The iodine and bromine addition products are used in the identification of oils, as the proportion of olein, and hence the amount of iodine or bromine capable of being absorbed, varies in the different oils.

Dibasic Acids.

By the oxidation of glycol, shown on page 25, oxalic acid, the simplest acid containing two carboxyl groups, is produced. The presence of two carboxyl groups gives this acid the power to yield, upon ionization, two positive hydrogen ions; hence it is the first acid in the dibasic series. This series, like the other homologous series studied is built up by the addition of CH_2 groups.



Each of these dibasic acids is referable to a diatomic alcohol and frequently may be formed from it, as glycol by oxidation may yield oxalic acid. The general method for the synthetic preparation of all organic acids, by treatment of a nitril with water, applies to the dibasic acids if a cyan-substituted acid is used in place of the alkyl cyanide. For example:



Oxalic acid, which may be considered as a type of the dibasic acids, occurs as small, colorless crystals (four- or six-sided prisms) containing two molecules of water of crystallization ($H_2C_2O_4.2\,H_2O$); it is but slightly efflorescent, and, if carefully crystallized, is suitable for the preparation of standard acid solution. Salts of oxalic acid occur in many plants; the acid potassium oxalate, "salt of sorrel," is found in common red sorrel (Rumex acetora) and in wood sorrel (Oxalis acetocella). Oxalic acid in various combinations, often with lime, is widely distributed in articles of vegetable diet, particularly rhubarb, spinach, and asparagus; grapes, apples, tomatoes, and cabbages also carry oxalates but in smaller amounts.

The source of oxalates in the system is twofold, — the ingested oxalates and those produced by oxidation, incident to metabolism, the exact nature of which has not been clearly demonstrated (see Calcium and Sodium Oxalates, under Urine and Saliva).

Oxalic acid was previously made commercially by the action of strong nitric acid on starch or sugar; it is now prepared by heating cellulose (in form of sawdust) with a mixture of potassium hydroxide and sodium hydroxide, precipitating the acid as CaC_2O_4, and decomposing the salt by sulphuric acid. The acid is then purified by repeated crystallization.

Malonic acid, $COOH-CH_2-COOH$, is an oxidation product of malic acid (from apples), and is comparatively unimportant.

Succinic acid, $COOH(CH_2)_2-COOH$, occurs in amber, from which it takes its name (Latin, *succinum*, amber). It has been detected in the urine after asparagus and some fruits have been eaten. It occurs as colorless crystals, soluble in water, and ony slightly soluble in ether. Succinic acid may be obtained by the saponification of ethylene cyanide, $C_2H_4(CN)_2$, and is a dibasic acid containing four carbon atoms. It is a constituent of some transudates and cyst fluids. It occurs in the spleen and thyroid gland, and has been found in sweat and in the urine (Hammarsten).

Pyro-tartaric acid, glutaric acid, formed by the distillation of ordinary tartaric acid, is one of four isomers of formula $C_5H_8O_4$,

and is of interest only in its relation to some of the amino acids which result from protein digestion. Formula for pyro-tartaric acid is $CH_3-CHCOOH-CH_2-COOH$.

Carbonic acid, $O=C\begin{matrix}/OH\\ \\ \backslash OH\end{matrix}$, is dibasic in that it contains two atoms of replaceable hydrogen, though not two carboxyl groups. It is claimed that a molecule of this sort cannot exist because a single carbon atom cannot hold more than one hydroxyl group in combination. This acid has never been isolated, all attempts to separate it in the pure form resulting in the formation of carbonic acid gas and water. Its compounds (carbonates) are very common and very important, both in organic and inorganic chemistry. Organic salts of carbonic acid may be made by treating silver carbonate with alkyl iodide.

$$CO\begin{matrix}/OAg\\ \\ \backslash OAg\end{matrix} + 2\,C_2H_5I = CO\begin{matrix}/OC_2H_5\\ \\ \backslash OC_2H_5\end{matrix} + 2\,AgI.$$

Oxyacids.

Hydroxy-acids, or alcohol acids, contain hydroxyl in place of one or more hydrogen atoms of the fatty acids. Thus we may consider

Carbonic acid as hydroxy-formic acid, $HO-COOH$;

Glycollic acid as hydroxy-acetic acid, $\begin{matrix}CH_2OH\\ |\\ COOH\end{matrix}$;

Lactic acid as hydroxy-propionic acid, $\begin{matrix}C_2H_4OH\\ |\\ COOH\end{matrix}$;

Malic acid (from apples) as hydroxy-succinic acid, $\begin{matrix}CHOH-COOH\\ |\\ CH_2-COOH\end{matrix}$

Tartaric acid as di-hydroxy-succinic acid. $\begin{matrix}CHOH-COOH\\ |\\ CHOH-COOH\end{matrix}$

Citric acid, from lemons, limes, etc., is in a class by itself. It is a tribasic acid (has three carboxyl groups and one hydroxyl); the formula is $C_3H_4OH-(COOH)_3$.

Glycollic acid occurs in nature in unripe grapes, and possibly as antecedent to oxalates in the system (Dakin, Journal of Biol. Chem., 3.57). Glycollic acid is formed from glycol by oxidation, and from glycocoll, by action of nitrous acid.

Nitric acid will oxidize glycollic acid to oxalic acid.

Lactic Acid. — α-hydroxy – propionic acid, or *i**-ethylidene lactic acid, $CH_3-CHOH-COOH$, is ordinary lactic acid produced by fermentation of milk-sugar, etc. It occurs in the gastric juice and in contents of the intestine, "particularly during a diet rich in carbohydrates," possibly in muscle (Chapter XV) and brain tissue (Foster). It is not volatilized at temperatures below 160° C.

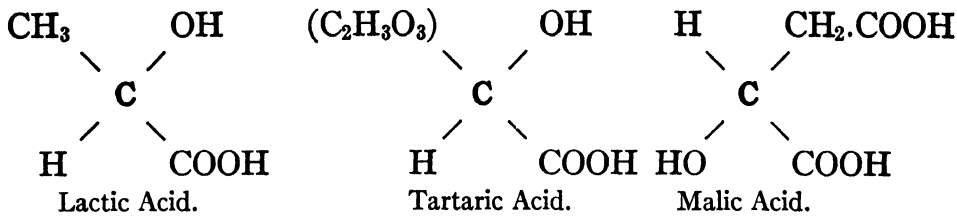
The optical inactivity of the lactic acid obtained from these sources is due to the fact that it is a *racemic* acid, that is, it consists equally of d-rotary and l-rotary† compounds, which have a neutralizing effect on each other.

Sarcolactic Acid. — This is the lactic acid more commonly met with, in physiological chemistry, perhaps, and it may be considered as the dextro-rotary portion of the racemic acid. It occurs in meat extract, and its presence causes the acid reaction of dead muscle, possibly of contracted muscle. It occurs in the blood and at times in the urine, and it is probable that it is this modification that may be found as lactates and acid lactates in the saliva and urine, the crystalline forms of which have been identified by Dr. E. C. Kirk of Philadelphia, by the use of the micropolariscopic method of Dr. Joseph P. Michaels of Paris. See Chapters XVIII and XXI.

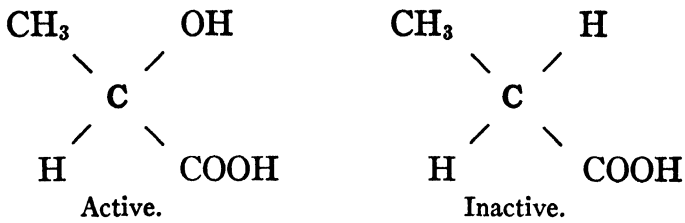
The optical activity of the lactic acids depends upon the presence of an *asymmetric* carbon atom. This asymmetric carbon, as the name implies, is one holding four *different* groups or atoms, as illustrated by the following compounds.

* Optically inactive.

† dextro-rotary, levo-rotary.



The truth of the above statement regarding the optical activity of these substances may be demonstrated quite readily by the reduction of the hydroxyl group in sarcolactic acid, as the inactive propionic acid results.



The optical activity consists in the power of the substance to turn the ray of polarized light to the right or to the left.

Both of these acids form characteristic crystalline salts of zinc and of calcium. In cold water the zinc sarcolactate is more soluble than zinc lactate; on the other hand, the calcium sarcolactate is rather less soluble than calcium lactate.

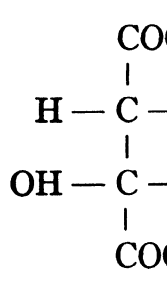
β-Oxybutyric acid, $CH_3-CHOH-CH_2-COOH$. — If there is introduced into butyric acid, $CH_3-CH_2-CH_2-COOH$, an OH group, an oxybutyric acid results. If this alcohol group (OH) occupies the secondary or β position (i.e., attached to the carbon atom twice removed from the carboxyl), the acid is the β-oxybutyric as above.

By oxidation of the compound, the alcohol group is broken up and hydrogen withdrawn to form water, leaving a keto acid, $CH_3-CO-CH_2-COOH$, known as diacetic acid. This in turn may give off carbon dioxide and become dimethyl ketone, or acetone, $CH_3-CO-CH_3$. These three substances, β-oxybutyric acid, diacetic acid, and acetone, are classed in von Noorden's " Autointoxication," and in the works of other recent writers, as " the acetone bodies," and by this convenient term

we may refer to them collectively. They occur in diabetic urine and, according to von Noorden, in other cases of perverted oxidation (not sufficient oxidation).

Tartaric acid is a dihydroxy-succinic acid, $COOH-(CHOH)_2-COOH$, obtained from grape juice.

We see by an examination of the graphic formula of this acid that it contains two asymmetric carbon atoms.



By placing the hydrogen or the hydroxyl on similar or opposite sides of the chain, we see how it might be possible to obtain a new form of isomerism depending on the relative position of the atoms in space and not at all upon their attachment to other atoms of the molecule. This is found to be the fact and this sort of isomerism, resulting only in differing physical properties, such as optical activity, has been called physical isomerism or stereo-isomerism.

A mixture of equal weights of these two kinds of tartaric acid crystallized together give an example of what is known as *di*-forms or racemic compounds (optically inactive).

The double tartrate of sodium and potassium (Rochelle salt), $KNaC_4H_4O_6$, is much used in medicine.

Tartaric acid combines with potassium and antimony to form tartar emetic $(KSbOC_4H_4O_6)_2\ H_2O$.

The "*scale salts of iron*," "ferri et ammonii tartras" and "ferri et potassii tartras," are prepared by dissolving freshly precipitated ferric hydroxide in the acid tartrate of ammonia or potash, and, after evaporation to thick syrup, solidifying in thin layers on glass plates.

Potassium bitartrate, or acid tartrate, $KHC_4H_4O_6$, is cream of tartar, and one of the few salts of potassium only sparingly soluble in water. Its commercial source is the wine vat.

Amino Acids.

Amino acids are characterized by an NH_2 group in place of one hydrogen of the alkyl radical of the acid. Thus we have:

Amino formic, NH_2COOH, carbamic acid
Amino acetic, CH_2NH_2COOH, glycocoll or glycine
Amino propionic, CH_3CHNH_2COOH, alanine

In the digestion and metabolism of protein substances amino acids are of prime importance. By hydrolytic enzyme action in the digestive tract, proteins are broken up eventually into amino acids which are capable of being absorbed into the blood stream. Here de-aminization takes place and urea is subsequently formed. (See Chapter XX.)

Of the amino acids which we are considering, all are derived from protein except the amino formic;* all except the first two, amino formic and amino acetic, contain one or more asymmetric carbon atoms. While, as just stated, these acids are derived from protein, a number of them do not occur or have not as yet been found in animal muscle, which is our common source of protein substance. For example, neither glycine nor its methyl derivative, sarcosine, occur in meat as such.

The amino acids are white, crystalline compounds, easily soluble in water, their solutions usually being sweet and in most cases giving a neutral reaction toward indicators. This characteristic is due to the fact that the amino group neutralizes the carboxyl group. The acid and basic properties are shown in the ability of an amino acid to react either with acids, forming such salts as glycine hydrochloride, or with metals, forming metallic salts such as copper glycine.

Although amino acids occur naturally as products of the cleavage of the protein molecule, it is exceedingly difficult to separate them from this source. They may be prepared in general, however, by the action of a substituted halogen acid

* Amino formic, or carbamic acid, $\begin{matrix} NH_2 \\ | \\ COOH \end{matrix}$, is a hypothetical acid consisting simply of an amino group, NH_2, united to a carboxyl group, COOH. By the union of ammonia and carbon dioxide the ammonium salt of this acid is formed,

$$2\,NH_3 + CO_2 = \begin{matrix} NH_2 \\ | \\ COONH_4 \end{matrix}$$

Ammonium carbamate is a constituent of commercial ammonium carbonate and an antecedent of ammonium carbonate in the hydrolysis of urea.

with alcoholic ammonia. The ammonium salt of the acid is formed.

All of these acids obtained from protein are α-amino acids; that is, the NH_2 group is joined to the carbon atom next to the carboxyl group, and may be grouped as:

(1) Glycocoll, amino-acetic, CH_2NH_2COOH

(2) *Amino-propionic acid derivatives*

Alanine, α-amino-propionic, CH_3CHNH_2COOH

Phenyl alanine, α-amino-β phenyl propionic
$CH_2C_6H_5.CHNH_2.COOH$

Serine, α-amino-β-hydroxy-propionic $CH_2OH.CHNH_2COOH$

Tyrosine, α-amino-β-para-hydroxy-phenyl-propionic
$CH_2(C_6H_4OH).CHNH_2COOH$

Tryptophane, α-amino-β-indol-propionic
$CH_2(C_8H_6N)CHNH_2COOH$

Histidine, α-amino-β-imidazol-propionic
$CH_2(C_3H_2N_2)CHNH_2COOH$

(3) *Di-amino acids*

Ornithine di-amino normal valeric
$CH_2NH_2CH_2CH_2CHNH_2COOH$

Lysine α-ϵ-di-amino-normal-caproic*
$CH_2NH_2(CH_2)_3CHNH_2COOH$

Cystine di-amino-dithio lactic

$$\begin{array}{l} CH_2SCHNH_2COOH \\ \quad\quad | \\ CH_2SCHNH_2COOH \end{array}$$

(4) *Dibasic acids*

Aspartic, amino-succinic
$CHNH_2COOH\ CH_2COOH$

Glutaminic, amino-glutaric
$COOH.CH_2CH_2.CHNH_2.COOH$

* It will be noted that caproic acid is the next higher homologue to valeric acid of the fatty acid series, and contains six carbon atoms.

PLATE I. — ORGANIC CHEMISTRY.

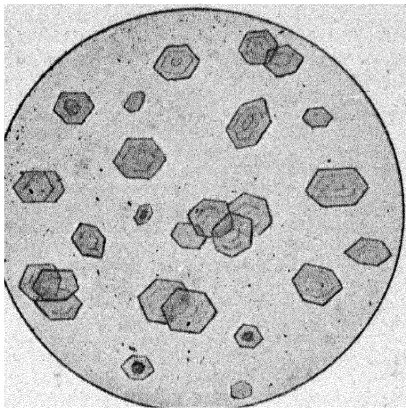


FIG. 1.
Cystin.

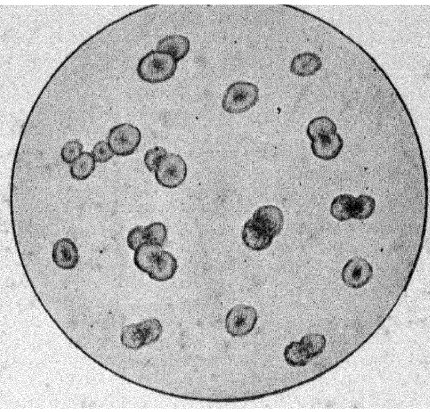


FIG. 2.
Leucin.

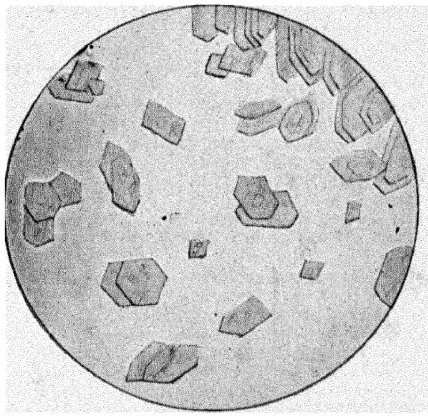


FIG. 3.
Urea Nitrate.

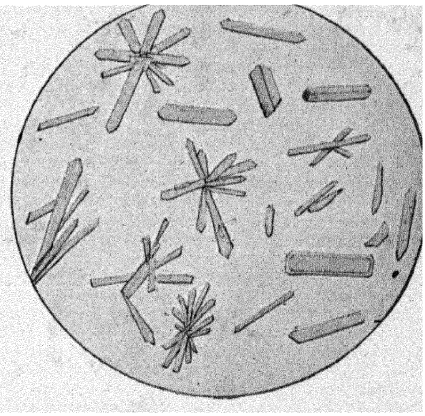


FIG. 4.
Hippuric Acid.

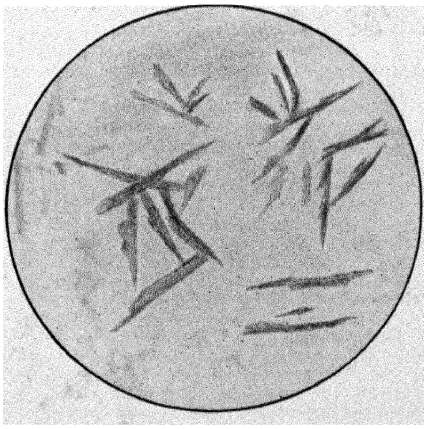


FIG. 5.
Benzoic Acid (sublimed).

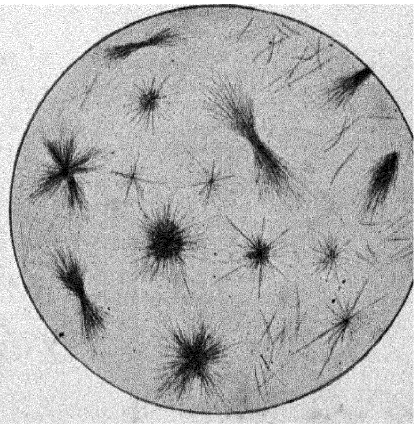
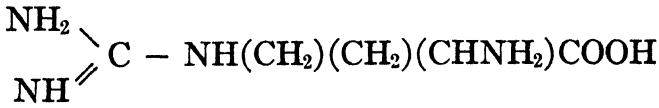
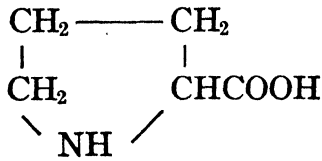


FIG. 6.
Tyrosin.

In addition to the above acids three others may be included: Leucine, α-amino-iso-caproic $(CH_3)_2CHCH_2CHNH_2COOH$; Valine, α-amino-iso-valerianic $(CH_3)_2CH.CHNH_2COOH$; Arginine, δ-guanidino-α-amino normal valeric



Proline, pyrrolidine carboxylic



The properties of the individual amino acids will be considered in Chapter XIV.

Esters.

Ester is the term applied to compounds in which an alkyl group has taken the place of replaceable hydrogen of the acid.* They are produced by the action of the acid upon the alcohol, which is as nearly free from water as possible.

Such action by the halogen acids would produce the alkyl haloids already considered; for example, $CH_3OH + HCl = CH_3Cl + H_2O$. As the water produces alcohol and hydrochloric acid by action on CH_3Cl, it must be removed as the experiment proceeds.

Other methods of preparation are (1) the action of an organic salt and the alkyl halides:

$$CH_3COOK + C_2H_5I = CH_3COOC_2H_5 + KI.$$

(2) the action of the acid chloride on the alcohol:

$$CH_3COCl + C_2H_5OH = CH_3COOC_2H_5 + HCl$$

(acetyl chloride)

The acetyl group, CH_3CO, is the group remaining when the hydroxyl of the carboxyl group of acetic acid has been removed. Such a radical in general is called an *acyl* group, and the termination "yl" is commonly added to the name of the acid to differentiate between them, as *acetyl*, *propionyl*, etc.

* The term ester is also applied to any organic salt in which either the positive or the negative ion is organic.

(3) the action of the acid anhydride with an alcohol, producing the ester and an acid.

$$\begin{matrix} CH_3CO \\ \qquad\diagdown O \\ CH_3CO\diagup \end{matrix} + C_2H_5OH =$$
$$CH_3COOC_2H_5 + CH_3COOH.$$

Anhydrides. — The acid anhydride of a monobasic organic acid may be considered as an oxide in which two *acyl* groups are joined to the oxygen atom. *Ethers*, in contrast, are oxides having two *alkyl* groups combined with the oxygen.

Acetic anhydride, to which reference has been made under acetic acid, is typical of organic acid anhydrides. It may be prepared from potassium acetate and acetyl chloride:

$$CH_3COOK + CH_3COCl = \begin{matrix} CH_3CO \\ \qquad\diagdown O \\ CH_3CO\diagup \end{matrix} + KCl.$$

The acetyl chloride is often produced from phosphorus oxychloride and the alkaline acetate, and a second molecule of the acetate is then allowed to react with the acetyl chloride, as given in the above reaction.

Acetic anhydride with alcohol, as just shown, produces ethyl acetate; with ammonia it produces acetamide.

These reactions are general for all organic acid anhydrides: with *alcohols* they form *esters*; with ammonia they yield *amides*; and with water, of course, the acid is produced.

Various esters will be considered as occasion requires, throughout the course. A few are given below.

Ethyl nitrite, $C_2H_5NO_2$, is a colorless liquid, boiling at 17° C. It is used in medicine as Sweet Spirits of Niter, which is an alcoholic solution containing traces of the ethyl nitrate, various oxidation products, and not less than 3.5 per cent nor more than 4.5 per cent of the ethyl nitrite. It is prepared from alcohol, sulphuric acid and sodium nitrite, as given in Exp. 64. It is

insoluble in water, but by action of boiling water or dilute alkalies becomes ethyl alcohol, $C_2H_5NO_2 + KOH = C_2H_5OH + KNO_2$.

Ethyl acetate, $CH_3-COO.C_2H_5$, is formed by heating ethyl alcohol, sulphuric acid, and acetate of sodium. This reaction constitutes a qualitative test for acetic acid or acetates, the odor of the ester being sufficiently characteristic to furnish a delicate test.

The acetic ether of the U. S. P. is "a liquid composed of about 98.5 per cent of ethyl acetate and 1.5 per cent alcohol."

Ethyl Butyrate, $CH_3-CH_2-CH_2-COOC_2H_5$. — This ester dissolved in 10 parts of alcohol forms pineapple essence. It may be made in a manner similar to the preparation of ethyl acetate, i.e., by heating together alcohol, butyric acid, and concentrated sulphuric acid. The production of the ester is likewise used as a qualitative test for the presence of the acid, and employed in the examination of gastric contents, as follows: "Heat 10 c.c. of contents with 5 c.c. of strong sulphuric acid and 4 c.c. of 95 per cent alcohol; odor of pineapple indicates butyric acid." (Hewes.)

Amyl acetate and amyl butyrate may be obtained by heating the respective acids with amyl alcohol ($C_5H_{11}OH$) and strong sulphuric acid. These esters may also be used in detecting the presence of the acid, amyl alcohol being used in place of ordinary alcohol. Amyl acetate gives the odor of pears, amyl butyrate that of bananas.

Amyl nitrite, $C_5H_{11}NO_2$, is a compound used in medicine to a considerable extent, usually administered by inhalation. The U. S. P. preparation contains about 80 per cent of amyl nitrite. It is very soluble and inflammable.

Fats are esters of glyceryl, C_3H_5, also called tritenyl, propenyl, etc. This radical forms with hydroxyl (OH) the propenyl alcohol, $C_3H_5(OH)_3$, which is ordinary glycerin or glycerol.

Glyceryl butyrate or butyrin $(CH_3-(CH_2)_2-COO)_3C_3H_5$, constitutes (together with smaller quantities of the glyceryl esters of capric, caproic, and caprylic acids) about 7 per cent of butterfat. These esters are readily saponified by treatment with

alcoholic potash; then, by decomposition of the potassium salts with H_2SO_4, the acids, being volatile, may be separated by distillation. The amount of volatile fat acids thus obtained is a valuable test for the genuineness of the butter.

For further consideration of fats see Chapter XIII.

CHAPTER V.

CYANOGEN COMPOUNDS. SULPHUR COMPOUNDS.

Cyanogen, C_2N_2, is an intensely poisonous gas, colorless, heavy (specific gravity 1.81), and inflammable. It is very easily soluble in water or alcohol, forming unstable solutions, which, upon decomposition, give rise to various nitrogen compounds, among them ammonia, hydrocyanic acid, and urea.

Cyanogen may be prepared by heating the cyanides of silver, mercury, or gold, or by the dry distillation of ammonium oxalate.

Hydrocyanic acid, HCN, may be produced by the fermentation of the glucoside amygdalin from bitter almonds; also from the kernel of peach-stones, cherry-laurel leaves, etc. Hydrocyanic acid may be formed by direct synthesis of C_2H_2 (acetylene) and nitrogen. The synthesis is induced by passing electric sparks through the mixed gases. It is conveniently prepared in the laboratory by distilling a mixture of dilute sulphuric acid with potassium ferrocyanide, $K_4Fe(CN)_6 + 5\ H_2SO_4 = 6\ HCN + FeSO_4 + 4\ KHSO_4$. Hydrocyanic acid is a colorless, poisonous liquid, boiling at 26.5° C., with a characteristic odor often designated as a peach-stone odor. It is soluble in water, and a 2 per cent aqueous solution constitutes the acidum hydrocyanicum dilutum of the pharmacopœia, also known as prussic acid.

Potassium cyanide (KCN or KCy) occurs in trade as a white solid, sometimes granular, more often as a powder. It is intensely poisonous owing to the dissociation of the salt and the activity of the free cyanogen.

Potassium cyanide is decomposed by carbonic acid of the air with liberation of hydrocyanic acid. The aqueous solution of potassium cyanide hydrolyzes in two distinct ways: the most easily apparent at ordinary temperature is that resulting in the formation of hydrocyanic acid and potassium hydroxide, giving the solution an alkaline reaction:

$$KCN + H_2O = HCN + KOH.$$

Upon boiling a solution, the second hydrolysis may be demonstrated, whereby ammonia and potassium formate are produced:

$$KCN + 2\,H_2O = HCOOK + NH_3 \text{ (Exp. 70.)}$$

The organic cyanides are known as *nitrils* or *isonitrils* (carbylamines) according as the hydrocarbon radical is attached directly to the carbon or to the nitrogen of the cyanogen group. That is, methyl cyanide would be represented by CH_3-CN, while the isocyanide would be CH_3-NC (methyl carbamine); the nitrogen atom being in the first place trivalent, in the second quinquivalent.

Of these two classes of compounds, the isocyanides are of much greater interest to the student of dental medicine, owing to their relation to the isocyanates and to urea.

Phenyl-isocyanide, C_6H_5NC, also known as isobenzonitril, is produced by warming aniline ($C_6H_5NH_2$) with alcoholic potash and chloroform. The intensely disagreeable odor of phenyl-isocyanide is utilized as a test for chloroform or chloral hydrate (page 81); or, with chloroform and potassium hydrate, the production of this isocyanide may become a test for aniline, acetanilide (antifebrin), and other derivatives of aniline.

Potassium ferrocyanide, yellow prussiate of potassium, $K_4Fe(CN)_6$, is obtained by heating animal refuse with a little over one-third its weight of potassium carbonate and scrap iron. The mixture is covered so as to exclude the air, and after cooling the resulting mass is boiled with water and filtered. Upon evaporation of the filtrate, potassium ferrocyanide will separate as yellow, four-sided crystals with a formula $K_4Fe(CN)_6 . 3\,H_2O$. The complex acid ion ($Fe(CN)_6$) is not regarded as poisonous but can be made to dissociate by the addition of acid. See Exp. 73. By the action of strong sulphuric acid the radical is broken up and carbon monoxide is evolved. Dilute sulphuric acid will yield hydrocyanic acid according to the reaction on page 45.

Potassium ferricyanide, red prussiate of potassium, $K_3Fe(CN)_6$, contains iron in the ferric condition and may be made by oxidizing the ferrocyanide by the action of chlorine gas.

Cyanic acid, HCNO, may be made by distillation of its polymer, cyanuric acid $(HCNO)_3$. Cyanic acid cannot be made in the usual way by decomposition of its salts with mineral acids, since in the presence of water cyanic acid becomes ammonium carbonate.

Potassium cyanate may be prepared by direct oxidation of potassium cyanide with lead oxide.

Ammonium cyanate passes, upon heating, directly into urea. See Exp. 79.

Isocyanic acid, O=C=N−H (carbimide) is supposed to be the acid of ordinary potassium and ammonium cyanates.

Fulminic acid (C≡N−O−H), isomeric with cyanic acid N≡C−O−H and isocyanic acid (O=C=N−H), is important only because of its relation to the fulminates, which are explosive compounds of the acid, with some of the heavy metals, such as silver and mercury.

Thiocyanic Acid or Sulphocyanic Acid. — In this acid and its salts, the atom of sulphur replaces the oxygen of cyanic acid in the empirical symbol (HCNS); but, graphically, the sulphur is attached to the basic element (metal or hydrogen) rather than to carbon: thus, K−S−C≡N, that is, the sulphocyanate is not an iso-compound. For occurrence and relations of HCNS in the human body, see Chapters XVIII and XXI.

Sulphur Compounds.

Mercaptan, an organic sulph-hydrate. The name mercaptan comes from two Latin words signifying "taking mercury" (mercurium captans), because of compounds readily formed with mercuric oxide. Representatives of this class of compounds are found as derivatives of both the open and the closed-chain hydrocarbons.

Ethyl mercaptan, thio-alcohol, C_2H_5SH, is a type of this class It is a colorless liquid, with bad odor, slightly soluble in water, boils at 37° C., and is used in the preparation of sulphonal.

The mercaptans may be prepared by action of KHS on the alkyl haloids:

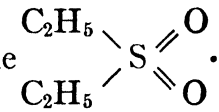
$$C_2H_5I + KHS = C_2H_5SH + KI.$$

The thio-alcohols form potassium and sodium compounds similar to common alcohol,

$$C_2H_5SH + K = C_2H_5SK + H.$$

Mercaptol, a name which has been applied to the thioketones. The simple compounds of this class are not known, as they form polymers very readily. A dimethyl-diethyl compound is produced in the process for preparation of sulphonal.

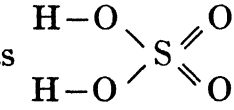
Thio-ethers are organic sulphides prepared in a manner analogous to that employed in the preparation of the thio-alcohols, the inorganic sulphide being used in place of the sulph-hydrate; for example: $2\ C_2H_5Br + K_2S = (C_2H_5)_2\ S + 2\ KBr.$

Sulphones are oxidation products of organic sulphides: as, for example, ethyl sulphone  .

Sulphonal is a complex derivative of mercaptan as previously stated. It may be prepared by the action of acetone and ethyl mercaptan with hydrochloric acid and subsequent oxidation of the resulting product. It possesses hypnotic properties.

Sulphonic acids as a class may be obtained by the oxidation of an organic sulph-hydrate (mercaptan). This oxidation may be produced by the action of nitric acid or potassium permanganate, and may be written as follows:

$$C_2H_5SH + 3\ O = C_2H_5.SO_2.HO.$$

A simple method for remembering the structure of the sulphones and sulphonic acids is to remember them in relation to sulphuric acid. If we consider the structure of sulphuric acid as  , then a sulphone is produced by the replacement of *both* hydroxyl groups with alkyl radicals, while a sulphonic acid is produced by the replacement of *one* hydroxyl with an alkyl radical.

Taurine is an important sulphonic acid of the paraffin series. Its graphic formula shows it to be an amino ethyl sulphonic acid,

$C_2H_4\begin{<}{HSO_3}{NH_2}$. Taurine is derived from taurocholic acid by hydrolysis. This acid is representative of one of the two principal acid groups occurring in the bile, the salts of which may be found in pathologic conditions in the urine, or, according to Dr. J. P. Michaels and others, in the saliva.

CHAPTER VI.

AMINES OR SUBSTITUTED AMMONIAS.

If one or more of the hydrogen atoms of ammonia, NH_3, be replaced by a hydrocarbon group, the resulting compound is an amine; thus CH_3-NH_2 is methylamine, and $(CH_3)_2NH$ is dimethyl amine. Trimethyl amine, $(CH_3)_3N$, has been found among the decomposition products of fresh brain, human liver, and spleen.*

When one hydrogen atom only has been substituted in NH_3 the amine is known as a primary amine or amino compound (containing the NH_2 group). These may be prepared in a number of ways, two of which we shall consider.

If alkyl iodides or bromides are heated with alcoholic ammonia, compounds are produced analogous in composition to the ordinary ammonium salts:

$$CH_3I + NH_3 = NH_2CH_3.HI.$$

Upon distilling the methyl ammonium iodide (of this reaction) with caustic alkali, the amine results:

$$NH_2CH_3HI + KOH = NH_2CH_3 + KI + H_2O.$$

The second method is by the action of nascent hydrogen upon alcoholic solution of the nitrils:

$$CH_3CN + 2\,H_2 = C_2H_5NH_2.$$

The disagreeable odor of carbylamine constitutes a characteristic test for the primary amines. This is known as Hofmann's Carbylamine Reaction and may be easily brought about by warming the amine with a little chloroform and alcoholic potash.

The reaction which takes place is analogous to that given on

* Vaughn and Novy, Cellular Toxins.

page 13 as a test for chloroform, using aniline, which may be regarded as phenyl-amine.

The secondary amines are those in which two hydrogen atoms of ammonia have been replaced, as in dimethyl amine $(CH_3)_2NH$. These compounds have also been called imines (imides) or imino (imido) compounds because they contain the "imino" group (NH).

Imides are formed with a number of the dibasic organic acids. The one of greatest interest is perhaps the imide of succinic acid which may be produced by the following reaction. Ammonium succinate subjected to heat splits off $2\ H_2O + NH_3$, becoming $\begin{matrix} CH_2.CO \\ | \qquad \diagdown \\ CH_2.CO \qquad \diagup \end{matrix}$ NH. The hydrogen of the imide group may be replaced by metals such as potassium, silver, or mercury. Succinimide may also be produced by heating succinic acid, carbonic anhydride, and ammonia. This with mercuric oxide will give a white powder soluble in water, which is the mercuric succinimide largely used for the treatment of pyorrhea.

The secondary amines may be produced by further action of alkyl iodides and the primary amines. By action of sodium nitrite and hydrochloric acid upon fairly strong solution of a secondary amine, a nitrosamine is formed which, when mixed with phenol and strong sulphuric acid, gives a dark green solution which becomes red upon dilution with water. This in turn becomes blue or green upon neutralization with a fixed alkali.

Trimethyl amine, formed with the methyl and dimethyl amines, is a liquid with a not unpleasant odor.

Diamines are derived from two molecules of ammonia, as ethylene diamine, $C_2H_4 \begin{matrix} \diagup NH_2 \\ \diagdown NH_2 \end{matrix}$.

To this class of compounds belong many of the "**ptomaines,**" produced by the putrefaction of organic matter, as putrescine (butylene diamine), $CH_2NH_2-(CH_2)_2-CH_2NH_2$, and cadaverine (penta-methylene diamine), $CH_2NH_2-(CH_2)_3-CH_2NH_2$.

According to Matthews, the so-called carboxylase bacteria have the power of splitting off carbon dioxide from amino acids.

If this bacterial change takes place *before* de-aminization of the amino acid, amines which are highly toxic are produced. Such amines are classed as *ptomaines.*

AMIDES.

If the hydrogen of ammonia be replaced by an acyl group, an amide results; thus $NH_2(C_2H_3O)$ is acetamide, or this compound may be regarded as acetic acid, CH_3-COOH, in which the OH has been replaced by NH_2.

It may be easier for the student to remember an amide as an organic acid with the OH of its carboxyl replaced by the " amido " or amino group NH_2.

Acetamide may be prepared by the action of strong ammonia upon ethyl acetate:

$$CH_3COOC_2H_5 + NH_3 = CH_3CONH_2 + C_2H_5OH.$$

It forms colorless crystals soluble in both alcohol and water.

Cyanamide (NH_2 in place of the hydroxyl of cyanic acid), $NCNH_2$, is prepared by the action of ammonia on cyanogen chloride. The calcium compound is of commercial importance as a means of utilizing atmospheric nitrogen for agricultural purposes. CaC_2 heated with N_2 becomes NCNCa; this in a crude state is used as fertilizer. The calcium cyanamide by action of carbon dioxide, water, and soil bacteria becomes first urea, then ammonium carbonate. See page 54.

Formamide, $CHO.NH_2$, is a liquid miscible with both alcohol and water. It boils with partial decomposition at about 200° C. Upon heating quickly, it splits into carbon monoxide and ammonia. (Bernthsen.)

Phenyl-formamide, $CHO.NHC_6H_5$, known as formanilide, occurs as yellow crystals soluble in water and in alcohol.

Phenyl-acetamide, $CH_3CONHC_6H_5$ (see acetanilide).

HYDRAZINES.

From diamide, NH_2-NH_2, or hydrazine, may be derived such substitution products as methyl-hydrazine, $CH_3-NH-NH_2$; ethyl-hydrazine, $C_2H_5-NH-NH_2$; and phenyl-hydrazine, $C_6H_5NH-NH_2$.

This last-named compound forms, with the monosaccharids and with many of the disaccharids, yellow crystalline compounds, known as osazones, which are precipitated in characteristic crystalline forms, recognizable upon microscopical examination and by their melting-points (see under Carbohydrates, page 99).

CHAPTER VII.

UREA AND URIC ACID.

This substance forms about 50 per cent of the total solids and about 85 per cent of the nitrogenous matter contained in the urine. When we consider that only 5 per cent of the nitrogenous waste passes off in the feces and 95 per cent in the urine, the importance of urea as an index of the nitrogen excreted and of protein metabolism becomes apparent.

Urea was the first organic substance synthesized from inorganic compounds. This was accomplished by producing a molecular rearrangement of ammonium isocyanate. The reaction is conveniently brought about by the double decomposition of potassium cyanate and ammonium sulphate and subsequent evaporation of the solution to dryness:

$$2\ CNOK + (NH_4)_2SO_4 = 2\ OCN.NH_4 + K_2SO_4.$$

Then $O=C=N-NH_4$ (ammonium isocyanate) + heat =

$$HN:C\begin{cases} NH_3 \\ | \\ O \end{cases} \text{(urea).}$$

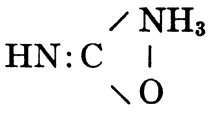
Urea was formerly considered as the amide of carbonic acid, $CO(NH_2)_2$, and from this type has been explained the rapid transformation of urea into ammonium carbonate in stale urine. $O=C\begin{cases} NH_2 \\ NH_2 \end{cases}$ with one molecule of H_2O becomes $O=C\begin{cases} ONH_4 \\ NH_2 \end{cases}$ or ammonium carbamate, and this, by addition of a second molecule of water, becomes $O=C\begin{cases} ONH_4 \\ ONH_4 \end{cases}$ or ammonium carbonate, $(NH_4)_2CO_3$. The last part of the reaction takes place whenever commercial "ammonium carbonate"

[really a mixture of carbamate ($NH_4-NH_2-CO_2$) and acid carbonate (NH_4HCO_3)] is dissolved in water.

Urea crystallizes in long needle-shaped crystals of the rhombic system. It is insoluble in water, somewhat soluble in alcohol, and nearly insoluble in ether. It fuses at 132°, and at a somewhat higher temperature it gives off ammonia and ammonium carbonate, and at 160° leaves a residue of ammelide, cyanuric acid, and biuret. Urea is decomposed by solutions of the alkaline hypochlorites or hypobromites, being broken up into N, CO_2 and H_2O, as follows:

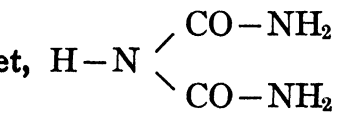
$$CO(NH_2)_2 + 3\ NaOBr = CO_2 + N_2 + 2\ H_2O + 3\ NaBr.$$

The cyclic formula for urea was suggested by Werner in 1914, based on the action of urea and some closely allied compounds. In static condition and in neutral solution, according to Werner, the molecule of urea may be best represented by the formula:



In the presence of strong acid, the equilibrium of the molecule is disturbed, and a hydrogen atom from the NH_3 group goes to the oxygen, forming a hydroxyl group: Isourea . Werner also suggested that under some conditions, though he has not been able to determine them, the hydrogen atom may pass to the NH group, giving the carbamide formula to which we are accustomed.

Cyanuric acid, $N_3C_3O_3H_3$, is a polymer of cyanic acid (NCOH), which is, at first, formed in the above decomposition.

Biuret,  , may be obtained by heating urea. When pure, it occurs as white, needle-shaped crystals. With NaOH and 1 per cent $CuSO_4$ it gives the characteristic violet and rose-red shades obtained in the biuret reaction (Piotrowski's protein test). Exp. 155, page 276.

Urea nitrate may be precipitated from fairly concentrated urine by addition of HNO_3. It separates in hexagonal crystals

or plates, easily recognizable under the microscope (Plate I, Fig. 3, opposite page 40).

Urea Oxalate. — Upon addition of a solution of oxalic acid to concentrated urine, crystals of oxalate of urea are precipitated. They are rather more easily obtained in characteristic forms (Plate II, Fig. 5, opposite page 77) than are the crystals of nitrate, and, in consequence, treatment with oxalic acid constitutes a better method for the qualitative detection of urea in the body fluids than the nitric acid test formerly used. These crystals polarize light, and the use of the micropolariscope facilitates their detection.

Substituted Ureas. — A hydrogen atom may be replaced by an alkyl radical in either one of the amino groups of urea, if we use the former carbamide formula, thus producing a methyl or ethyl urea (carbamide), $O=C\begin{smallmatrix}/\ NH_2\\ \backslash\ NHCH_3\end{smallmatrix}$, or in the hydroxyl group of *isourea* as $HN=C\begin{smallmatrix}/\ OCH_3\\ \backslash\ NH_2\end{smallmatrix}$ thus producing *methyl isourea.* Both methyl urea or methyl carbamide and methyl isourea have been known in the free state.

Or by introducing an acid radical a *ureide* may be formed as acetyl urea represented formerly as $O=C\begin{smallmatrix}/\ NH_2\\ \backslash\ NH(C_2H_3O)\end{smallmatrix}$ but now more correctly represented as $HN=C\begin{smallmatrix}/\ NHCOCH_3\\ \backslash\ OH\end{smallmatrix}$

In the case of a dibasic acid, such as oxalic $(COOH)_2$, entering into the reaction, one or both (OH) groups may be split off, forming in the first instance a ureide acid, $O=C\begin{smallmatrix}/\ NH_2\\ \backslash\ NH.CO.COOH\end{smallmatrix}$

More correctly represented perhaps as $HN=C\begin{smallmatrix}/\ NH.CO.COOH\\ \backslash\ OH\end{smallmatrix}$

oxaluric acid

or, in the second case, a ureide, as $O=C\begin{matrix}\diagup NH-C=O\\ \diagdown NH-C=O\end{matrix}$ parabanic acid.

If the residue of *two* molecules of urea enter into the composition of the new molecule, the compound is a diureide. Of this class one of the most important is:

Uric acid, trioxypurin, $C_5H_4N_4O_3$. — Its relation to urea may be shown by the graphic formula

$$O=C\begin{matrix}NH-CO\\ |\quad\ |\\ NH-C=NH\\ \quad\ \|\\ \quad C-NH\end{matrix}\diagup\diagdown C=O$$

Uric acid is also referable to "purine," by the use of which the relationship of xanthin, hypoxanthin, and other "purine" or nuclein bases is easily demonstrated.

These bases are of great physiological interest, in that they form an unquestioned link between the decomposition products of the proteins, nuclein, etc., on the one hand, and uric acid and the urates on the other.

Uric acid normally occurs in the urine combined with alkaline bases, also with traces of calcium and magnesium. It is insoluble in alcohol, ether, or dilute acids; practically insoluble in water, but much more soluble in solutions of urea or of glycerin. A solution of uric acid does not redden blue litmus.

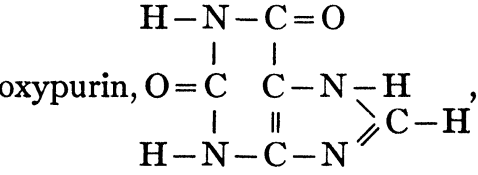
Purine is a white, crystalline substance melting at 216° C., soluble in water and of weak basic character but not sufficiently basic to turn litmus blue (Holleman-Walker). Its formula is $C_5H_5N_4$, or graphically as

$$\begin{matrix}N=C-H\\ |\quad\ |\\ H-C\quad C-N-H\\ \|\quad\ \|\quad\ \diagdown\\ N-C-N\quad\ C-H\end{matrix}$$

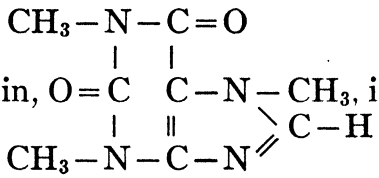
. If we now break all double bonds except those linking two carbon atoms (4 and 5), we obtain a graphic nucleus,

$$\begin{matrix}1-N-C^6\\ \quad |\quad\ |\\ 2=C\quad C^5-N-7\\ \quad |\quad\ \|\quad\ \diagdown\\ 3-N-C^4-N-9\end{matrix}\quad C=8,$$

by numbering the atoms of which we may easily designate any structural formula of the group; thus, 2–6–8, trioxypurin, is uric acid as above, while xanthin is 2–6, dioxypurin,



and 1 – 3 – 7, trimethyl-xanthin,



is caffein and thein, alkaloids from coffee and tea.

Traces of xanthin (2.6 dioxypurin), hypoxanthin (6 oxypurin), guanin (2 imino, 6 oxypurin), adenin (6 amino purin), and heteroxanthin (7 methyl xanthin) have been found in urine, and, in cases of leukemia, many of them in increased amounts, notably xanthin, hypoxanthin, and adenin (Witthaus).

Upon heating uric acid, urea and cyanuric acid may be obtained; NH_3 and CO_2 are given off. We are not to infer from this decomposition that the uric acid is an antecedent of urea in the animal body; for such is not the case, except possibly to a limited extent.

Uric acid produces, upon oxidation, a variety of compounds, according to the temperature and the oxidizing agent employed.

Chlorine, hot, yields cyanuric acid, $C_3N_3(OH)_3$. Chlorine or bromine, cold, forms oxalic acid, alloxan $CO\begin{matrix}\diagup \\ \diagdown\end{matrix}\begin{matrix}NHCO \\ NHCO\end{matrix}\begin{matrix}\diagdown \\ \diagup\end{matrix}CO$, parabanic acid $CO\begin{matrix}\diagup \\ \diagdown\end{matrix}\begin{matrix}NH-CO \\ NH-CO\end{matrix}$ and ammonium cyanate. HNO_3 in the cold, forms alloxan, alloxantin, and urea (Witthaus).

Uric acid may be detected by the murexide* test. See Exp. 84, page 263, also by the more delicate phosphotungstic acid test, page 264.

While uric acid is practically insoluble in H_2O and the acid

* *Note.* — Murexide is a definite chemical compound ($C_8H_8N_6O_6$) and may be produced from alloxantin, an oxidation product noted above.

urates only sparingly soluble, the uric acid in the system is apparently held in solution as an acid urate ($NaH\overline{U}$) by the presence of the sodium phosphates, NaH_2PO_4 and Na_2HPO_4, possibly also aided by the presence of some unknown organic combination.

$NaH\overline{U} + NaH_2PO_4$ forms, at 38° C., a solution with an acid reaction; if, however, the mixture is cooled to room temperature, the reaction becomes alkaline from Na_2HPO_4, and uric acid is precipitated (Bunge):

$$NaH\overline{U} + NaH_2PO_4 = Na_2HPO_4 + H_2\overline{U}.$$

Na_2HPO_4 is a normal constituent of the blood, and a tendency to precipitate uric acid may be met by the following reaction: $Na_2HPO_4 + H_2\overline{U} = NaH_2PO_4 + NaH\overline{U}$. Because the acid urate of lithium is much more soluble in water than any of the other monometallic urates, lithium salts have long been used as uric acid solvents. But the fact that lithium solutions will precipitate from solutions of Na_2HPO_4 crystals of Li_2HPO_4, has been made the basis for a claim that such use of lithium salts is without effect other than to decompose and render insoluble the alkaline phosphate, which has been acknowledged a valuable factor in keeping uric acid in solution. While the disodic phosphate is regarded by many as superior to lithium salts as a uric acid solvent, the fact of comparative insolubility of Li_2HPO_4 can hardly be regarded as conclusive evidence that lithium compounds are not effective.

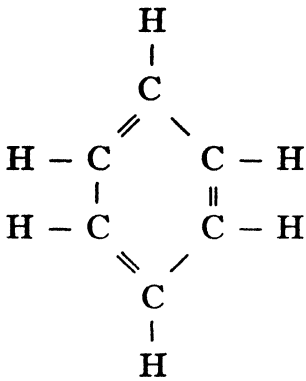
The following in regard to our need for " sarsaparilla " in the spring is given by Dr. E. C. Hill, of the University of Denver, in his text-book of chemistry, page 370: " Reduced alkalinity of the blood, as in winter from eating meats freely, throws uric acid out of solution to collect in the more acid tissues (spleen, liver, and joints). With the vernal tide of alkalinity (due to freer sweating, with excretion of fatty acids) these deposits are swept out in the blood-current, irritating the nerves and giving rise to ' that tired feeling.' "

CHAPTER VIII.

CLOSED-CHAIN HYDROCARBONS.

In illustrating the simpler relationships of organic compounds we have, as far as possible, carefully avoided reference to the closed-chain or aromatic compounds, as the characteristic groupings are more easily seen by the use of simple formulæ. The distinguishing feature of the aromatic (also called cyclic) compounds is a nucleus consisting of a closed chain of atoms; this chain may contain three, four, five, six, or seven members, but the six-carbon ring is by far the most important, and the only one that we are to consider.

The hydrocarbons of the aromatic series have, for a general formula, C_nH_{2n-6}, the simplest being *benzene* or benzol, C_6H_6; and we may consider that the aromatic compounds are derived from this. The structure of the benzene molecule is represented by Kekulé's benzene ring. Note that there are three double bonds, which of course permit of addition products, as $C_6H_6Cl_2$, benzene di-chloride, etc. The substitution products are, however, of far greater importance.



The principal source of the hydrocarbons of the aromatic series is coal-tar. When this substance is distilled, water, ammonium compounds and some of the lighter hydrocarbons are found in the first portions of distillate, coming over at a temperature below 105° C. The distillate obtained from 105° to 210° is known as "light oil," and in it may be found, among other substances, the first three members of the closed-chain hydrocarbons — benzene, toluene, and xylene.

Benzene, C_6H_6 (benzol), is a colorless liquid from the "light oil" obtained by distillation of coal-tar. It boils at 80°, has a

gravity of 0.899, is soluble in ether, alcohol, and chloroform, but insoluble in water. It may be made pure by distilling an intimate mixture of benzoic acid and quicklime, and at a temperature of about 5° C. may be obtained as a crystalline solid, $C_6H_5COOH + CaO = CaCO_3 + C_6H_6$. (See Exp. 92, page 264.)

Benzene may be considered as phenyl-hydride, C_6H_5H, and, as in the case of the straight-chain hydrocarbons, two of these phenyl groups may be made to combine, giving a hydrocarbon $C_{12}H_{10}$, known as diphenyl. Reaction $2\ C_6H_5Br + 2\ Na = C_{12}H_{10} + 2\ NaBr$.

Toluene, (toluol). — The next higher homologue of the series will be C_7H_8; this is methyl benzene ($C_6H_5CH_3$) or toluene.

The hydrocarbons of this series may be prepared in a manner similar to that used in the preparation of the hydrocarbons of the paraffin series.

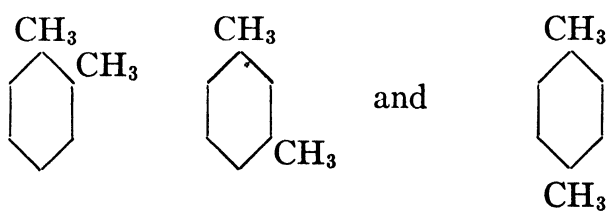
Toluene may be made by the action of metallic sodium upon a mixture of brombenzene and methyl iodide.

$$C_6H_5Br + CH_3I + Na_2 = C_6H_5CH_3 + NaBr + NaI.$$

Toluene is a colorless liquid boiling at 110° C., and yielding upon oxidation a benzene derivative; i.e., the CH_3, or so-called side chain, is the part of the compound changed by oxidizing agents, rather than the benzene ring,

$$C_6H_5CH_3 + 3\ O = C_6H_5CO_2H + H_2O.$$

Xylene, C_8H_{10} (xylol) or dimethyl benzene, the next hydrocarbon of this series, exists in coal-tar as a mixture of three isomeric compounds which may be graphically represented as follows:



These three possible positions of the *second* substitution are known as ortho-, meta-, and para-; thus, the first representation

at the left will be ortho-xylene, or ortho-dimethyl benzene. The other two will be meta-xylene and para-xylene respectively.

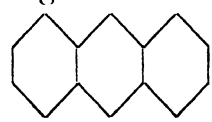
A tri-substituted benzene may be "adjacent," if the substituted element or group is attached to the carbon atoms 1 – 2 – 3, or "unsymmetrical" 1 – 2 – 4, or "symmetrical" 1 – 3 – 5.

A fourth isomer of dimethyl benzene would be an ethyl benzene, $C_6H_5C_2H_5$. This, upon oxidation, yields benzoic acid, in a manner similar to toluene. (Bernthsen.)

Mesitylene, C_9H_{12}, is a trimethyl benzene. Only two isomers are possible. It can be prepared by dehydrating acetone by the use of sulphuric acid:

$$3\ C_3H_6O - 3\ H_2O = C_9H_{12}.$$

As the distillation of coal-tar proceeds to a temperature of 210–240°, the distillate is known as "carbolic oil" and contains some of the hydroxyl derivatives of benzene (phenols), and a hydrocarbon with a structural formula based upon the union of two benzene rings, *naphthalene* ($C_{10}H_8$). This is a white, crystalline solid, which melts at 80° and boils at 218°. From 240° to 270°, the so-called "creosote oils" are obtained; in these oils are found hydroxyl derivatives of toluene (cresol) and naphthalene (naphthol).

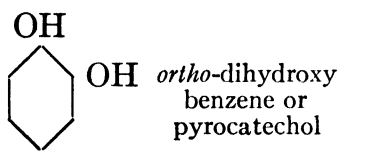
Above 270° a third benzene ring links itself to the two of naphthalene, giving us *anthracene*  ($C_{14}H_{10}$), colorless plates melting at 213°. The residue is pitch, used in preparation of roofing materials, etc.

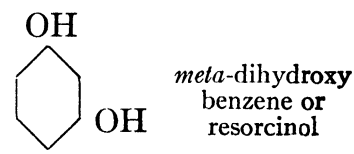
Of course, it is not to be understood that the various distillates contain only the substances indicated above. There are many others, and constituents of one fraction are pretty sure to be found in lesser quantities in the fractions above and below; e.g., the light oil contains some of the phenols, and some naphthalene comes over above 240°.

Hydroxy Derivatives of the Aromatic Hydrocarbons.

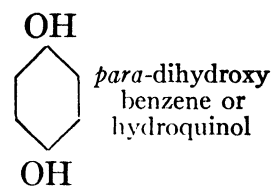
Phenol, carbolic acid, or oxybenzene, C_6H_5OH, is obtained from the distillation of coal-tar, and used as an antiseptic and disinfectant. For properties and test, see page 88. Phenol acts like an acid, in that it forms salts with the metallic bases, C_6H_5OK, potassium phenolate, but it does not have an acid reaction on litmus paper or other indicators, i.e., it does not have free hydrogen ions when in solution. It may be made in the laboratory from salicylic acid, Exp. 104.

The three di-hydroxy-benzenes are all of interest and are graphically represented as follows:





and



The ortho-compound is **pyrocatechol**. Its ethereal sulphate (acid sulphate) is given by Hoppe-Seyler as a constituent of normal urine, and its monomethyl ether, **guaiacol,** $C_6H_4OH-O-CH_3$, is obtained from beech-wood creosote, of which it constitutes the greater part (60 to 90 per cent U. S. D.). Guaiacol and various compounds produced from it have been widely recommended for tubercular diseases.

Pyrocatechol has been found to be the most practical reagent for the detection of oxidizing enzymes in the saliva.

Resorcinol is a white crystalline solid, becoming more or less colored upon exposure to the light. It melts at 118° C., and, in solution, gives a purple color with ferric chloride. Heated with sodium nitrate, it produces a substance known as "Lacmoid" which is used to a considerable extent as an indicator.

The **hydroquinol,** or hydrochinon, is a white powder melting at 169° C., and is largely used as a photographic developer.

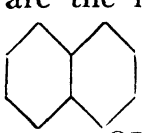
Pyrogallol, or tri-hydroxy-benzene, $C_6H_3(OH)_3$ (1 – 2 – 3), may be made by heating gallic acid, and because of this fact is usually called pyrogallic acid. It is a white, silky crystal which, like hydroquinol, is used as a photographic developer. Dissolved in a solution of caustic potash, it absorbs oxygen to a marked degree, and may be used as a reagent for the quantitative determination of oxygen in gas analysis.

Phloroglucinol is another tri-hydroxy-benzene, isomeric with pyrogallol but with the hydroxyl groups occupying positions 1 – 3 – 5 in the ring. The formula is $C_6H_3(OH)_3$ (1 – 3 – 5). It crystallizes in rhombic prisms, soluble in water, alcohol and ether. This is used in physiological chemistry as a reagent with vanillin as a test for free hydrochloric acid.

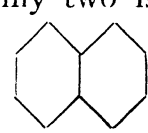
Thymol (3 methyl-6 isopropyl-phenol), $C_6H_3OH_{(1)}CH_{3(3)}C_3H_{7(6)}$, is a solid of the nature of camphor, melting at 44° C., and is obtained from various volatile oils, particularly from the oil obtained from Thymus Vulgaris. It is very sparingly soluble in water. The addition of a little alcohol increases the solubility. It is largely used in the preparation of antiseptic dental preparations, mouth washes, etc.

Eugenol is synthetic oil of cloves with a formula of $C_6H_3OH_{(1)}-O-CH_{3(2)}-CH_2CH=CH_{2(4)}$, largely used as an antiseptic in the preparation of dental " cements."

Naphthols. — Important hydroxyl derivatives of naphthalene are the naphthols, $C_{10}H_7OH$. Only two isomers are possible,



the alpha-compound and



the beta. Properties of the naphthols are not unlike those of the phenols.

Alpha naphthol melts at 95° C., and is used in physiological chemistry as a test for the presence of carbohydrates. (Exp. 126.)

Cresol, $C_6H_4CH_3OH$, is a hydroxy-toluene. Three isomeric compounds of this formula are obtained from the distillation of coal-tar between 200° and 210°C. The ortho- and para-cresols

are solid at ordinary temperatures, the ortho compound melting at 31° C., the para at 36° C. Meta-cresol is a liquid which does not solidify except under extreme conditions of cold and pressure.

The cresols are similar to phenol, not only in composition but also in physical and therapeutic properties; hence, cresol has been called cresylic acid, just as phenol has been called carbolic acid.

A mixture of the cresols, said to be composed of meta-cresol 40 per cent, ortho- 35 per cent, and para-cresol 25 per cent, constitutes the tri-cresol very largely used in dentistry as a germicide and antiseptic similar to carbolic acid.

An emulsion of cresol, obtained by the solution of resin soap as an emulsifying agent, is known as creolin. Cresol is also a constituent of the disinfectant lysol.

Tri-cresol is miscible with formalin in all proportions, and the mixture is recommended in the treatment of root canals. See Buckley's "*Formo-cresol*," page 83.

Aromatic Alcohols, Aldehydes and Acids.

The various hydroxyl derivatives above considered are not true alcohols, in that the $-OH$ is attached directly to the carbon nucleus, the characteristic alcohol groups, $-CH_2OH$ and $-CHOH$, are lacking, and these derivatives yield neither aldehydes nor ketones upon oxidation.

A type of the true aromatic alcohols is benzyl alcohol, $C_6H_5CH_2OH$. Benzyl alcohol is a liquid only slightly soluble in water. It gives none of the phenol reactions (Holleman), but may be oxidized to an aldehyde, like the primary straight-chain alcohols.

Benzaldehyde (C_6H_5CHO), or "True oil of bitter almonds," is the aldehyde constituting about 85 per cent of the natural oil. Benzaldehyde may be made by oxidation of toluene by methods which fall short of acid production. By action of KOH it is "saponified," producing the alcohol $2\ C_6H_5CHO + KOH = C_6H_5CH_2OH + C_6H_5COOK$. Benzaldehyde is quite easily oxidized to benzoic acid, the simplest of the aromatic acids.

Benzoic acid, C_6H_5COOH, was originally produced from gum benzoin, but may be made from hippuric acid (q.v.), which

(from urine of horses) formerly constituted a commercial source. It is chiefly prepared, however, from toluene; (see Exp. No. 113) it crystallizes in colorless plates or long prismatic crystals (from solution). It is sparingly soluble in cold water, more soluble in hot water, easily soluble in alcohol. It sublimes and is inflammable, burning without residue. (Plate I, Fig. 5.)

Benzoates of sodium, ammonium, lithium, and lime are all used in medicine. Benzoated or benzoinated lard is prepared by digesting gum benzoin in hot lard. This is much used as a base for ointments, and keeps well.

Salicylic acid, ortho-hydroxy-benzoic acid, $C_6H_4-OH.COOH$, is a white, crystalline powder, odorless, irritating to mucous surfaces, soluble in alcohol and ether, and in about 450 parts of water at 15° C. (U. S. D.). Salicylic acid may be made by action of carbon dioxide on sodium phenate and subsequent decomposition of the sodium salicylate. By heating rapidly the acid may be changed into phenol and carbon dioxide.

Acetyl salicylic acid, $C_6H_4.C_2H_3O_2.COOH$, known in medicine as aspirin, may be obtained by heating salicylic acid with acetyl chloride. It occurs as white needles, slightly soluble in water, soluble in alcohol and ether. Aspirin is decomposed in the intestine, salicylic acid appearing in the urine twenty to thirty minutes after administration of aspirin.

Salicylates have been used to considerable extent in various uric-acid diseases. Methyl salicylate constitutes 90 per cent of natural oil of wintergreen (Gaultheria). The alcoholic solution is essence of checkerberry.

Salol is phenyl-salicylate, $C_6H_4OH.COO(C_6H_5)$, a white, crystalline powder, practically insoluble in water and not decomposed by the dilute acids of the stomach juices; in the intestine it becomes salicylic acid and phenol, as follows:

$$C_6H_4.OH.COOC_6H_5 + H_2O = C_6H_4OH.COOH + C_6H_5OH.$$

Gallic acid, a tri-hydroxy-benzoic acid, $C_6H_2(OH)_3COOH$, (1 : 2 : 3 : 5), is prepared from tannic acid by action of dilute sulphuric acid, or by oxidation by exposure of powdered galls. It forms slightly brownish crystals; if pure, the crystals are

colorless. At ordinary temperatures one part of acid is soluble in about one hundred parts of water, five parts of alcohol or twelve parts of glycerine.

Tannic acid, or tannin, sometimes called di-gallic acid because its composition, $C_{14}H_{10}O_9$, corresponds to two molecules of gallic acid less one molecule of water, occurs in galls, in many astringent drugs and bark from various trees, as hemlock and oak. Tannic acid causes dark-colored precipitate with ferric chloride, and precipitates gelatin, albumin and starch, differing in all of these particulars from gallic acid. (U. S. D.)

Hippuric acid, benzoyl glycocoll, $C_6H_5CO.NH.CH_2-COOH$, occurs in traces in human urine, to a considerable extent in the urine of the herbivora, but not at all in that of the carnivora. It crystallizes in prismatic needles (Plate I, Fig. 4, page 40), often resembling crystals of ammonium magnesium phosphate; but as these latter only occur in neutral or alkaline urine and hippuric acid usually in acid urine, there is little danger of confounding the two substances. Hippuric acid is hydrolyzed by the urease of fermenting urine, forming benzoic acid and glycocoll (amino-acetic acid):

$$C_6H_5CO-NH-CH_2-COOH + H_2O = C_6H_5COOH + CH_2NH_2COOH$$

Tryosin, $C_6H_4OH-CH_2CH(NH_2)-COOH$, may be crystallized as fine, silky needles. It is an amino acid, formed from protein substances, particularly casein and fibrin, both by the action of proteolytic enzymes and by putrefactive processes. It rarely occurs in urinary sediment; when found it is in bundles or sheaves (Plate I, Fig. 6, page 40), and is usually indicative of acute liver disease, phosphorus poisoning, etc.

Phthalic acid, $C_6H_4\begin{<}{COOH}{COOH}$, occurs in the form of rhombic crystals. By heating phthalic acid, phthalic anhydride may be obtained.

Phthalic anhydride, $C_6H_4\begin{<}{CO}{CO}O$, heated with phenol and

sulphuric acid, will give phenolphthalein, a valuable and familiar indicator in volumetric analysis.

Sulphanilic acid, $C_6H_4\begin{cases}HSO_3\\NH_2\end{cases}$, is made by treating aniline with concentrated sulphuric acid. It is a strong acid, occurring as white crystals, is soluble in water, and is used in the manufacture of aniline dyes and also with naphthylamine as a reagent for the detection of nitrites.

Phenyl-sulphuric acid, $C_6H_5HSO_4$, occurs only in combination, the acid being unstable if attempt is made to isolate it. Its potassium salt is present in the urine as a product of intestinal putrefaction.

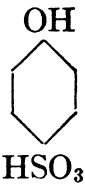
Phenyl-sulphonic acid may be made by action of oxygen upon the sulph-hydrate, as in the process described on page 48.

$$C_6H_5SH + 3\,O = C_6H_5SO_2HO.$$

The potassium salt of this acid, heated with potassium hydroxide, is a commercial source of phenol.

$$C_6H_5.SO_3K + KOH = C_6H_5.OH + K_2SO_3.$$

Phenol-sulphonic acid. — When phenol is treated with several times its volume of cold, strong sulphuric acid, phenol sulphonic acid,

or 

, results. If the mixture is heated for some time over a water-bath, the di-sulphonic acid results. When this acid is warmed with a nitrate and the mixture treated with excess of ammonia, ammonium picrate is produced. This constitutes a delicate test for nitrates present in drinking water.

Phenol-sulphonic acid has been used in dentistry as a therapeutic agent (as antiseptic and otherwise). Such use is discussed in detail by Herman Prinz, M.D., D.D.S., in the *Dental Cosmos* for April, 1912, with the conclusion that the ortho compound is several times more active than either the meta or para compounds; that a one per cent solution is about equal in antiseptic strength

to a one per cent phenol solution; but that in this strength it decalcifies the tooth structure, discolors the teeth, and should not be used in the mouth on account of its pronounced acid character.

Nitrogen Derivatives.

Benzidine, a di-para-diamino derivative of diphenyl is made by the reduction of di-nitrophenyl; it is a solid substance melting at 122° C., and is used as a reagent in testing for blood.

Nitrobenzene, $C_6H_5NO_2$, may be produced by treating benzene with a mixture of nitric and sulphuric acid at reduced temperature. (Exp. 98, page 265.) It is a yellow, oily liquid, with the odor of bitter almonds, commercially known as oil of mirbane, and used in the manufacture of aniline.

Aniline or Amino-benzene, $C_6H_5NH_2$. — By reaction of nitrobenzene with nascent hydrogen, the NO_2 group becomes an NH_2 group and aminobenzene or aniline is produced. Aniline, a colorless liquid, also called aniline oil, is important from a commercial rather than from a medical standpoint, as it forms the basis of the aniline dyes. When pure it is a colorless liquid, but changes quite rapidly when exposed to the light. It is used in testing for chloral and chloroform. It is slightly soluble in water, and easily soluble in alcohol and ether. At 8° C. it becomes a crystalline solid.

Toluidine, aminotoluene ($C_6H_4.CH_3.NH_2$).

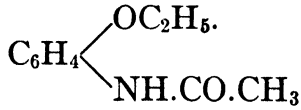
By treatment of toluene with HNO_3 and H_2SO_4 nitro derivatives, analogous to nitrobenzene are produced, and these by reduction will form the amino compounds.

Ortho-toluidine is a liquid boiling at about 200°. Para-toluidine is a solid melting at 45°.

Diphenyl-amine, $(C_6H_5)_2NH$, is formed by the substitution of the phenyl group for one of the amino hydrogens of aniline. It crystallizes from petroleum ether in white crystals which melt at 54° C.

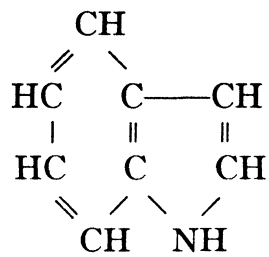
Acetanilide, $C_6H_5.NH.COCH_3$, also known as antifebrine, may be produced by heating aniline and glacial acetic acid; it crystallizes in colorless plates which melt at 115° C. Exp. 77.

Amino-phenol may be formed by the reduction of nitro-phenol by the action of nascent hydrogen (tin and hydrogen chloride). The para compound forms an ethyl ester which by action of glacial acetic acid gives phenacetine or para-acet-phenetidine,



Picric acid is tri-nitrophenol, $C_6H_2.OH.(NO_2)_3$. It may be formed by action of strong nitric acid, or mixture of sulphuric acid and nitric acid on phenol. It occurs as yellow plates slightly soluble in water, easily soluble in alcohol and ether, and is used in Esbach's reagent for the estimation of albumin in urine and as an alkaloidal precipitant.

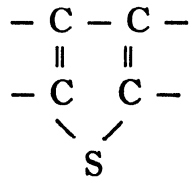
Salvarsan, (606), arsenobenzol, more accurately para-diamino-dioxy-arsenobenzene hydrochloride, is an arsenic derivative of benzene used in medical practice as a specific for syphilis.

Indol, C_8H_7N,  , is produced from protein

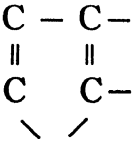
by the putrefaction occurring in the small intestine, also by action of the proteolytic enzyme of the pancreatic juice (trypsin). The indol, by oxidation (after absorption from the intestines) becomes indoxyl, C_8H_6NO, which, with potassium sulphate, forms indoxyl-potassium sulphate, $C_8H_6NKSO_4$, and, as such, is eliminated (in part) by the kidneys. (See page 201.) This substance is a type of the so-called ethereal or conjugate sulphates, skatoxyl-potassium sulphate (skatol) and phenol-potassium sulphate being other compounds of this class. The ethereal sulphates are not precipitated by barium chloride in alkaline solutions, but may be decomposed by prolonged boiling with hydrochloric acid and then precipitated as usual.

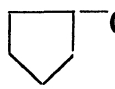
Heterocyclic Compounds.

Of the compounds containing elements other than carbon in the nucleus, we shall consider only a few. One of the simple compounds of this character *Thiophene*, C_4H_4S, is a liquid constituent of the benzene distillate from coal-tar, boiling at 84°.



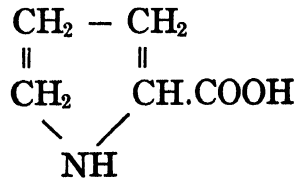
It forms nitro derivatives and reacts chemically with somewhat greater ease than does benzene.

Furfuran, C_4H_4O  , obtained from pine-tar, is a volatile liquid boiling at 32° and of much less importance than some of its derivatives, notably furfuraldehyde and pyromucic acid.

Furfuraldehyde, $C_4H_3O.CHO$, is a liquid boiling at 162°. It gives a red color with aniline and hydrochloric acid and is responsible for the color reactions of the aldehydes with milk and sulphuric acid, of carbohydrates and alpha naphthol, etc. Furfuraldehyde, by action of alcoholic KOH or Ag_2O, will yield the corresponding acid  , known as pyromucid acid, also furfuryl alcohol, .

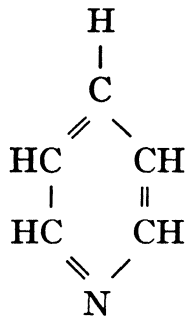
Pyromucic acid may be made by action of heat, dry distillation, of mucic acid. It is white, crystalline, and will sublime unchanged. Upon distillation of the ammonium salt of pyromucic acid (or of mucic acid), pyrrol results.

Pyrrol, C_4H_4NH, is of interest because of its relationship to the proteins.

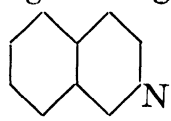
Prolin is pyrrolidine-carboxylic acid 

found among the decomposition products of proteins generally (with exception of the protamines), particularly among those resulting from decomposition of casein and the vegetable proteins, hordenin and gliadin.

Pyridine, C_5H_5N, obtained from the light oil of the coal distillate and also from bone oil, is a colorless liquid, miscible with water. It has a boiling point of 115° C., a faintly alkaline reaction, and, like benzene, resists the action of chemical agents to a marked degree.

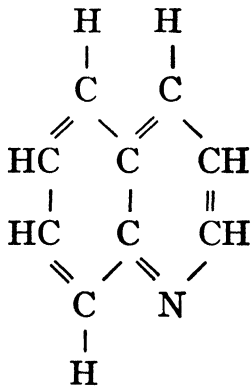


Quinoline may be regarded as a condensation product of benzene and pyridine. It is a colorless liquid with a high boiling point, 236° C. Quinoline and its isomer, isoquinoline,



Isoquinoline

are of interest because of their relation to a number of important alkaloids.



Quinoline

The alkaloids are complex vegetable constituents frequently representing the active medicinal properties of the plant. They are basic in character and form salts with the common acids.

Caffeine and theobromine, already considered in connection with uric acid, are purine derivatives obtained from coffee and cocoa respectively

Nicotine is a liquid alkaloid found in tobacco leaves combined with malic and citric acids. Its empirical formula is $C_{10}H_{14}N_2$. It contains both the pyrol and pyridin groups.

Morphine, $C_{17}H_{19}NO_3$, and *narcotine,* $C_{22}H_{23}NO_7$, are both derived from opium and contain the isoquinoline group.

Heroin, an acetyl derivative of morphine, has been considered on page 84.

Quinine, $C_{20}H_{24}N_2O_2$, perhaps the alkaloid best known outside of the scientific world, represents the active principle of cinchona or Peruvian bark. There are more than twenty alkaloids that have been separated from cinchona, quinine being the most important and occurring in the largest quantity. Quinine is a quinoline derivative.

Strychnine, $C_{21}H_{22}N_2O_2$, a very powerful alkaloid, is associated with brucine in the seeds of Strychnos nux vomica, the so-called "Quaker button." Strychnine is quite easily detected by the "fading purple test" obtained by drawing a minute crystal of potassium dichromate *through* a drop of concentrated sulphuric acid and *over* the strychnine residue contained in a small porcelain capsule obtained as follows:

To an unknown aqueous solution or mixture, from which the fat has been removed, add acetic acid, producing a salt of the alkaloid. In this form the alkaloid is insoluble in chloroform. Agitate gently at intervals for several hours, with chloroform, in order to remove extraneous coloring matter which might interfere with the test. Remove this "acid" chloroform and change the reaction of the solution by adding a decided excess of ammonia. This precipitates the alkaloid as such. A repetition of the treatment with chloroform will now dissolve out the strychnine. By separation of the chloroform and evaporation over a water-bath, the strychnine residue referred to above is obtained.

PART II.

MICROCHEMICAL ANALYSIS.

CHAPTER IX.

METHODS.

The advantages of microchemistry are many, as claimed by its enthusiastic advocates, and there are two particulars in which microchemical methods strongly recommend themselves to the dental practitioner: (1) Microchemical analysis deals with exceedingly minute portions of matter, making the examination of very small particles of substance easily possible. (2) Three or four one-ounce " drop-bottles " and a few two-drachm vials will contain all necessary reagents, and in consequence three feet of bench-room will furnish ample laboratory space.

The principles of microchemical analysis are, of course, the same as those of any analysis, but the processes employed are quite different and need some explanation. In microchemical analysis the production of crystals of characteristic form furnishes perhaps the most rapid method of detection of an unknown substance, and in this we are greatly aided by the use of polarized light, which not only helps in the differentiation of crystals but often makes it possible to see and distinguish small or transparent crystals which might otherwise escape notice altogether.

Use of Microscope. — For the examination of the crystals mentioned in this chapter, also for the work required on saliva or urine, lenses of comparatively low power are sufficient. For most of the microchemical tests, a No. 3 Leitz or a 16-mm. Bausch & Lomb objective will be found satisfactory. For a few microchemical tests and for urine, an 8-mm. Bausch & Lomb or a No. 5 Leitz objective will give better results in the hands of a beginner than one of higher power.

In using the microscope for microchemistry, the preparation should *always* be covered with a cover glass and the examination be made with the low-power lens if possible. The object in covering is to prevent any action by reagent upon the objective. As a further precaution, it is well to form the habit of first lowering the objective and then focusing by upward movement of the draw-tube.

Formation of crystals may be brought about two ways: *first*, by precipitating insoluble crystalline salts by use of reagents, as in ordinary qualitative analysis; *second*, by allowing salts to crystallize by spontaneous evaporation of the solvent.

If the first method is to be employed it is essential to have the dilution fairly constant in order to obtain crystals comparable with those obtained at other times or by other individuals. The tendency of strong solutions is to give amorphous precipitates. Sometimes the precipitate will be amorphous when first thrown down, but upon standing will assume crystalline form. To secure the uniformity of results necessary to correct deductions, the following method of procedure should be *exactly* followed *every* time.

The reagent should be of uniform strength, usually one or two per cent. Place on a clean microscope-slide a small drop of the solution to be tested, and as close as possible without touching it, one of about equal size of the reagent to be used. Now bring the drops together by tapping the slide or with a small glass rod. If a precipitate forms immediately, *cover with a cover-glass* (this must *always* be done) and examine with the microscope. If the precipitate is crystalline, note the form, and in any case, whether crystalline or not, repeat the test after diluting the unknown solution one-half. If the second test gives an amorphous precipitate, or crystals of different shape from the first, *continue the dilution of the unknown* till a point is reached when admixture with the drop of reagent gives *no immediate* precipitate, but one appearing in a few seconds' time (five to thirty). In this way we have produced the precipitate under standard conditions or as nearly so as is possible with unknown solutions.

Until thoroughly familiar with the forms obtained by drying

the various reagents, it is well to evaporate a small drop of the reagent alone, on the same slide on which a test is made, for the sake of subsequent comparisons.

Filtration in microchemical examinations, when perhaps only a few drops of solution are to be had, may be effected in a very satisfactory manner and without appreciable loss by absorption as follows:

Cut a filter-paper about 1 cm. wide and 6 cm. long, double it and crease the middle so that it assumes the shape of an inverted V. Put the solution to be filtered in a small watch-glass placed at a slight elevation above a microscope slide; now place one " leg " of the strip of filter-paper in the watch-glass, allowing the end of the other to touch the slide. By capillary attraction the clear solution will follow over the bend in the strip of paper and a drop or two of perfectly clear filtrate suitable for the test will be found upon the slide.

Evaporation of a *solution* is best effected on a small watch-glass *held in the fingers* and moved back and forth over a low Bunsen flame, or else placed over a water-bath.

The purpose of the microchemical tests here outlined is not so much a method of general qualitative analysis, to which they are not suited, as it is a specific application of well-known reactions to concrete examination of substances, the uses and probable composition of which are known. The details of the various tests will be given under classification furnished by the substances investigated.

Our study may include alloys and amalgams, teeth, tartar, dental anesthetics, cement, mouth-washes, antiseptics, disinfectants, and sediments obtained from the saliva and from the urine.

The following crystals are selected as among those most frequently met with in the analysis of the above substances, or best suited for the study of microchemical processes. The student should make each test here indicated and carefully draw the crystals produced.

1. Calcium oxalate from 2 per cent $H_2C_2O_4$ and $CaCl_2$ solutions (Plate II, Fig. 1).

PLATE II. — MICROCHEMICAL ANALYSIS.

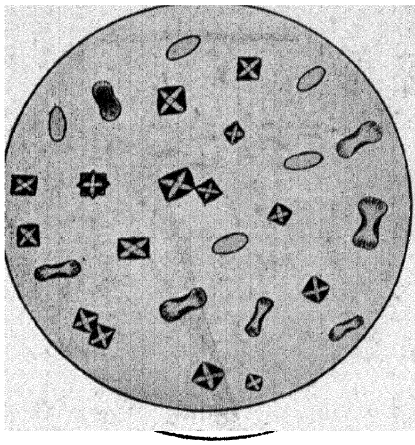


FIG. 1.
Calcium Oxalate.

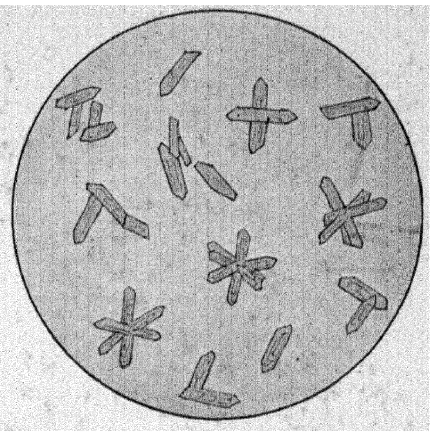


FIG. 2.
Cadmium Oxalate.

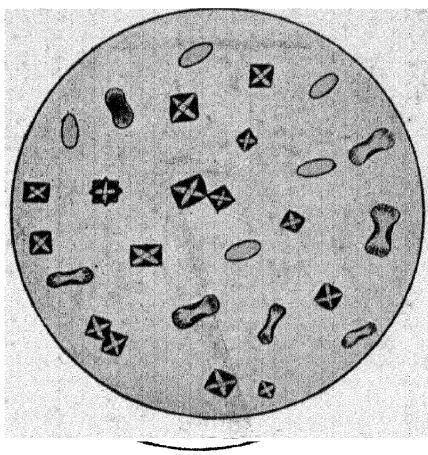


FIG. 3.
Strontium Oxalate.

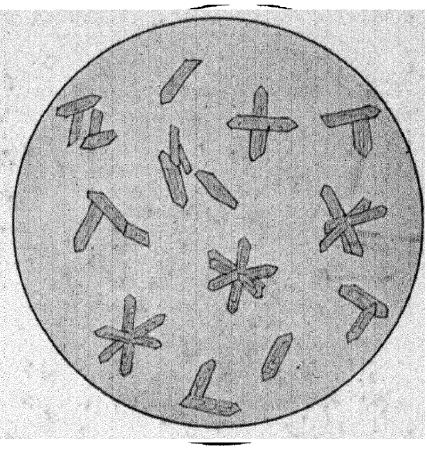


FIG. 4.
Sodium Oxalate (P.L.).

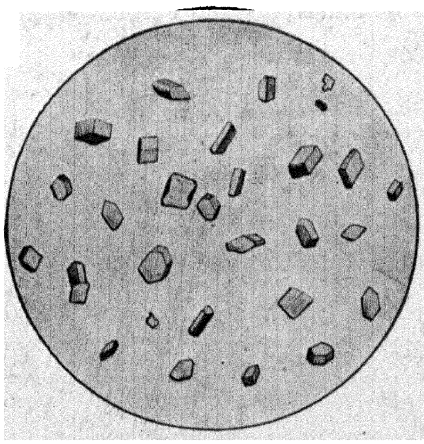


FIG. 5.
Oxalate of Urea.

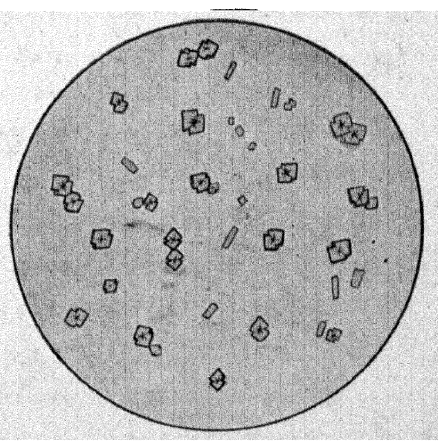


FIG. 6.
Zinc Oxalate.

PLATE III. — MICROCHEMICAL ANALYSIS.

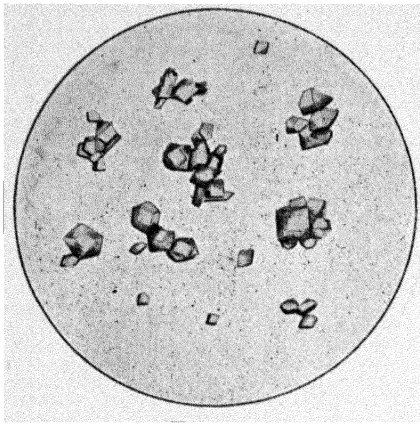


FIG. 1.
Ammonium Platinic Chloride.

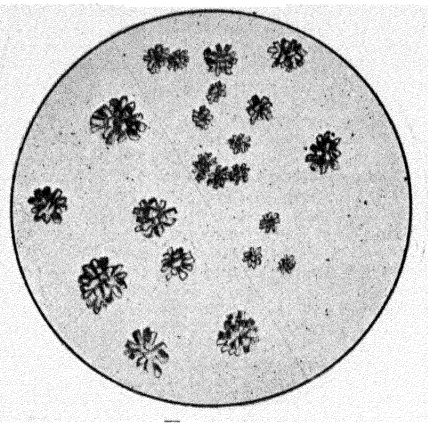


FIG. 2.
β Eucaine and Platinic Chloride.

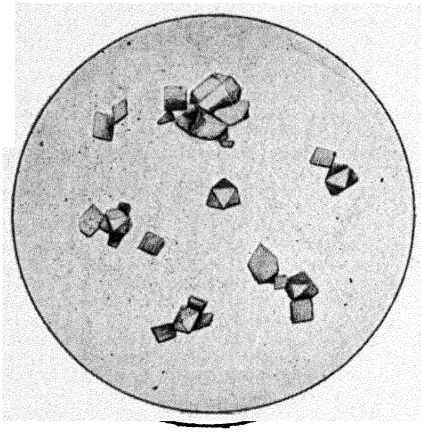


FIG. 3.
Potassium Platinic Chloride.

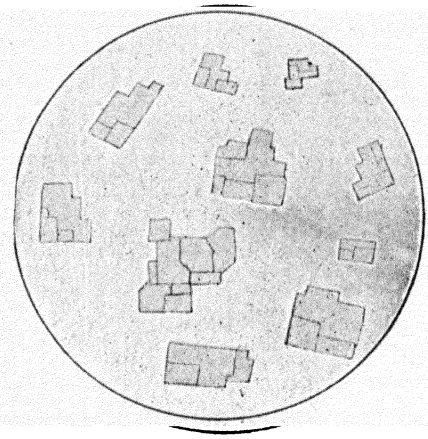


FIG. 4.
Cocaine and Potassium Permanganate.

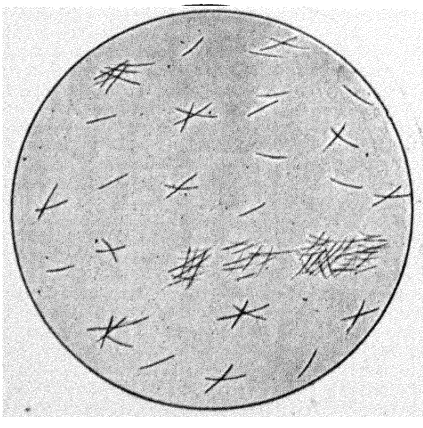


FIG. 5.
Tri-brom-phenol.

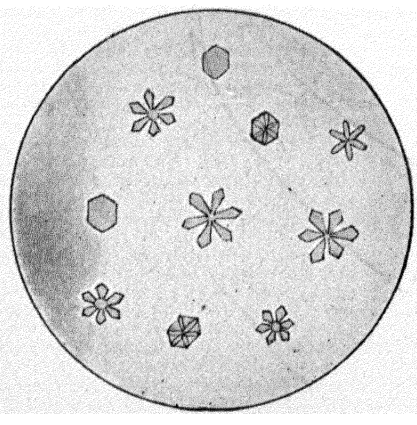


FIG. 6.
Iodoform.

PLATE IV.—MICROCHEMICAL ANALYSIS.

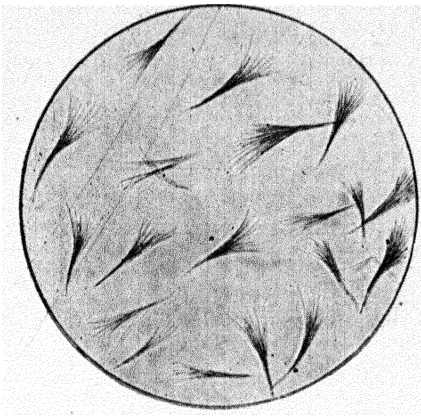


FIG. 1.
Morphine and Marme's Reagent.

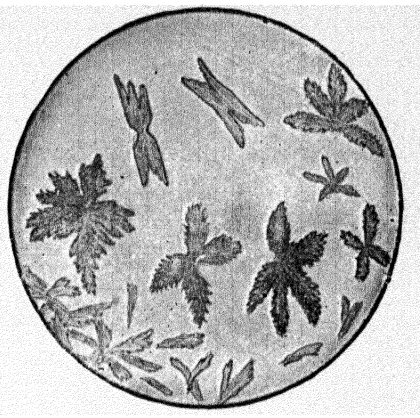


FIG. 2.
Magnesium Ammonium Phosphate.

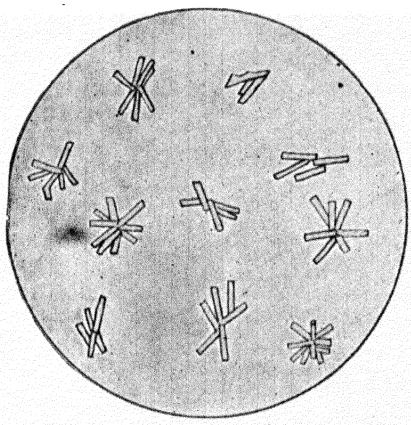


FIG. 3.
Cocain with Tin Chloride.

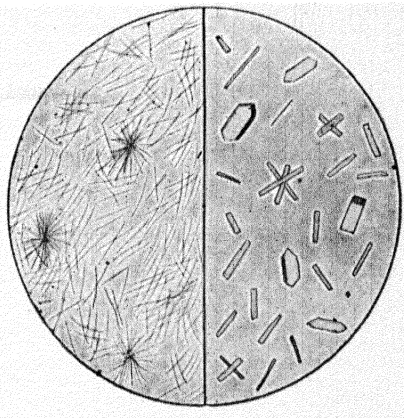


FIG. 4.
Morphine.

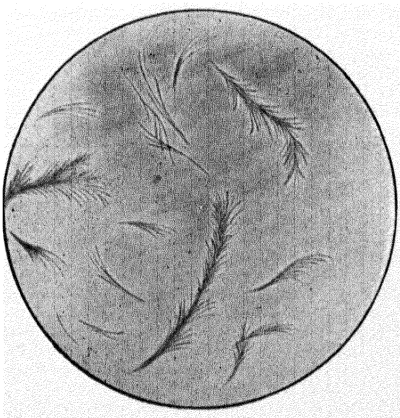


FIG. 5.
Palmitic Acid.

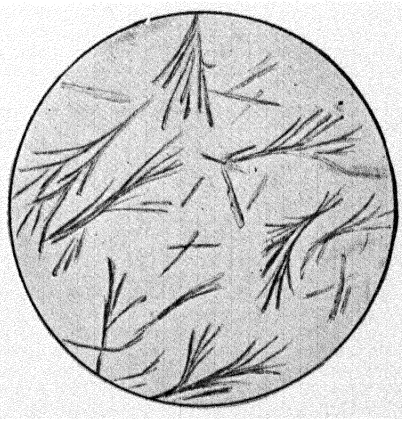


FIG. 6.
Alypin and Potassium Iodide.

2. Cadmium oxalate from 2 per cent $H_2C_2O_4$ and $CdSO_4$ solutions (Plate II, Fig. 2).

3. Strontium oxalate from 2 per cent $H_2C_2O_4$ and $Sr(NO_3)_2$ solutions (Plate II, Fig. 3.)

4. Sodium oxalate by evaporation of aqueous solution, also by evaporation of urine containing $Na_2C_2O_4$ (polarized light) (Plate II, Fig. 4).

5. Urea oxalate from 2 per cent $H_2C_2O_4$ and urea solution (Plate II, Fig. 5).

6. Ammonium-magnesium-phosphate from magnesium mixture* and sodium phosphate (Plate IV, Fig. 2).

7. Ammonium platinic chloride (Plate III, Fig. 1). For preparation of crystals see Vol. I.

8. Potassium platinic chloride, K_2PtCl_6 (Plate III, Fig. 3). For preparation of crystals see Vol. I.

9. Sodium urate, from urine sediment (Plate IX, Fig. 3 opp. page 214).

10. Crystals formed from cocaine and potassium permanganate (Plate III, Fig. 4).

11. Crystals formed from phenol and dilute bromine water (tri-brom-phenol) (Plate III, Fig. 5).

12. Crystals formed from morphine solutions and ammonia (morphia) (Plate IV, Fig. 2).

13. Crystals formed from morphine and Marmé's reagent (Plate IV, Fig. 1).

14. Platinum chloride and β-eucaine (Plate III, Fig. 2).

15. Alypin and KI (Plate IV, Fig. 6).

16. Iodoform from acetone and iodine solution in potassium iodide (Plate III, Fig. 6).

The list may be extended to include the crystals produced by various alkaloidal salts with the common reagents, also substances usually employed in the manufacture of the various dental preparations.

* Magnesium mixture as used in urine analysis to precipitate phosphates contains $MgCl_2$ (or$MgSO_4$), NH_4Cl, and NH_4OH.

CHAPTER X.

DENTAL PREPARATIONS.

Among dental preparations, anesthetics are perhaps the most important. In considering the chemistry of local anesthetics we may divide them into two classes as follows:

First, those of definite or well-known compositions; and

Second, preparations of a proprietary nature, the composition of which is always problematical.

In the first class will be found cocaine, eucaine, tropacocaine, acoin, ethyl chloride, etc., which will be later alphabetically considered. The second class contains a large number of preparations of all degree of value, among them some of exceeding merit and largely used, others of doubtful worth, some worthless if not dangerous. Many of the preparations of this class contain cocaine as the anesthetic, and frequently a little nitroglycerin as a cardiac stimulant to counteract the depressant effect of the alkaloid. Carbolic acid and oil of cloves are also frequently used.

Many of the constituents of this class of anesthetics may readily be identified by the processes of microchemical analysis to which previous reference has been made; others may be detected by special tests, some of which are given under the various substances in the following list. This list has been extended to include a considerable number of preparations of common occurrence.

Acoin, a synthetic compound, chemically diparanisyl-monophenetyl-guanidine hydrochloride $\left(C \begin{matrix} \diagup (NC_6H_4OCH_3)_2 \diagdown \\ \diagdown (NC_6H_4OC_2H_5) \diagup \end{matrix} HCl \right)$ soluble in both alcohol and water. It is strongly antiseptic and a valuable anesthetic, especially in conjunction with cocaine. Acoin should be used only in solution and this should be kept in a dark place.

Adrenalin, a valuable hemostatic and frequently used in conjunction with dental anesthetics, is the active principle of the suprarenal gland or capsule. It occurs as very small white crystals which are not very stable and only slightly soluble in water; hence, the article is usually sold in solution with sodium chloride, according to the following formula taken from a commercial sample:

Adrenalin chloride, 1 part; normal sodium chloride solution (with 0.5 per cent chloretone), 1000 parts. This solution is usually diluted with the normal (0.6 per cent) salt solution. According to the *Druggists' Circular,* preparations similar to the above are also marketed under the names of adrenol, adnephrin, hemostatin, suprarenalin (Armour & Co.), suprarenin, etc. (See Epinephrine.)

Alypin. — Benzoyl - dimethylamino - methyl - dimethylamino-butane hydrochloride, white crystalline, hygroscopic, melts at 169° C. Soluble in water and alcohol.

Alypin can be sterilized without decomposition, is not half so poisonous as cocaine and is cheaper. It is used in 2 per cent solution. The solution should be freshly made and prolonged boiling avoided. Alypin is sometimes used with adrenalin. (*Cosmos*, 1908, p. 889.)

Alypin nitrate occurs as a white, crystalline powder melting at 159° C., readily soluble in ether. Mfrs.: Farbenfabriken of Elberfeld, Elberfeld (Germany) and New York. (Mod. Mat. Med., page 2.)

Test. — Alypin gives needle-shaped crystals with potassium iodide, easily produced. (Plate IV, Fig. 6.)

Ammonium bifluoride is strongly recommended as a solvent for tartar by Dr. Joseph Head of Philadelphia. In *Items of Interest,* Vol. 31, page 174, Dr. Head gives the following method for its preparation. Hydrofluoric acid is neutralized with ammonium carbonate, the solution filtered and evaporated to half its bulk, the original volume restored by adding more hydrofluoric acid, and then the resulting mixture is again concentrated to half its volume by evaporation.

Anesthol, or Anæsthol, is a mixture of ethyl chloride and methyl chloride, used as a local dental anesthetic. The name is also applied to a general anesthetic given by inhalation and consisting of a mixture of ethyl chloride 17 parts, chloroform 35.89 parts, and ether 47.1 parts.

Anæstheaine, a local anesthetic, contains 5 grains of stovaine to the fluid ounce.

Argyrol, a protein compound of silver, occurs as dark brown crystals containing 30 per cent of silver. It is easily soluble in water. It does not precipitate chlorine nor coagulate albumin, and is recommended for use in place of ordinary silver nitrate.

Aristol is given by the U. S. D. as a synonym for dithymol-diiodide which contains 45 per cent of iodine and is used as an antiseptic in the same way as iodoform.

Atropine, an alkaloid obtained from belladonna, is usually used, combined with sulphuric acid, $(C_{17}H_{23}NO_3)_2H_2SO_4$; the alkaloid is only sparingly soluble in water but the sulphate is easily soluble, dissolving in about one-half part of water at ordinary temperature. A one per cent solution is said to produce complete insensibility of the nerves in cases in which an artificial tooth is inserted in a living root. (U. S. D., page 249.)

Tests. — Atropine may be separated from a local anesthetic by first rendering the mixture alkaline with ammonia and shaking with chloroform. Upon evaporation of the chloroform solution on a watch-glass, the resulting residue may be tested by adding a drop or two of sulphuric acid and a trace of potassium bichromate and a little water. The odor of bitter almonds is produced. A more conclusive test is to convert the alkaloid, which has been dissolved by the chloroform, into a salt, by the addition of a few drops of acetic acid, evaporating to *complete* dryness, taking up in a few drops of distilled water and placing one or two drops of this solution in the eye of a cat. If atropine is present, a dilation of the pupil occurs in from fifteen minutes to an hour and a half, according to the amount present.

Borax. — Sodium tetraborate, $Na_2B_4O_7$, is used in antiseptic solutions and may be detected as follows: evaporate a little of the solution to dryness, add a little HCl, evaporate to dryness

a second time, then add a very dilute HCl solution containing tincture tumeric. When this mixture is dried, a beautiful pink color appears. If much organic matter is present it may be burned off in the Bunsen flame *before* the addition of any acid.

Carbolic Acid. — See Phenol.

Chloral hydrate, $CCl_3CHO.H_2O$, a crystalline solid composed of trichlor-aldehyde, or chloral, with one molecule of water (U. S. P.), easily soluble in water, may become with alcohol a chloral alcoholate comparatively insoluble in water.

Tests. — Chloral may be detected by adding to the suspected mixture a few cubic centimeters of fairly strong alcoholic solution of KOH or NaOH with one drop of aniline oil, and heating. Isobenzonitril, which has a peculiarly disagreeable and characteristic odor, is thus produced. This test is also given by chloroform, which is produced by heating chloral hydrate with caustic alkali. If more than traces of chloral are present this latter reaction may be a sufficient test.

Chloretone, $CCl_3COH(CH_3)_2$, is the commercial name of acetone-chloroform or tertiary trichlor-butyl alcohol. Made from chloroform, acetone, and an alkali, it occurs as small white crystals, with taste and odor like camphor. It is dissolved by alcohol and glycerol and to a slight extent by water.

Chloroform, trichlor-methane, $CHCl_3$, is prepared by action of chlorinated lime on acetone. Chloroform is a heavy colorless liquid with a specific gravity of 1.490 at 15° C. It is very volatile and used as a solvent for gutta-percha, caoutchouc, many vegetable balsams, camphor, iodine, bromine, and chlorine; it also dissolves sulphur and phosphorus to a limited extent.

Tests. — It may be detected by its odor, when heated, or by the isobenzonitril test, to which reference has been made under chloral hydrate.

Cocaine is the alkaloid obtained from erythroxylon coca. The hydrochlorate, $C_{17}H_{21}NO_4HCl$, is the salt most usually employed. This is easily soluble in water and very largely used as a dental anesthetic in a 1 or 2 per cent solution.

Tests. — Cocaine solutions respond to the usual alkaloidal reagents. With 1 per cent solution potassium permanganate

gives pink plates resembling chloesterol (Plate III, Fig. 4) in form but not in color.

Dilute cocaine solution with picric acid gives a yellow precipitate which becomes crystalline on standing. Quite characteristic crystals may also be obtained from dilute cocaine solutions and stannous chloride in the presence of free HCl.

Creosote. — This is a mixture of phenols derived from the destructive distillation of wood tar. It is a heavy oily liquid, acting when pure as an escharotic. It is analogous in many respects to carbolic acid and may be used for similar purposes. To distinguish between creosote and carbolic acid, boil with nitric acid until red fumes are no longer given off. Carbolic acid will give yellow, crystalline deposit; creosote will not. An alcoholic solution of creosote is colored emerald green by an alcoholic solution of ferric chloride. Phenol is colored blue.

Cresol is the next higher homologue to phenol, having a formula $C_6H_4CH_3OH$, boiling at 198° C. It is largely used, usually together with allied compounds from coal-tar, as antiseptic and disinfectant solutions.

Ektogan is peroxide of zinc, ZnO_2, designed for external use.

Epinephrine. — This is the active principle in the suprarenal glands. Chemically it is an *o*-dihydroxyphenyl-ethanolmethylamine, $C_6H_3(OH)_2.CHOH.CH_2NHCH_3$. It is a weak base which combines with hydrochloric acid to form the hydrochloride, in which form it is usually used in dilutions of one part to a thousand. It acts as a cardiac stimulant, causing rise in blood pressure with slower heart action, acting somewhat in the same way as digitalis.

Ethyl chloride, monochlor-ethane, C_2H_5Cl, is a gaseous substance at ordinary temperature, but when used as a dental anesthetic it is compressed to a colorless liquid which has a specific gravity of 0.918 at 8° C., is highly inflammable and usually sold in sealed glass tubes of 10 to 30 grams each.

β-Eucaine is the hydrochlorate of benzoylvinyl-diacetonealkamine, and occurs as a white, neutral powder, soluble in about thirty parts of cold water. It is used like cocaine as a local anesthetic, and is claimed to be less toxic, and sterilizable by

boiling without danger of decomposition. It is usually applied in 1 to 5 per cent solutions, which are conveniently prepared in a test-tube with boiling water. It is also marketed in the form of 1½ and 5-grain tablets. (*Druggists' Circular.*)

Test. — β-Eucaine gives characteristic crystals with platinic chloride. (Plate III, Fig. 2.)

Eucain Lactate. — "Eucain lactate is used in 2 to 5 per cent solution as a local anesthetic in ophthalmic and dental practice, and in 10 to 15 per cent solution when used in the nose or ear." (*Review of American Chemical Research*, page 97, 1905.)

Eudrenin is a local anesthetic marketed in capsules of 0.5 c.c. containing 1/12 grain of eucain and 1/4000 grain of adrenalin hydrochloride. It is used as a local anesthetic, chiefly in dentistry. The contents of one or two capsules, according to the number of teeth to be extracted, is injected into the gums ten minutes before extraction. Mfrs.: Parke, Davis & Co., Detroit, Mich. (Mod. Mat. Med., page 147.)

Eugenol, $C_{10}H_{12}O_2$, synthetic oil of cloves, is miscible with alcohol in all proportions. Exposure to air thickens and darkens it. It should be kept in well-stoppered amber-colored bottles (U. S. D.).

Europhen is recommended by Dr. J. P. Buckley as a substitute for iodoform (*Dental Review*, Vol. 21, page 1284).

Di-iso-butyl-cresol is described as a bulky, yellow powder of faint saffron odor and containing 28 per cent of iodine. (Mod. Mat. Med., page 152.)

Formaline, formol, formine, etc., are commercial names for a 40 per cent aqueous solution of formaldehyde, HCHO, prepared by the partial oxidation of methyl alcohol. Formaline is a powerful disinfectant, very generally used. (For test see page 254, Exp. 28).

Formo-Cresol. — Buckley's Materia Medica combines cresol with formaline in proportion of three to one, under the name of formo-cresol.

The putrescent pulp contains bacteria which produce hydrogen sulphide, ammonia, and certain amino acids, all of which will combine chemically with formaline. The cresol somewhat

retards the action, tends to make it more complete, and aids materially in the study of sterilization of the pulp chamber.

Glycerol is a triatomic alcohol, $C_3H_5(OH)_3$, a colorless liquid of syrupy consistency and sweetish taste, specific gravity 1.250 at 15° C. It is easily soluble in either water or alcohol.

Tests. — Upon heating with acid potassium sulphate (solid) it is decomposed, giving off odor of acrolein, which is usually sufficient for its identification. A further test may be made by moistening a borax bead on a platinum wire with the suspected solution (after concentration) and holding in a non-luminous flame, to which it will give a deep green color which does not persist. Glycerol when present is apt to interfere with characteristic crystallization of many precipitates.

Gram's solution, Kuhne's modification, contains 2 grams of iodine, and 4 grams of potassium iodide in 100 c.c. of water.

Gutta-percha. — The name signifies scraps of gum. It is obtained as a milky exudate from a number of tropical trees. It is soluble in ether, chloroform, carbon disulphide, toluene, and petroleum ether. It may be freed from impurities by shaking the solution with calcium sulphate, which will mechanically carry coloring matter and other impurities with it as it slowly settles out from the mixture. It is not soluble in alcohol or in water.

Heroin is a diacetic ester of morphine. It is usually obtained as the hydrochloride and occurs as a white powder, soluble in two parts of water. Its action is similar to that of morphine; it answers to the usual color tests for morphine, but may be distinguished from it by the fact that it will yield acetic ether upon heating with alcohol and sulphuric acid.

Howe's Silver Nitrate Solution. — The silver nitrate solution used in Dr. Howe's method for sterilization of root canals is a nearly saturated ammoniacal solution. It is made by taking 3 grams silver nitrate, 1 c.c. water and 3 c.c. strong ammonia. The ammonia should be poured into the silver nitrate slowly, and the solution shaken constantly.

The formaline solution, applied a few minutes after the silver solution in the treatment, is made by taking 40 per cent U. S.

formalin solution 1 part, and 3 parts water. At present eugenol is used by a great many dentists in place of the formalin.

Hopogan (also known as biogen) is a peroxide of magnesium, MgO_2, recommended as a non-poisonous and non-astringent intestinal germicide.

Hydrogen Peroxide, or dioxide, H_2O_2, is, when pure, a syrupy liquid without odor or color. It is sold under various trade names in aqueous solution containing about 3 per cent and yielding upon decomposition about 10 volumes of oxygen gas. It is used also as an escharotic in ethereal solutions containing 25 to 50 per cent H_2O_2. Peroxide solutions may be concentrated by heat without decomposition if kept *perfectly* free from dirt or traces of organic matter. Hydrogen peroxide is readily prepared by treatment of metallic peroxides, as BaO_2 with dilute acids.

$$BaO_2 + H_2SO_4 = BaSO_4 + H_2O_2$$

or

$$BaO_2 + H_2O + CO_2 = BaCO_3 + H_2O_2.$$

This latter reaction has the advantage of producing an insoluble barium compound and at the same time introducing no objectionable acid. The peroxides of sodium, calcium, magnesium, and zinc may also be used; ZnO_2, however, is comparatively expensive and is used in powder form as an antiseptic dressing rather than as a source of H_2O_2. Na_2O_2 is valuable as a bleaching agent, because for this purpose an alkaline solution is required and the solution of Na_2O_2 in water produces both alkali and H_2O_2 according to the following reaction:

$$Na_2O_2 + 2\,H_2O = 2\,NaOH + H_2O_2.$$

Sodium perborate (page 89), also sold as euzone, is a powder which will produce H_2O_2 in water. Commercial H_2O_2 solutions are usually acid in reaction, as such solutions are more stable than if neutral or alkaline.

Test. — Add to a solution of H_2O_2 a few drops of bichromate of potassium solution and a little dilute H_2SO_4. Shake cold with a little ether in a test-tube. The ether should be colored blue. (For further tests see experiments.)

Lugol's caustic iodine is made of iodine and potassium iodide, 1 part of each dissolved in 2 parts of water.

Lugol's Iodine Solution. — See appendix under Iodine Solution.

Menthol is the stearopten obtained from the oil of peppermint. It is a volatile, crystalline substance having a formula $C_6H_9OHCH_3C_3H_7$. Menthol is but slightly soluble in water but freely soluble in alcohol, ether, chloroform, or glacial acetic acid. The presence of menthol may usually be detected by its odor. If the odor should be suggestive but not distinctive it is well to place a little of the substance on a filter-paper, rub it between the thumb and finger, thereby obtaining a "fractional evaporation," when the more easily volatile substance will pass off first, thus producing a partial separation of substances.

Mercuric chloride, corrosive sublimate, $HgCl_2$, is soluble in about 16 parts of water and 3 parts of alcohol. It is a powerful antiseptic, in aqueous solution 1/1000 to 1/5000, but should never be used in mouth-washes.

Tests. — A drop of the suspected solution with a trace of potassium iodide will give a red precipitate of mercuric iodide soluble in excess of either reagent. With lime-water or fixed alkaline hydroxides a black precipitate is produced. A drop of mercurial solution placed on a bright copper plate will leave a tarnished spot due to the reduction of the mercuric salt and subsequent amalgamation of the metal.

Methethyl. — Ethyl chloride mixed with a little methyl chloride and chloroform is said to be the composition of a local anesthetic sold under the name of methethyl (U. S. D.).

Methyl chloride, CH_3Cl, is a colorless gas which condenses to a liquid at $-23°$ C. Methyl chloride is easily soluble in alcohol, somewhat in water, and is used in a similar manner to ethyl chloride.

Morphine, $C_{17}H_{19}NO_3$, alkaloid from opium. Solutions for use are made from the sulphate, hydrochlorate, or acetate. The alkaloid itself is insoluble in water; its salts are easily soluble.

Morphine may be separated from solutions containing it by making the solution alkaline with ammonia, and shaking out

the precipitated alkaloid with warm ethyl acetate. Upon evaporation of the solvent the residue may be tested with Fröhde's reagent (sodium molybdate, 1 per cent, in strong sulphuric acid). The color obtained should be a *violet*, changing usually to brown; a pure blue color is not distinctive for morphine. If the morphine solution is of sufficient strength the addition of ammonia will produce minute crystals of the alkaloid, as shown on Plate IV, Fig. 4. Dental anesthetics containing morphine will give precipitates with the usual alkaloidal reagents. Marmé's reagent (CdI_2) gives crystals represented on Plate IV, Fig. 1.

Nirvanin, hydrochloride of diethyl-glycocoll-*p*-amino-*o*-oxybenzoic-methylester, has the formula

$$\begin{array}{l}(CH_2N) = (C_2H_5)_2HCl \\ \quad | \\ CO.NH.C_6H_3(OH)COOCH_3.\end{array}$$

White prisms, soluble in water and in alcohol, melt at 185° C., and give violet reaction with ferric chloride.

Nitroglycerin, $C_3H_5(NO_3)_3$, is used as a cardiac stimulant in alcoholic solution, the U. S. P. Spiritus Glonoini, containing 1 per cent by weight of the substance.

Test. — Extract the dry substance, or the evaporated residue, with alcohol. Filter and evaporate to dryness. Add 1 c.c. of sulphuric acid and 1 c.c. of phenol-disulphonic acid. Heat over a water-bath for five minutes; add water and excess of ammonia. A deep yellow color of ammonium picrate indicates nitrates in the original substance. Exp. No. 110, p. 266.

Novocaine, discovered by Uhlfelder and Einhorn, is a hydrochloride *p*-aminobenzoyl-diethylamino-ethanol. It occurs as thin, colorless needles; melts at 156° C., soluble in 1 part water and 30 parts alcohol. It is seven times less toxic than cocaine, and three times less toxic than stovaine. It can be sterilized by boiling, and is used in 1/2 to 2 per cent solution, often with adrenalin 1/1000. (Mod. Mat. Med., page 275.)

Novocaine, if intended to represent a solution which is isotonic with the blood corpuscles, must be dissolved in a 0.92 per cent sodium chloride solution. (*Dental Cosmos*, 1910, page 605.)

Oil of cloves, oil of Gaultheria, and other essential oils may be detected by the same process of fractional evaporation as suggested for menthol. In testing for the presence of any substance by its odor, it is usually necessary to make a comparative test on known samples, using the same methods.

Orthoform, $C_6H_3OH(NH_2)COOCH_3$, methylpara-amino-meta-oxybenzoate, used as an anesthetic and antiseptic, is without odor, color, or taste, is slightly soluble in water, and easily soluble in alcohol or ether.

Phenol. — Carbolic acid, C_6H_5OH, is obtained from the destructive distillation of coal-tar. It is a light, oily liquid of specific gravity 0.94–0.99. Carbolic acid is usually obtained as a white, crystalline mass, soluble in 20 parts of water. The pure acid turns pink with age, but does not suffer deterioration on account of this change of color. The addition of 5 to 8 per cent of water will result in liquefaction of the crystals and cause the preparation to become permanently liquid. It is easily soluble in glycerol, and strong solutions may thus be prepared. Carbolic acid is sometimes added to local anesthetics with the intent of rendering the solution sterile, but, as shown by Dr. Endelman (*Dental Cosmos*, Vol. 45, page 44), it would be necessary, in order to prevent the development of micro-organisms, to add the acid in proportion that would render the solution unfit for hypodermic purposes.

Tests. — Phenol may be detected in the majority of preparations by the addition of bromine-water, which gives white crystals of tri-bromphenol (see Plate III, Fig. 5). See also Exp. 107.

Phenol Compound. — The following is Dr. Buckley's formula for treatment of root canals: menthol 1.3 grams, thymol 2.6 grams, and phenol 12 c.c.

Potassium hydroxide, KOH, gives an alkaline reaction to litmus paper and may be detected by the ordinary methods of inorganic analysis.

Rhigolene is a light, inflammable liquid, obtained from petroleum, boiling at about 18° C., used as a spray for the production of low temperature, in the same manner as methyl or ethyl chloride. It is readily inflammable, and the vapor, mixed with

certain proportions of air, is explosive. It should be kept in a cool place.

Ringer's solution, which is used as a solvent for novocaine and other anesthetics, has the formula:

Sodium Chloride	0.50
Calcium Chloride	0.04
Potassium Chloride	0.02
Distilled water	100.00

Saccharin. — Saccharin is official in the ninth revision of the Pharmacopœia as benzosulphinidum. It is a derivative of toluene, having a formula of $C_6H_4COSO_2NH$, being benzoyl-sulphonimide. It is a white, crystalline powder, melting at 219° to 222° C.

It is said to be at least three hundred times sweeter than cane sugar and is used in mouth-washes, tooth-paste, etc., as a flavor and an antiseptic.

Test. — Add a few drops of potassium hydroxide solution to a little saccharin; heat for a few minutes. Acidify with hydrochloric acid; add a few drops of ferric chloride; a reddish brown or purplish color is thus produced.

Silver nitrate, $AgNO_3$, crystallizes in colorless plates without water of crystallization; it is used as an antiseptic, disinfectant, or escharotic. It is freely soluble in water and may be detected by the ordinary methods of qualitative analysis.

Sodium chloride, NaCl, is a constituent of many preparations designed to be used hypodermically. Experience has proved the value of such addition; a possible reason for its desirability is given by Dr. G. Mahe, of Paris, in the *Dental Cosmos* for September, 1903, in the statement that sodium chloride added in excess to a toxic substance diminishes its toxicity by one-half, and this has been demonstrated particularly with cocaine.

Sodium perborate is a powder having the composition $NaBO_3$. $4\,H_2O$, which will furnish 10 per cent of available oxygen and produce H_2O_2 with water; it is very stable and is recommended as a bleach-powder.

Sodium perborate may be made by thoroughly mixing sodium

peroxide (Na_2O_2) with crystallized boric acid, and stirring the mixture gradually into cold water. The proportions recommended by V. E. Miegeville in the *Dental Cosmos* for 1905, page 1381, are 78 grams of the sodium peroxide, 248 grams of the boric acid, and 2 liters of water. The sodium perborate is formed spontaneously and separates from the solution as a white, crystalline powder. Its solubility is increased by addition of weak organic acids, citric or tartaric.

Sodium peroxide, Na_2O_2, is a white powder, easily soluble in water, usually with evolution of more or less oxygen and formation of hydrogen dioxide.

Somnoform is a general anesthetic, administered in a manner similar to chloroform; it was introduced by Dr. Rolland, of Bordeaux, and consists of 60 per cent ethyl chloride, 35 per cent ethyl bromide, and 5 per cent methyl bromide. (*Dental Cosmos*, Vol. XLVII, page 236.)

Stovaine. — Benzoyl-ethyl-dimethyl-amino-propanol-hydrochloride, $C_{14}H_{21}O_2N.HCl$, closely related to alypin, occurs in small shining scales, freely soluble in alcohol or water. It is incompatible with alkalies and all alkaloidal reagents. It can be sterilized by boiling. (Mod. Mat. Med., 2nd edition.)

It melts at 175° C., is very soluble in water, and gives reaction similar to cocaine, which is also a benzoyl derivative. (U. S. D., page 1661.)

It is less powerful than cocaine and physiologically incompatible with adrenalin. (*Dental Cosmos*, 1905, page 146.)

Test. — Stovaine gives rather irregular but characteristic crystals with platinic chloride.

Suprarenal Glands. — The official preparation consists of dried glands obtained only from animals used for food by man. The glands must contain not less than 0.4 per cent nor more than 0.6 per cent of epinephrine.

Tannic acid, or tannin, sometimes called gallotannic acid, is an astringent organic acid obtained from nut-galls. It may be obtained as crystals carrying two molecules of water, $HC_{14}H_9O_9.2\,H_2O$. Tannic acid is a white or slightly yellowish powder, soluble in about 1 part water or 0.6 part alcohol. It is

used as an alkaloidal precipitate, also in astringent washes. It may be detected by the addition of ferric solutions, which form with it a black tannate of iron of the nature of ink.

Thymol, $C_6H_3(CH_3)(OH)(C_3H_7)$ 1 : 3 : 4. This is a phenol which occurs in volatile oils of thymus vulgaris (Linne). It melts at 44° C.; is sparingly soluble in water, easily in alcohol and ether.

Tests. — It may usually be detected by its odor or by dissolving a small crystal in 1 c.c. of glacial acetic acid; whereupon, if 6 drops of sulphuric acid and 1 drop of nitric acid be added, the liquid will assume a deep bluish-green color. (U. S. D.)

Thymol iodide, di-iodo-dithymol, $(C_6H_2.CH_3.C_3H_7OI)_2$, is a valuable antiseptic containing 43 per cent of iodine. It is a brown powder, insoluble in water, slightly soluble in alcohol, easily soluble in chloroform or ether.

Thymophen is a mixture of equal parts of thymol and phenol.

Thyroids. — The dried, powdered, thyroid glands of animals used for food by man, freed from connective tissue and fat, containing not less than 0.17 per cent or more than 0.23 per cent of iodine, constitute the official preparation used as a remedy in myxedema and other cases of perverted metabolism.

Trichlor-acetic acid occurs as deliquescent crystals, readily soluble in water. It distils at 195° C. and is a powerful caustic. Dilute solutions are recommended for treatment of pyorrhea.

Tropa-cocaine is an alkaloid, originally isolated by Giesel from the leaves of the small-leaved coca-plant of Java and introduced by Arthur P. Chadbourne, Harvard Medical School. It is used hypodermically in normal salt solution. It is probably superior to cocaine, but rather more expensive. It is obtained as an oil which, when quite dry, solidifies in radiating crystals, melting at 49° C. It is easily soluble in alcohol.

A number of commercial mouth-washes and local anesthetics will be given to the class for identification, the object being to familiarize the student with the more easily made tests for the principal ingredients of these preparations. Complete analysis will rarely be attempted. The following table, taken from the *Druggist's Circular* of June, 1910, may be helpful.

DIFFERENTIATION OF COCAINE AND ITS SUBSTITUTES.

	Iodine potassium iodide.	Bromine water.	Sodium hydroxide.	Potassium permanganate.
Eucaine — *a*.	Yellow-maroon precipitate, soluble on boiling.	Yellow precipitate, soluble on heating.	White precipitate, insoluble in excess and on boiling.	Violet precipitate, blackening quickly.
Eucaine — *b*.	Deep-red precipitate, soluble on boiling.	Yellow precipitate, slightly soluble on heating, reprecipitated white on boiling.	White precipitate, insoluble in excess and on boiling.	No precipitate immediately; color persists for a day.
Cocaine.....	Yellow-maroon precipitate, soluble on boiling.	Yellow precipitate, soluble on heating.	White precipitate, insoluble in excess and on boiling.	Violet precipitate, color persists for one hour, then deposits MnO_2.
Novocaine.....	Deep-red precipitate, soluble on boiling.	Yellow precipitate, soluble on heating.	White precipitate, insoluble in excess and on boiling.	Violet precipitate, blackening quickly.
Stovaine.....	Deep-red precipitate, soluble on boiling.	Yellow precipitate, soluble on heating.	White precipitate, insoluble in excess; aromatic odor on boiling.	Violet precipitate blackening almost immediately.
Nirvanin.....	Deep-red precipitate, soluble on boiling.	Yellow precipitate, soluble on heating, but the liquid becomes red and gives an agreeable fruity odor.	Precipitate, very soluble in excess of the reagent.	Precipitate, first maroon, then brown.
Alypin.....	Yellow-maroon precipitate, insoluble on boiling; orange-red deposit.	Yellow precipitate, soluble on gentle heating.	White precipitate, insoluble in excess and on boiling.	Bluish-violet precipitate, slowly blackening.

PART III.

PHYSIOLOGICAL CHEMISTRY.

CHAPTER XI.

FERMENTS OR ENZYMES.

Physiological chemistry treats of the substances that go to make up the animal body, the changes which these substances undergo in the process of digestion and assimilation, and the final products of metabolism.

This subject, like others, will receive our attention in outline, simply with a view to enabling the student to understand the conditions which at present seem to have the most direct bearing on dental science. The changes produced by the class of bodies known as ferments are of great importance and will be the first to be considered.

If yeast is allowed to grow in a sugar solution of moderate strength, the sugar molecule is split into carbonic-acid gas and alcohol. The process is one of fermentation; the yeast is the ferment. There are various substances which cause similar splitting of complex molecules into simpler compounds.*

The distinction between the organized and the unorganized ferments is no longer recognized, as it has been proved that the activity of an organized ferment is due to the presence of the unorganized ferment or enzyme. We shall, by preference, refer to these substances as enzymes.

The enzymes, as a class, possess certain general properties which should be remembered:

First. Their action is limited to a very few substances; i.e., the enzyme from yeast, referred to above, will convert a

* Occasionally fermentation may produce a synthesis (putting together) rather than an analysis (pulling apart).

few sugars only, as indicated. It will not act in any other way nor upon other substances.

Second. The enzymes act only at ordinary temperatures, usually showing the greatest activity at about the temperature of the animal body, 37° to 40° C.

Third. Enzymes act only within very narrow limits as regards the chemical reaction (acid or alkaline) of the media.

Fourth. Enzymes are destroyed (killed) by the heat of boiling water.

Fifth. In regard to the nature of their composition, many of the enzymes are closely allied to the proteins.

Hydrolytic Enzymes. — An enzyme may be classified according to the sort of work it does. Many of the chemical changes involved in the utilization of food consist of breaking up a complex molecule and, by the use of a molecule of water, forming new and simpler compounds. This sort of change is called hydrolysis and an enzyme that will produce it is a hydrolytic enzyme. By hydrolysis or hydrolytic cleavage, the molecule of cane-sugar, $C_{12}H_{22}O_{11}$, becomes two molecules of a simpler sugar, such as glucose, $C_6H_{12}O_6$. $C_{12}H_{22}O_{11} + H_2O = 2\ C_6H_{12}O_6$.

Hydrolysis is not dependent upon enzyme action, as the same change is produced by prolonged boiling with very dilute mineral acids.

De-aminizing Enzymes. — These are characterized by their ability to split off the amino group. They are found in the blood and tissue cells, and their particular function is the de-aminization of the amino acids which are absorbed.

Oxidizing Enzymes. — Another large and very important class of enzymes consists of those which produce oxidative changes. They may be divided into the oxidases, which produce direct oxidation, and the peroxidases, which produce oxidation only in the presence, or by the aid, of peroxide.

Oxidases have been found to exist in saliva, milk, blood, nasal mucus, tears, and semen, in many of the organs, and also in the muscular tissue. They exist, moreover, in the vegetable kingdom, in which the subject of oxidizing enzymes was first

studied by Bertrand and Bourquelot.* It is said that the urine, bile, and intestinal secretions do not contain any ferment of this kind.

Besides the classification of enzymes by the character of the work they do, the name of the substance acted upon may also be used to designate an enzyme: thus, a proteolytic enzyme produces a cleavage of protein substances, a lipolytic enzyme (lipase) splits the fat molecule, etc.

Several of the digestive enzymes, notably the proteolytic or flesh-digesting enzymes, such as pepsin, trypsin, etc., exist in the animal cell, not as active agents, but as inactive parent enzymes which are called pro-enzymes or zymogens. Enzymes of this class are set to work (liberated from the parent substance) by a class of substances known as " activators " (illustrated by the enterokinase of the intestine, page 151).

Neither the zymogen nor the activator has of itself any digestive action whatever; a provision which results in the prevention of autodigestion (autolysis) of the cells containing them.

In addition to the *exocellular* enzymes occurring in the digestive tract, there is a large class of so-called *endocellular* enzymes, occurring throughout the body cells. This class, of which the de-aminizing enzymes are an example, is chiefly responsible for the metabolic processes taking place in the animal body.

Catalase is a term which has been applied to enzymes similar in action to the peroxidases, i.e., enzymes that destroy a peroxide with the formation of molecular oxygen; although, according to Hammarsten, they differ from both the oxidases and peroxidases in giving no reaction whatever with guaiac.

The name of a specific enzyme usually ends in " -ase ": as zymase, the enzyme contained in yeast; lipase, a fat-splitting enzyme; urease, the urine ferment.

* "Enzymes and their Application," Effront: Prescott's translation. This work is also authority for statement immediately preceding regarding the source of oxidizing enzymes.

CHAPTER XII.

CARBOHYDRATES.

The carbohydrates predominate in the vegetable kingdom. As the name signifies, they are compounds of carbon, hydrogen and oxygen, in which the oxygen and hydrogen usually occur in the same proportion as in water. There are a few carbohydrates in which this proportion does not hold; and there are a number of substances — acetic acid, for example — in which the proportion holds true though the substance is manifestly not a carbohydrate.

As a rule, the carbohydrates are white, solid substances, some of them crystalline, many of them sweet, and some of them tasteless, as starch and glycogen.

The carbohydrates are synthesized almost exlusively by the vegetable body. The exact manner of this synthesis is an open question, various theories having been suggested to explain it. Baeyer has suggested that formaldehyde, formed from carbon dioxide and water by the action of sunlight on the chlorophyll of the plant,* will condense and eventually produce $C_6H_{12}O_6$.

$$CO_2 + H_2O \rightarrow CH_2O + O_2$$
$$(CH_2O)_3 \rightarrow C_3H_6O_3$$
$$(C_3H_6O_3)_2 \rightarrow C_6H_{12}O_6$$

It is known that formaldehyde in a slightly alkaline solution will, by condensation of the molecules, become formose, a mixture of sugars. The plant, although practically neutral in reaction, seems to possess a catalytic agent capable of bringing about this condensation very rapidly. Different plants produce different carbohydrates; and it is therefore reasonable to assume that the

* The exact action of the chlorophyll is not clear, but Priestly and Usher have demonstrated that extracted chlorophyll, with sunlight, CO_2 and water, can produce formaldehyde.

condensation taking place in the individual plant is regulated and controlled by an inherent property of the plant.

By the action of *electricity*, formaldehyde has been converted into glycol aldehyde; and from this, sugar has been produced in the laboratory.

However, although the exact nature of the processes which take place in the plant is at present unknown, it is established beyond question that the vegetable organism absorbs carbon dioxide, gives off oxygen and produces carbohydrates.

Classification of Carbohydrates.

Sugars	Arabinose, Xylose	Pentoses.
	Dextrose, Levulose, Galactose	Monosaccharides or monoses.
	Saccharose, Maltose, Lactose	Disaccharides or dioses.
Starch	Starch, Glycogen	Polysaccharides or polyoses.
Gum, Cellulose	Dextrin	

Characteristics. — The monosaccharides are reducing bodies of either the aldehyde or the ketone type. The termination "ose" is applied to all sugars, and may also be used in designating the type; thus dextrose is an "aldose," while levulose is a "ketose;" i.e., dextrose is an aldehyde, containing the characteristic $-CHO$ group, while levulose is a ketone containing the $\begin{matrix} | \\ C=O \\ | \end{matrix}$ group.

The pentoses ($C_5H_{10}O_5$) are represented by two important compounds, arabinose and xylose. The first of these occurs occasionally in the urine (pentosuria), and can be prepared by boiling gum arabic with dilute mineral acids. The second,

xylose, has been obtained from the pancreas, but may be prepared more easily from bran or straw by boiling with dilute hydrochloric acid (Exp. 124, page 270).

The pentoses, as a class, boiled with dilute mineral acid (hydrochloric or sulphuric), yield furfuraldehyde by splitting off the elements of three molecules of water:

$$C_5H_{10}O_5 - 3\,H_2O = C_5H_4O_2.$$

The formation of furfuraldehyde can be easily demonstrated by various color reactions as given in experiment 124, page 270.

The hexoses, $C_6H_{12}O_6$, also called monoses, occur quite generally in nature (not true of the pentoses). They constitute the various fruit sugars, and may be obtained by hydrolysis of the dioses and polyoses.

They all reduce Fehling's copper solution (galactose less easily than the others), and they are all fermented by yeast (galactose more slowly than the others.)

Dextrose or **glucose,** $C_6H_{12}O_6$, also known as grape-sugar and as diabetic sugar, occurs in grapes, honey, etc. It is formed by the action of diastatic ferments on the disaccharides; also from many of the polysaccharides. Glucose thus occurs in the processes of digestion and constitutes the sugar of diabetic urine. It may be obtained commercially as a white solid, and also as a thick, heavy syrup, known as confectioners' glucose. The commercial glucose is prepared by the action of dilute acids on starch, hydrolysis taking place, as follows:

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

Glucose is an aldose and may be represented graphically: $CH_2OH.CHOH.CHOH.CHOH.CHOH.CHO$. The presence of the aldehyde group is responsible for many of its characteristic reactions.

Dextrose can be oxidized first to gluconic acid ($CH_2OH.(CHOH)_4.COOH$), and by further oxidation to dibasic saccharic acid:

$$COOH.(CHOH)_4.COOH.$$

PLATE V. — PHYSIOLOGICAL CHEMISTRY.

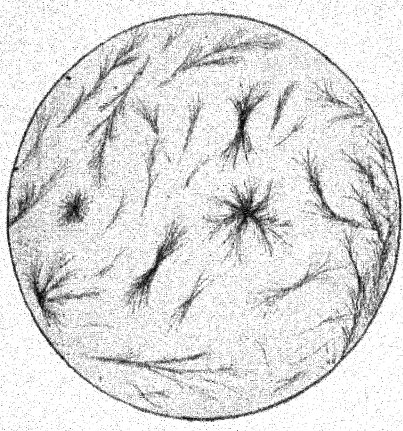


FIG. 1.
Glucosazone.

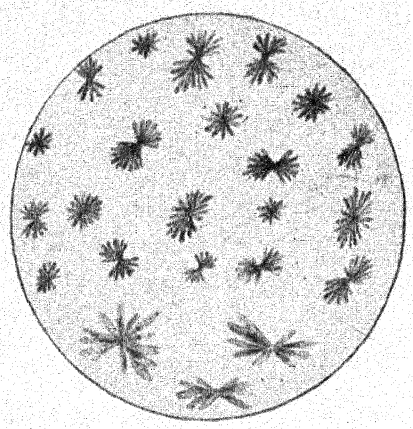


FIG. 2.
Maltosazone.

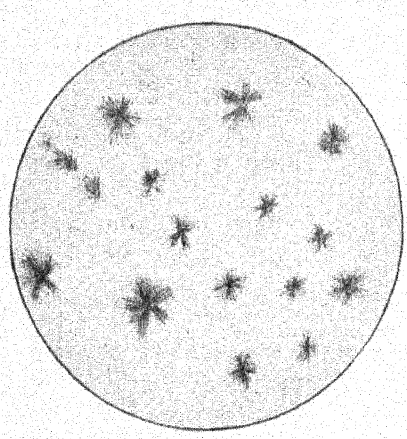


FIG. 3.
Lactosazone.

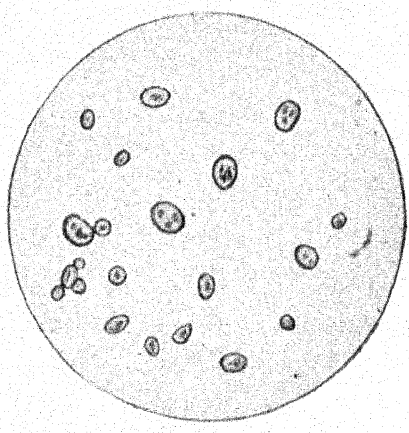


FIG. 4.
Wheat Starch.

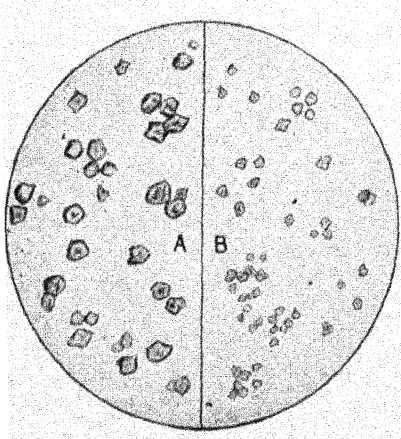


FIG. 5.
A, Corn starch; *B*, Rice starch.

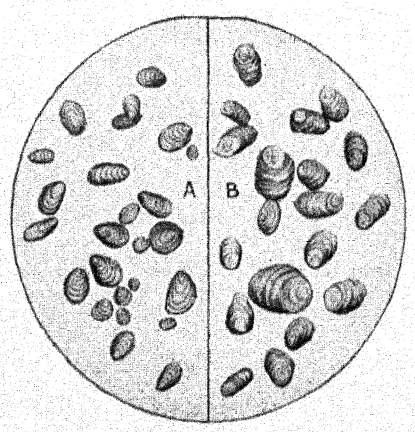


FIG. 6.
A, Potato starch; *B*, Arrowroot starch.

This oxidation can be effected by the use of nitric acid. Saccharic acid forms a definite soluble salt with calcium. Whether the fact has any bearing whatever on the relation between poor teeth and excessive use of candy has not been demonstrated.

Tests. — Glucose boiled with Fehling's solution precipitates the red suboxide of copper (Cu_2O).

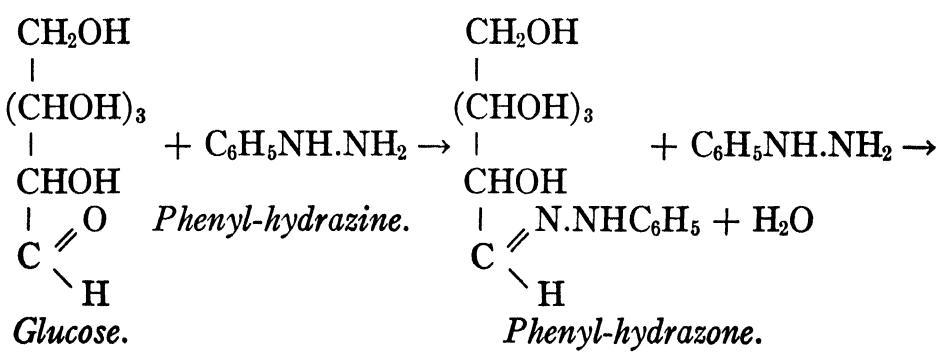
Benedict's reagent with glucose gives a turbidity which is claimed by many to be more delicate than Fehling's test and in some instances more characteristic.

Glucose responds to Molisch's test for carbohydrates, which is made with an alcoholic solution of α-naphthol and concentrated sulphuric acid (Exp. 126). The monosaccharides, of which glucose is a convenient representative, may be distinguished from the other carbohydrates by heating with Barfoed's solution (copper acetate in dilute acetic acid), which is reduced with precipitation of cuprous oxide.

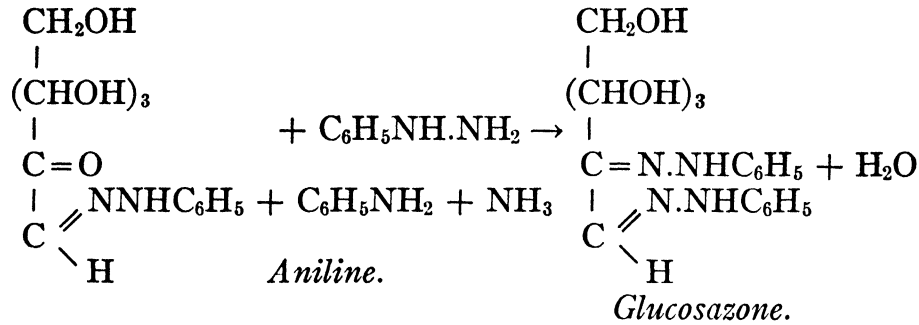
Heated with phenyl-hydrazine solution nearly to the boiling-point of water, glucose forms phenyl-glucosazone, which crystallizes, as the mixture cools, in characteristic yellow needles usually arranged in bundles or sheaves. (Plate V, Fig. 1.)

Osazones are the various compounds formed by the different sugars and phenyl-hydrazine, when treated as above. They crystallize in fairly distinctive forms and furnish valuable tests for the sugars. The phenyl-hydrazine test is considered at least ten times more delicate than Fehling's test.

The formation of the osazones with glucose may be expressed by the following reactions:



Glucose. *Phenyl-hydrazone.*



Glucose readily undergoes alcoholic fermentation, yielding C_2H_5OH and CO_2. (See Exp. 134, page 271.)

Levulose, $C_6H_{12}O_6$, or fruit-sugar, turns the ray of polarized light to the left, and to a greater degree than glucose turns it to the right. It occurs in honey and in many fruits, and is produced with glucose by hydrolysis of cane-sugar. Levulose forms an osazone not to be distinguished from glucosazone. It reduces copper solutions in a manner similar to glucose, and, like it, is easily fermented by yeast.

Levulose differs from glucose in that it is a ketose rather than an aldose. The keto group is joined to one of the CH_2OH groups, and it may be represented graphically thus:

$$CH_2OH.CHOH.CHOH.CHOH.CO.CH_2OH.$$

The position of the keto group, between a primary and a secondary alcohol group, accounts for the fact that levulose possesses reducing properties, page 22.

Galactose is the product of the hydrolysis of lactose, or milk-sugar, and some other carbohydrates. It is a crystalline substance which reduces Fehling's solution and ferments slowly with yeast.

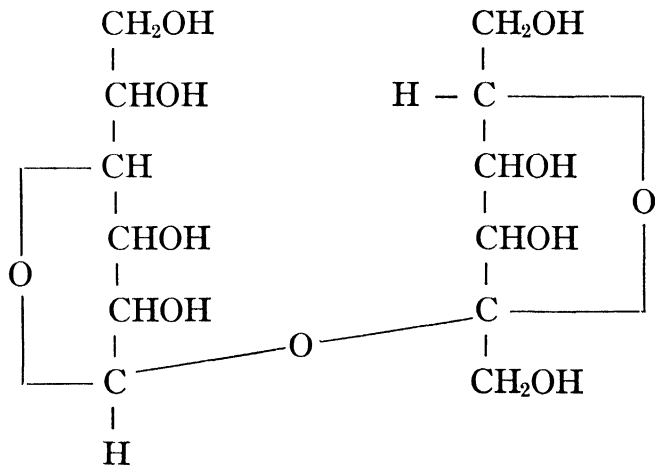
Disaccharides or Dioses.

Disaccharides have the general formula $C_{12}H_{22}O_{11}$. They are converted into the monosaccharides by hydrolysis, brought about either by action of enzymes or by boiling with mineral acid.

Cane-sugar, $C_{12}H_{22}O_{11}$, sucrose or saccharose, is obtained from the sugar-cane (various varieties of sorghum), also from the sugar-beet (*Beta vulgaris*) and the sugar-maple (*Acer saccha-*

rinum). Cane-sugar is a white, crystalline solid, soluble in about 1/2 part of water and in 175 parts of alcohol (U. S. P.). It does not reduce copper solutions, nor does it form an osazone with phenyl-hydrazine; but it is easily hydrolyzed with the formation of dextrose and levulose, and then, of course, the reactions peculiar to these substances may be obtained. It does not ferment directly, but, by the action of invertin contained in yeast, it takes up water, becoming glucose and levulose as above, these latter sugars being easily fermentable.

The explanation of its failure to respond to the usual sugar tests is shown by its graphic formula, which is probably:



Note the *lactone* structure, i.e., the joining of the α and δ carbon atoms through oxygen. Note also the absence of either aldehyde or ketone grouping.

Maltose, $C_{12}H_{22}O_{11}$, or malt-sugar, is an intermediate product in the hydrolysis of starch, and by further hydration becomes two molecules of dextrose: $C_{12}H_{22}O_{11} + H_2O = 2\ C_6H_{12}O_6$. It is formed in the fermentation of barley by diastase (the ferment of malt), and with phenyl-hydrazine it produces an osazone distinguished from glucosazone and lactosazone by its microscopical appearance (Plate V, Fig. 2) and its melting-point.

Lactose, $C_{12}H_{22}O_{11}$, obtained from milk, is a disaccharide with far less sweetening power than sucrose. It forms an osazone which crystallizes in small burr-shaped forms (Plate V, Fig. 3), often covered with long hair-like appendages.

It reduces Fehling's solution, but does not reduce Barfoed's solution. It resists fermentation in a marked degree. Upon hydration it is converted into dextrose and galactose.

Polyoses — Polysaccharides.

Starch. — This well-known and widely distributed plant-constituent, is a carbohydrate represented by $C_6H_{10}O_5$, the actual molecule, however, being many times this simple formula. The microscopical appearance of the starch granule is quite characteristic, and recognition of the more common starches by this method is not at all difficult (see Plate V, page 99).

Starch is not soluble in cold water; but in hot water, or in solutions containing "amylolytic" enzymes, or in solutions containing certain chemical substances, as chloride of zinc or of magnesium, dilute hydrochloric or sulphuric acid, capable of forming hydrolytic products, the starch granules swell up, and ultimately dissolve, being converted into dextrose. The conversion, however, takes place in several well-defined steps, as follows: Soluble starch is first formed, answering the same chemical test with iodine (Exp. 213, page 286); next, *erythrodextrin*, which gives a red color with iodine solution; then α, β, and γ achroodextrins (maltodextrin), which give no color with iodine, but react slightly with Fehling's copper solution; then *maltose*, also negative with iodine, but reacting strongly with Fehling's solution; and finally *dextrose*.

Dextrin ($C_6H_{10}O_5$) is a yellowish powder, also known as British gum; it is formed from starch, as indicated above, and constitutes to a considerable extent the "crust" of bread. It is soluble in water, the solution giving a red color with iodine, and is also distinguished from starch by its failure to give a precipitate with solution of tannic acid.

Glycogen, or animal starch, is a carbohydrate, with the general formula $C_6H_{10}O_5$, occurring *principally* in the liver, and to a lesser extent in nearly all parts of the animal body. Freshly opened oysters are a convenient source of the substance for

laboratory demonstration. It occurs in horse-flesh in considerably larger proportions than in human flesh.

Properties. — Glycogen is a white powder without odor or taste. It dissolves in water, producing an opalescent solution. It is closely allied to the starches of vegetable origin in that the products of its hydrolysis are dextrin and ultimately dextrose. It differs in its ready solubility in water, and in the fact that it is precipitated by .66 per cent alcohol, also in its power of rotation, which is much stronger than that of starch.

Physiology. — Glycogen is formed by the liver, and stored by this same organ for future use. It is derived principally from carbohydrates, but may also be derived from proteins. It disappears during starvation. In dead liver or muscle it rapidly undergoes hydrolytic change with the production of a reducing sugar.

Cellulose, $C_6H_{10}O_5$, is a carbohydrate which occurs as a principal constituent of woody fiber, and which may be found in the laboratory in a nearly pure state, as absorbent cotton or Swedish filter-paper. It is insoluble in water, alcohol, or dilute acids; it may be dissolved, however, by an ammoniacal copper solution, also by Schweitzer's* reagent, and by a concentrated solution of antimony chloride or tin chloride. It is converted into monosaccharides by acids, only after first treating with concentrated sulphuric acid, which partially dissolves it. Cellulose aids digestion in a purely mechanical way by separating the digestible matter and allowing easier access of digestive ferments. The celluloses may be divided into three classes: first, those resisting hydrolysis and consequently lacking nutritive value, such as flax, cotton fibers, and hemp; second, those which hydrolyze slightly, which include the ligno-celluloses and may be utilized as food by herbiverous animals; and third, the pseudo celluloses, or hemicelluloses, which hydrolyze, but instead of forming sugars give intermediate products such as the pentosans or hexosans, which, of course, will yield respectively pentose or hexose.

The galactans, another class of these intermediate substances, are widely distributed in nature, and when pure will yield galac-

* A hydrated solution of cupric oxide in ammonia.

tose upon hydrolysis. An important example of this class is agar-agar. As a constituent of diet it absorbs moisture and prevents drying of the residual fecal matter found in the intestine. In consequence of this property it tends to prevent constipation.

CHAPTER XIII.

FATS AND OILS.

Natural fats and oils of animal or vegetable origin are mixtures of several glyceryl esters (see page 43); they may be separated, by cold and pressure, into two portions, one solid with comparatively high melting-point, and the other liquid at ordinary temperatures. The solid portion is known as the stearopten, and the liquid, as the eleopten, of the fat. Thus, from beef-fat, we may express a fluid eleopten consisting largely of olein and obtain as a residue a stearopten, stearin. The stearoptens of the volatile or essential oils are classed as camphors, on account of their resemblance to ordinary camphor. Menthol, from oil of peppermint, and thymol, from oil of thyme, are examples of this class of compounds, both of which are largely used in dental practice.

Properties. — Fats are insoluble in water, easily dissolved by ether, chloroform, and carbon disulphide, less easily by alcohol, crystallizing on evaporation of the solvent. (Plate VI, Fig. 3, page 132.) They are emulsified by mechanical subdivision of the fat globules, in the presence of some agent which prevents their reuniting. The vegetable mucilages, soap, jelly, etc., are such emulsifying agents. On exposure to the air, fats and oils are more or less easily oxidized, which causes a separation of the fat acid. This produces an unpleasant odor or taste, and the fat is said to become rancid.

Chemistry. — The principal organic acids entering into the composition of fat are stearic acid, $HC_{18}H_{35}O_2$, solid, white, without odor or taste, melting at 70° C.; palmitic acid, $HC_{16}H_{31}O_2$, resembling stearic acid in its physical properties but melting at 62° C.; oleic acid, $HC_{18}H_{33}O_2$, containing two CH= groups with double-bonded carbons in the middle of the chain. This last acid is fluid at ordinary temperatures and predominates in the

softer animal fat. Its glyceryl ester, olein, constitutes 70 to 85 per cent of human fat (percentage said to increase with age) and 36 per cent of butter.

Physiology. — Fats are not digested to any appreciable extent until they reach the intestine; here they are broken up by a fat-splitting enzyme, emulsified, and to a slight extent saponified, after which they may be absorbed by the system (see Pancreatic Digestion).

Glyceryl palmitate, $C_3H_5(C_{16}H_{31}O_2)_3$, tripalmitin; **glyceryl stearate,** $C_3H_5(C_{18}H_{35}O_2)_3$, tristearin, and **glyceryl oleate,** $C_3H_5(C_{18}H_{33}O_2)_3$, triolein; these in varying proportions make up the greater part of animal and vegetable fats and oils.

The prefix "tri" is used because the "mono" and "di" compounds, as monopalmitin, $C_3H_5(OH)_2-C_{16}H_{31}O_2$, etc., are possible and may be prepared by synthesis. Triolein is liquid at ordinary temperature, solidifies at − 6° C., and is a "double-bonded" compound; hence it forms addition products with the halogens as stearin and palmitin cannot do, since they are "saturated hydrocarbons."

The amount of chlorine, bromine, or iodine which a fat or oil can thus absorb is an index of the proportion of unsaturated fatty acids contained in it, and hence becomes a valuable method of identification. For example, the iodine-absorption number of butter is 33.3, while for lard it is 55 and for cottonseed oil 109.5. For detail of determination see Exp. 149. Olive oil and lard oil contain large amounts of olein.

Tripalmitin melts at 66° C., and is usually obtained from palm oil. Tristearin melts at 72° C., and occurs with palmitin and olein in beef-fat, mutton-tallow, etc., the consistency of the fat being dependent upon the proportions of the constituent esters.

The fats, stearin for example, may be split into glycerol and fatty acid by steam under pressure, as follows:

$$C_3H_5(C_{18}H_{35}O_2)_3 + 3\ H_2O = C_3H_5(OH)_3 + 3\ HC_{18}H_{35}O_2.$$

A partial result of this sort is brought about by the fat-splitting enzyme (lipase) of the pancreatic juice (see Steapsin).

Saponification is the term applied when a glyceryl ester is acted upon by caustic alkali. When the glyceryl ester is an ester of palmitic, stearic or oleic acid, or a mixture of these esters, soaps similar to ordinary soap are produced.

Saponification of the fats by caustic alkali takes place as follows:

$$C_3H_5(C_{18}H_{35}O_2)_3 + 3\ KOH = C_3H_5(OH)_3 + 3\ KC_{18}H_{35}O_2.$$

The potassium salts of the fatty acids constitute the soft soaps, while the sodium salts are in general the hard soaps. The " salting-out " process in soap manufacture brings about a double decomposition, resulting in the production of ordinary soap.

When any determination of the fatty acids present in a fat is desired, the fat is usually saponified, and the fatty acids are thus fixed as soaps. A procedure frequently employed for determining the purity of butter-fat is the *Reichert-Meissel* determination of the volatile fatty acids. The fatty acids are first fixed as soaps; sulphuric acid is then added; and then, by distillation, the volatile acids are separated. See Exp. 148. Butter contains a much higher percentage of volatile fatty acids than animal fats, giving a Reichert-Meissel number of approximately 28.

Emulsification consists in producing a more or less permanent intimate mixture of liquids not otherwise miscible — e.g. oil and water. The emulsification is effected by means of emulsifying agents which mechanically hold apart the very finely divided particles of oil and prevent their reuniting. Gum arabic solution and albumin are examples of emulsifying agents. Milk is a natural emulsion.

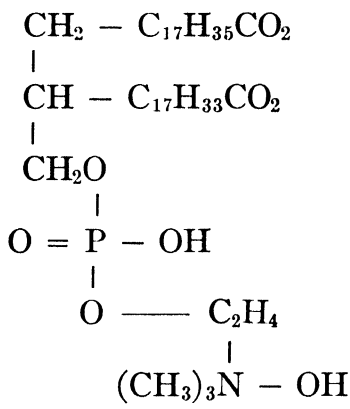
Volatile oils contain, instead of the glyceryl base, a group of hydrocarbons known as the " terpenes." The formula is $(C_5H_8)_2$, and the most important of the group is $C_{10}H_{16}$ from oil of turpentine and many of the essential oils.

The odor of the volatile oils seems to be dependent upon the presence of water and air; for example, oil of clove distilled over lime and in atmosphere free from oxygen has little odor. The

presence of oxygen and moisture restores the characteristic odor.

Lanolin or wool fat, obtained from sheep's wool, constitutes about 45 per cent of the crude substance. It is a mixture of cholesterin esters. The fat may be partially saponified, and from the alkaline solution three alcohols have been separated, all of which are white, crystalline powders. One of these, with formula $C_{12}H_{33}OH$, is known as lanolin alcohol and by oxidation with CrO_3 may be converted into lanolinic acid, $C_{12}H_{22}O_3$. (U. S. D.)

Lecithin has been classified as a phosphorized fat; it occurs in nervous tissue, and in the bile, and is obtained in considerable quantity from the yolk of eggs. It contains two fat acid radicals combined with glycerol, phosphoric acid and choline. Lecithin is soluble in chloroform, alcohol, ether and benzene, and may be obtained in crystalline form from the alcoholic solution. The fatty acid radicals are not always the same or necessarily alike. Lecithin may be represented by the following formula:



and its decomposition by the following reaction:

$$C_{44}H_{90}NPO_9 + 3\,H_2O = 2\,C_{18}H_{36}O_2 + C_3H_9PO_6 + C_5H_{15}NO_2$$

Lecithin		Stearic acid	Glycero-phosphoric acid	Choline

Cholesterol, as its name implies, is an alcohol containing one hydroxyl group and one pair of double-bonded carbon atoms. It is an important member of a group of sterols or " solid al-

cohols." It is insoluble in water or dilute acids or alkalies, but is soluble in bile or solutions of bile salts.

Cholesterol occurs in brain and nerve tissue, in bile, yolks of eggs and various glandular tissues. It is soluble in ether, chloroform, and hot alcohol, from which it may be recrystallized in characteristic plates with one corner more or less imperfectly formed. Exp. No. 239.

Cholesterol responds to a number of color tests, of which Exp. 151, and the following will furnish sufficient illustration:

L. Kaplenberg, in the *Journal of Biological Chemistry*, May, 1922, page 225, says, " In arsenic chloride, brain cholesterol or gall stone dissolves yielding a pink solution which gradually turns to a cherry red on standing, more rapidly on heating. Color is discharged by addition of benzene, toluene or chloroform."

CHAPTER XIV.

PROTEINS.

Protein is a general term used to designate the nitrogenized bodies which constitute the greater proportion of animal tissue.

While meat and "protein" are usually associated, it must not be forgotten that meat is not the exclusive source of protein, for we usually find protein in vegetable substances, often to a considerable extent.

Unlike that of the other two great divisions of food substances (carbohydrates and fats), the structure of the protein molecule is so complex that, with a few exceptions of the simplest kind, its representation has not been attempted.

The protein molecule contains nitrogen (often as the amino group NH_2) in addition to the carbon, hydrogen, and oxygen of the carbohydrates and fats. It frequently contains sulphur, often phosphorus, and occasionally the metallic elements, particularly iron.

As examples of the complexity of protein molecules, the following proposed formulae are given in Hawk's "Physiological Chemistry."

Serum albumin $C_{450}H_{720}N_{116}S_6O_{140}$.

Oxyhemoglobin, $C_{658}H_{1181}N_{207}S_2FeO_{210}$.

The protein molecule has been likened to a bundle of rods more or less loosely bound together, the several rods representing the several amino acids of which the protein molecule is largely composed. When the binders are broken, by process of digestion, the rods or amino acids fall more or less completely apart and are ready for absorption. There are about eighteen of these amino acids entering into the composition of the protein molecule which seem to be important, but not all of these acids are present in all proteins. For example, the protein zein, found in maize, or Indian corn, is lacking in the amino acid lysin; gelatin is lacking

in tyrosine, cystine and tryptophane. It has been shown that animals forced to depend on such deficient proteins do not thrive; hence, the *character* of the protein contained in the food must be considered.

Of great interest in this connection is the following paragraph, taken from page 65 of "The Newer Knowledge of Nutrition," Second Edition, by Dr. E. V. McCollum.

"It has been abundantly demonstrated by several workers, that the simplest of the amino acids, glycocoll, is readily synthesized by the body tissues. Casein contains no glycocoll, yet it is a complete protein, and can meet all the requirements of an animal for nitrogen in the form of amino acids. From these results it is safe to conclude that the tissues of the higher animals are capable of synthesizing certain amino acids, and that these are made use of in some way to conserve the body proteins, even though the list which can be so synthesized is incomplete. It appears certain that tyrosine, tryptophane, and probably all the other cyclic amino acids and cystine cannot be synthesized by the mammal."

We shall not take time to study the structure of all of these necessary amino acids; but a few, because of their importance as factors of nutrition, as above suggested, or because of importance in certain laboratory tests, will be considered.

For general properties see page 39.

Glycocoll, amino acetic acid, $CH_2NH_2.COOH$, is the simplest obtained from protein, and may, as above stated, be synthesized within the body. (For relation to hippuric acid see page 67.)

Alanine, α-amino propionic acid, $CH_3CHNH_2.COOH$, occurs in protein in comparatively small amounts, but related to it — perhaps derived from it — are serine, phenylalanine, tyrosine, and cystine.

Phenylalanine is α-amino-β-phenyl propionic acid, $CH_2C_6H_5.CHNH_2.COOH$.

Serine is α-amino-β-hydroxy propionic acid, $CH_2OH.CHNH_2.COOH$.

Tryptophane is α-amino-β-indol propionic acid, $CH_2(C_8H_6N).CHNH_2.COOH$. The putrefaction of proteins containing tryp-

tophane gives rise to indican in the urine. (See page 201.) This acid has been shown to be a growth-promoter in small animals. It is lacking in zein and gelatin.

Tyrosine is α-amino-β-parahydroxy-phenyl propionic acid, $CH_2(C_6H_4OH)-CHNH_2.COOH$. Tyrosine may be obtained from old cheese; it crystallizes in tufts of needle-shaped crystals and occurs rarely in urinary sediments, in cases of phosphorus poisoning and some other acute conditions.

Lysine is α-ϵ-diamino-normal caproic acid, $CH_2NH_2(CH_2).CHNH_2.COOH$. Lysine is one of the amino acids that are indispensable for growth, but is lacking in both zein, from maize, and gelatine.

Classification of Proteins.

A chemical basis for the nomenclature of proteins seems at present impossible, but the following suggestions and groupings, based on the properties of the protein substances, are generally accepted.

The word protein designates that class of substances which consist, so far as is known at present, essentially of α-amino acids and their derivatives: e.g., α-amino acetic acid, or glycocoll; α-amino propionic acid, or alanin; β phenyl-α-amino propionic acid, or phenylalanin; guanidine-amino valerianic acid, or arginine, etc. Proteins are, therefore, essentially polypeptides.

I. Simple Proteins.*

Protein substances which yield, on hydrolysis, only α-amino acids or their derivatives.

Although no means are at present available whereby the chemical individuality of any protein can be established, a number of simple proteins have been isolated from animal and vegetable tissues, and have been so well characterized by constancy of ultimate composition and uniformity of physical properties that they may be treated as chemical individuals until further knowledge makes it possible to characterize them more definitely.

* The classification and definitions herewith given are taken from the recommendations of the Committees of the American Physiological and Biochemical Societies as printed in Science, Vol. 27, No. 692, page 554.

The various groups of simple proteins may be designated as follows:

(*a*) *Albumins.* — Simple proteins soluble in pure water and coagulable by heat; e.g., ovalbumin, serum albumin, lactalbumin, vegetable albumins.

(*b*) *Globulins.* — Simple proteins insoluble in pure water, but soluble in neutral solutions of salts of strong bases with strong acids;* e.g. serum globulin, ovoglobulin, edestin, and other vegetable globulins.

(*c*) *Glutelins.* — Simple proteins insoluble in all neutral solvents but readily soluble in very dilute acids and alkalies;† e.g., glutenin.

(*d*) *Alcohol-soluble Proteins* (*Prolamines*). — Simple proteins soluble in relatively strong alcohol (70 to 80 per cent), but insoluble in water, absolute alcohol, and other neutral solvents;‡ e.g., zein, gliadin, and hordein.

(*e*) *Sclero Proteins, Albuminoids.* — Simple proteins which possess essentially the same chemical structure as the other proteins, but are characterized by great insolubility in all neutral solvents;§ e.g., elastin, collagen, keratin, and reticulin.

(*f*) *Histones.* — Soluble in water and insoluble in very dilute ammonia and, in the absence of ammonium salts, insoluble even in an excess of ammonia; yield precipitates with solutions of other proteins and, on heating, a coagulum which is easily soluble in very dilute acids. On hydrolysis they yield a large number of amino acids, among which the basic ones predominate; e.g., globin, thymus histone, scombrone.

(*g*) *Protamines.* — Simpler polypeptides than the proteins included in the preceding groups. They are soluble in water, un-

* The precipitation limits with ammonium sulphate should not be made a basis for distinguishing the albumins from the globulins.

† Such substances occur in abundance in the seeds of cereals and doubtless represent a well-defined natural group of simple proteins.

‡ The sub-classes defined (*a*, *b*, *c*, *d*) are exemplified by proteins obtained from both plants and animals. The use of appropriate prefixes will suffice to indicate the origin of the compounds, e.g., ovoglobulin, myoalbumin, etc.

§ These form the principal organic constituents of the skeletal structure of animals and also their external covering and its appendages. This definition does not provide for gelatin, which is, however, an artificial derivative of collagen.

coagulable by heat, have the property of precipitating aqueous solutions of other proteins, possess strong basic properties and form stable salts with strong mineral acids. They yield comparatively few amino acids, among which the basic amino acids greatly predominate; e.g., salmine, sturine, clupeine, scombrine.

II. Conjugated Proteins.

Substances which contain the protein molecule united to some other molecule or molecules, otherwise than as a salt.

(*a*) *Nucleo-proteins.* — Compounds of one or more protein molecules with nucleic acid; e.g., nucleo-histone.

(*b*) *Glyco-proteins.* — Compounds of the protein molecule with a substance or substances containing a carbohydrate group other than a nucleic acid; e.g., mucins and mucoids (osseomucoid, tendomucoid.)

(*c*) *Phospho-proteins.* — Compounds of the protein molecule with some, as yet undefined, phosphorus-containing substance other than a nucleic acid or lecithins; e.g., casein, vitellin.

(*d*) *Hemoglobins.* — Compounds of the protein molecule with hematin or some similar substance; e.g., hemoglobin, hemocyanin.

(*e*) *Lecitho-proteins.* — Compounds of the protein molecule with lecithins; e.g., lecithans, phosphatides.

III. Derived Proteins.

1. *Primary Protein Derivatives.* — Derivatives of the protein molecule apparently formed through hydrolytic changes which involve only slight alterations of the protein molecule.

(*a*) *Proteans.* — Insoluble products which apparently result from the incipient action of water, very dilute acids or enzymes; e.g., myosan, edestan.

(*b*) *Meta-proteins.* — Products of the further action of acids and alkalies whereby the molecule is so far altered as to form products soluble in very weak acids and alkalies, but insoluble in neutral fluids.

This group will thus include the familiar " acid proteins " and

" alkali proteins," not the salts of proteins with acids; e.g., acid meta-proteins (acid albuminate), alkali meta-protein (alkali albuminate).

(*c*) *Coagulated Proteins.* — Insoluble products which result from (1) the action of heat on their solutions, or (2) the action of alcohols on the protein.

2. *Secondary Protein Derivatives.** — Products of the further hydrolytic cleavage of the protein molecule.

(a) *Proteoses.* — Soluble in water, uncoagulated by heat, and precipitated by saturating their solutions with ammonium sulphate or zinc sulphate;† e.g., protoproteose, deuteroproteose.

(*b*) *Peptones.* — Soluble in water, uncoagulated by heat, but not precipitated by saturating their solutions with ammonium sulphate;‡ e.g., antipeptone, amphopeptone.

(*c*) *Peptides.* — Definitely characterized combinations of two or more amino acids, the carboxyl group of one being united with the amino group of the other, with the elimination of a molecule of water;§ e.g., dipeptides, tripeptides, tetrapeptides, pentapeptides.

General Color Reactions of Proteins.

Although there is wide variation in the structure of the protein molecule, proteins in general will respond to several *color reactions* based on the presence of certain amino acid groupings in the protein molecule.

Biuret Reaction, Piotrowski's Test.

This reaction is brought about by making the protein solution alkaline with sodium hydroxide and then adding a drop or two of

* The term *secondary hydrolytic derivatives* is used because the formation of the primary derivatives usually precedes the formation of these secondary derivatives.

† As thus defined, this term does not strictly cover all the protein derivatives commonly called proteoses; e.g., heteroproteose and dysproteose.

‡ In this group the kyrins may be included. For the present we believe that it will be helpful to retain this term as defined, reserving the expression peptide for the simpler compounds of definite structure, such as dipeptides, etc.

§ The peptones are undoubtedly peptides or mixtures of peptides, the latter being at present used to designate those of definite structure.

very dilute *copper sulphate*. Upon standing for a few minutes a *violet* coloration is observed if protein is present.

The shade of color produced varies from a *blue* violet in cases of simple proteins to a *rose* violet when proteoses or peptones are present.

This reaction, although typical, is not distinctive for proteins, several other substances responding positively to it. The substances that do give this reaction have been observed to be those containing at least one $CONH_2$ group in which the *amino group* is *free* and one or more substituted amide groups. Also, according to Schiff, any amide of a dibasic acid in which the amide groups are attached to different carbon atoms will react positively, oxamide being an example, $NH_2-CO-CO-NH_2$. Only *one* free amino group seems to be distinctly necessary, however.

Xanthoproteic Test.

If a solution of protein is made acid with concentrated nitric acid and heated, a yellow color is produced which changes to deep *orange* upon the addition of alkali. The alkali, generally ammonium hydroxide, should not be added until the solution is cool. This reaction is dependent upon the *benzene ring*, present in phenylalanine, tyrosine and tryptophane.

A positive xanthoproteic test will show, therefore, the presence of those amino acid groupings in the protein. The yellow color produced by the addition of nitric acid is indicative of the formation of a nitrobenzene product. This, upon the addition of ammonia, is converted into the salt formation which gives the orange coloration.

Millon's Reaction.

Millon's reagent, as given in the Appendix, consists of a mixture of mercuric nitrite and nitrate. When put in contact with protein and tested, it causes the protein to be precipitated; if allowed to stand, the precipitate will turn red.

Millon's reaction seems to be dependent on the presence of a *monohydroxy* phenyl group and is consequently a distinguishing test for *tyrosine*.

Hopkins-Cole Reaction.

This reaction is effected by treating the protein solution with glyoxylic acid and underlaying the solution with concentrated sulphuric acid. In the presence of the indol grouping, tryptophane, a condensation takes place producing a purple color. The exact chemistry of this reaction is not wholly known.

Lieberman's Reaction.

This reaction may be used as a test for a protein molecule containing both tryptophane and a *carbohydrate* grouping. It is made by treating the protein solution with strong HCl.

The action of the acid on the carbohydrate forms aldehydes which, we may assume, react with the tryptophane grouping to give a violet color.

The carbohydrate grouping may also be detected by Molisch's α-naphthol test, as given under carbohydrates.

Protein Precipitants.

The substances generally classed as protein precipitants may be grouped conveniently as *acids*, *metals*, and *salts*. Among the acids that produce a more or less insoluble compound with proteins are phospho-tungstic, phospho-molybdic, tannic, picric, chromic, and bichromic. Their precipitating action is now explained on the assumption that proteins contain several *free amino groups*, making them possess basic properties, and that the precipitate produced by action of an acid is an insoluble salt formation of that acid, as protein tannate, protein picrate, etc.

Similarly, when any of the precipitating metals, as mercury, lead, copper, platinum, manganese, iron, or aluminium, reacts with a protein, an insoluble *metal proteinate* is produced. The acid properties of the protein, which account for its behavior with metals and other basic substances, are presumably due to the fact that proteins contain, in addition to the free amino groups, *free carboxyl* groups. They are consequently amphoteric substances.

Hardy has shown that proteins in acid solution become electro-

positively charged, while proteins in alkaline solution are negatively charged. From this it follows that if the precipitating ion is the negative part of the molecule, or the anion, as is the case with the acid precipitants, the protein solution must be electro-positively charged, or *acid* in reaction, to bring about complete precipitation. Likewise, if the precipitating ion is positive, cation, as with the metal precipitants, in order to secure precipitation of the protein the solution must be negatively charged, or *alkaline.*

Mathews gives some exceptions to this rule, which may be noted. Some of the most strongly acid proteins,* as casein, are not affected by slight amounts of alkali, and hence will sometimes react electro-positively in an alkaline solution. The reverse holds true for those proteins strongly basic in reaction.

If we consider proteins as colloidal substances, we see that these statements are in direct accord with what was said in the first chapter in Vol. I, in regard to the manner of precipitating colloids; i.e., a negatively charged colloid is precipitated by the positive ion of the precipitating reagent, and vice versa.

As the third class of protein precipitants, we include neutral salts. The most important salt of this group, and the one generally used in laboratory work, is ammonium sulphate. All proteins, with the exception of the peptones and peptides, may be precipitated by completely saturating the solution with this salt. The form in which the protein is precipitated makes it easily soluble, and hence this precipitating reagent is well adapted for use in studying the properties of the protein. Magnesium sulphate and sodium chloride are also used as protein precipitants, but their action is not general.

When acted upon by salts, proteins behave like any colloid, as suggested above; the precipitating ion of the salt being dependent on the electrical charge of the protein solution.

The changes occurring during precipitation and solution of proteins are now regarded from a purely chemical viewpoint. Löeb and others have demonstrated the true ionization of proteins and have shown their characteristic reactions to be de-

* Those which contain several comparatively strong acids in the molecule.

pendent on their ionization, just as is the case with inorganic substances. The ionization of the protein is also stated to be responsible for its viscosity, and if the ionization of the protein is at a minimum the tendency will be for the viscosity to be at a minimum too.

Another protein precipitant of practical importance is alcohol, 95 per cent. Proteins, including peptones, are insoluble in strongly alcoholic solutions. The action of alcohol in bringing about protein precipitation may be considered as a process of dehydration. In this process, however, the protein is very apt to become denatured, so that for practical work on proteins it is inadvisable to make the precipitation with alcohol. The precipitation is increased by the addition of a few drops of an electrolyte, as 10 per cent acetic acid.

Isoelectric Protein.

The term isoelectric protein means a protein in a perfectly pure state, in which it is chemically inactive; that is, a state in which it will combine neither with the anion nor the cation of a substance, and if placed in an electric field its particles will not be attracted to either pole. The point at which protein acts in this way is usually at a very definite hydrogen-ion concentration, designated as the isoelectric point of the protein. If a given protein is then in a solution with a hydrogen-ion concentration greater than its isoelectric point, it is capable of combining only with the cation of any solution which may be added. On the other hand, a protein in a solution having a hydrogen-ion concentration less than its isoelectric point can combine only with the anion of any compound. This accounts for the fact that protein salts are formed in some cases, and in other cases metal proteinates.

For application to formation of tartar, see page 143.

Albumins.

The albumins are conveniently represented by egg-albumin and serum-albumin. They are soluble in water, respond to the general protein reactions (Exp. 152, page 276, etc.), and may be

completely precipitated by saturation of the solution by ammonium sulphate. Albumin is coagulated by heat (75° to 80° C.).

Egg-albumin differs from serum-albumin in that it is not absorbed when injected into the circulation, but appears unchanged in the urine. Egg-albumin is readily precipitated from aqueous solution by alcohol, while serum-albumin is precipitated only with difficulty. Albumins in general form, with acids or with alkalies, *derived proteins* known as acid or alkali albumins or albuminates (acid or alkali metaproteins). An acid albumin derived from myosin is known as syntonin. It differs but slightly from other acid albumins. The acid and alkali albumins are both precipitated by neutralization, but neither of them are coagulated by heat.

Albumin normally occurs in all the body fluids except the urine. The amount in milk is extremely slight; the amount in saliva seems to vary in inverse proportion to mucin. Albumin occurring in urine in appreciable quantity is always abnormal, although in many cases it has no serious significance unless persistently present in more than the slightest possible trace.

Globulins.

Globulins occurs in both plants and animals, and crushed hemp seed may be used as a convenient source for laboratory experiment. It is also associated with albumin in blood-plasma, and may be separated from it by half saturation with ammonium sulphate, which precipitates the globulin only; but it is not to be distinguished by the ordinary protein tests and reactions. The albumin of albuminous urine always consists of a mixture of these two proteins, globulin and albumin, not, however, always in the same proportion. The globulins are not soluble in distilled water as the albumins are, but a very small quantity of neutral salt, such as sodium chloride, will serve to effect the solution. Globulin is thrown out of solution by action of carbon dioxide as a white flocculent precipitate. By dialysis the inorganic salts necessary for its solution will be removed and the protein will be precipitated. It is also thrown out by saturation of sodium chloride or magnesium sulphate. Globulin is coagu-

lated by heat at practically the same temperature as serum-albumin; i.e., 75° C.

Sclero-Proteins.

Albuminoids or sclero-proteins are the simple proteins characterized by pronounced insolubility in all neutral solvents, and the common examples are keratin, from nails and hoofs, etc.; collagen, from bone and connective tissue; and elastin, from tendons and ligaments.

The differences in these substances are slight, the keratin being less soluble and less easily acted upon by digestive ferments than either of the other two. Keratin also contains more sulphur. It is the principal constituent of horn, nails, hair, feathers, egg membrane, and some shells, such as turtle and tortoise. The sulphur content of these various sources differs considerably, ranging from about 5 per cent in hair, about 3 per cent in nail and horn, to 1.4 per cent in egg membrane.

The *keratins* are characterized by the fact that the sulphur which they contain is loosely combined, i.e., easily separated by the formation of hydrogen sulphide and other sulphur compounds, as proved by Exp. 207. The keratins are insoluble in dilute acids and unaffected by any of the digestive ferments; they do, however, dissolve in the caustic alkali solutions, and may be used as the source of leucin, tyrosine, cystin, and other well-known products of protein digestion.

Keratins heated with water, under pressure, to 150° C. will decompose with the formation of mercaptan, hydrogen sulphide, and a substance resembling the proteoses.

Collagen, upon hydrolization with boiling water, produces gelatin, which is a characteristic property of this class of proteins. It may be dissolved by both the gastric and pancreatic juices, especially if previously treated with warm acidulated water. Collagen contains less sulphur than keratin and is obtained particularly from the *tendo Achillis* which contains about 32 per cent of this albuminoid and 63 per cent of water. Collagen responds to the general color tests for the proteins.

Elastin contains the least sulphur of any of the three sub-

stances which we have considered. It may be obtained from the *ligamentum nuchæ* of an ox, which contains about 31½ per cent of elastin and 58 per cent of water, by chopping the ligament finely and extracting for two or three days with *half*-saturated solution of calcium hydroxide. Like collagen, it is dissolved upon prolonged treatment with proteolytic ferments.

Reticulin occurs as a fibrous part of lymph glands. It is insoluble in water and is not digested by pepsin or trypsin. It does not respond to Millon's test for proteins, indicating the absence of the hydroxy-phenyl group of tyrosin.

Gelatin is made by hydrolysis of ossein or collagen brought about by *prolonged* boiling with dilute mineral acids. Gelatin, if first treated with cold water till soft, may be dissolved in hot water. The solution is precipitated by mercuric chloride, alcohol, tannic, and picric acids. It responds but feebly to the general protein reactions, but, by digestion with either pepsin or trypsin, compounds are obtained analogous to those resulting from similar protein digestion.

Gelatin solutions respond to the biuret test, not to Millon's nor to the Hopkins-Cole test. This fact shows the absence of any tyrosine or tryptophane grouping in the molecule of gelatin.

Derived Proteins.

Meta-proteins — Acid Meta-protein. — The digestive action of the gastric juice on protein substances is the formation of an acid meta-protein, formerly called acid albuminate. The meta-proteins are characterized by the fact that they are precipitated on neutralization and are not coagulated by heat. They may also be precipitated by saturation with common salt.

The Alkali Meta-protein or alkali albuminate is the stronger of these two classes of compounds when considered from a chemical standpoint; that is, the reactions are more marked, and some compounds will be formed with the alkali albuminate which are not produced when the acid albuminate is treated in a similar way. The acid meta-protein from the digestion of meat is known as syntonin.

The Proteoses (albumoses) may be considered as the next well-defined product of protein digestion following the albuminate. That is, leaving out the many intermediate products between which sharp lines of demarcation cannot be drawn, the decomposition of albumin brought about by enzymes or digestive ferments gives, first, acid albumin; second, albumose; and third, peptone. Albumose may be taken as a type of this second class of digestive products. Other proteoses, such as globulose, etc., are the substances derived from other proteins at a corresponding point of decomposition or peptic digestion. Albumose may be coagulated by heat at a temperature ranging upwards from 56° C., but, as the temperature approaches the boiling-point, certain varieties of albumose go again into solution, and at a boiling temperature may be separated from albumin by filtration. In these cases, as the filtrate cools, the albumose will again precipitate. Albumose is also precipitated by nitric acid, by ferrocyanide of potassium and acetic acid (the precipitate in both cases being dissolved by heat), and the other general protein precipitants. The biuret test gives a distinctive color with proteoses and peptones, it being a marked reddish shade rather than the violet or blue obtained with other proteins.

Peptones are the final products of *peptic* digestion of the proteins. They are soluble substances which give the biuret test similarly to the proteoses, but are not precipitated by heat, by nitric acid, by potassium ferrocyanide and acetic acid, nor by saturation with ammonium sulphate. Peptones may be precipitated by phospho-tungstic acid, phospho-molybdic acid, absolute alcohol and tannic acid. An excess of the reagent may dissolve the precipitate.

Peptides.

The peptides differ from the peptones in that the peptide does not possess protein characteristics to any marked extent. They are hydrolytic products of the peptone and seem to be merely bunches of amino acids. Upon decomposition or hydrolytic splitting of peptides, the simpler amino acids without any protein characteristics result.

Conjugated Proteins.

These are substances which contain the protein molecule united to some other molecule or molecules, otherwise than as a salt. The conjugated proteins which we shall study are mucin, a type of glyco-protein, yielding upon decomposition a substance containing a carbohydrate group; casein (from milk), a phosphorus-containing substance; and hemoglobin (from blood).

The glyco-protein, mucin, a selected type of this class of protein substance, occurs in various forms in saliva, in urine, bile, and other body fluids.

True mucins have been separated and examined from the secretion of the submaxillary glands, from snails, from mucous membranes of the air passages, from synovial fluid, and from the navel cord.

Mucin is quite readily converted to meta-protein by boiling with dilute acid, and, by action of strong acid, will yield a number of the simpler amino acids. Mucin itself is acid in reaction, but is, like all proteins, an amphoteric substance producing metal mucinates in solutions with hydrogen-ion concentrations above its isoelectric point, and mucin salts in solutions with acid concentrations less than its isoelectric point.

The mucins are insoluble in pure water, but dissolve upon the addition of traces of alkali. The solution thus obtained will give the usual color reactions for the proteins. It is in reality a solution of the alkali mucinate which has been formed. From such a solution mucin may be precipitated by acetic acid. If pure isoelectric mucin is desired, a very dilute hydrochloric acid is preferable as a precipitating agent.

Mucin in the saliva and its possible relations to bacterial growth, placque formation, and tartar deposit are discussed on pages 143.

Closely allied to the true mucins are the so-called *mucin substances*. These are mucilaginous substances occurring in some of the lower forms of animal life, but they differ in their chemical reactions from the true mucins. They are not precipitated from alkaline solutions with acetic acid.

Casein, the second conjugated protein which we shall consider,

is the principal nitrogenous constituent of milk and will be studied as such.

Milk.

Milk is the characteristic secretion of mammals and contains the three great classes of food material, viz.: proteins, carbohydrates, and fats. The fat is held as a permanent emulsion in so-called milk plasma.

The plasma consists of water holding in solution casein, albumin with a trace of globulin, milk-sugar (lactose), and mineral salts.

Specific Gravity. — Milk contains two different sorts of substances influencing the gravity: first, the fat, being lighter than the water, tends to decrease the gravity; second, the solids-not-fat, which are heavier than water, tend to increase the gravity of the milk. Consequently, it may happen that a very poor milk and a very rich milk will have the same specific gravity; e.g., the normal gravity of whole milk is about 1.031, while the gravity of skim milk will be about 1.035 or 1.036, and that in which cream occurs in large amount may be as low as 1.015 or 1.020. It can be easily seen that, starting with whole milk, the addition of cream or the addition of water will both alike reduce the gravity. Hence, taken alone, the gravity tells little or nothing as regards the quality of milk; but, if the gravity is taken together with the fat content, the *two* factors oftentimes give sufficient information.

The relation between the gravity of the fat and the total solids is approximately constant, and the following formula will give the amount of total solids usually within 0.10 or 0.15 of 1 per cent.

$$\text{Total solids} = \frac{\text{Fat} \times 6}{5} + \frac{\text{Sp. gr.}}{4} + 0.46.$$

Reaction. — The reaction of cow's milk, when perfectly fresh, is amphoteric to litmus; i.e., it will both redden blue litmus paper and turn red litmus blue, at the same time. This double

reaction is due to the presence of various salts, probably the acid and alkaline phosphates.

Cow's milk is acid to phenolphthalein, and this acidity is naturally increased by the multiplication of various acid-forming bacteria, which produce lactic acid by hydrolysis of the milk sugar. When the acid strength has increased sufficiently, the casein (English caseinogen) is decomposed, and paracasein (English casein) is produced and precipitated.

This paracasein constitutes the curd, and the process is the ordinary souring of milk.

Lactic acid is not the only acid produced in the spontaneous fermentation of milk, as traces of formic, acetic, butyric, and succinic acids have been demonstrated by different investigators.

The degree of acidity of milk is conveniently determined as suggested by W. Thorner (*Chem. Zeit.*, 1891, page 1108, abst. analyst XVI, 200): 10 c.c. of milk with an equal volume of water and a few drops of phenolphthalein as indicator, are titrated with N/10 alkali and every tenth of a degree of alkali used is considered as representing one " degree " of acidity.

By experimenting on samples kept under various conditions, Thorner found that milk coagulates on boiling when the acidity reaches 23°. Adopting 20° as the permissible limit of acidity, he proposes the following test: 10 c.c. of milk, 20 c.c. of water, a few drops of indicator, and 2 c.c. of decinormal alkali are thoroughly mixed; if any red color, however weak, results, the milk will not coagulate upon boiling.*

This method is given partly for its own sake and partly because exactly the same method is used by Dr. Eugene S. Talbot of Chicago and many others for the determination of acidity of urine. By slight modification it may be used for saliva. The record of slight amounts of acidity made in degrees in this way has several practical points in its favor.

Casein is the principal protein found in milk. It exists in combination with calcium salts. This combination is broken up and paracasein precipitated by the action of rennin and other enzymes, by acids, and by certain inorganic salts.

* From Allen's 'Commercial Organic Analysis,' Vol. 4.

Casein is classified as a pseudo-nucleo-albumin. The nucleo-proteins, so named because true nuclein may be obtained from them, are constituents of the cell nuclei, and differ in composition from ordinary proteins by containing from 0.5 to 1.6 per cent of phosphorus. Casein from cow's milk contains, according to Hammarsten, 0.85 per cent of phosphorus. It has been classified as a *pseudo*-nucleo-albumin because, upon digestion with pepsin, pseudo-nuclein rather than true nuclein is obtained.

Casein is practically insoluble in water, but dissolves readily in dilute alkaline solutions. Its precipitation as curd is dependent upon the presence of calcium salts.

Lactalbumin is the only other *protein* substance worthy of note in milk. This may be found in the filtrate after separating the casein. The total proteins contained in human milk average from 1.5 to 2.5 per cent while in cow's milk the proteins are 3.0 to 4.5 per cent. This difference, together with the variation of reaction and sugar-content, makes it necessary to "modify" cow's milk when it is used as an infant food.

The modification usually consists in the addition of lime-water (to change the reaction), of water (to reduce percentage of proteins), and of cream and milk-sugar (to increase fat and lactose).

The following table shows comparative composition:

	Reaction.	Total solids.	Proteins.	Sugar.	Fat.	Ash.
Human milk..	Alkaline	13.00%	2.70%	6.10%	4.00%	0.20%
Cow's milk...	Acid	14.00%	4.15%	4.90%	4.25%	0.70%

Fat. — The fat of milk exists as microscopic globules apparently inclosed in a protein-like membrane separating substance, the presence of which seems a necessary theory to account for the behavior of milk-fat toward various solvents, such as ether. The milk-fat, or butter-fat, consists largely of olein and palmitin with a slight amount of butyrin and traces of several other fatty acids.

Milk, as has already been stated, undergoes lactic acid fermen-

tation readily and this may be induced by a considerable number of microörganisms. It is not, however, liable to alcoholic fermentation except under peculiar circumstances. Alcoholic fermentation may be induced by certain ferments, such as the Kephir grain used quite largely in the East, the product being known as Kumiss or milk wine. Kumiss originally was produced from mare's milk, but the name has also been applied to any milk which has undergone alcoholic fermentation.

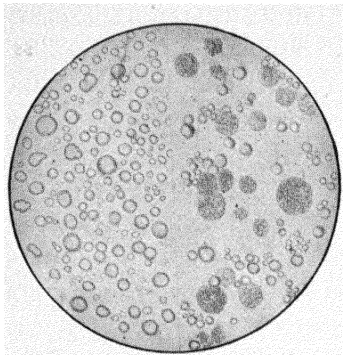


FIG. 2. Milk and Colostrum.

Colostrum is a peculiar substance occurring at the very earliest stages of lactation. Its specific gravity is considerably higher than that of milk, being 1.040 to 1.060. It contains much more protein substance and is characterized by the presence of granular corpuscles known as colostrum corpuscles. (Fig. 2.)

CHAPTER XV.

BLOOD AND MUSCLE.

Blood.

The blood, carrying oxygen and other forms of nutrition to all parts of the body, and returning carbon dioxide and the waste products of cellular activity, is an exceedingly complex substance. Its complexity is not surprising when we consider that it is the channel through which all internal exchanges are made.

As very well expressed by Mathews* the function of the blood is fourfold.

"1. It carries food from the intestine to the tissues; and gaseous food, or oxygen, from the lungs to the tissues. 2. It removes waste products from the tissues and carries them to the kidneys, lungs, intestine, and skin, the excretory organs of the body. 3. It provides for the metabolic co-ordination of the body, in that it distributes the internal secretions from each organ to other organs which utilize them. It thus keeps tissues, which may be far separated, in metabolic co-ordination or exchange with each other; it is the internal medium of exchange. 4. It plays a very important part in the defense of the organism against the invasion of parasites."

Blood normally is slightly alkaline, P_H 7.35, and is remarkable for the constancy of its alkalinity, the P_H being only slightly lowered in severe cases of *acidosis*. Emphasis should be laid on the fact that *true acidity* of the blood never occurs, the term signifying in this case merely a lessened alkalinity. It has been stated by Hawk that a change in the P_H of the blood as great as that occurring between the P_H of distilled water and the P_H of tap water would be fatal to the individual.

* Mathews' "Physiological Chemistry," 3rd Edition, page 459.

Composition of the Blood.

The composition of the blood itself, however, may be roughly described as a fluid (plasma) carrying in suspension the cellular constituents, red and white corpuscles. The plasma contains solid matter to the extent of about 8.9 per cent. This is largely protein, consisting of serum globulin, serum albumin, a slight amount of nucleo-protein, and fibrinogen; also a fibrin ferment, known as thrombase or thrombin.

The last two constituents play an important part in the formation of the *blood clot.* Many theories* have been suggested in explaining this property of the blood, and although at present no theory has been technically proven the following seems to be well grounded and is quite extensively accepted.

By action of a zymogen known as *prothrombin* with the calcium salts present in the blood, the fibrin ferment or *thrombin* is produced. The *thrombin* thus formed acts with the *fibrinogen* and produces the *fibrin* or blood clot. An interesting addition has been made to this theory by Howell,† who suggests that the prothrombin is held in combination by another substance called antithrombin. It is liberated only by the action of thromboplastin, which comes from the disruption of the *blood platelets*, on the antithrombin. The " clot " mechanically carries down the corpuscles, and as the clot contracts, the " serum " separates as a clear, amber-colored liquid, consisting of serum globulin (paraglobulin), serum albumin, and the fibrin ferment.

Although their exact function may not be clearly understood at present, the blood platelets seem to be essential in the formation of the blood clot. They may be considered as oval colorless disks about one-third the size of the red corpuscles.

The clotting of blood is obviously brought about by contact with any foreign body and may be hastened by increasing the calcium content; the tendency to clot is decreased by the presence of oxalates, citrates and a few other inorganic salts.

* For a detailed discussion the student is referred to Mathews' 'Physiological Chemistry,' 3rd Edition, pages 514–538.

† Howell, Am. Jour. of Physiology, 29, 187, 1911.

Fibrin. — The fibrin may be obtained free from corpuscles by whipping fresh blood. Under this treatment the fibrin separates as shreds, while the remaining fluid constitutes " defibrinated blood."

Fibrin, as usually obtained, is in the form of brown, stringy, and " fibrinous " masses, which are kept under glycerin for laboratory use. It is insoluble in water or alcohol. In dilute acid, (HCl), or alkali solutions, it swells and ultimately dissolves, although it may be several days before solution is effected. The fibrins from the blood of different animals differ in composition, as indicated by marked differences in solubility.

In addition to the protein content of the plasma, just described, the blood plasma contains various other substances, the most important of which are glucose, urea, uric acid, creatinine, fat, amino acids, enzymes, lecithin and cholesterol, dissolved gases and mineral salts. Acetone is normally present in very slight traces, and its increase, as is true of many of the constituents, is indicative of a pathological condition.

The chemistry of the red and white corpuscles is more complex and not so well known as the chemistry of the plasma, which we have considered. The red corpuscles consist of a frame of protoplasm, also called stroma, which contains lecithin, cholestrin, nucleo-albumin, and a globulin. (Hammarsten.) Upon and all through the stroma is the hemoglobin, which, together with its oxygen compound, oxyhemoglobin, is responsible for the *color* of the blood. Oxyhemoglobin may be obtained as silky, transparent crystals of blood-red color.

Hemoglobin. — Hemoglobin may be separated from blood by shaking with a little ether and water and allowing to stand twelve hours on ice; or sometimes crystals may be obtained by simply allowing a drop of defibrinated blood to partially dry on a microscope slide. The hemoglobin from different animals crystallizes in more or less distinctive forms: for example, from human blood the crystals will be diamond-shaped or rectangular; those from the blood of guinea pigs will be tetrahedrons or octahedrons resembling the crystals of white arsenic; and those from the blood of squirrels, six-sided plates.

The composition of hemoglobin has been given as 96 per cent globin (a histone), and the remainder hemochromogen.

Hemoglobin forms compounds with various gaseous substances and furnishes a good example for the study of the law of mass action. In the lungs, excess of oxygen slowly drives other gases, particularly carbon dioxide, out of combination, and forms oxyhemoglobin, while in the capillaries excess of carbon dioxide in venous blood replaces the oxygen. Hydrogen sulphide, nitric oxide, nitrous oxide, and carbon monoxide all form compounds with hemoglobin of various degrees of stability, the most stable being formed by carbon monoxide which acts as a poison by preventing the formation of oxyhemoglobin. Blood containing carbon monoxide hemoglobin is of a bright red color, which darkens in the air much more slowly than ordinary blood.

Hemoglobin is a conjugated protein, acid in reaction, decomposing into *globin* and *hemochromogen*, which is the iron-containing radical of the hemoglobin.

Oxyhemoglobin is similar in its properties, breaking into *globin* and hematin, the hematin being an oxidation product of hemochromogen and, like it, containing iron. Hematin, to which the formula $C_{34}H_{32}N_4O_4Fe$ has been assigned, is a brown, amorphous substance, acid in reaction, and forms with hydrochloric acid hematin hydrochloride, or *hemin*, which crystallizes in characteristic form. See Plate VI, Fig. 2.

If hematin is heated with strong hydrochloric acid, the iron is removed in the form of ferrous chloride, and iron-free hematin, or *hematoporphyrin*, is produced.

Hematoporphyrin is a dark purplish powder, with the properties of an acid and a base and is *isomeric* with *bilirubin*. It may be considered as the source of the bile pigments.

These relationships may be expressed thus:

$$\text{Hemoglobin} + \text{Oxygen} = \text{Oxy hemoglobin}$$

$$\text{Globin} + \overbrace{\text{Hemochromogen} + O = \text{Hematin}}^{\text{Hematin}} \begin{cases} + HCl = \text{Hemin} \\ -Fe = \text{Hematoporphrin} \end{cases} + \text{Globin}$$

PLATE VI. — PHYSIOLOGICAL CHEMISTRY.

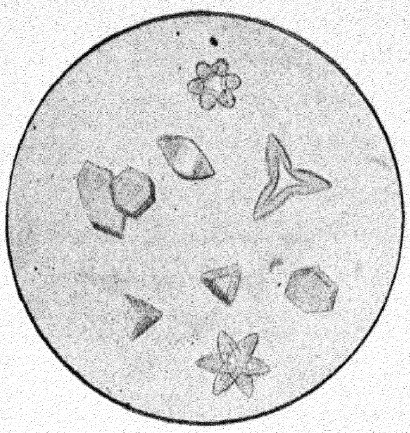


FIG. 1.
Edestin.

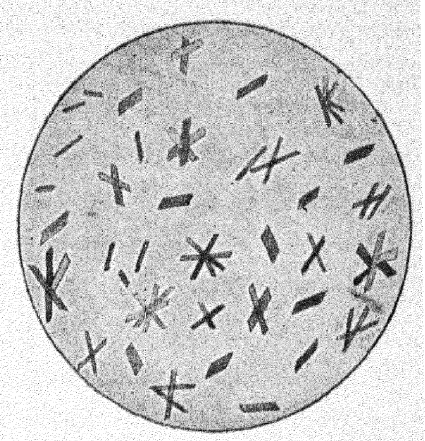


FIG. 2.
Teichmann's Hemin Crystals.

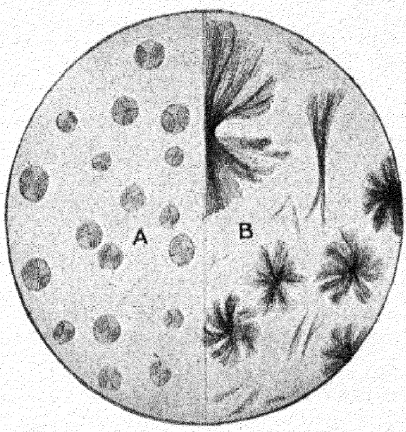


FIG. 3.—Fat Crystals.
A, Butter Crystals; *B*, Lard Crystals.

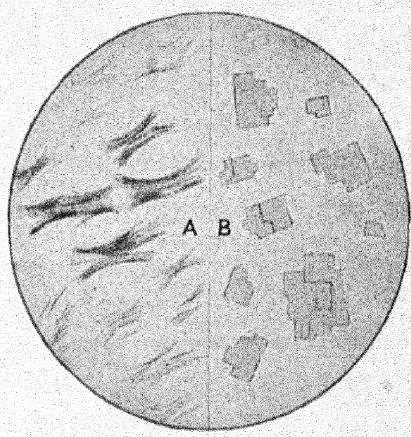


FIG. 4.
A, Fat Acid; *B*, Cholesterin.

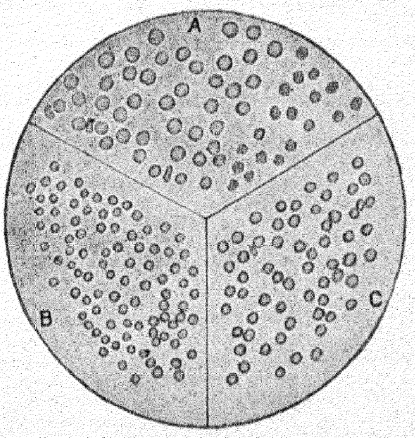


FIG. 5.
A, Human Blood; *B*, Horse Blood;
C, Dog Blood.

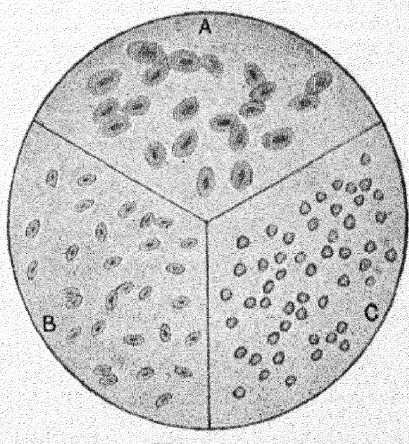


FIG. 6.
A, Frog Blood; *B*, Chicken Blood;
C, Fish Blood.

The iron from the blood may, by decomposition of the pigment and subsequent combination with sulphur (FeS), cause discoloration of teeth. This is the theory of Dr. E. C. Kirk, and in the author's opinion is perfectly sound, and far more probable than other explanations which have been offered, but which do not recognize the formation of a sulphur compound.

The form of the red corpuscle is that of a biconcave disk without nucleus; by action of water it becomes swollen, and the hemoglobin may be washed away, leaving the " stroma." This is what takes place when blood is " laked, " i.e., when hemolysis takes place. The diameter of the red corpuscles of human blood is about 1/3200 of an inch. Of the domestic animals, the corpuscles of the dog approach most nearly to the measurement of the human. The sheep, horse, and ox have smaller corpuscles than man, while those of birds, cold-blooded animals, and reptiles are larger (see Plate VI, Figs. 5 and 6).

The white corpuscles are rather larger than the red, and occur in much smaller numbers, a cubic millimeter containing about 5,000,000 red to 7500 white. The white corpuscles present a much greater diversity of character than do the red. They contain one to four nuclei, and are capable of amœboid movements. The white corpuscles are also called leucocytes, and aggregations of them constitute pus. The leucocytes are divided histologically into various classes, — lymphocyte, neutrophiles, eosinophiles, etc., — according as they are acted upon by different staining-fluids or fulfill some particular office; but these classes are not to be distinguished chemically.

Blood Tests.

Teichman's Hemin Test.

If to a drop of fresh blood on a microscope slide 2 drops of glacial acetic acid are added and the mixture is covered with a cover glass and gently warmed, upon cooling, dark brown, rhombic crystals of hematin hydrochloride, or hemin, are formed. These may be seen, frequently crossed, under the microscope. See Plate VI, Fig. 2. Hemin crystals can also be produced from

dried blood, provided a small crystal of sodium chloride is added. These crystals are easily made from human blood and also from the blood of many other animals which fact tends perhaps to lessen the value of the test.

Benzidene Reaction.

This is considered one of the most delicate tests for blood. It consists in the development of a bluish-green color when a saturated benzidene solution and hydrogen peroxide are brought in contact with the sample. Equal volumes of the benzidene solution, prepared in glacial acetic acid or alcohol, according to the method given in the Appendix, and commercial hydrogen peroxide are used. Exp. 206. The hydrogen peroxide is decomposed by the hemoglobin of the blood, and the oxidation of the benzidene, due to the liberation of oxygen, gives the blue color.

The blood of several different species of animals responds positively to this reaction, as to the hemin test.

Guaiac Test.

See Exp. 205. This test, according to Hawk, is of value if carefully performed, although it has lost favor with many because of its positive reaction with so many substances. The precautions necessary are that the unknown solution be boiled twenty seconds before the addition of the guaiacum and hydrogen peroxide solutions, and that the blue color does not develop until *after* the addition of the peroxide. Under these conditions it seems to be a fairly distinctive test for blood.

Bordet Reaction.

This test is regarded as the most satisfactory distinctive test for *human blood.* An antiserum, which will produce a precipitate with human blood and *with the blood of no other species,* is prepared from rabbits who have previously been injected with human defibrinated blood or blood serum.

Analysis of Blood *adapted from Folin.*

For a complete technique of blood analysis the student is referred to Folin's 'Laboratory Manual.' Only a small part of the procedure is outlined here, in the belief that it may be of value to the dentist.

Precipitation of the Protein.

The first step necessary in any blood analysis is the precipitation of the protein material and the subsequent formation of the clear, colorless filtrate, which is protein free. Folin accomplishes this by means of tungstic acid, the procedure being as follows:

The oxalated blood* is laked with seven volumes of water, and to this one volume of 10 per cent sodium tungstate (the tungstate must be pure) is added. Then one volume of two-thirds normal sulphuric acid is added slowly and with constant shaking. The flask which contains the solution is then stoppered and shaken thoroughly. After it has stood for five minutes, if the coagulation is complete, the color of the precipitate becomes dark brown. If the red color continues it usually indicates too much oxalate and it is necessary to add 10 per cent sulphuric acid, *one* drop at a time, with vigorous shaking, until the brown color is obtained and all foaming has ceased. The precipitate may then be filtered, and if the filtration is performed slowly and carefully the first filtrate will be clear and colorless.

Determination of Non-Protein Nitrogen.

This determination of the total nitrogen present, as urea, amino acid, ammonia, creatinine, uric acid, and other substances, has recently become one of the most valuable determinations made from a clinical standpoint. The nitrogen is determined by Folin's micro-Kjeldahl method, using the phosphoric sulphuric acid mixture† to bring about digestion.

Place 5 c.c. of the blood filtrate in a large dry pyrex test-tube

* Blood which has been collected over potassium oxalate to prevent coagulation.

† See Appendix.

and add 1 c.c. of diluted acid mixture (1 volume acid with 1 volume water). Boil vigorously from three to seven minutes, or until the tube is nearly filled with dense fumes; then quickly reduce the flame until boiling almost stops. Cover with a watch crystal and continue heating until oxidation is complete, i.e., until the solution is practically colorless. This usually happens in less than two minutes. Remove flame and let mixture cool for little more than a minute, then dilute with 15 to 25 c.c. of water. The solution may then be nesslerized and the color obtained compared with a standard ammonium sulphate solution. To obtain reliable results by this method the student needs practice in handling the colorimeter, and to give accurate results the colorimeter itself must be a fairly good one. The use of this method thus involves a considerable expense.

Equally accurate results may be obtained quite as easily by the inexperienced, by distilling off the ammonia, which has been formed from the digestion, into standard acid and titrating the excess of acid. Because of the small quantities used the distillation is not a long process. N/50 acid and alkali are recommended.

The normal non-protein nitrogen is 25–30 mg. per 100 c.c. There is a marked increase in nephritis.

Determination of Chlorine.

Chlorides present in the blood filtrate may be determined directly by titration with potassium sulphocyanate by the same procedure as that given for chlorine in the saliva. Normal blood (whole blood) chlorine is 450–500 mg. per 100 c.c. — about .45 per cent. The plasma content is slightly higher.

Determination of Glucose.

Reagents required:

(1) Standard sugar solutions

(a) 1 per cent glucose preserved with toluene, or made in a nearly saturated benzoic acid solution.

(b) 5 c.c. of solution (a) diluted to 500 c.c. — contains 1 mg. of glucose per 10 c.c.

(c) 5 c.c. of solution (a) diluted to 250 c.c. — contains 2 mg. of glucose per 10 c.c.

(2) Alkaline copper solution.

In a 1000 c.c. flask dissolve 40 grams of anhydrous sodium carbonate in 400 c.c. water. Add 7.5 grams of tartaric acid and, when dissolved, 4.5 grams of crystallized copper sulphate. Mix and fill to the mark with distilled water.

(3) Molybdate phosphate solution.

Place 5 grams of sodium tungstate and 35 grams molybdic acid in a large beaker (1000 c.c.). Add 200 c.c. 10 per cent NaOH and an equal volume of water and boil the solution vigorously for thirty or forty minutes. When cold, add water to make volume about 350 c.c., and then add 125 c.c. conc. phosphoric acid. Dilute to 500 c.c.

Procedure.

For this determination a special tube (Fig. 3) is necessary. Place in the tube 2 c.c. of the blood filtrate and 2 c.c. of the alkaline copper solution, making the volume of the solution such that it comes up to just the narrow part of the tube. In two other similar tubes place respectively 2 c.c. of standard sugar solutions (b) and (c) and to each add 2 c.c. of the alkaline copper solution. Heat the three tubes (for six minutes) in a boiling water-bath; then cool and add 2 c.c. of the molybdate phosphate solution to each tube. This will quickly dissolve the cuprous oxide formed. The blue solutions are then diluted to the 25 c.c. mark, and the flask stoppered and shaken. The color obtained in the unknown is compared colorimetrically with the standard which matches it most nearly.

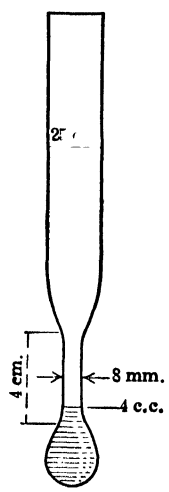


Fig. 3.
Folin Sugar Tube.

$$\text{Calculations} - \frac{\text{Reading of standard usually 20}}{\text{Reading of unknown}} \times \frac{\text{Mg. of glucose in standard}}{2} =$$

mg. of glucose per 100 c.c. blood.

Uric Acid Determination

The Folin phospho-tungstic test for uric acid is given under saliva. By this test it has been found that the normal content of uric acid in the blood is 4–5 mg. per 100 c.c. These figures are somewhat higher than those usually given and are not correct if the older and less delicate methods are used.

The value of blood analysis to physicians has long been known, but only recently has the importance to the dentist of a knowledge of systemic conditions been emphasized. That a relationship, and often a very close one, exists between oral disease and systemic conditions, is now a recognized fact; and in making this relationship clearer, blood analysis is essential.

Dr. Toren* makes some interesting statements in regard to the value of the *microscopical* blood examination.

" We wish to urge more thorough and painstaking work in the histological examination of blood. This work should not be delegated to an assistant or technician, but should be done by the physician or dentist himself." And further:

" It was about ten years ago that we first noticed, in the course of the general diagnostic examination of patients, that a particular type of leucocyte was present in the blood of patients having infections around the teeth."

MUSCLE.

Muscle forms, in the adult, a little less than 45 per cent of the body weight; and of all the metabolic processes taking place in the animal body probably from 50 to 75 per cent take place in the muscle. These facts make the chemistry of muscle very essential and, at the same time, decidedly complex.

The muscle changes rapidly upon the death of the animal, so much so that the liquid which may be expressed from living muscle (or from muscle frozen immediately upon the death of the animal) has been called muscle plasma, to distinguish it

* Diagnosis of Oral Infection by Blood Examination, by Julius A. Toren, M.D.: *Dental Cosmos*, Sept. 1922, p. 917.

from muscle serum, which is obtained in the same manner from dead muscle. The chemical reactions and the composition of these solutions differ considerably.

Muscle contains about 75 per cent water and about 25 per cent solid matter, the latter being chiefly made up of protein substances.

The two proteins of muscle plasma are given by Halliburton as paramyosinogen 25 per cent, and myosinogen 75 per cent. Of these the paramyosinogen seems to be a globulin, while the myosinogen, having many of the properties of a globulin, is soluble in pure water and is rather a mother protein from which the clot from muscle serum is produced. The protein of the muscle clot is known as myosin or myogen. Myosin may be precipitated from muscle serum by saturation with sodium chloride or magnesium sulphate. It has many of the properties of the globulins, but differs in the very important particular of not being precipitated by dialyzation.

In contrast with the soluble proteins of the muscle plasma, the proteins of the stroma, as the residue from which the plasma has been expressed is called, seem to be of an insoluble albuminoid character.

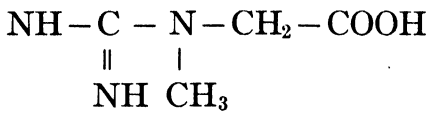
According to Mathews, the stroma also contains nucleoproteins, and phosphorus in the form of phospholipins, probably combined with a protein substance.

Muscle Extractives.

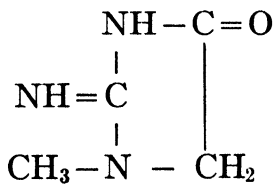
When muscle is treated with boiling water several organic substances are dissolved out of the muscle substance. These so-called extractives are of importance, because from studying them we get some idea of the metabolism of the muscle cells, and we may believe that some of these extractives give rise to substances excreted in the urine.

Nitrogenous Extractives. — This group includes creatine and its anhydride, creatinine, as well as xanthine, hypoxanthine, uric acid, urea, guanine, methyl guanidine, taurine, and a few others. Most of these are white solids which crystallize more or less easily with characteristic forms.

The exact source of creatine and creatinine has been the subject of much study, and it is still a disputed question. Creatine is methyl-quanidine acetic acid with the following formula:



If creatine is dehydrated by means of boiling with dilute mineral acid, creatinine is produced.



In the muscle, creatine seems to be a product of cell metabolism, diffused into the blood in small amounts. It has recently been suggested that it plays an important part in maintaining muscle tone. Creatinine, likewise a product of cell metabolism, may be present in living muscle in small amounts. It is found in the blood and urine, however, in much larger percentages than is creatine; the content seems to vary with the age, sex, and health of the individual, but is not appreciably altered by the protein intake. This fact is significant as regards its source, and points directly to it as a product of the vital processes of the muscle cell.

The purine derivatives, pages 57 and 58, found among the extractives of muscle are derived from the nuclei of the cells. Muscle tissue contains less nuclear material than most other body tissue, and therefore gives off a smaller amount of purines. These purine derivatives, according to Mathews, are diffused into the blood stream, particularly during muscular activity.

Non-nitrogenous Extractives

Under this group may be included glycogen, dextrin, lactic acid, sugars, inosite, and some fat.

Glycogen exists in the muscle as stored carbohydrate, and is converted to glucose and oxidized as the muscle needs it. Lactic acid, commonly classed as a fatigue product of muscle, probably comes from the carbohydrate present. It is the α-hydroxy-lactic

acid, known as sarcolactic acid, that is responsible for the acid reaction of dead muscle. Inosite, $C_6H_{12}O_6 + H_2O$, is a hexa-hydroxy-benzene, $C_6H_6(OH)_6$. It has a sweet taste and was formerly erroneously classed with carbohydrates. Inosite may occur in the urine of diabetic individuals.

The chief inorganic constituent derived from muscle is the acid phosphate of potassium. Like lactic acid and carbon dioxide, this may be considered as a fatigue product of the muscle. Chlorides of sodium, iron, magnesium and calcium are also found present.

Muscle Metabolism.

In considering the metabolism of the muscle we must think of it in two distinct ways: the metabolism which is concerned with the maintenance of the muscle itself, or, as Mathews calls it, " formative metabolism "; and that which produces muscular activity, or " energy metabolism."

The former is similar to that taking place in all of the body tissues and is the process of building new protein material by re-synthesizing amino acids. The exact nature of the synthesis is as yet merely conjecture. Unlike many other body tissues, muscle is capable of self-destruction; that is, it apparently contains proteolytic enzymes which must be constantly held in check. This checking is easily accomplished when there is no lack of food, but during starvation the muscle may be used as food for the more essential organs of the body.

The so-called energy metabolism is unfortunately no more clearly known than the formative metabolism. The glycogen is converted to glucose by enzyme action, and energy and heat are produced by the oxidation of the glucose. Lactic acid, carbon dioxide, acetone, traces of alcohol, and traces of many other substances have been found in muscle, and different theories have been advanced as to their origin. However, no proven information is available at the present time. Further, it has been shown by Cannon that some secretions of the ductless glands, especially adrenalin, have a marked effect on the glycogen content of the muscle.

CHAPTER XVI.

TEETH AND TARTAR.

The teeth consist of enamel, dentine, cement, and the pulp chamber, the first three of which may be considered chemically. The composition of the cement is practically that of true bone, the dentine and enamel differing principally in the proportion of organic matter which they contain. In all of these, the presence of lime, phosphoric acid, carbonic acid, and traces of magnesium and calcium fluoride may be demonstrated. The tartar contains a greater proportion of carbonic acid, less calcium phosphate, and much less organic matter than the teeth, taken as a whole, or than dentine, but about the same as enamel. According to Berzelius, sodium chloride and sodium carbonate may also be found.

The composition of the different parts of the tooth substance has been given as follows:

	Organic Matter.	Ash	$Ca_3(PO_4)_2$.	$MgHPO_4$.	$CaCO_3$.
Dentine	23.2	76.8	70.3	4.3	2.2
Cement	32.9	67.1	60.7	1.2	2.9
Enamel	3.1	96.9	90.5	traces	2.2

Also traces of magnesium carbonate, calcium sulphate, fluorides, and chlorides. An increase in the percentage of calcium phosphate or fluoride increases the hardness of the tooth, while an increase of calcium carbonate decreases the hardness.

Potassium sulphocyanate, ferric phosphate, sulphites, and uric acid have been found in tartar, as additional chemical constituents, while after the solution of the mineral matter the presence of epithelium cells, mucus, and the leptothrix may be demonstrated by the microscope.

According to Vergness, *Du tartre dentaire*, quoted by Gamgee, the tartar from incisor teeth and that from molars show decided

difference in their content of iron and calcium phosphates, the analysis being as follows:

	Tartar of Incisors.	Tartar of Molars.
Calcium phosphate	63.88–62.56	55.11–62.12
Calcium carbonate	8.48– 8.12	7.36– 8.01
Phosphate of iron	2.72– 0.82	12.74– 4.01
Silica	0.21– 0.21	0.37– 0.38
Alkaline salts	0.21– 0.14	0.37– 0.31
Organic matter	24.99–27.98	24.40–24.01

Deposition of Tartar Under Various Systemic Conditions.

The presence of oxalates and urates has been reported in the black tartar from pyorrhea cases. The deficient oxidation and high acidity usually occurring in such cases is conducive to the production of large amounts of oxalic or uric acids in the system, not necessarily on the teeth, whether these substances have etiological relations to pyorrhea or not.

The formation of ordinary hard tartar, consisting principally of phosphate and carbonate of calcium, is accounted for by Dr. Percy R. Howe* as follows: "An excess of calcium salts in the blood must be granted as one of the causes of calcification. These calcium salts are held in solution by two distinct factors: first, the excess of carbon dioxide; and second, by the presence of colloidal substances in suspension."

The colloidal substances consist largely of mucin and it has been determined that the isoelectric point of mucin, or the P_H at which mucin is most easily precipitated, is low, ranging with that of serum albumin, (P_H 4.7)† and serum globulin (P_H 5.4),† thus putting the isoelectric point of the salivary proteins below the P_H of any ordinary saliva. We know then *why* the calcium of the saliva may always be considered, as existing, in part at least, in the form of a calcium proteinate, and why we may disregard the occurrence of mucin as a protein salt (page 119). In the deposition of tartar, i.e., the precipitation of calcium phosphate and carbonate from saliva, the protein of the calcium

* *Dental Cosmos*, 1915, p. 307.

† Michaelis' 'Wasserstoffionenkonzentration,' Berlin, 1914.

proteinate will be acted upon only by electro-positive ions (Vol. I), furnished in the saliva by the alkaline salts. (Na_2HPO_4, $NaHCO_3$) which are always present. The calcium is thus left to combine with the anions of phosphoric and carbonic acids.

We should then expect that the higher the P_H of the saliva the more liable it would be to the formation of tartar. This has been shown to be not only possible but highly probable, by work done in the authors' laboratory. (*Journal Dental Research*, Vol. IV, No. 1, March, 1922.) Modifying factors which must be taken into consideration are, of course, the relative quantities of calcium, phosphoric acid, and carbonic acid. The foregoing statements are in relation to the effect of P_H only on tartar formation; large amounts of carbon dioxide would tend to keep calcium in solution and, of course, lower the P_H accordingly.

The important thing, however, is not exactly that the oral conditions which are most advantageous for the precipitation of tartar should be known, but rather that when this subject is under investigation all of the several factors influencing it should be considered.

Barille holds that calcium phosphate occurs in the blood as an unstable carbonate-phosphate which tends to decompose into calcium acid phosphate and bicarbonate, and that in saliva we find both these salts held in solution by carbon dioxide as follows:

$$Ca_3(PO_4)_2 + 4\,H_2CO_3 = H_2O + P_2O_8Ca_2H_2.2\,CO_3(CO_3H)_2Ca.$$

Upon the escape of the carbon dioxide, the calcium precipitates as the tri-metallic phosphate if the solution is alkaline, and as dicalcic phosphates if the solution is acid; and, of course, the loss of carbon dioxide will at the same time result in the precipitation of the neutral carbonate ($CaCO_3$).

That the general systemic condition is also a factor in the deposition of tartar is indicated by the experience of Dr. Wright of the Harvard Dental School, who has watched for a succession of years the fairly uniform increase in tartar deposits from October to June, and has found the vacation period marked by smaller amounts of deposit.

Lactic and other organic acids have been found in minute quantities in tartar, but these, as well as the qualitative tests for urates, will be considered more in detail under the Chemistry of Saliva.

Analysis of Teeth and Tartar.

The substance for analysis should be reduced to a moderately fine powder by crushing in a mortar, and a fair sample of the whole taken for each test.

Moisture may be detected by the closed-tube test (Vol. I.) and may be determined by accurately weighing out one gram of the substance in a counterpoised platinum dish or crucible and drying at 100° C. to constant weight.

Inorganic matter may be determined by careful ignition of dried substance; raise the temperature slowly till full red heat is reached; cool in a desiccator and weigh.

Organic matter may be ascertained by difference.

Lactates and other organic acids may be detected by careful crystallization and examination with the micropolariscope.

The several inorganic constituents may be demonstrated as follows:

Phosphoric Acid. — Dissolve a little of the powdered substance in dilute nitric acid; then to a few drops of the clear solution add an excess of ammonium molybdate in nitric acid. A yellow crystalline precipitate of ammonium phospho-molybdate will separate. Avoid heating above 60° C., as the ammonium molybdate may decompose and precipitate a yellow oxide of molybdenum.

Carbonic acid may be detected by liberation of carbon dioxide and passing the gas into lime-water, as described in Vol. I, or with closed tube and drop of baryta-water.

Chlorine may be detected in the dilute nitric acid solution by the usual silver nitrate test.

Calcium and magnesium may be separated and identified by the usual methods of analysis in the presence of phosphates.

Test for calcium and magnesium as follows: Add to the hydrochloric acid solution an excess of ammonia; calcium phos-

phate and magnesium phosphate are precipitated, white. Filter and to the filtrate add ammonium oxalate; a white precipitate shows lime, not as phosphate. Wash the precipitate produced by ammonium hydroxide, dissolve in dilute hydrochloric acid, and add ferric chloride carefully till a drop of the solution gives, when mixed with a drop of ammonium hydroxide, a yellowish precipitate. Nearly neutralize with sodium carbonate and add barium carbonate, which precipitates ferric phosphate. Filter, heat the filtrate, precipitate the barium with dilute sulphuric acid, and filter again. From the filtrate calcium is precipitated as white calcium oxalate, by making it alkaline with ammonium hydroxide and adding ammonium oxalate as long as a precipitate is formed. Filter and add to the filtrate sodium phosphate, which precipitates magnesium as ammonio-magnesium phosphate, white.

LABORATORY EXERCISES may consist of the examination by microchemical methods of one or more samples of tartar.

BONE.

If all organic matter is burned off from bone, there remains the bone-earth, so-called, made up of the phosphates and carbonates of lime and magnesia, with slight amounts of chlorine, fluorine, and of sulphates, the proportion being practically the same as given for dentine, under Teeth, on page 142. Because in some diseases, in which the bones are softened or decalcified (as osteomalacia), the relation of the calcium oxide and phosphorous pentoxide remains unchanged, it has been claimed that these substances exist in the bone in the form of a definite phosphate-carbonate containing three molecules of the tribasic phosphate to one of carbonate: $3\ Ca_3(PO_4)_2.CaCO_3$.

If, by treatment with dilute hydrochloric acid, the mineral constituents are entirely dissolved out of bone, there remains a substance from which glue (gelatin) is derived, of similar composition to the collagen from connective tissue, and known as *ossein*. Neither of these (ossein or collagen) is soluble in water or in dilute acids.

Bone marrow is of two sorts, red and yellow. The red marrow contains erythrocytes, fat, lecithin, protein substance consisting of a globulin, a nucleo-protein, fibrinogen, traces of albumin and proteose.

The yellow marrow is similar in composition, except that it contains fewer erythrocytes, more fat and more olein in the fat.

CHAPTER XVII.

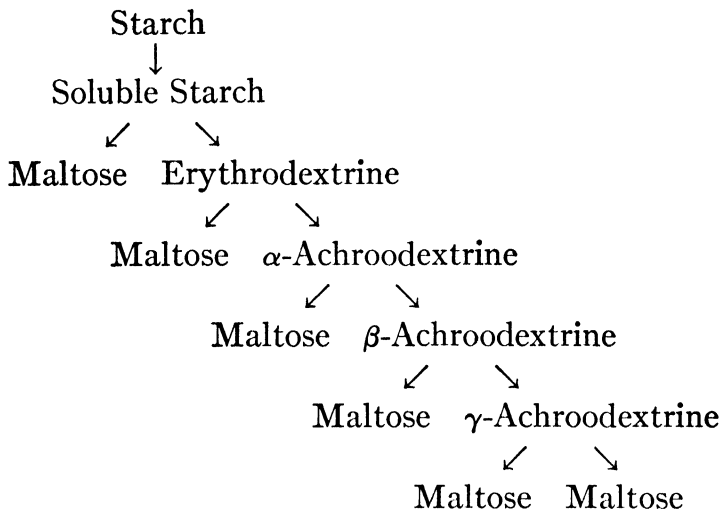
DIGESTION.

Salivary Digestion.

Digestion begins with the action of the saliva upon the carbohydrates, and if mastication is sufficiently prolonged, the ptyalin may convert an appreciable quantity of starchy food into a more soluble form before it reaches the stomach. In the stomach the amylolitic action of the saliva is stopped by the contact with the gastric juice. A certain amount, however, of salivary digestion takes place within the stomach, due to the fact that considerable time necessarily elapses before the acid of the gastric juice has been secreted in sufficient quantity to completely permeate and acidify the mass of food received from the esophagus. As has been previously shown, a very feeble degree of acidity is conducive to the activity of the amylolytic ferment. The average alkalinity of the saliva, calculated as Na_2CO_3, is about 0.15 of one per cent.

Saliva carries the digestion of starches to the *maltose* stage through action of the enzyme *ptyalin*. The first substance formed is *soluble starch*, which splits into *erythrodextrin* and maltose. The erythrodextrin hydrolyzes to give *α-achroodextrin* and *maltose* and the α-achroodextrin hydrolyzes in turn to β- and γ-achroodextrin, some maltose also being produced each time. The further cleavage of the γ- achroo-dextrin yields only maltose. Diagrammatically these changes may be represented as shown on the following page.

These digestive changes may be indicated by the action of saliva on starch, using iodine as an indicator (see page 286); the color of the iodine test changes from a decided blue with the starch to purple and red with dextrine, finally becoming negative with maltose.



The amylolytic action of the saliva is best adapted to a neutral or slightly alkaline solution. It will act, however, in an acid solution, *provided the acid* is in combined form, i.e., as in the form of a protein salt; in the presence of *free* acid the action is prevented.

In addition to the ptyalin of the saliva there is present also a small amount of *maltase* which hydrolyzes the maltose to glucose. By far the greater part of this hydrolysis, however, does not take place until the food comes in contact with the maltase of the intestinal juice.

According to some authorities, the saliva also contains a trace of proteolytic enzyme; but in the author's opinion there has been no substantial experimental evidence differentiating between the action of such a proteolytic enzyme and bacteria.

Gastric Digestion.

The first step in the gastric digestion is probably the union of the stomach hydrochloric acid with the proteins, forming meta-proteins or allied bodies which are changed by pepsin, which is the active digestive ferment of the stomach, into the proteoses, and slight amounts of the various peptones, following practically the changes produced experimentally on page 288.

Pepsin is an active proteolytic enzyme occurring in the cells of the stomach-wall, probably as pepsinogen; this latter is

decomposed by the hydrochloric acid, with the formation of free pepsin. Pepsin works only in faintly acid solutions, and in the stomach carries the digestion of proteins but little beyond the stage of the proteoses.

The activity of pepsin is dependent on the hydrochloric acid, and for this reason the hydrochloric acid of the gastric juice is classed with those substances known as *activators*. They are not enzymes but are essential to the action of the enzyme.

Hydrochloric acid is obtained from the fundus glands by an interchange of radicals between alkaline chlorides and the carbonates of the blood. The quantity present varies from nothing to 0.3 per cent, the degree of acidity most favorable for peptic activity being about 0.18 per cent, P_H 1.5–2.0.

Aside from HCl, various organic acids may be present in the stomach contents; lactic acid, butyric acid, and acetic acid are the most important of this class, tests for which are referred to under analysis of gastric contents, page 288.

Hydrochloric acid combines with protein substances of the food, forming a rather unstable compound, in which condition the acid is known as combined hydrochloric acid in distinction from the free hydrochloric acid which the gastric juice may also contain. The combined acid possesses only in modified form the properties of the free acid, and hence is less likely to stop the digestive action of ptyalin from the saliva.

Rennin is a second enzyme found in the stomach. This, like pepsin, also exists as a zymogen, and is liberated or developed by the presence of acid. Its action is particularly the curdling of milk, i.e., the decomposition of casein (Exp. 182), and consequent coagulation of the paracasein, the curd.

This process involves a splitting of the casein into a slight amount of a peptone-like body, and soluble casein. From this latter substance the insoluble curd is produced by the action of the calcium salts contained in the milk.

The activity of rennin is greatest in a slightly acid medium, P_H 5.0, although it will act in a neutral or slightly alkaline solution. If the alkalinity becomes equal to that of the blood the enzyme is destroyed.

It is interesting to note the difference in the optimum P_H of pepsin and rennin, the two proteolytic enzymes of the stomach. In adult life the normal gastric acidity is very close to the P_H best adapted for pepsin activity; while in the child the acidity is much less, and in very early life we find the pepsin virtually inactive and the condition to be that most favorable for the action of rennin.

Gastric lipase, or stomach steapsin, a fat-splitting enzyme, also exists in the stomach, in very small quantities. Its action is comparatively weak and of but slight importance.

It is to be noted that the digestive action of the stomach is only partial, the proteins being split into proteoses and to some extent into peptones, while further action is left for the more active ferments of the pancreatic and intestinal juices.

Pancreatic Digestion.

It may be an aid, in remembering the various digestive ferments, to note that in the saliva we have one principal ferment, ptyalin; in the stomach we have two, pepsin and rennin; in the pancreatic juice, three, trypsin, amylopsin, and steapsin. In addition to these, the pancreatic juice contains a ferment similar to rennin, known as chymosin.

Trypsin is the proteolytic enzyme of the pancreatic juice. It is a much more energetic digestive agent than pepsin, converting the proteoses into peptones, tyrosin, leucin, and other amino acids. It also differs from pepsin in that it acts in an alkaline medium rather than an acid. Trypsin exists, like other proteolytic enzymes, as a parent enzyme, trypsinogen, which in itself is not a digestive ferment, but which is rendered active (activated) by another substance known as enterokinase.

The enterokinase, another one of the activators, occurs in the intestinal juice, and seems to be secreted only as it is needed for the activation of the trypsinogen. Enterokinase does not in itself possess digestive power, but its action is destroyed by heat and in this it resembles the enzymes.

Amylopsin, or pancreatic amylase, is the starch-digesting enzyme of the pancreatic juice. Here, again, we have an enzyme much more energetic in its action upon carbohydrates than the ptyalin of the saliva. It converts starch into maltose and to some extent to dextrin. The amylopsin is active in faintly alkaline or very faintly acid solution; more acid, however, retards its action.

The starch-splitting enzyme of the pancreas is dependent upon the presence of electrolytes; if these are removed by dialysis a juice results which is devoid of starch-splitting power. A halogen ion, chlorine or bromine, is apparently essential to the activity of this enzyme.*

Steapsin, lipase, is the fat-splitting enzyme of the pancreatic juice, inactive until it comes in contact with constituents of the bile. It splits the fat, as indicated on page 106, into glycerol and fatty acids, and also acts as an emulsifying agent. The free fatty acids thus formed unite with the alkaline bases found in the intestines, to form soaps, which are also active emulsifying agents.

Chymosin, or pancreatic rennin, has practically the same action upon casein as the gastric rennin.

The pancreatic juice and the bile enter the duodenum in very close proximity, and the digestive action of each is dependent, to a considerable extent, upon the presence of the other.

The secretion of the pancreatic juice is brought about by a substance called secretin, secreted by the mucous membrane of the intestine. Secretin, according to some authorities, exists as *prosecretin* and is converted into secretin by the acidity produced in the intestine with the passage into the duodenum of the acid stomach contents. It belongs to that class of substances known as *hormones*, and differs from the activators in that it starts specific chemical action. Very little is known about the action of hormones, but that they are essential to the function of various glands is a recognized fact. Hormones are secreted into

* Journal of the American Chemical Society, Vol. 32, p. 1087, Kendall and Sherman.

the blood, and the presence in the blood of these so-called " chemical messengers " seems to act as a co-ordinating agent between the various glands.

The intestinal juice contains a number of substances playing an important part in the preparation of food material for assimilation. Among them is erepsin (erepase). This is a protein-splitting enzyme acting upon the products of tryptic digestion. It has little power upon the simple proteins, but will split the peptones into amino acids. There are also in the intestinal juice certain amylolytic enzymes, sucrase, lactase, and maltase, which continue the digestive action started by amylopsin or by ptyalin of the saliva. Their action is, respectively, the conversion of sucrose to glucose and levulose; of lactose to glucose and galactose; and of maltose to two molecules of glucose.

Bile. — This is a secretion produced by the liver and stored in the gall-bladder, from which it is delivered to the intestines, where it aids materially in emulsification and absorption of the fats.

Composition of Bile. — The composition of bile is very complex, as it contains a portion of the waste products of metabolism as well as substances playing an important part in digestion and designed to be re-absorbed into the circulation.

Among the first class are the two principal bile pigments: the bilirubin (bile red) and its oxidation product, biliverdin, (bile green). The bile-pigments are derived from the coloring matter of the blood. The appearance of either of these or of their derivatives, in either urine or saliva, is indicative of pathological conditions either of the liver- or bile-ducts, causing obstructions to the outflow of the bile or a destruction of the red-blood corpuscles.† The blood pigments, according to Michaels, are easily demonstrable in the desiccated saliva by means of polarized light.

Cholesterol ($C_{27}H_{45}OH$?) may also be considered a waste product of the bile. It is excreted with the feces; when retained it is likely to produce " gall stones," which are often found to consist of fairly pure cholesterol with a little coloring matter.

† Ogden.

DIGESTION IN THE ALIMENTARY CANAL.

Location.	Gland.	Juice.	Ferment.	Acts upon	Changes into	Action.	Second ferment.	Converted into
Mouth	Salivary	Saliva	Ptyalin	Carbohydrates (starch)	Maltose / Dextrose	Amylolytic	Maltase or Glucase	Glucose
Stomach	Gastric	Gastric	Pepsin	Proteins by HCl into Metaproteins	Proteoses (albumoses) / Peptones, leucine / Tyrosine, etc.	Proteolytic	.....	.....
			Rennin	Casein (phosphoprotein)	Casein (Neucleoprotein)	Proteopyknotic [1]	.....	.....
			Gastric Lipase	Fat	Emulsion	Lipolytic [4]	.....	.....
Duodenum	Pancreas	Pancreatic	Trypsin	Proteoses	Proteoses / Peptones, Peptides	Proteolytic	.....	.....
			Amylopsin [2]	Carbohydrates (starch)	Maltose, Dextrin	Amylolytic	Maltase	Glucose
			Steapsin [3]	Fats	Emulsion	Lipolytic	.....	.....
			Pancreatic Rennin	Casein of milk	Casein (proteose-like body)	Proteopyknotic	Lactase	Glucose / Galactose
	Liver	Bile	Alkali activators (emulsifying agent)	Fat	Emulsion (glycerol and fatty acid)	Lipolytic	.....	.....
Intestine	Intestine	Intestinal (Succus Entericus)	Erepsin (erapase)	Tryptic digestion	Amino-acids	Proteolytic	.....	.....
			Invertase [5]: Sucrase	Sucrose	Glucose [8] / Levulose [8]	Saccharalytic [7]	.....	.....
			Invertase [5]: Lactase	Lactose	Glucose / Galactose	"	.....	.....
			Invertase [5]: Maltase	Maltose	Glucose	"	.....	.....
			Enterokinase [6]	Trysinogen	Trypsin	Activation	.....	.....
			Lipase	Fat	Emulsion	Lipolytic	.....	.....

[1] Protein-coagulating or milk-curdling enzyme. [2] Pancreatic amylase. [3] Pancreatic lipase. [4] Fat-splitting. [5] Ferment obtainable from intestine and yeast. [6] Activator for trypsin. [7] Sugar-splitting. [8] Invert-sugar.

Two important acids of the bile are taurocholic and glycocholic, existing principally as sodium or potassium salts. Glycocholic acid upon hydrolysis splits into a simpler acid (cholic) and glycocoll, glycocoll being an amino-acetic acid (page 111), which is undoubtedly an antecedent of urea.

Taurocholic acid, on the other hand, splits into cholic acid and taurine, taurine being an amino-ethyl sulphonic acid (page 49).

CHAPTER XVIII.

SALIVA PROPERTIES, CONSTITUENTS, AND ANALYSIS.

The saliva is a mixed secretion from the parotid, submaxillary, and sublingual glands, together with a slight amount obtained from the smaller buccal glands. The chemical composition of the secretion from these various sources differs considerably, but from a dental standpoint we are much more interested in the mixed saliva and its constituents than the differences in the products of the various glands. The notable differences are that the mucin is practically wanting in the parotid saliva. The alkaline salts seem to be in smaller proportion in the parotid saliva than in the other two. Potassium sulphocyanate is a constituent of all varieties of saliva, although more constantly present in the submaxillary and in the sublingual than in the parotid. The parotid, on the other hand, contains a larger proportion of dissolved gases. The data on the composition of these varieties differ to a considerable extent and comparisons are not wholly satisfactory.

The mixed saliva contains, according to Professor Michaels, all the salts of the blood which are dialyzable through the salivary glands, and hence furnishes a reliable index of metabolic processes which are being carried on within the system. In order that this fact may be of practical value, two things are obviously of prime importance: First, methods of analysis which are not too complicated and which are at the same time conclusive; second, a knowledge regarding the source of the various constituents found, which will enable us to make a rational interpretation of the results obtained. In both of these fundamentals we are very much hampered by lack of knowledge; as yet there is much to be desired in the way of practical clinical tests for the various salivary constituents, and very much to be learned

as to their meanings, in order to make deductions which shall be conclusive. We are led to believe, from the work of an increasing number of specialists, that this subject of salivary analysis promises much and is worthy of careful investigation.

The quantity of saliva secreted in twenty-four hours is variously estimated from a few hundred c.c. to 1500; 1200–1500 c.c. is the more probable amount. As the measurement of the twenty-four hour quantity is practically impossible, Ferris has used the quantity secreted in twenty minutes as a basis for quantitative estimation. The quantity is diminished in fevers, severe diarrhea, diabetes, and nephritis, by fear and anxiety, and by the use of atropine. It is increased by smoking, by mastication, by the use of mercury, potassium iodide, or pilocarpin. The flow of saliva is also increased by the action of the sympathetic nervous system, during pregnancy, and by local inflammatory processes.

The methods outlined here for the analysis of saliva have been selected with two objects in view; first, to furnish some tests sufficiently simple to make them of use to the dental practitioner; and second, to give some determinations, usually of greater accuracy, suitable for the demands of investigators with the facilities of large chemical laboratories at their command. In some instances several methods will be given.

Physical Properties. — The physical properties of saliva include its appearance, specific gravity, color, odor, and viscosity.

Appearance. — The appearance is clear, opalescent, frothy, or cloudy; normal saliva is usually opalescent. It may become turbid by precipitation of lime-salts caused by the escape of carbon dioxide.

Specific Gravity. — Specific gravity ranges from 1.002 to 1.009, the total solids being only from 0.6 to 2.5 per cent.

Color. — Saliva is usually colorless when fresh, but upon standing for twenty-four hours may assume various tints, which are developed from constituents derived from bile. (Professor Michaels.) Saliva may be colored red or brown by the presence of blood or blood pigments, but in such cases the source of the color is usually local and easily discovered.

Odor. — Normal saliva is practically odorless. In cases of pyorrhea there is usually a peculiar fetid odor, easily recognized. In other pathogenic conditions the odor may be slightly ammoniacal, or may occasionally resemble the odor of acetone or garlic.

In any analysis *physical properties* of the saliva should first be noted. The color and appearance of the perfectly fresh sample is to be carefully compared with the appearance and color after standing for forty-eight hours in a small, tightly covered vial. The color may be yellowish, greenish, or brown, according to the variety of the derivative of biliverdin from which the color is obtained.* The general appearance may also change, independently of any color. A saliva that is hypoacid in character, when fresh, is usually markedly opalescent and of offensive odor after forty-eight hours, while a hyperacid saliva may have become clear or cloudy but without odor.

We may add to this examination a viscosity test which will be of value as indicating the amount of mucin, as the mucin content affects the viscosity more than any other one constituent.

The **viscosity** may be determined by use of the apparatus pictured in Fig. 4.

The essential features of the viscosimeter are a straight graduated tube with the *constriction* (*C*) jacketed so that the conditions under which a given sample will pass through the opening will always be under absolute control.

The apparatus is standardized by partly filling with distilled water in which the bulb of a thermometer is immersed.

The temperature of the distilled water is brought to 25° C. The thermometer is removed to facilitate reading, and from 5 to 10 c.c. of the liquid is allowed to run out, the time consumed being accurately determined by a stop watch.

The viscosity of saliva is determined in the same way, care being taken that only a perfectly clear solution is used, as fine particles will clog the opening at *C*. The use of the stop cork as pictured in Fig. 4 is undesirable; in fact, it has been found

* Dr. Joseph P. Michaels. S. S. White's reprint of paper read before International Dental Congress, Paris, 1900.

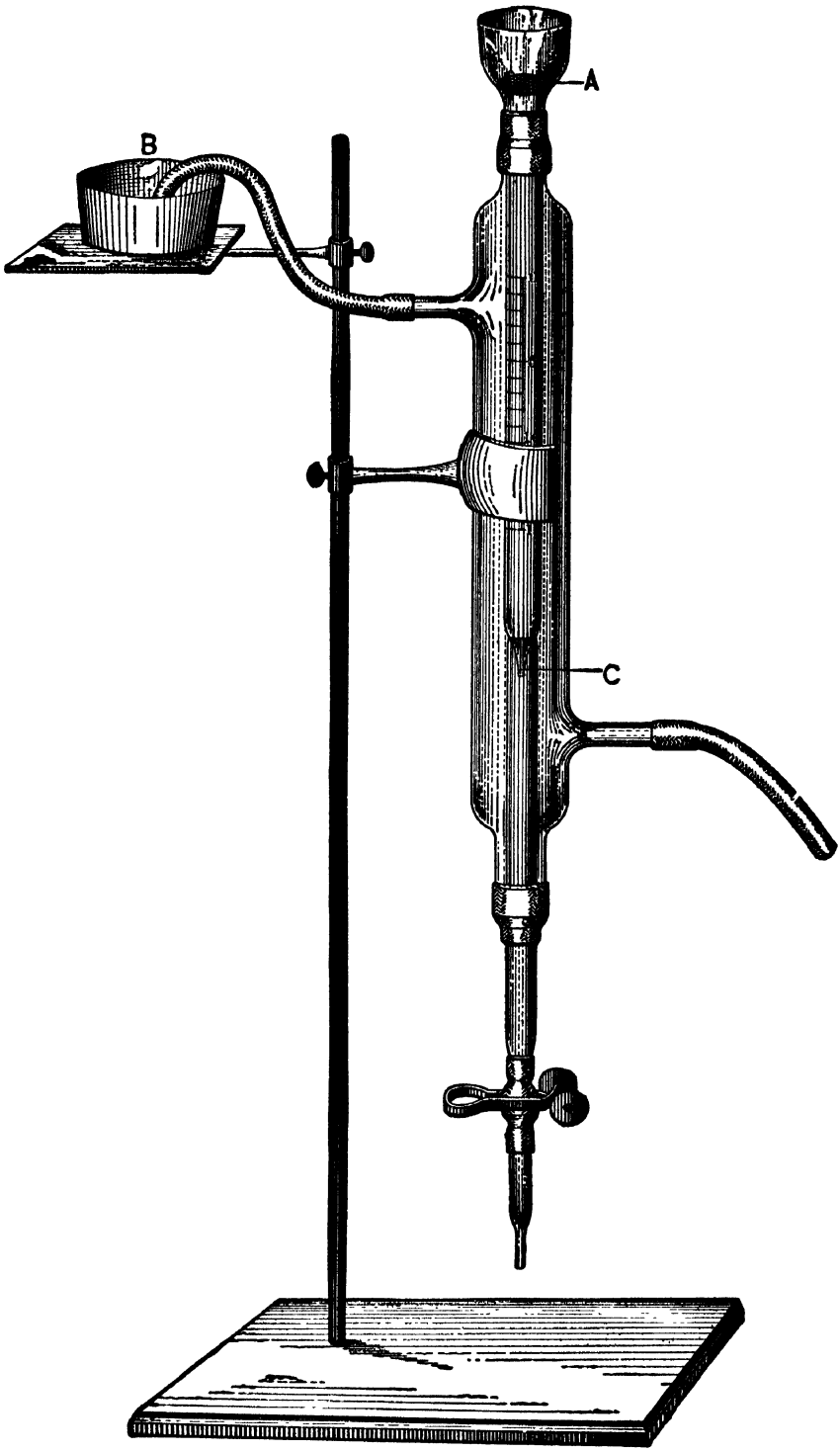


FIG. 4.

that straining the saliva, filtering through paper, or even centrifugalizing in order to separate the solid portions, will occasion a variation in the results obtained. The first determination should be carefully made and used, as repeated determinations result in a regular diminution of the viscosity figure, due to mechanical changes brought about by passing the saliva through the very small opening at *C*.

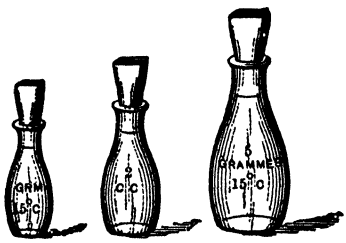


FIG. 5.

If the constriction of the graduated tube is sufficiently great, i.e., if the opening is sufficiently small, comparison may be made by counting drops delivered in a given time. This is not advised, as there is much greater difficulty in obtaining the saliva so free from suspended particles as not to clog the tube.

The inner tube should always be filled to the same mark in the determination as that used in the standardization of the instrument.

Specific gravity may be taken by an ordinary urinometer or a specific gravity bulb if the quantity is sufficient, the reading to be made from beneath the surface of the liquid. If the quantity of the saliva is small, it may be diluted with an equal volume of water, and the last two figures multiplied by two will give the gravity of the undiluted sample, or the gravity may be taken by the pyknometer, in which the bulb of the instrument is filled with saliva accurately to the mark *M* (Fig. 6). The reading on this instrument, of course, will be from the bottom up, and the lower the bulb sinks the greater will be the gravity of the sample. This method, devised by S. A. De Santos Saxe, M. D., for use in examination of urine, has been suggested by Dr. Ferris and adopted by the National Dental Association as an official method.

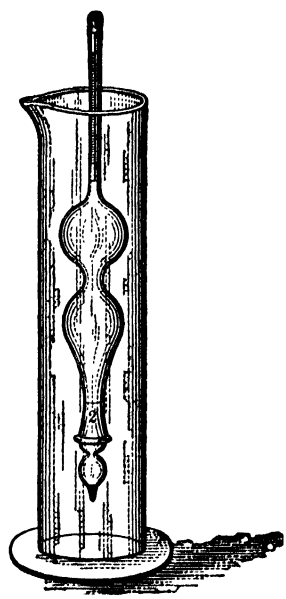


FIG. 6. Pyknometer.

For very accurate work the use of specific gravity bottles is recommended. These may be obtained holding 1, 2, and 5 cubic centimeters (Fig. 5), and with an accurate balance the gravity can be accurately obtained.

Chemical Properties

The first *chemical property* to be noted is the reaction. Saliva is normally alkaline to litmus paper or to lacmoid. Normal saliva, however, fails to give an alkaline reaction with phenolphthalein owing to the presence of free carbon dioxide which may be present to the extent of nineteen parts in a hundred by volume.

Acidity of Saliva.

Perhaps the one determination which is of the most clinical importance to the dentist is the determination of the acidity of the saliva. The value of this determination lies in its simplicity and in its significance in regard to the general systemic condition of the patient.

In discussing acidities in Vol. I, Chapter I, it will be remembered that they were divided into two kinds — actual acidity and titratable acidity. A complete analysis of saliva includes the determination of both.

Hydrogen-ion Concentration.

The expression of the actual acidity, hydrogen-ion concentration, or P_H, is very clearly explained by Leon S. Medalia in his article on " Color Standards for the Colorimetric Measurement of H-Ion Concentration, P_H 1.2 to P_H 9.8."* He says, " The accumulation of free hydrogen-ions present in a given solution, i.e., the H. I. C. of that solution, can be measured to the minutest amount and has been expressed in terms of " normal solutions." The amounts are so minute that they run up to the billionth or trillionth normal, since the acid strength or the hydro-

* " Color Standards " for the Colorimetric measurement of Hydrogen-ion Concentration P_H 1.2 to P_H 9.8, by Leon S. Medalia: Jour. of Bacteriology, Vol. V, No. 5, Sept. 1920.

gen-ion content of neutral or even alkaline solutions is measurable. In order to overcome the unwieldiness of the figures necessary to express the H. I. C., Sörensen suggested the symbol P_H to express one-tenth normal beginning on the acid side and going up in negative multiples of one-tenth towards alkalinity. Thus P_H 1 equals N/10 acid; P_H 2 equals N/10 × N/10 = N/100; P_H 3 equals N/100 × N/10 = 1/1000 normal, etc. The lower the P_H of a given solution, therefore, the more acid, or the higher its H. I. C., and the higher the P_H the less acid, or the lower is its H. I. C."

The hydrogen-ion concentration may be found either electrolytically or colorimetrically. The latter method, though not quite as accurate as the former, is sufficiently accurate for all general purposes and is by far the simpler and more convenient.

Principle of Method.

In 1917, Clark and Lubs developed a series of indicators giving a range of P_H from 1.2–9.6, each indicator being extremely sensitive to the hydrogen-ion concentration of solutions coming within its field. The range of P_H and the color changes of these indicators are given by Clark and Lubs as:

1. Thymol blue, acid range	Red —Yellow	P_H	1.2–2.8
2. Brom-phenol blue	Yellow—Blue	P_H	3.0–4.6
3. Methyl red	Red —Yellow	P_H	4.4–6.0
4. Brom-cresol purple	Yellow—Purple	P_H	5.2–6.8
5. Brom-thymol blue	Yellow—Blue	P_H	6.2–7.8
6. Phenol red	Yellow—Red	P_H	6.8–8.4
7. Cresol red	Yellow—Red	P_H	7.2–8.8
8. Thymol blue, alkaline range	Yellow—Blue	P_H	8.0–9.6

Medalia noted the fact that each indicator covered a range of a P_H of 1.6, so that by dividing this color range into eight equal parts he obtained a series of color changes at intervals of .2 P_H. This was accomplished by taking a series of sixteen tubes, eight of which contained 10 c.c. acid and eight 10 c.c. alkali. Then, starting with .8 c.c. of the indicator in the acid tubes, he decreased the amount of indicator in each tube by .1 c.c., while in the tubes containing the alkali he started with .1 c.c. of indicator and

increased to .8 c.c. Then by placing the acid tubes behind the alkaline, arranging them as shown (Fig. 7), so that each pair contained .8 c.c. of indicator, he succeeded in forming a series of tubes that gave the range of P_H for any specific indicator at intervals of .2 P_H. By matching the color obtained with an unknown with one of these pairs, one may determine the P_H of the unknown.

Procedure for P_H Determination of Saliva.

The most convenient apparatus is the " Comparator Black," illustrated in the accompanying diagram. Shell tubes of equal

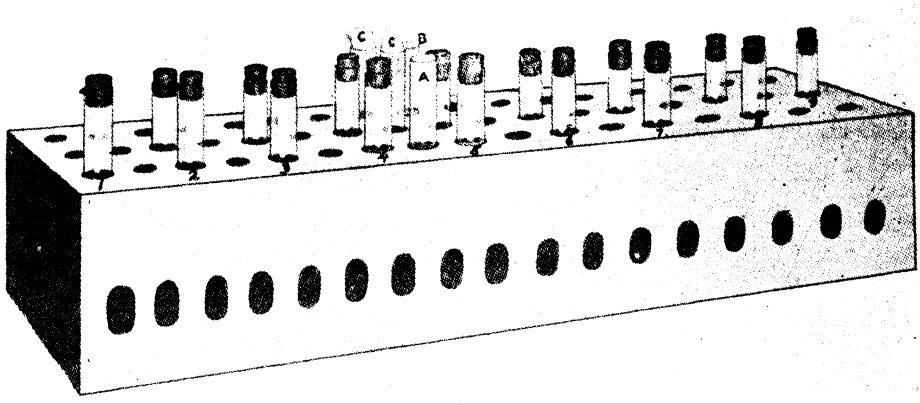


FIG. 7.

A contains the 1 c.c. sample, 4 c.c. distilled water and .4 c.c. indicator.
B contains the 1 c.c. sample and 4 c.c. distilled water.
C contains 5 c.c. distilled water.

Each pair of tubes represents a definite hydrogen-ion concentration and the observer matches the color of the unknown with that of the known by looking through the holes in the front of the box. B and C are used so that the depth of liquid is the same in each case.

diameter are used, and it has been found simpler in saliva work to use just half of the quantities given above; that is, to let each acid tube contain 5 c.c. HCl (.1 per cent, made from .1 c.c. concentrated HCl and 100 c.c. distilled water) and each alkali tube 5 c.c. N/20 NaOH. The quantity of indicator is also halved; that is, .4 c.c. is used and the amount is decreased or increased by .05 c.c. each time. Each pair of tubes, as shown in the diagram, will then contain .4 c.c. indicator divided between them differently, and the color change in the series will follow the table

given on page 162, depending on the indicator used. Brom-cresol purple and brom-thymol blue will cover the range of P_H of practically all salivas, and these standard series will keep three months if carefully made.

After the standards are prepared, the actual determination of the P_H of an unknown solution is extremely simple. In each of two shell tubes similar to those used in the standard sets, 4 c.c. of distilled water and 1 c.c. of the centrifugalized saliva are placed. Then to one of these tubes .4 c.c. of the indicator is added, usually brom-thymol blue for saliva. The tubes are gently rotated and the one containing the indicator placed beside the tube in the standard set which matches its color best. In order to have the same depth and density of liquid to look through, two tubes containing 5 c.c. of distilled water are placed behind the unknown and, to insure an equal coloration in each if the unknown is slightly colored, the tube containing 4 c.c. distilled water and 1 c.c. of unknown is placed behind the acid and alkali pair with which the P_H of the unknown is being compared.

The hydrogen-ion concentration of resting saliva gives a P_H of 6.5–7.0, while activated saliva is normally 7.0–7.3.

Titratable Acidity.

The total titratable acidity of saliva is determined in a similar manner to that described under urine, page 194.

Five c.c. of the centrifugalized sample is titrated with N/40 NaOH, using phenolphthalein as an indicator. Calculating in degrees as explained on page 126 (1° being equivalent to each tenth of N/10 NaOH used when 10 c.c. of unknown is used) the normal acidity for *activated saliva* is 2°–4°. The resting saliva will usually give a slightly higher degree of acidity and a slightly lower P_H.

The increased alkalinity of the activated saliva is due to the increased alkaline phosphate present. The acid phosphate of the blood is constantly reacting with the acid carbonate

$$NaH_2PO_4 + NaHCO_3 = Na_2HPO_4 + H_2O + CO_2$$

yielding the alkaline phosphate and carbonic acid. It is reasonable to suppose that an increased activity of the glands increases the quantity of this salt which is dialyzed into the saliva.

The acidity of saliva, referred to its behavior to phenolphthalein, is in large part due to the presence of free carbon dioxide.

The sources of carbon dioxide in saliva are probably three: carbon dioxide dialyzed through the salivary glands, traces from carbohydrate fermentation, and more or less absorbed from contact with expired air.

The saliva obtained by chewing paraffin (a process calculated to furnish the maximum amount from the last two sources), may yield several times the amount of free carbon dioxide that another sample taken from the same patient by a saliva ejector will give.

Acidity of saliva may be temporary, in which case it may be entirely removed by drawing air through the heated (not boiled) sample. The permanent acidity may be determined by titration of the sample after removal of carbon dioxide.

Determination of Carbon Dioxide.

Method I. — The apparatus pictured in Fig. 8 has been used by the author for this acidity determination.

The air is drawn from left to right, first through a potash bulb (*A*) to absorb atmospheric carbon dioxide, next through 10 c.c. of saliva diluted with 20 c.c. of water contained in a small Soxhlet flask (*B*) whereby the carbon dioxide from the saliva is carried through the " test-tube " condenser and collected in baryta-water in the Erlenmeyer flask (*C*) at the left. This in turn is connected with a suction pump or aspirator. The " drip cup " (*D*) has been found necessary when working with very viscid samples. The thistle tube (*E*) holds water for maintaining the volume in (*B*) if the condenser is not used.

The amount of free carbon dioxide may be determined by adding a standard carbonate solution (N/100 Na_2CO_3) to a volume of baryta-water equal to that used in the Erlenmeyer flask and then comparing the degree of turbidity obtained. This may be done by viewing through flat-bottom tubes (shell

tubes) of about 20 c.c. capacity, or, in many cases, better, by use of the Duboscq colorimeter used in other determinations (Fig. 9, page 176), or better still by the use of the nephelometer made with the Duboscq colorimeter after the method of Dr.

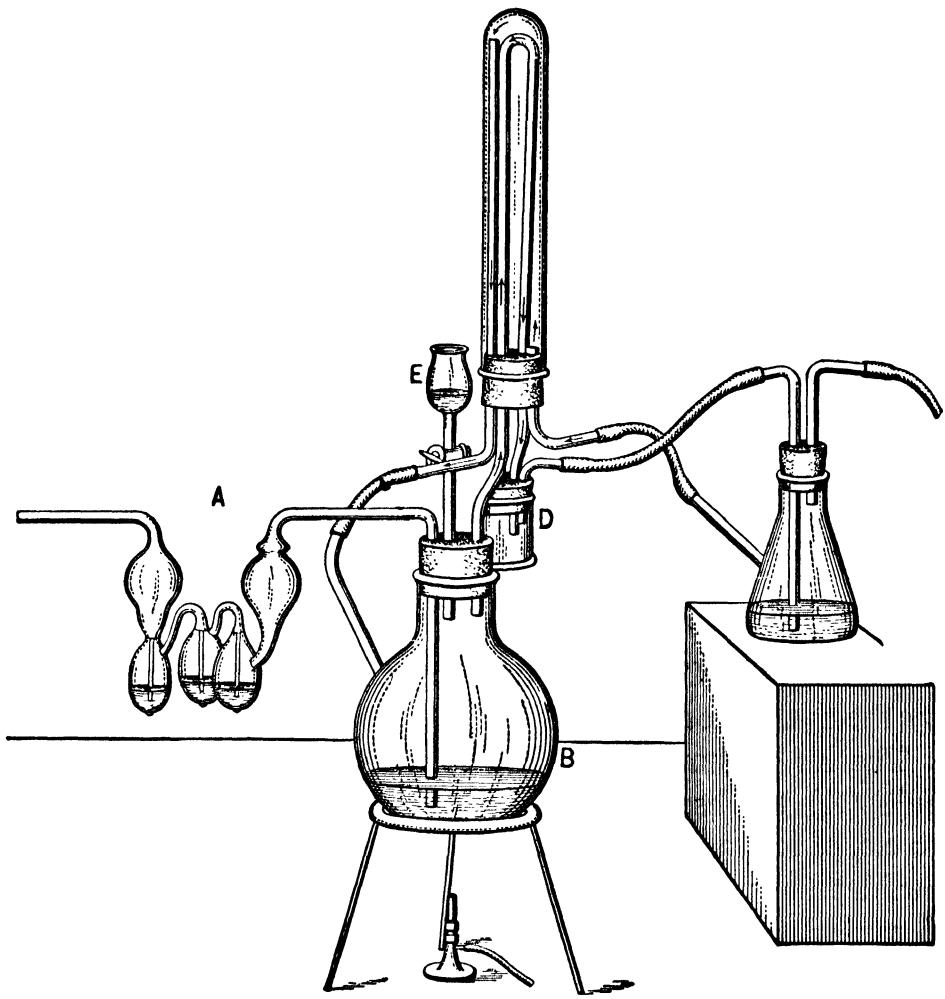


FIG. 8.

Bloor. (*Journal of Biological Chemistry*, Vol. 22, p. 145, 1915.) This apparatus may also be used to advantage in the determination of calcium in saliva, or acetone bodies in urine. The nephelometer differs from the Duboscq colorimeter in that it makes use of reflected rather than transmitted light.

Method II. — Another method consists in passing carbon dioxide, as above, into a measured volume of standardized

baryta-water (N/20) and titrating excess of barium hydroxide with N/20 oxalic acid. The end-point is determined by " spotting " on to fresh tumeric paper. When the paper ceases to turn brown-red the end of the reaction has been reached.

Permanent Acidity.

If the acidity of saliva is due to the presence of carbon dioxide, the carbon dioxide may be driven off by boiling the sample for ten minutes, and the resulting saliva will then be alkaline to phenolphthalein. In the activated sample this so-called *temporary acidity* is normal. In nearly all cases of resting saliva (saliva collected upon rising in the morning) and in many other cases which the writer considers pathological, a permanent acidity has been observed. Certain types of erosion and pyorrhea will as a rule yield salivas with a permanent acidity.

The cause of this acidity is at present rather hypothetical, probably differing more or less with various samples. In several instances it has been shown to be at least partly due to lactic acid, and a qualitative test for lactic acid is therefore of considerable importance in the analysis of all samples showing a permanent acidity. The determination is most easily carried out by evaporating a few cubic centimeters of the sample with dilute hydrochloric acid, extracting with ether, evaporating the ether solution to dryness, dissolving the residue in distilled water, and making a careful test for lactic acid according to Exp. 60. A control test should always be made with water and the same amount of ferric chloride.

Some salivas upon boiling will show a constantly increasing degree of acidity, which has been explained by Gies as probably due to decomposition of unknown compounds of the soluble calcium acid phosphate, $CaH_4(PO_4)_2$, with organic matter from the saliva.

Alkalinity.

Saliva normally is alkaline to litmus owing to the presence of various alkaline salts which are not neutralized by the acid factors present. The value of determining the alkalinity of saliva lies

perhaps in the relationship existing between it and the titratable acidity.

In the *Pacific Dental Gazette* for 1915, pages 335–345, Professor John A. Marshall of California published his technique for the determination of a "*Salivary Factor*," representing the difference in neutralizing power of resting and activated saliva from the same patient as an indication of immunity from, or tendency toward, dental caries. The factor was determined by obtaining the alkalinity (*A*) as described just below, and the acidity (*B*) as described on page 164, using N/100 acid and alkali in each case; then by adding (*B*) to (*A*) the so-called neutralizing power of the saliva was determined. This process was repeated with both the resting and the activated saliva, and by means of the following ratio the factor was obtained.

$$\frac{\textit{neutralizing power resting saliva} \times 100}{\textit{neutralizing power activated saliva}} = \textit{Salivary Factor}$$

Professor Marshall claimed that a low factor, below 80, meant immunity, while 80 or above indicated a carious condition.

It has been shown from experiments with dialyzed saliva that the inorganic constituents of the saliva are chiefly responsible for its neutralizing power. A high neutralizing power does not of necessity mean a high salivary factor, but it is usually associated with high inorganic constituents and a low percentage of proteins.

Too much emphasis has apparently been placed on the relation of the Salivary Factor to the development of caries. The neutralizing power of the saliva, determined in some such manner as that adopted by Professor Marshall, is not at all unlikely to become a useful method in a comprehensive study of the saliva, but perhaps with a rather different significance from that thus far suggested.

The alkalinity of a given sample may be easily determined by titration. To 5 c.c. of centrifugalized saliva, 10 c.c. of N/40 HCl and a few drops of paranitrophenol are added. The excess acid is then titrated with N/40 NaOH, and the difference indicates the number of cubic centimeters of the standard acid

necessary to neutralize the alkalinity of the saliva. Calculated in degrees, the alkalinity of normal saliva is between 15° and 20°.

Constituents. — We should here distinguish carefully between saliva proper and sputum. The constituents of sputum are derived from the air-passages rather than from the salivary glands, and are not at present under consideration. Among the *normal* constituents of saliva are included mucin, albumin, ptyalin, also oxidizing enzymes, ammonium salts, nitrites, potassium sulphocyanate, alkaline phosphates, and chlorides, with traces of carbonates, urea, creatinine and in fact practically all normal constituents of the blood; and, in the sediment, epithelium cells, occasional leucocytes, and fat globules. The *abnormal* constituents will include glycogen, dextrin, rarely sugar, cholesterin, derivatives from bile, lecithin, xanthin bodies or alkaline urates, acetone, lactic acid, and crystalline elements resulting from insufficient oxidation or perverted glandular function. These latter are recognizable by the micropolariscope. Mercury and lead may also be found in saliva in cases of poisoning by salts of these metals.

Considerable work has been done in comparing some of the salivary constituents with those of the blood of the same patient, and the author is convinced that there may be a significant similarity between the two analyses. Particularly has this been observed to be true in cases of nephritis, where almost invariably a rise of urea nitrogen or creatinine in the blood will mean a corresponding rise in the saliva.

Proteins.

Mucin. — The secretion from the parotid gland contains practically no mucin, but the sublingual saliva contains large amounts. Mucin is, according to Simon, the most important constituent of the saliva, not excepting ptyalin. The various glands contributing salivary mucin do not in all probability furnish just the same kind of protein; moreover, the mucin from different individuals seems to vary in composition and properties, some yielding more abundant acid decomposition

products than others (see article by W. D. Miller, in *Dental Cosmos* for November, 1905), while, according to Professor Michaels, the mucin varies greatly in the same individual in health and disease. The changes in the characteristics of salivary mucin have been studied but little, and the investigation of these changes, as indications of diathetic states, promises much.

An excess of mucin in the saliva tends to an increase of bacterial growth, from the fact that it furnishes increased facilities for multiplication; it has been suggested that it may also give rise to mucic acid, and thereby be a possible factor in tooth erosion. (Dr. G. W. Cook, in *Dental Review*, May, 1906, page 461.)

Mucin may be separated, after taking the gravity, by the addition of a little acetic acid. It should then be filtered off, but it will be necessary to dilute and agitate, in order that a fairly clear filtrate may be obtained.

A quantitative result may be obtained by weighing the precipitated mucin after drying it. This result will of necessity be somewhat inaccurate as the character of the mucin is altered by both the acid and any heat to which it may be subjected.

Albumin. — Albumin is present in very small quantities, increased during mercurial ptyalism, usually in cases of pyorrhea, and, according to some authorities, in various albuminurias. It may be detected by the usual methods after the separation of mucin.

"According to Vulpian, the quantity of albumin is increased in the saliva of albuminurics of Bright's disease. The saliva of a patient with parenchymatous nephritis had mucin 0.253 and albumin 0.182 per cent. The saliva of another patient, with albuminuria of cardiac origin, contained mucin 0.45, albumin 0.145 per cent. In a healthy man there was found mucin 0.320, albumin 0.05 per cent. This fact has been confirmed by Pouchet, who found these substances in greater quantities."*

Albumin may be demonstrated in the filtrate, from which mucin has been separated by underlaying with strong nitric

* Dr. Joseph P. Michaels. S. S. White's reprint of paper read before International Dental Congress, Paris, 1900.

acid. This is Heller's test for albumin in the urine, and is best performed in a small wine-glass with round bottom and plain sides.

Enzymes

Ptyalin. — Ptyalin is the principal ferment of the saliva; it converts starch, by hydrolysis through the various dextrins (page 149), to maltose. The maltose in turn is converted into glucose by a second ferment, known as maltase, which exists in saliva in very small quantities.

The activity of ptyalin is greatest at a temperature of 40° C. Very faintly acid saliva is the best medium. Neutral and faintly alkaline salivas are next in order.

The amylolytic power of a given sample of saliva may be determined by the action on dilute starch paste. In making comparative tests it is essential that the conditions under which the ptyalin is allowed to act should be exactly the same, especially as regards the temperature and duration of the process. A slight variation in the strength of the starch solution is of no consequence, as starch is supposed to be in excess. (See Exp. 213 on page 286, also method on page 172.)

Proteolytic Enzymes. — Upon incubation with certain products of protein digestion (dipeptides), proteolytic action of saliva has been noted; whether this action is due to an enzyme or to bacteria is an open question. (See eighth edition of Hawk's "Physiological Chemistry," pages 56 and 57.)

Oxydases. — As a result of the work of Dr. C. F. MacDonald in the author's laboratory, the following conclusions were reached regarding these enzymes:

First. That human mixed saliva contains an oxidizing enzyme distinct from ptyalin.

Second. That the enzyme exhibits the properties of both an oxydase and a peroxydase.

Third. That it is a product of the body (probably glandular) metabolism and may be increased in quantity or activity by mastication.

Fourth. That it is more resistant to heat than ptyalin, but more easily destroyed by acids.

Fifth. That the color obtained with a freshly prepared 1 per cent solution of pyrocatechol is sufficient test for this enzyme in saliva.

The test for oxidizing enzymes may be made with the pyrocatechol as given on page 173, also by the use of phenolphthalin (reduced phenolphthalein). This last reagent has recently been rendered available by the work of Dr. H. L. Amoss, Harvard Medical School, who has given us a concise and simple method for its preparation. (*Jour. Biolog. Chem.*, 1912.)

Determination of Enzymes.

Amylolytic Enzymes.*— Preparation of starch paste. Put 15 c.c. of distilled water to boil. Meanwhile, weigh out 3 grams of sterile starch and mix with 6 c.c. of cold distilled water. Add drop by drop under constant stirring to the boiling water, then rinse out with 5 c.c. of distilled water any particles of starch adhering to the dish and add to the boiling starch solution. Boil one minute under constant stirring. Cool to blood temperature and add gradually 4 c.c. of N/100 iodine solution.

This makes 30 c.c. of a 10 per cent starch solution, which, when colored, is of a dark blue, and can be kept several days in the ice-box.

Filling the Tubes. — Suck up the paste into glass tubes of 1.5 mm. diameter, and cool in the ice-box. Just before using, make a file mark 1 cm. from the end of the tube and break off the piece of tubing so that it is full of the blue starch paste. Be sure that this small tube is broken so as to leave each end square and full of paste. Examine under low-power microscope.

Determination of Enzyme. — Immediately after delivery of the specimen, measure 2 c.c. of saliva into a test-tube. Place it in the small tube of starch paste, and heat the whole in a thermostat at from 37° to 38° C. for half an hour. The enzyme of the saliva will dissolve the paste from the ends of the tube,

* Note — Method taken from Dr. Ferris' Saliva Analysis, *Dental Cosmos*, Nov., 1911, page 1295.

leaving a blue column of paste unchanged in the center of the glass tube. After half an hour, measure with a micrometer gauge the total length of the tube and the length of the blue starch paste column remaining undissolved. The difference between these two measurements represents the amount of starch digested by the enzyme. Since the quantity of ferment in any fluid varies with the square of the length of the column digested, the quantity of ferment in the saliva is found by squaring this difference. Multiply by 100 to give the enzymic index.

Oxidizing Enzyme. — (Oxydase.) Treating 5 c.c. of saliva, diluted with an equal volume of water, with about 1 c.c. of a 1 per cent solution of pyrocatechol. The color obtained is a characteristic brown, developing within thirty minutes.

SALTS OF THE SALIVA.

Phosphates and Carbonates. — These salts are probably present in both acid and neutral forms; that is, the phosphate may exist as Na_2HPO_4, also as NaH_2PO_4, and at times both of these may be present at once. The acid carbonate, $NaHCO_3$, is an undoubted constituent, while the neutral carbonate is probably not present at all. Chittenden says that mixed human saliva contains normally no sodium carbonate whatever.

As explained by Dr. Kirk, the normal reaction by which overacidity of the blood is taken care of by renal epithelium is $H_2CO_3+Na_2HPO_4=NaH_2PO_4+NaHCO_3$, and when conditions are such as to produce larger quantities of carbonic acid than the kidneys can eliminate in accordance with the above reaction, there is an increased acidity of the saliva as well as of the urine.* In the hypoacid individual, the so-called alkaline sodium phosphate, Na_2HPO_4, is present in the greater quantity.

Determination of Phosphates. — Five c.c. of saliva are heated with a few drops of 10 per cent sodium acetate, and the hot solution titrated with standard uranium solution (for preparation see page 197). The end-point is obtained by " spotting " with $K_4Fe(CN)_6$ until a permanent brown color is obtained. Each

* International Dental Journal, February, 1904.

cubic centimeter of uranium solution used is equivalent to .005 gram of phosphate.

A less accurate end-point may be obtained by using cochineal as an indicator. The appearance of a permanent green color denotes the end-point.

Potassium Thiocyanate represents the salts of HCNS found in saliva. It occurs only in very slight traces in other body fluids, and in saliva only to the extent of 0.001 to 1.02 per cent. Dr. Michaels considered the proportion of thiocyanates relative to the ammonia to be of importance, and states that in health the ammonium salts and the thio-cyanates are present in very slight amounts, and the color tests, with Nessler's solution* and with ferric chloride, respectively, are of about equal intensity. In the hyperacid state the sulphocyanates are in excess of ammonia, while in hypoacid conditions, the ammonia exists in the greater quantity. Sulphocyanate is detected by means of ferric chloride, and distinguished from meconates and acetates, as indicated by Exp. 215, page 287.

As we shall see in a subsequent chapter the intensity of color produced by ferric chloride and thiocyanate is not necessarily an index of the quantity of HCNS present; hence the above conclusions are of questionable value.

The sulphocyanates are normal constituents of saliva, and consequently always present. According to A. Mayer (*Deutsch. arch. f. klin. med.*, Vol. 79, No. 394), the sulphocyanates, without doubt, result from the decomposition of proteins, and exist in the urine in quantities variously estimated from 20 to 80 milligrams per liter, while in saliva they have been estimated at 60 to 100 milligrams per liter. Professor Ludholz of the University of Pennsylvania says that the sulphocyanates are eliminated in increased amounts in conditions where there is a lack of oxygen in the system, thus corroborating statements of Professor Michaels (see Ammonia). Dr. Fenwick (*Lancet*, 1877, Vol. II, page 303) demonstrated that the quantity of KCNS was directly dependent upon the bile salts in the blood. He found an increase

* See page 300.

of the salt in liver disorders attended with increase of bile salts in the blood, and marked increase in jaundice. In gout, rheumatism, and conditions producing pyorrhea, it is also claimed to be present in considerable quantity.

The sulphocyanates are usually present in more than normal quantity in the saliva of people addicted to smoking tobacco.* The claim has been made for this salt that it exerts a specific antiseptic action toward bacteria.

While the sulphocyanates, or, in fact, any salt in sufficient concentration, will have an inhibitory action on the growth of bacteria, it is rather doubtful if this is the particular office of KCNS in the saliva.

Thiocyanate (Sulphocyanate) *Tests.* — To a large drop of saliva on a white porcelain surface, add about half as much 5 per cent ferric chloride, acidified with hydrochloric acid. A reddish coloration indicates the presence of thiocyanate.

Quantitative Method (Ferris).†

Into a 10 c.c. cylinder measure 2 c.c. of saliva, 5 c.c. N/10 HCl, and 5 c.c. of 5 per cent ferric chloride. Dilute to the mark and mix. Similarly, make up a standard solution, using in place of the saliva 1 c.c. of standard ammonium thiocyanate solution (.5 gm. C.P. NH_4CNS dissolved in one liter of water). One c.c. of this solution contains .5 mg. NH_4CNS.

Compare colorimetrically by placing a few cubic centimeters of the standard solution in one cup of a colorimeter (Fig. 9), and a few cubic centimeters of saliva in the other. Set the standard at 20 and then adjust the other cup so that the disk is of uniform intensity of color when observed through the lens.

Calculation:

$$\frac{20}{\text{reading}} \times .5 \times 50 = \text{mg. of } NH_4CNS \text{ per 100 c.c. saliva.}$$

* See article by Dr. J. Morgan Howe in Jour. of the Allied Societies, Vol. 4, p. 183.

† Jan. Journal of the American Dental Association, 1923. "Composition of Human Saliva," Ferris.

Nitrites. — That nitrites exist in most salivas is without question. So far as we know at present, the nitrites are apparently incidental, and occur as intermediate products in the oxidation

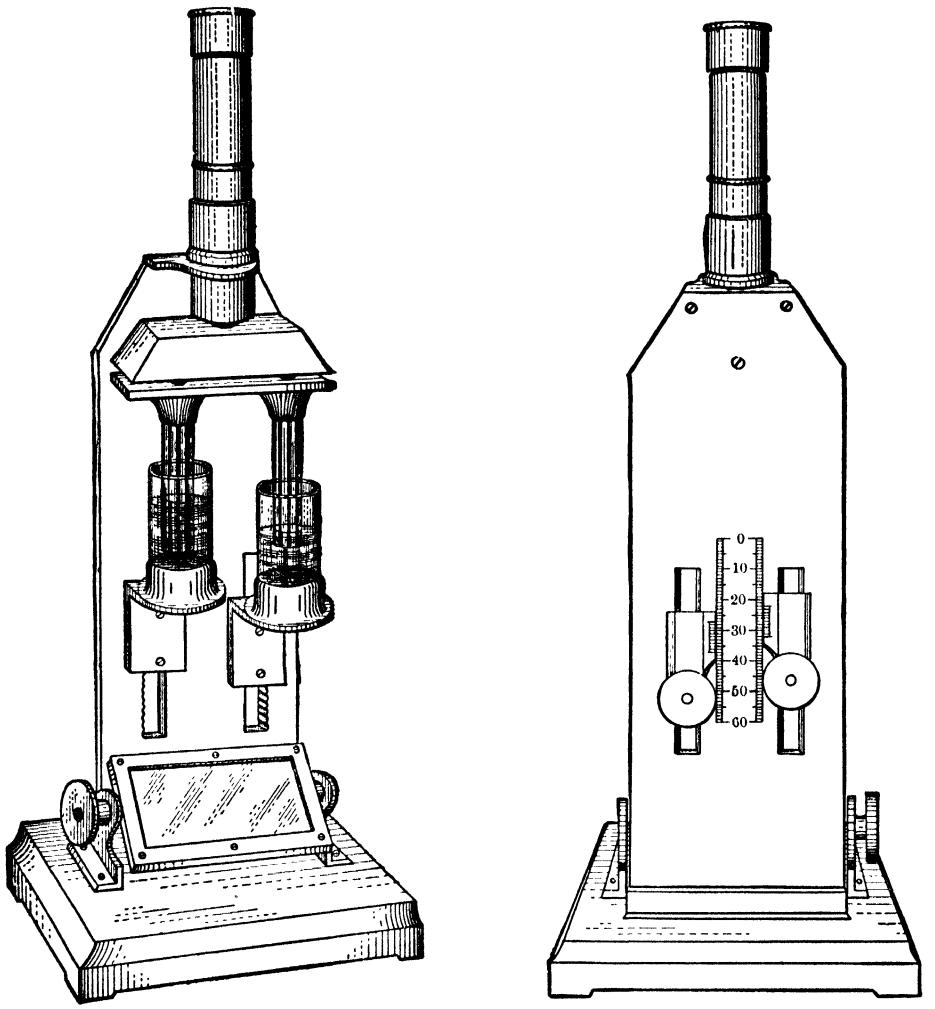


FIG. 9. Colorimeter.

of ammonia to nitrates, just as they do otherwise in nature outside of the animal body.

It is not at all improbable that the proportion of nitrates is dependent upon activities of the oxidases. This has, in some cases at least, been proven to be the case, as the same sample of saliva has frequently given steadily diminishing quantities of nitrites until they have wholly disappeared in cases containing active oxidizing enzymes.

Considerable work* has been done recently in an attempt to prove the above statements, but the results have not been very satisfactory. It was conclusively shown, however, that in fresh saliva nitrites and oxydases were present and that if nitrates were present at all they were not in sufficient quantity to be detected. In the course of two or three weeks' time a slight positive test for nitrates was obtained, while the nitrites were diminished. That the oxidation was brought about by the oxydase was demonstrated by the fact that no change seemed to occur in the quantity of nitrites and there was no test for nitrates in samples in which the enzyme had been destroyed by boiling.

Nitrites may be detected by adding to a large drop of saliva on porcelain a few drops of freshly prepared reagent, made by dissolving a very little naphthylamine chloride and an equal amount of sulphanilic acid in distilled water strongly acidulated with acetic acid. A pink coloration is a test for nitrites.

This method could be made quantitative in a manner similar to the colorimetric methods for ammonia, or thiocyanate of potassium; but, at the time of the present writing, there seems to be no particular reason for undertaking this amount of work.

Chlorides.

Chlorides are always present in the saliva, usually as alkaline salts, but in very variable quantities. As a rule, the chlorine content of the saliva is less than that of blood, there being from 75 to 125 mg. per 100 c.c., as compared with about 500 mg. per 100 c.c. of blood. This difference may be accounted for by the fact that during the process of dialysis from the bloood the salt content of the lymph is increased, thereby diminishing the quantity in the saliva.

Chlorine may be easily and accurately determined by taking 5 c.c. of centrifugalized saliva, adding 10 c.c. of N/40 $AgNO_3$ and titrating back with N/40 KCNS, using ferric alum as an indicator. If for any reason the sample is not clear, it may be ashed

* Thesis work of G. H. Leatherman, Harvard Dental School, 1924.

with a few grams of calcium oxide. This treatment serves to fix the chlorine as calcium chloride, which may be dissolved in water. The chlorine content may then be determined in the manner described above.

Calcium may be determined by the following volumetric method, recommended by Dr. Percy R. Howe, *Dental Cosmos*, April, 1912. To 5 c.c. of saliva, add as much more distilled water and a slight excess of oxalic acid or ammonium oxalate (5 c.c. of normal solution will be sufficient). Add ammonium water to alkaline reaction, heat nearly to the boiling-point, and allow to stand for twenty to thirty minutes. Filter through a hardened filter paper into a small beaker which is allowed to stand on a piece of black glazed paper. Under these circumstances, a slight rotary motion of the beaker will show if any of the white precipitate of calcium oxalate is passing through the paper.

After filtration is complete, wash five times in hot distilled water; then place the precipitate, together with the paper, into a small beaker, add about 30 c.c. of dilute sulphuric acid, and heat nearly to the boiling-point; then titrate with N/20 permanganate solution.

Gravimetric Calcium Determination (McCrudden).

To 50 c.c. of saliva add 10 c.c. of N/2 HCl and 10 c.c. of $2\frac{1}{2}$ per cent oxalic acid. Boil for fifteen minutes, during which time most of the calcium is precipitated. Cool and add 10 c.c. of 3 per cent ammonium oxalate and 6 c.c. of 20 per cent sodium acetate. This aids in decreasing the acidity and in precipitating the calcium oxalate in large crystals.

Filter through ashless filter-paper, washing the precipitate with cold 1 per cent ammonium oxalate and then with small portions of distilled water. Dry the filter-paper containing the precipitate and ignite, using a platinum evaporating dish if possible. Weigh carefully and calculate result as calcium oxide.

Normally the calcium content of saliva is 10–20 mg. per 100 c.c.

This method is accurate and fully as easy of manipulation as

the volumetric method. However, it is often impossible to obtain sufficient saliva to make a gravimetric determination feasible.

Determination of Urea Nitrogen Plus Ammonia.

Measure 2 c.c. of centrifugalized saliva into a heavy-walled test-tube fitted with a two-hole rubber stopper, one hole of which is connected with an aerating tube while the other one holds a delivery tube going into an Erlenmeyer flask. Place in the flask 10 c.c. of N/100 HCl, colored pink with methyl red, and a few drops of petroleum to prevent bumping. Put with the saliva about 2 c.c. of freshly prepared 5 per cent urease solution (Arlco-Urease) and allow digestion *without heat* to take place for twenty minutes. Then add 10 c.c. of saturated potassium carbonate and a few c.c. of petroleum to the digestion mixture, and blow air through the apparatus for fifteen or twenty minutes. This serves to blow over the ammonia formed from the urea into the standard acid, which fixes it as ammonium chloride. The excess acid is then titrated with N/100 alkali and from the difference the amount of urea nitrogen and ammonia nitrogen is calculated.

$$\frac{.00014 \times (\text{difference}) \times 100}{2} = \text{grams of urea nitrogen + ammonia nitrogen per 100 c.c. saliva.}$$

Normally the total urea and ammonia nitrogen is 10–15 mg. per 100 c.c.

Ammonia.

Qualitatively, the presence of ammonia may be shown by treating one drop of saliva with one drop of Nessler's reagent. The intensity of the yellow color obtained gives an approximation of the amount of ammonia present. According to Dr. Michaels, a high quantity of ammonia tends toward a carious condition of the teeth with a general hypoacid diathesis. Little value is now attached to his statement, however.

Determination of Ammonia — *Quantitative.*

Follow the directions outlined for urea nitrogen plus ammonia, omitting the addition of urease. This eliminates the necessity

of digestion, and the ammonia may be blown over directly after the action of the potassium carbonate has taken place. The calculation is the same as that given for urea nitrogen plus ammonia nitrogen.

The ammonia content varies, but, calculated as nitrogen, is usually from 5–7 mg. per 100 c.c.

Determination of Creatinine.

Place 5 c.c. of centrifugalized saliva and 5 c.c. of distilled water in a small Erlenmeyer flask. In another similar flask place 5 c.c. of diluted standard creatinine solution* and 15 c.c. of water. Make a fresh alkaline picrate solution by mixing together saturated picric acid 5 parts and 10 per cent sodium hydroxide 1 part. Add to the flask containing the saliva 5 c.c. of the freshly prepared alkaline picrate solution, and to the flask containing the standard creatinine add 10 c.c. Allow to stand four minutes and compare colorimetrically, setting the standard at 20 (Fig. 9).

Calculation:

$$\frac{20}{\text{reading}} \times .0075 \times 20 \times 2 = \text{mg. of creatinine per 100 c.c.}$$

Creatinine is normally present in very small amounts in saliva (.3 to .7 mg. per 100 c.c.). It has been found to be increased in cases of nephritis, where the blood creatinine has increased, and it has also been demonstrated that *smoking* directly before or during collection of the sample will cause a tremendous apparent increase.† That this is due to some action of the tobacco smoke on the picrate solution has been recently shown by drawing smoke through distilled water and obtaining a seemingly high creatinine content. Experiments have also been conducted with pure nicotine and have given nearly negative results, in-

* This standard consists of the diluted standard referred to in the appendix (page 296) 1 part and distilled water 3 parts. Five c.c. then contain .0075 mg. creatinine.

† Thesis Work — W. E. Crocker, H. J. Cox, J. M. Collins, Harvard Dental School, 1924.

dicating that it is some constituent of tobacco smoke, rather than of nicotine, that causes the error in creatinine determinations. This error is likely to be very large, unless care is taken to prevent it.

Determination of Uric Acid.

To 2 c.c. of saliva in a small beaker add 1 c.c. of diluted phospho-tungstic acid.* Filter carefully into a 25 c.c. graduate, washing the precipitate repeatedly. Add *from a burette* 2 c.c. of $7\frac{1}{2}$ per cent sodium cyanide solution and allow to stand two minutes. Simultaneously prepare a standard by using 5 c.c. of standard uric acid† solution, 1 c.c. diluted phospho-tungstic acid and 2 c.c. sodium cyanide. After both solutions have stood two minutes at room temperature transfer them to two test-tubes and place in boiling water for ninety seconds. Then replace them in the original cylinders and dilute them to volume. Mix and compare colorimetrically, placing the standard as usual at 20.

Calculation:

$$\frac{20}{\text{reading}} \times .02‡ \times 50 = \text{mg. of uric acid per 100 c.c.}$$

The normal content is from 1–1.5 mg. per 100 c.c. See also page 245.

Total Solids and Ash.

(Method I.) These should be determined immediately upon the arrival of the specimen, to avoid error through evaporation of moisture.

Use a platinum or fused silica dish of constant weight, which has been kept in a desiccator over sulphuric acid. Weigh the dish accurately and rapidly, then introduce $2\frac{1}{2}$ c.c. of the well-mixed specimen and heat in a drying oven, not over 100° C., for two hours. Then place in the desiccator over sulphuric acid for twelve hours or longer, and weigh accurately and rapidly.

* Prepared by using 2 parts phospho-tungstic acid, Appendix, page 301, and 1 part distilled water.

† Dilute standard for use referred to in Appendix, page 302.

‡ Weight of uric acid contained in 5 c.c. of standard used.

The difference between these weights represents the weight of total solids. To calculate the percentage, divide by two and one-half times the specific gravity.

Add to the dish two or three drops of fuming nitric acid, and heat over a flame, keeping the dish two inches above the top of the flame, until the black color has become white. Heat in the direct flame until glowing, place at once in desiccator to cool for one or more hours, and weigh. Calculate the percentage of ash in same manner as that of total solids.

(Method II.) Total solids and ash are best obtained as follows: evaporate over a water-bath 5 grams of the sample thoroughly mixed with a weighed amount (half a gram) of ignited magnesium oxide. The weight of residue (less the magnesia) obtained by drying at 100° C., gives the total solids. These may be ignited until white ash is obtained, and again weighed. The second weight (less magnesia) gives the ash.

The use of the magnesium oxide serves to retain carbonates and chlorides in the total solids and the chlorides in the ash. It also obviates the necessity of oxidation with nitric acid, which would decompose many of the inorganic constituents of the ash.

To determine weight of sediment, obtain total solids as above; then if a portion of the saliva is carefully filtered and the solids determined in the clear filtrate by the same method, the difference between the two determinations of solids will be the weight of sediment, epithelium, leucocytes, etc.

Abnormal Constituents.

Acetone is of quite frequent occurrence in the saliva. In diabetic patients this substance is often present in comparatively large amounts, sometimes sufficient for the detection of the acetone by its characteristic odor. Acetone may appear in the saliva when it is not present in the urine. In such cases it has usually resulted from disordered digestion and a consequent faulty metabolism. (For further consideration of acetone, see Urine.)

Acetone is most satisfactorily detected by the *Gunning's Iodoform Test,* which may be performed as follows:

To about 2 c.c. of saliva add a few drops of Gram's reagent and a few drops of dilute ammonia. This forms a black precipitate of nitrogen iodide, which reacts *slowly* with the acetone to form iodoform. The iodoform will be observed as a yellowish sediment which upon microscopic examination will show the characteristic hexagonal iodoform crystals (Plate III, Fig. 6, page 77).

Sugar. In diabetic patients, sugar has very rarely been found in the saliva. One case coming under the observation of the author was that of a woman of middle age, with diabetes of long standing, with 8 per cent of sugar in the urine; from this case there were obtained a very few osazone crystals by subjecting a considerable quantity of saliva, after concentration, to the phenylhydrazine test.

Cholesterin and *lecithin* have been found by Professor Michaels in pathological saliva, and leucin has been found by him in a case of lupus. According to Novey, leucin has also been found in a case of hysteria.

Of the *crystalline salts* which may be separated by evaporation of dialyzed saliva, the sodium oxalate and the lactates and acid lactates of lime and magnesia are of the most importance and have been the most thoroughly studied. As these salts may likewise be separated from urine, their significance will be studied under that head.

Lactic Acid has been considered: *butyric* and *acetic acids* may each be tested for, qualitatively, by the methods given under gastric digestion.

Mercury. — A very delicate test may be made for this metal as follows: Collect as large a sample of saliva as possible, dilute with an equal volume of water, acidify with a few drops of hydrochloric acid, throw in a few very small pieces of copper-turnings which have been recently cleaned in dilute nitric acid, and boil for at least one-half hour, keeping up the volume by occasional additions of water. Remove the copper-filings, dry thoroughly on filter-paper, and place in a large-sized watch-glass (3 inches). In another watch-glass of similar size place one drop of solution of gold chloride, and quickly invert so that

the drop remains hanging on the under side of the glass. Now place this watch-glass directly over the one containing the copper, so that the chloride of gold is suspended directly above the turnings and perhaps half an inch from them; then gently heat the lower watch-glass with a very small flame, when the slightest trace of mercury, which may have been deposited upon the copper, will be volatilized, reducing the chloride of gold, and causing a purplish ring to appear around the edge of the drop. If no reduction of the gold occurs, mercury is absent.

Lead, which occasionally occurs in saliva, may be detected by the methods given under urine.

Microscopical examination of the sediment should be made in every instance. Normal saliva will contain epithelium from various parts of the oral cavity, an occasional leucocyte, and occasional mold fungi, leptothrix, etc. Constituents which perhaps are not properly classed as normal, and at the same time are not pathological, are fat globules, a rare blood-corpuscle, sarcinæ, extraneous material, as food particles, starch granules, muscle fibers, etc. An excessive amount of blood, fat, pus, or micro-organisms would, of course, indicate pathogenic conditions. The bacteriological investigation of samples of saliva is always of interest, and may be necessary, but the detailed methods of such investigation do not lie within the scope of this work.

Crystals from the Dialyzed Saliva.

To obtain characteristic crystals, as has been explained in considering the subject of micro-chemistry, uniformity as to conditions under which the crystallization takes place is a necessity. In the case of saliva, however, we are not producing new compounds, but simply searching for compounds, already formed and existing in unknown proportions in the samples tested. It is therefore necessary to make several preparations of each sample, in order that we may obtain the widest range of possibility for characteristic crystallizations. The following method of procedure will usually give satisfactory results: For a dialyzer use a fairly wide glass tube, over one end of which

PLATE VII. — ANALYSIS OF SALIVA.

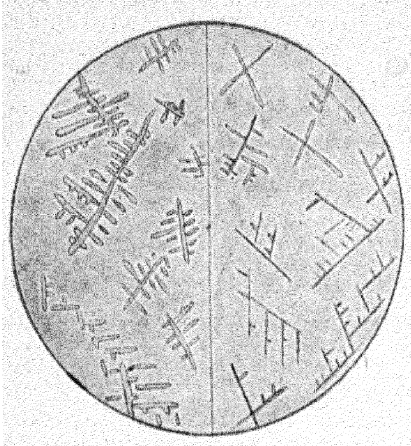


FIG. 1.
Ammonium Chloride.

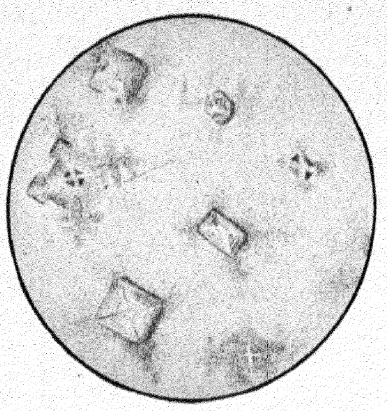


FIG. 2.
Sodium Chloride, $\frac{1}{8}\%$.

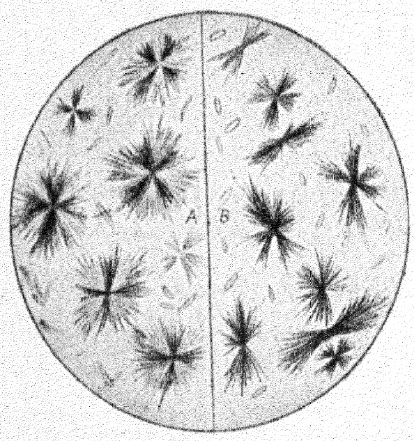


FIG. 3.
A, Magnesium Lactate (P. L.).
B, Calcium Lactate (P. L.).

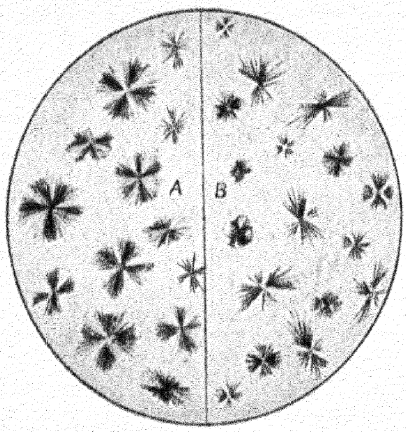


FIG. 4.
A, Magnesium Acid Lactate.
B, Calcium Acid Lactate.

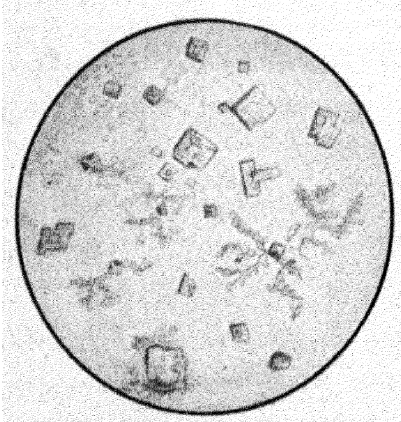


FIG. 5.
Potassium Chloride, $\frac{1}{8}\%$ Solution.

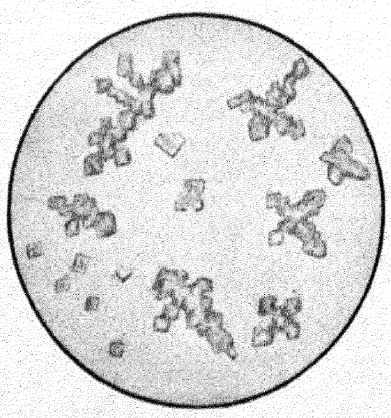


FIG. 6.
Potassium Chloride, $\frac{1}{4}\%$ Solution.

has been tightly tied a piece of parchment (Fig. 10), or, better, a small dialyzing tube made entirely of parchment. Place about 15 c.c. of saliva in the dialyzing tube, and suspend it in a small beaker or wine-glass which contains an equal volume of distilled water. At the end of twenty-four hours the distilled water will contain the dialyzable salts in nearly the same concentration as existed in the original saliva. Take four previously prepared cell-slides (microscope slides on which a ring of Bell's or other microscopical cement has been placed) and fill each cell full of the dialyzed saliva. Put number *one* in a warm place that it may evaporate rapidly, leave number *two* exposed to the air at the room temperature and it will dry in from half to three-quarters of an hour. Place number *three* under a large beaker, or small bell-jar, and cover number *four* with a cover-glass, and from time to time examine the crystals that may be formed. Numbers *three* and *four* will probably take several hours, perhaps several days, before crystallization is complete. When the crystals have appeared, the preparation may be preserved by mounting in xylol balsam. In attempting to obtain crystals from the saliva before dialyzation, results are usually unsatisfactory, owing to the presence of mucin and other organic substances which interfere with the crystallization. The crystals obtained by this method are principally chlorides of the alkali metals, particularly ammonium, and frequently sodium oxalate, lactates, and acid lactates of lime and magnesia, and rarely urates of the alkalies. (For forms of these crystals see Plate VII, Figs. 3 and 4, page 185 and Plate II, Fig. 4, page 77.)

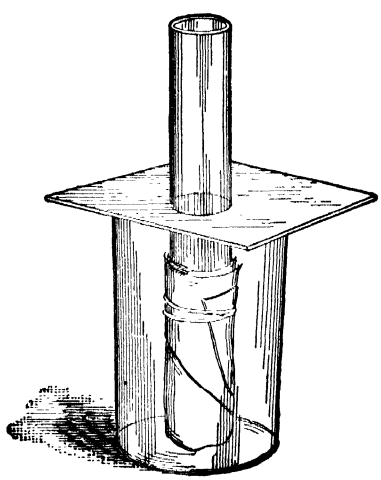


FIG. 10.

The following is the type of analysis blank used for recording results.

NO. **Date** **NAME**

Saliva Analysis by

Appearance	Odor		Sp. Gr.	CO_2
Acidity — Total	Resting / Activated	P_H	Resting / Activated	Alkalinity
Acidity permanent			temporary	
Mucin	Albumin		Chlorine	
Ammonia	H C N S		Urea N.	
Creatinine	Nitrites		Oxydase	
Acetone	P_2O_5		Viscosity	
Lactic Acid	Uric Acid			
Soluble Salts				
Sediment				
Remarks				

CHAPTER XIX.

URINE.

Urine is a solution of waste products from the blood. It contains, normally, certain coloring matter, urea, uric acid in combination with alkaline bases, various organic constituents in slight amounts, including, perhaps, albumin and sugar, chloride of sodium, sulphates and phosphates of the alkalies and the alkaline earths. Abnormally, the urine may contain albumin, sugar, uric acid as such, bile, salts of the heavy metals, lead, mercury, and arsenic; also occasionally albumose, peptones, lactates, acid lactates, oxalates, carbonates, hippuric acid, also organic compounds, resulting from insufficient or imperfect oxidations, as amino acids, leucin, tyrosin, and acetone bodies.

We are to study the urine, not primarily with a view to the diagnosis of renal disease, which is more particularly the province of the physician, but to detect irregularities or deficiencies in the body metabolism: and, as far as possible, we are to study the methods whereby we may correct and regulate the malnutrition which lies at the foundation of many diseases of the oral cavity. It has been well stated that, if there are diseases of the oral cavity which may have their etiology in some systemic derangement not easily apparent, and if such diseases are to receive the attention of the dentist, he should obtain all possible light on every case, and at present a quantitative analysis of the urine is of greater value than any other laboratory aid except the analysis of the blood. In examining a sample of urine to obtain information as above indicated, it is essential that the sample be a portion of the *mixed* twenty-four-hour quantity, and that the total amount of the twenty-four-hour excretion be known. In collecting samples for such analysis a convenient method is to give the patient a one- or two-dram vial, nearly filled with water, and containing three or four drops of a commercial formaldehyde solution, with instructions to empty this into a bottle, or other receptacle, in which the twenty-four-hour sample is to be col-

lected. Formaldehyde if used in this amount has no effect on the subsequent analysis and is a sufficient preservative.

Physical Properties.

Quantity. — The quantity of urine passed in twenty-four hours is normally about 1200 to 1400 c.c. for an adult female and 100 to 200 c.c. more than this for the male. The amount is increased in Bright's disease, in diabetes, and various other pathological conditions, also in cold weather when less moisture is given off from the skin. Normally, the quantity passed during twelve day hours, as 8 A.M. to 8 P.M., will exceed the amount overnight from 8 P.M. to 8 A.M. In cases of chronic interstitial nephritis the twelve-hour night quantity exceeds the day, hence it is desirable in collecting a twenty-four-hour sample to divide the time as suggested, and measure the amounts separately, especially if there is any suspicion of chronic kidney disease. A diminished quantity of urine may indicate simply a diminished amount of water taken into the system. The urine is diminished pathologically in acute conditions, such as fevers, etc., but such samples rarely reach the dental practitioner.

Color. — The normal color of the urine is usually given as straw color or pale yellow. If lighter than this the color is regarded as pale; if darker than normal it is regarded as high. The urine may also be colored by various abnormal constituents; it may be bright red from the presence of blood, or chocolate colored with a so-called coffee-ground sediment from decomposed blood coloring matter. It may be brown to yellow, bright blue or green, as a result of the ingestion of various drugs. If bile is present in any quantity in the urine, it will have a dark or smoky appearance, and, upon shaking, the foam will have a distinctly yellowish or yellowish-green tint.

Appearance. — In addition to the colors mentioned above, urine may sometimes have a smoky appearance, due to the presence of hematoporphyrin or iron-free hematin, often found in cases of lead-poisoning. It may have a milky appearance, due to presence of finely divided fat globules, as in chylous urine, due to the presence of chyme. It may be cloudy from four

principal causes: first, amorphous urates; second, amorphous phosphates; third, pus; and fourth, bacteria. These may easily be distinguished. The application of a slight degree of heat (insufficient to cause coagulation of albumin) will redissolve the urates, and clear a urine which is cloudy from this cause. A deposit of phosphates is increased by the application of heat, but clears easily upon the addition of a few drops of acetic acid. A urine cloudy from the presence of pus is not cleared by either of these methods, but the cloud settles with comparative rapidity and pus corpuscles are easily recognized by microscopical examination of the sediment. If bacteria are present in sufficient quantity to cause cloudiness, the sample is apt to be alkaline in reaction and will not clear upon filtering. If it is necessary to obtain a clear solution, a little magnesium mixture may be added to the urine, then a little sodium phosphate; warm gently with agitation, when the precipitated ammonium magnesium phosphate will mechanically carry down the bacteria, and a filtrate may be obtained which, after acidifying with dilute acetic acid, will be suitable for an albumin test.

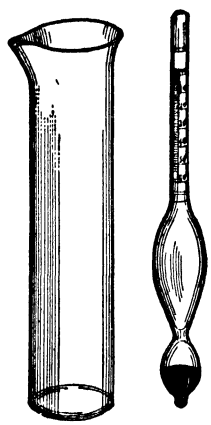


FIG. 11.

Specific Gravity. — The gravity is most conveniently taken with a urinometer (Fig. 11). Care should be taken in the selection of this instrument so that the scale graduation may be accurate. The fact that the instrument will sink in distilled water at the proper temperature (usually 60° F., 15½° C.) to the zero mark, is not a sufficient proof of its accuracy, as many cheap instruments will do this, and give erroneous readings at the higher markings of the scale. Distilled water is represented by 1000, and the relative increase in the comparative gravity of urines will be easily represented on the scale ranging from 1000 to 1050. As the first two figures of the specific gravity are always the same (10) they are usually omitted from the scale which is made to read from 0 to 50 or 60. The reading should be made, if possible, from underneath the surface of the liquid, as the liquid is usually drawn around the stem by adhesion, so that

accurate readings from the surface are difficult. The specific gravity of normal urine is from 1018 to 1022; it decreases in cases where the quantity is much above the normal (polyurias), unless sugar is present. It is increased by the presence of sugar or by concentration, whereby the normal solids are relatively increased. In case the quantity of urine is too small for the determination of the gravity in the usual way, the urinopyknometer, devised and recommended by Dr. Saxe in his " Examination of the Urine," may be employed. (See page 160, Fig. 6.)

Reaction. — The reaction of urine is normally acid to litmus paper, because of the presence of acid sodium phosphate, and because of organic acid combinations, the composition of which is unknown. The degree of acidity is roughly indicated by the intensity of color produced with the carefully prepared litmus paper. More accurate results may be obtained by a regular volumetric examination (with N/20 alkali), or by the test for urinary acidities given by Freund and Topfer, who suggest the following method:

" To 10 c.c. of the urine add two to four drops of a 1 per cent solution of alizarin. If the resulting color is pure yellow, free acids are present; if deep violet, combined acid salts. If none of these colors appear, there are present acid salts of the type of disodic phosphate. The amount of one-tenth normal hydrochloric acid standard solution required to produce a pure yellow color represents the alkaline salts, while the amount of one-tenth normal sodium hydrate required to cause a deep violet represents the acid salts."

The average P_H of normal urine is about 6.0, and this determination should be made whenever practical. Note that the greater the hydrogen-ion concentration the stronger the acidity, but the lower the P_H figure. Hawk says that the hydrogen-ion concentration is increased in most pathological conditions. (Hawk's " Physiological Chemistry," 6th Ed., p. 511.)

NORMAL CONSTITUENTS OF URINE.

The more important normal constituents of the urine are urea, uric acid (combined as urates), chlorides, phosphates, sulphates, indoxyl, coloring matters; traces of mucin, organic

acids, carbonates, hippuric acid, creatine, and creatinine may also be present. The total normal solids are composed approximately of 50 per cent urea, 25 per cent chloride of sodium; at least one-half of the remainder are phosphates and sulphates. We see that the constituent which most influences the specific gravity is the urea, and in normal samples the specific gravity is an index of the amount of urea present. The total solids may be calculated by multiplying the last two figures of the specific gravity, taken at 25° C. by 2.6,* which will give approximately the number of grams of solids in one liter of urine; from this the solids in the twenty-four-hour amount may be easily calculated.

Urea.

The chemistry of urea has already been considered (page 54).

Detection. — A qualitative test for this substance is obviously superfluous, although such may be made by obtaining the crystals of urea nitrate or oxalate (page 56). The quantity of urea is of great importance, especially in cases where there is any question in regard to the body metabolism or the amount of nitrogen excreted. By far the greater proportion of all nitrogenous waste is eliminated by the kidneys in the form of urea, a comparatively slight amount as other nitrogenous constituents of the urine, a still smaller amount in the feces, and traces only by other avenues. The urea may be quantitatively determined by various methods, the hypobromite method being the most practical. See reaction on page 297.

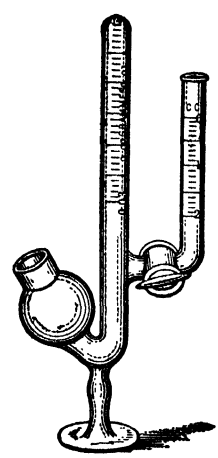


Fig. 12.

Quantitative Determination. — There are various forms of apparatus used in connection with this process.

The Doremus-Hinds apparatus shown in Fig. 12 gives a perfectly satisfactory method for the estimation of urea by the hypobromite method. The reagent, equal parts of bromine solution and 40 per cent NaOH (Appendix, page 297), is intro-

* Long's Coefficient.

duced into *R* and the tube completely filled. The tube *U* is next filled exactly to the zero mark; then by means of the stop-cock *S* 1 c.c. of urine is allowed to enter *T* a few drops at a time and slowly enough to prevent any escape of gas through *R*. The gas rises in small bubbles through a comparatively long tube and remains in contact with the reagent, which insures perfect absorption of CO_2, thus overcoming the greatest objection to the Squibb's apparatus.

The tube *T* is graduated to read centigrams of urea in 1 c.c. of urine.

A more accurate determination of urea depends upon the conversion of urea into ammonia by various methods which make quantitative application of the Kjeldahl determination

For detail of this test see page 280.

Uric Acid.

Uric acid and its antecedents, the xanthin bases, are derived from the decomposition of nuclein and nucleo-protein. See pages 57 to 59 and page 226. The uric acid is increased by a highly nitrogenous diet and certain vegetable substances which contain purine (page 58) derivatives, such as coffee, tea, and cocoa. Meats in general, and particularly those rich in nuclein, such as tongue, liver, sweet breads, etc., are regarded as the most abundant source of uric acid and urates. As previously suggested, uric acid does not occur as such in normal urine, but is combined with the alkaline bases.

Determination. — It is unnecessary to make a qualitative test in urine, as urates are always present. If a qualitative test is desired, the murexide test, as given on page 263, is available. Uric acid and allied constituents of the urine are conveniently determined quantitatively by the centrifugal method, as devised by Dr. R. Harvey Cook.* The detail of this method is as follows: Measure 10 c.c. of urine into a graduated tube, used in the centrifugal machine, add a few grains of sodium carbonate, and about 3 c.c. of strong ammonium hydrate. Place in the centrifuge, and allow to run for one or two minutes, then carefully

* Medical Record, Mar. 12, 1898, page 373.

decant the clear urine into another graduate tube, leaving the precipitate which consists of earthy phosphates. The bulk of this precipitate may be noticed and an idea obtained as to whether the earthy phosphates are present in normal quantities or not. To the clear urine add 2 or 3 c.c. of ammoniacal silver-nitrate solution ($AgNO_3$, 5 grams; distilled water, 80 c.c.; strong ammonia, 20 c.c.), and run in the centrifuge till the precipitate of silver urate has reached its lowest obtainable reading. The ammonia will prevent the precipitation of chlorides and, unless iodides or bromides are present, the precipitate will be fairly pure silver urate, each tenth of a cubic centimeter of the precipitate being equivalent to 0.001176 gram of uric acid in the 10 c.c. of urine used, or 0.01176 per cent.

The silver precipitate is by no means pure silver urate, many of the other nitrogenous bases in urine forming insoluble silver salts. These occur only in very slight traces; so, for clinical purposes, the method is available unless the sample contains bromides or iodides, when iodide or bromide of silver will be formed, insoluble in the amount of ammonia usually used. More accurate results may be obtained by either Hopkins' or Folin's method. These are somewhat similar and consist of precipitation of the uric acid as ammonium urate. One hundred to 200 c.c. of urine is used and the precipitation effected by a saturated solution of NH_4Cl (Hopkins' method) or 10 grams ammonium sulphate (Folin's method).

The precipitate is washed in the reagent and dissolved in boiling water and the amount of uric acid determined by titration with N/20 permanganate of potassium. Each cubic centimeter of $KMnO_4$ used is equal to 0.00375 gram of uric acid.

Ammonia Determination.

The amount of ammonia normally present in urine is about 0.7 gram in the twenty-four-hour amount. Ammonia is increased in any systemic condition resulting in an increase of acidic elements (Acidosis), or upon ingestion of ammonium salts of inorganic acids, i.e., salts not easily changed to urea.

Normally the quantity of NH_3 follows more or less closely

the urea and the protein metabolism, and amounts to about one-twentieth of one per cent (0.05 per cent) or about 0.7 gram in twenty-four hours. In acidosis, even of the mild type, the ammonia is increased at the expense of the urea; i.e., the proportion of ammonia to urea is greater than normal, and uric acid is often high while urea is low.

Formaldehyde Method. — Place 10 c.c. urine in a 250 c.c. Erlenmeyer flask, add 10 or 20 c.c. H_2O, titrate with N/10 NaOH with phenolphthalein as an indicator. The amount of NaOH used will represent total acidity of sample.

After exact neutralization add 10 c.c. of previously neutralized commercial formaldehyde solution and titrate *again* with N/10 NaOH. The *second* amount of alkali added represents ammonia as follows:

$$4\,NH_4Cl + 6\,CH_2O + 4\,NaOH = N_4(CH_2)_6 + 10\,H_2O + 4\,NaCl.$$

As the ammonium salts and the caustic soda react molecule for molecule, it is possible to make calculation for quantity of NH_3 by multiplying the N/10 factor (0.0017) by the number of cubic centimeters of N/10 NaOH used.

In cases of diabetes, when the ammonia reaches a comparatively large amount the figures obtained by this process will be found to be a little high, as amino acids are also acted upon by the NaOH, and will be calculated as ammonia, but for ordinary work of clinical comparisons this method is very simple and sufficiently accurate.

This method is not affected by urea, uric acid, creatine, creatinine, purine bases, or hippuric acid.*

Zeolite Method for Ammonia (Folin).

This method is based on the property of zeolite powder (commercial permutit, a synthetic aluminate silicate) to absorb ammonia. The powder may be used repeatedly if it is carefully

* Dr. Hans Malfatti in Zeit. für Anal. Chemie, 47, page 273.

Note. — See also the Vacuum Distillation Method, giving very exact results when properly carried out:

H. Björn Andersen und Marius Lauritzen, Zeit. für Physiol. Chemie, 64, page 21.

washed first with water and then with 2 per cent acetic acid and finally with water.

Procedure: In a 100 c.c. volumetric flask, place about 2 grams of zeolite. Add 5 c.c. water and 2 c.c. urine. Add a little more water, 1–5, in order to rinse down the urine, and then shake gentl for *five minutes*. Rinse the powder to the bottom of the flask with 25–40 c.c. water, and decant the supernatant liquid. Repeat. Then add a few c.c. of water to the powder, 1 c.c. 10 per cent NaOH, shake gently and let stand for a few minutes.

Prepare a standard solution as follows: In another 100 c.c. flask place 5 c.c. standard $(NH_4)_2SO_4$* solution, 1 c.c. 10 per cent NaOH. Dilute to about 75 c.c. and mix thoroughly. Measure 10 c.c. of Nessler's reagent into a cylinder and add all at once to the solution in the flask *while the contents of the flask are in vigorous motion.* A *perfectly clear* solution should be obtained. Then dilute the urine flask to about 75 c.c. and add 10 c.c. of Nessler's solution as directed above. Compare the colors obtained in a colorimeter, setting standard at 20, and calculate result as follows: —

$$\frac{20}{\text{Reading of cup}} \times .5 \times 50 = \text{mg. of ammonia nitrogen per 100 c.c.}$$

Chlorides.

The chlorides are represented in the urine chiefly by sodium chloride. This is present to the extent of 12 to 20 grams in twenty-four hours. An increase above this quantity is unusual, although it simply indicates an increase in the ingested salt, and is without clinical significance. The chlorine is diminished in dropsy, acute stages of penumonia, and in fevers generally.

Determination. — The usual qualitative test with silver nitrate and nitric acid is employed for detection of chlorine in the urine. If one drop of a strong solution of silver nitrate (1 to 8) is allowed to fall into the wine-glass in which the albumin test has been made (q.v.), the appearance of the resulting precipitate will give a rough idea of the quantity of chlorine present. If a solid ball of silver chloride is formed which does not become

* For preparation see Appendix.

diffused upon gently agitating the contents of the glass, the chlorine is normal or increased. If the precipitate falls as a cloud distributed throughout the liquid, the chlorine is diminished. The chlorine may be determined by precipitation with silver nitrate in 10 c.c. of urine, and the precipitate settled in a centrifuge-tube to constant reading, but this method is not recommended, as the precipitate is a bulky one, and usually takes a long time for thorough settling.

An accurate titration of chlorine may be made as follows:

To 10 c.c. of urine contained in a porcelain dish add about 5 c.c. of saturated solution of ferric alum strongly acidified with nitric acid. Add 20 c.c. of an N/10 silver nitrate solution. This precipitates *silver chloride*, but silver urate, silver phosphate and any possible organic silver compounds are held in solution by the nitric acid. Titrate excess of silver nitrate with N/10 potassium thiocyanate, the ferric alum acting as an indicator.

Calculation: Subtract number of c.c. of thiocyanate used from 20, multiply result by .0035 and then by 10 to obtain per cent.

Phosphates.

The phosphates in the urine are of two kinds, the alkaline phosphates, Na_2HPO_4 and NaH_2PO_4, etc., and the earthy phosphates represented by the magnesium and the calcium phosphates. The phosphates are normally present to the extent of $2\frac{1}{2}$ to $3\frac{1}{2}$ grams, calculated as P_2O_5 (in twenty-four hours).

The triple phosphates, ammonium magnesium phosphates (Plate IX, Fig. 6, page 214), are the forms in which phosphoric acid is usually found in urinary sediment. Crystals of acid calcium phosphate are occasionally found, and resemble the acid sodium urate in form (Plate IX, Fig. 3, page 214), except that they are usually a little broader and more often occur in fan-shaped clusters. They may be distinguished by treatment with acetic acid, which dissolves the calcium phosphate promptly, while the urate is slowly dissolved and crystals of uric acid appear after a little time. The phosphates are deposited from neutral or alkaline urines, and when this precipitation takes

place within the body the crystals cause more or less irritation to the urinary tract and may form aggregations which result in calculi. Phosphates are supplied by either a cereal or meat diet. They may be much increased in diseases accompanied by nervous waste, or by softening and absorption of bone. Phosphates are diminished in gout, in chronic diseases of the kidney, and during pregnancy.

Determination. — A qualitative test for earthy phosphates (E.P.) may be made by taking a test-tube half full of urine, and making alkaline with ammonium hydrate. When the precipitate has thoroughly settled, if it is about $\frac{1}{4}$ to $\frac{1}{2}$ inch in depth, it represents normal earthy phosphates. If this mixture is now filtered, the alkaline phosphates (A.P.) may be determined in the filtrate by the addition to the solution of one-third its volume of magnesium mixture.* The precipitate after settling will be $\frac{1}{2}$ to $\frac{3}{4}$ of an inch in depth if normal. The total phosphates may be determined in the centrifugal machine by adding 5 c.c. of magnesium mixture to 10 c.c. of urine. Each tenth of a cubic centimeter of the centrifugalized sediment will be equivalent to 0.00225 gram of P_2O_5 in the 10 c.c. used.

A more accurate determination of the total phosphoric acid may be made by the titration with uranium nitrate or acetate solution as follows:

Reagents Required. — *First.* A standard uranium solution may be prepared as follows: Dissolve 35.5 grams of pure uranium nitrate or acetate in about 800 c.c. of distilled water; add 3 or 4 c.c. of glacial acetic acid and heat it enough to complete solution. Allow to stand over night, filter carefully, and make up to 1000 c.c. Standardize this solution against crystallized microcosmic salt by dissolving 14.721 grams of the pure salt ($NaNH_4\,HPO_4\,.\,4\,H_2O$) in sufficient water to make 1000 c.c. Then titrate 20 c.c. of this solution, to which has been added 30 c.c. of water and 5 c.c. of sodium acetate solution, with the uranium solution (method of titration is given under process below).

The uranium solution should then be adjusted (diluted) so

* See Appendix.

that it will take exactly 20 c.c. for this titration, when 1 c.c. of the uranium will be equivalent to 5 milligrams of P_2O_5.

Second. A sodium acetate solution containing 100 c.c. of 30 per cent acetic acid and 100 grams of sodium acetate in enough distilled water to make 1000 c.c.

Third. An indicator consisting of a saturated solution of potassium ferrocyanide.

Process. — Place 50 c.c. of urine with 5 c.c. of sodium acetate solution above described in a small Erlenmeyer flask and heat nearly to the boiling-point. Titrate, while hot (80° or above), with the standard uranium solution till a drop of the mixture placed on a white porcelain tile with a drop of the indicator $K_4Fe(CN)_6$, gives a distinct brown color. This method of determining the end-point is known as "spotting" and with a little practice gives very accurate results.

The number of cubic centimeters of uranium solution multiplied by 0.01 will give the weight of P_2O_5 in 100 c.c. of urine (1 c.c. of reagent being equal to 0.005 gram P_2O_5).

This same process may be used for saliva by diluting the reagent one part to five, and preparing the sample for titration as follows: Take from 2 to 5 c.c. saliva, add sufficient alcohol to make 10 c.c. of mixture, warm, and filter. This serves to separate the protein substance. Take 5 c.c. of the filtered solution and titrate with the diluted uranium solution as by the process given above for urine. In this case, of course, 1 c.c. of the standard uranium will represent 1 milligram of P_2O_5 rather than 5.

Sulphates.

The sulphates in the urine are present as alkaline sulphates, K_2SO_4 and Na_2SO_4; also as ethereal sulphates, represented by such compounds as indoxyl potassium sulphate, page 201.

Detection and Determination. — The sulphates may be detected by precipitation with barium chloride in hydrochloric acid solution. If the precipitate is obtained from 10 c.c. of urine and centrifugalized to constant reading, the per cent of sulphuric acid by weight will be one-fourth of the volume per

cent of the precipitate. The sulphates follow the urea rather closely, and their determination is not of great importance. They are increased in acute fevers, diminished in chronic diseases generally, and markedly diminished in carbolic-acid poisoning. (Ogden.)

Determination of Total Sulphur. — (J. Benedict, *Biol. Chem.*, 6, 363; W. Denis, *J. Biol. Chem.*, 8, 401.) To 25 c.c. of urine contained in a porcelain evaporating dish (10–12 cm. diameter) add exactly 5 c.c. of a solution containing 25 per cent copper nitrate, 25 per cent sodium chloride, and 10 per cent ammonium nitrate. Evaporate to dryness over a water-bath. Then heat over a flame, gradually increasing the heat until the dish is red hot, and continue heating for ten to fifteen minutes. Allow to cool. Add 20 c.c. dilute hydrochloric acid and warm gently. Rinse into a flask or beaker by means of about 100 c.c. hot water. Heat to boiling, and add drop by drop 25 c.c. of a 10 per cent barium chloride solution. Filter, wash, ignite, and weigh.

Coloring Matter.

Urochrome is a pigment to which the yellow color of urine is chiefly due. Uroerythrin and urorosein are less important, existing only in very slight quantities, but they are responsible for colors of some sediments and of decomposition products which are noticed in analysis. In an article on urochrome (K. F. Pelkon, *J. Biol. Chem.*, March, 1920) the statement is made that all evidence points to the fact that urochrome is derived from the protein in the diet. A low protein diet decreased the urochrome up to 50 per cent while a high protein diet increased it in all the experiments which the above investigator performed.

Urobilin, a less important coloring matter of the urine, exists as a parent substance, or chromogen, to which has been given the name urobilinogen. This undergoes decomposition by action of light with liberation of urobilin.

Urobilin is without doubt derived from the bilirubin of the bile, which, in turn, comes from the hemochromogen of the

blood. Dr. J. B. Ogden is authority for the statement that "it is safe to infer that the amount of urobilin in the urine is a measure of the destruction of the hemoglobin or blood pigment."

Another pigment closely resembling and possibly identical with urobilin is *hydrobilirubin*. It is considered as an oxidation product of bilirubin and is normally produced in the fecal matter during its passage through the large intestine.

Soluble Salts.

An examination of the soluble salts of the urine is easily and often profitably made by simply allowing a large drop to evaporate spontaneously and examining the residue with the micro-polariscope. The alkaline chlorides are often seen but they do not polarize light. Crystalline phosphates, sulphates, urates, and oxalates do polarize light and may frequently be detected by their characteristic forms. The value of determination of soluble oxalates in this way is suggested on page 246.

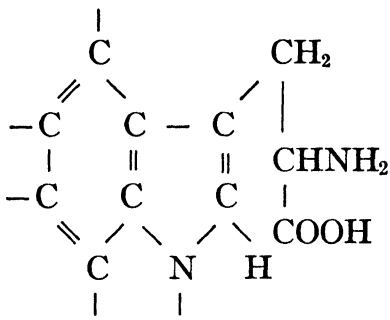
Indoxyl.

The test for indoxyl in the urine is of great importance as it indicates an excessive degree of nitrogenous putrefaction taking place in the small intestine. An increase of this nature may be induced by an acute inflammatory process of the peritoneal cavity, but in the majority of cases liable to come under examination in dental practice, the excess of indoxyl will mean intestinal indigestion. Ordinary constipation does not of itself cause an increase in the indoxyl.

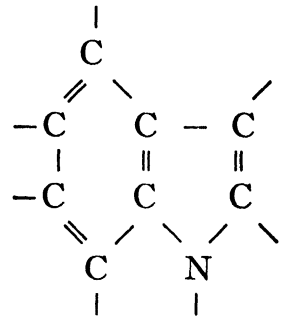
Detection and Determination. — Place 15 c.c. of strong HCl in a wine-glass, and add a single drop of concentrated nitric acid; then stir 30 drops of urine into the mixture. If indoxyl is present, an amethyst color develops in five to fifteen minutes. If the color is purple the indoxyl is increased. Variation of the amount of indoxyl within normal limits is rather wide, and the indoxyl may be reported as high or low, normal, increased, or diminished.

The particular constituent of the protein molecule responsible for appearance of indican in the urine is tryptophane which, it

will be remembered, is an α-amino-β-indol-propionic acid. The chemistry of the changes involved may be represented as follows:

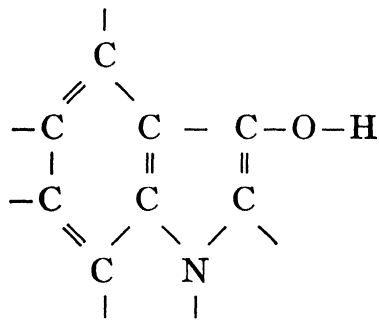


Tryptophane



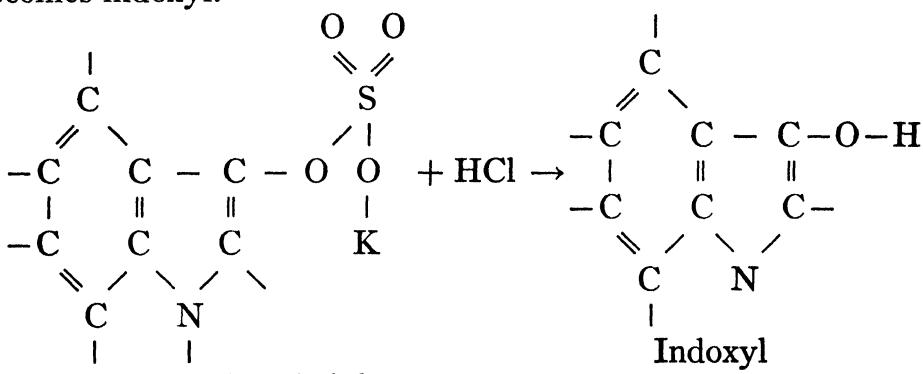
Indol

Tryptophane by putrefactive action becomes *Indol*
By oxidation the indol becomes *Indoxyl* C_8H_7NO or C_8H_6NOH



Indoxyl

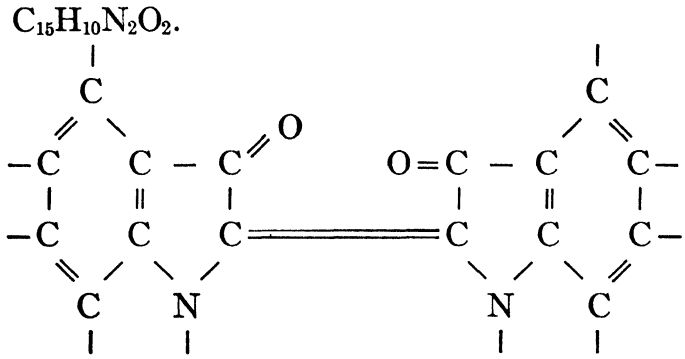
The indoxyl forms an ethereal sulphate, indoxyl potassium sulphate, or *indican*, and as such is eliminated by the kidney; i.e., the indican ($C_8H_6NSO_4K$) appears in the urine and then by action of the strong HCl and trace of HNO_3 of the test, again becomes indoxyl.



Indoxyl Potassium Sulphate

Indoxyl

Two molecules of indoxyl by action of the oxidizing agent become one molecule of indigo blue.



Indigo blue.

ABNORMAL CONSTITUENTS OF URINE

The principal abnormal constituents are albumin, sugar, acetone, bile, and various crystalline salts, discoverable either by microscopical examination of the sediment, or by evaporation of a clear fluid, and examination with the micropolariscope.

Metallic substances, arsenic, lead, and mercury, are occasionally present, and tests should be made for them when general symptoms or the conditions of the kidney indicate metallic poison. ALBUMIN is probably present in minute traces in the majority of urines. When in sufficient quantity to be detected by the usual laboratory methods, it is essential that we learn the source from which it has been derived, for the simple presence of even a considerable trace of albumin may be of but slight clinical importance. Albumin may indicate either a pathological condition of the kidney, which allows the entrance into the renal tubules of serum-albumin from the blood, or it may indicate a change in the composition of the blood, whereby the albumin passes more easily through the renal membranes, or its presence may be due to irritations from various sources of the urinary tract; and, as regards the bearing of albuminurias on dental disease, it is sufficient simply to determine whether renal disturbance is primary or secondary to some other trouble, such as heart disease; or purely local, as when caused by irritation due to crystalline elements.

Detection. — Albumin may be detected by either of two simple methods. It is often desirable to use both of these methods, thereby eliminating possible confusion from the presence of substances other than albumin, which may respond to one of the two tests, but not to both.

The first consists simply in underlaying about 25 c.c. of *filtered* urine in a wine-glass with concentrated nitric acid. The wine-glass should be tipped as far as possible and the acid allowed to run very slowly down the side. This method is preferable to the use of the apparatus known as the albuminoscope or Horismascope (Fig. 13). As this latter method does not provide for

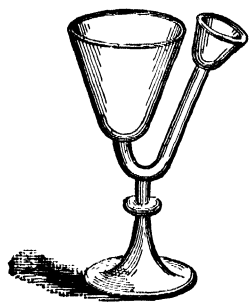


FIG. 13.

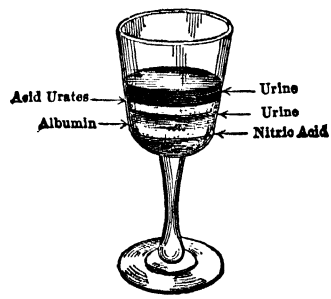


FIG. 14.

sufficient mixing of nitric acid with the sample, the albumin is shown by a narrow white ring *at* the plane of contact of the two liquids. A white ring *above* the plane of contact is not albumin, but is composed of acid urates, indicating an excess of urates in the sample (Fig. 14). The albumin, in distinction from this band, occurs directly above the acid and is usually reported as the slightest possible trace when just discernible; as a slight trace, when well marked, but not dense enough to be seen by looking through the liquid from above; as a trace, when the white cloud may be seen by looking down into the glass from above and a large trace if plainly visible in this way.

The acetic acid and heat method of testing for albumin is the other method referred to in the preceding paragraph. It is of about the same delicacy as the nitric acid test, and is less liable to respond to substances other than albumin. It is made as follows:

A test-tube is filled two-thirds full of perfectly clear filtered urine, one drop of acetic acid added and the upper half of the sample boiled. The tube can easily be held in the hand by the lower end. After boiling, if the tube is examined before a black background, a slight cloudiness or turbidity resulting from coagulated albumin can be easily detected in the upper part of tube. Anything more than a trace should be determined in the centrifugal machine by mixing 10 c.c. of filtered urine with about 2 c.c. of acetic acid and 3 c.c. of potassium ferrocyanide solution. Each tenth of a cubic centimeter of the precipitated albumin, when settled to constant reading, indicates one-sixtieth of one per cent albumin by weight. This factor is fairly correct up to four- or five-tenths of a cubic centimeter of precipitate; beyond this it is of little value, and the albumin is best determined quantitatively by measuring 50 or 100 c.c. of urine into a small beaker, adding a drop of acetic acid, and boiling, which will completely precipitate the albumin. It may then be filtered into a counterpoised filter, thoroughly washed, first in water, next in alcohol, and lastly in ether, dried at a temperature a little below the boiling-point of water, and weighed. Esbach's method may be of value in some instances, and is carried out as follows:

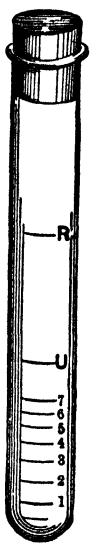


FIG. 15.

Fill the albuminometer (Fig. 15) with urine to the line *U*, and then add the reagent* to the line *R*; close the tube, mix the contents thoroughly, and allow to stand in an upright position for twenty-four hours. At the end of that time the depth of precipitate may be read by the figures on the lower part of the tube, these figures representing tenths of one per cent of albumin, or grams of albumin in a liter of urine. If a sample of urine contains more albumin than is easily estimated by the centrifugal or Esbach's method, approximate results will be obtained by diluting with several volumes of distilled water, until the quantity of albumin precipitated is within the limit

* Esbach's reagent consists of picric acid, 10 grams; citric acid, 20 grams, and distilled water sufficient to make one liter.

of the test. The proteoses occasionally occur in the urine, and are distinguished from albumin by the fact that they redissolve at a boiling temperature. If filtered while hot, albumin, which usually accompanies them, will remain on the paper, while albumose will separate from the clear filtrate as it cools.

Sugar.

Sugar in urine represents a perverted process of oxidation, for which the pancreas is largely responsible. The liver also often plays an important part in cases of diabetes, but just how this is done is not clearly known. Sugar in the urine does not of necessity indicate diabetes any more than albumin indicates Bright's disease. Many cases of glycosuria are of a temporary nature and respond readily to dietary treatment. Whenever sugar is found it is desirable to make tests upon both a fasting and an after-meal sample, such as might be obtained before breakfast and one hour after dinner. If the fasting sample is comparatively free from sugar, it indicates that the glycosuria is of a temporary nature and due to faulty metabolism, rather than to any organic disease of the liver or pancreas.

Detection. — Sugar in the urine may be detected by several general carbohydrate tests, as previously given.

Fehling's test. This test is very generally employed (Exp. 129, page 271). It is best, however, to modify it by bringing the Fehling's solution to active ebullition, adding from 5 to 30 drops of the suspected sample and allowing to stand without further heating. This prevents possible reduction of the sugar by xanthine bases or other occasional constituents of the urine, which might give misleading results if the mixture were boiled after addition of the sample. There is less danger of trouble of this sort if the gravity of the urine is below normal. If it is necessary to make a rapid test, the mixture may be boiled after the urine is added, and in case the result is negative there is no need of further test; if, however, a slight reduction of the copper solution takes place, it will be necessary to repeat the test, using the precaution above given. Quantitatively, sugar may be determined by the use of Fehling's solution as follows:

Fehling's Quantitative Method.

If the urine contains more than a trace of albumin, this substance should be removed by adding a drop of acetic acid and heating; after filtration the sample should be cooled and restored to original volume with distilled water. If specific gravity of the urine is more than 1025, it should be diluted to ten times its volume with distilled water (urine, 1 part; water, 9). If the gravity is less than 1025, dilute it to five times its volume, mix, and fill a 25 c.c. burette. In a 250 c.c. flask place 10 c.c. each of the alkaline tartrate and copper sulphate solutions (Fehling's solution), and add about 100 c.c. of distilled water. Place the flask over a Bunsen burner, and bring to a boil. If no change takes place after a minute or two of boiling, add the solution from the burette gradually, until the precipitate bceomes sufficiently dense to obscure the blue color of the solution. Continue to boil for one or two minutes, then remove from the flame and watch carefully the line directly beneath the surface of the liquid, which will appear blue until all of the copper has been reduced to the red suboxide. The solution should be kept at the boiling-point throughout the entire operation, except in making the examination of the meniscus between the additions of the diluted urine. These additions must be made very carefully, and as the process nears completion not more than one or two drops should be added at a time. When the blue color has entirely disappeared, and the line of meniscus has become colorless, note the number of cubic centimeters of dilute urine used, and calculate that in that quantity there is an equivalent of 0.05 gram of glucose; in other words, 0.05 gram of glucose will exactly reduce the amount of Fehling's solution used, and from this fact the amount of glucose in the entire twenty-four hour amount of urine is easily calculated. If the titration is carried beyond the proper "end-point" the meniscus will appear yellow instead of colorless.

Benedict's Sugar Determination.

Qualitative. — The following application of Benedict's solution to the detection of sugar in urine is taken from a paper

by Stanley R. Benedict in the *Journal of the American Medical Association*, October 7, 1911. "For the detection of glucose in urine about 5 c.c. of the reagent are placed in a test-tube and *eight to ten drops* (*not more*) of the urine to be examined are added. The mixture is then heated to vigorous boiling, kept at this temperature for one or two minutes, and allowed to cool spontaneously. In the presence of glucose the *entire body of the solution will be filled with a precipitate*, which may be red, yellow or greenish in tinge. If the quantity of glucose be low (under 0.3 per cent) the precipitate forms only on cooling. If no sugar be present the solution either remains perfectly clear, or shows a faint turbidity that is blue in color, and consists of precipitated urates. The chief points to be remembered in the use of the reagent are (1) the addition of a small quantity of urine (8 to 10 drops) to 5 c.c. of the reagent, this being desirable not because larger amounts of normal urine would cause reduction of the reagent, but because more delicate results are obtained by this procedure, (2) vigorous boiling of the solution after addition of the urine, and then allowing the mixture to cool spontaneously, and (3) if sugar be present, the solution (either before or after cooling) *will be filled from top to bottom with a precipitate*, so that the mixture becomes opaque. Since bulk, and not color, of the precipitate is made the basis of a positive reaction, the test may be carried out as readily in artificial light as in daylight, even when examining for very small quantities of sugar."

Benedict's Quantitative Sugar Titration.

Make a 1–10 dilution of the urine and fill a 25 c.c. burette with the diluted sample. In a large porcelain dish place 25 c.c. of Benedict's Quantitative Sugar Reagent (see Appendix) and about 10 grams of sodium carbonate, and some pumice stone to prevent bumping. Heat to boiling and allow the urine to run in from the burette. A heavy, white precipitate is produced. Continue allowing the sugar solution to run into the copper, which is kept at a boiling temperature throughout the determination, until the blue color has been entirely discharged.

Then note the reading on the burette and make calculations as follows:

The 25 c.c. of Benedict's solution is reduced by .05 gram of glucose. Therefore:

$$\frac{\text{Reading on burette}}{.05} = \frac{100}{X}$$

This method is preferable to the Fehling's determination because other substances do not react to it; a sharp end-point is attainable even by the inexperienced, and the titration is more quickly made.

The fermentation test (Exp. 134, page 271) may also be used to detect the presence of sugar and, approximately, the amount.

The fermentation test for sugar is a convenient and easily made qualitative test, it being only necessary to fill a fermentation tube (Fig. 26, page 271) absolutely full of urine to which a small portion of yeast has been added, and to allow the tube to stand in a warm place for several hours. Any collection of gas in the top of the tube will indicate the presence of sugar. This method may also be used as a quantitative test for sugar by taking two portions of the same sample, adding yeast to one, and using the other as a control. At the end of twenty-four hours, CO_2 is removed from fermented sample, the specific gravity of both samples is carefully taken, and the loss of density in the fermented sample is calculated as sugar by multiplying the number of degrees lost in gravity by 0.23, water being considered as 1000.

The phenyl-hydrazine test may be used as a confirmatory test or in cases where very minute quantities are suspected. This test is considered about ten times as delicate as the Fehling's test; consequently, it may show small amounts of sugar which are not detected by the more rapid process.

The optical analysis for sugar may be made with a polariscope, preferably constructed for use on urine. This determination depends upon the ability of glucose to rotate the plane of polarized light towards the right, the degree of rotation indicating the amount of sugar in a pure solution. Of course, allowance

or correction must always be made for the presence of any substances which will rotate the light in the opposite direction, such as albumin, levulose and β-oxybutyric acid.

For the detail of construction and use of the polariscope, the student is referred to the more complete works on urine analysis by Ogden, Holland, or Purdy.

Acetone.

Acetone may occur in the urine as a result of various pathological conditions and, according to von Noorden, they are all due to some one-sided perversion of nutrition. The acetonurias attendant on diabetes, scarlet fever, pneumonia, small-pox, etc., are of less practical interest to the dental practitioner than those which are more often overlooked by the medical profession, and which indicate improper diet, possibly resulting in serious malnutrition. The following points may be noted: In advanced stages of diabetes, acetone appears in the urine accompanied by diacetic acid. An increased ingestion of proteins may result in the appearance of acetone, in which case the direct cause is an "insufficient utilization of carbohydrates"* rather than the increase of protein.

It should be remembered that while acetone may appear because of insufficient utilization of carbohydrates, the acetone bodies are produced from neither protein nor carbohydrates but are derived from the perverted metabolism of fat, as suggested in the chapter on Metabolism, page 230.

Detection. Acetone may be detected in the urine by the production of iodoform, as described under analysis of saliva on page 182, but it is not in this case nearly so delicate a test on account of the odor and acid character of the urine. A more useful test is known as Legal's test and is made as follows: To a third of a test-tubeful of urine add a few drops of a freshly prepared and fairly concentrated solution of sodium nitroprusside; next add two or three drops of strong acetic acid, and then a considerable excess of ammonia. If the contents of the tube are mixed by a rather rapid rotary motion without inverting

* von Noorden's Diseases of Metabolism and Nutrition.

or violent shaking, the ammonia will not reach the bottom of the tube, and the presence of acetone will be indicated by a violet-red band above the layer of acid liquid. If much acetone is present a deep violet to purple color is obtained.

Diacetic Acid occasionally occurs in urine as an abnormal constituent, most commonly in advanced stages of diabetes, usually accompanied by acetone and β-oxybutyric acid. It may be detected by adding to the urine a little ferric chloride, when a dark wine-red color is produced. If a precipitate of ferric phosphate is obtained, filter the urine and examine the filtrate for color. This test may be made fairly distinctive for diacetic acid by boiling and cooling a second portion of the urine previous to making the test, when the result will be negative if the color at first produced was due to diacetic acid.

β-oxybutyric Acid. — This substance usually accompanies diacetic acid, as above stated. Determinations of the quantity present cannot be made by any simple method. Perhaps the most practical method is by Bloor's nephelometer, page 166. See also page 229.

Bile.

Bile may occur in the urine as such, and may be due to pathologic conditions of the liver or bile-ducts, as stated on page 153. The coloring matters of the bile may also occur from causes aside from lesions of the liver. A urine containing bile or bile-pigments is always more or less highly colored, and upon shaking, the foam will be of a yellow or greenish-yellow color. Albumin and high indoxyl accompany the presence of bile and there is also usually considerable renal disturbance. It may be detected by carefully adding to one-half a wine-glass of the suspected sample a few cubic centimeters of the alcoholic solution of iodine (tincture of iodine). A green color will be observed just beneath the line of contact of the two liquids (page 293). The test may be conveniently made by placing the iodine first in the wine-glass and then with a pipette introducing the urine beneath the iodine solution.

Metallic Substances.

Arsenic, mercury, and lead are the three metals which it may be necessary to look for in a sample of urine. The method for the detection of mercury, given on page 183, is applicable for this purpose.

Arsenic may be detected by the Marsh-Berzelius test (as given in Vol. I), after oxidizing all organic matter. The process may be carried out as follows: Evaporate to dryness a liter of urine, to which 200 c.c. of strong nitric acid has been added; add to the residue, while still hot, from 15 to 20 c.c. of concentrated sulphuric acid. This must be done in a large porcelain evaporating-dish, or else the acid must be added very slowly to prevent frothing over and loss of a portion of the sample. After the action has quieted down the whole mixture may be transferred to a 500 c.c. Kjeldahl flask and heat applied, gradually at first, and then more strongly. It will be necessary to add from time to time small portions of nitric acid and possibly a little more sulphuric acid; as the oxidation progresses the liquid in the flask becomes lighter in color and at the completion of the process is water-white, even when the temperature is increased so that sulphuric-acid fumes are given off. After cooling, the strongly acid liquid is diluted with four or five times its volume of water, filtered, if necessary, to remove excessive amounts of earthy sulphates, and is then ready for the arsenic test.

Lead. — The sample of urine to be tested for lead should measure at least 1000 c.c., and should be tested for iodine to establish the presence of potassium iodide to dissolve lead salts; otherwise a negative result may be obtained when lead is actually present and is poisoning the system. Oxidize the sample in precisely the same manner as when making the arsenic test, up to the point of diluting the strong acid solution with water; then, in this case, use rather less water for the dilution, allow to cool, and neutralize with Squibb's ammonia, acidify quite strongly with acetic acid, and pass H_2S gas into the solution. It is desirable to leave the solution saturated with H_2S for at least twelve hours. Then filter, and without washing dissolve the precipitate

in warm dilute nitric acid, evaporate the HNO_3 solution to dryness, add 5 c.c. of water, make alkaline with a drop or two of ammonia, and again acidify with acetic acid and add a solution of bichromate of potash.* Allow to stand several hours, filter off the chromate of lead, wash several times with distilled water, and lastly with H_2S water, whereupon the lead chromate will blacken from the formation of lead sulphide. This stain is a superficial one and disappears upon standing, but when the process is conducted in this way it constitutes a very delicate and satisfactory test for lead in either urine or saliva.

Urinary Sediments.

The sediment which settles from a sample of urine upon standing consists normally of a slight amount of mucin and epithelial cells. It may also contain bacteria and a considerable variety of extraneous matter, including starch grains, various vegetable spores, yeast cells, fibers from various fabrics, cotton, wool, flax from linen, etc., diatoms, scales from insects' wings, and other particles which may occur as dust (see Plate VIII, Fig. 6; also Plate IX, Fig. 4). Under abnormal conditions the sediment may contain crystalline elements, including uric acid and urates, phosphates, oxalates, cystin, tyrosin, leucin, etc., also organized elements such as epithelium, renal or other casts (Plate VIII, Fig. 4), blood globules, pus cells (Plate VIII, Fig. 3), spermatozoa (Plate VIII, Fig. 2), fat, mucin (Plate VIII, Fig. 5), etc. Urinary sediment may be thrown down from a fresh specimen by the use of a centrifuge, or the urine may be allowed to stand in a glass tube with rounded bottom for several hours, when the sediment settles to the bottom by gravity. If possible it is best to examine sediments settled in both of these ways, as the centrifuge will show elements, such as small casts, that would settle slowly, possibly not at all, by the gravity method. On the other hand, the sediment allowed to settle spontaneously will often give a more correct idea of comparative numbers of the various elements observed, than when settled in a centrifuge-

* Natural chromate of potash will precipitate copper, the acid chromate precipitates lead only of the second group metals.

PLATE VIII. — URINE.

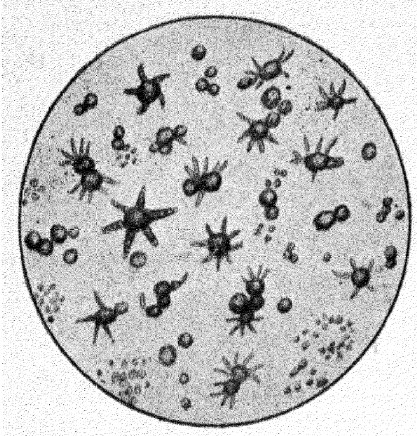


FIG. 1.
Ammonium Acid Urate.

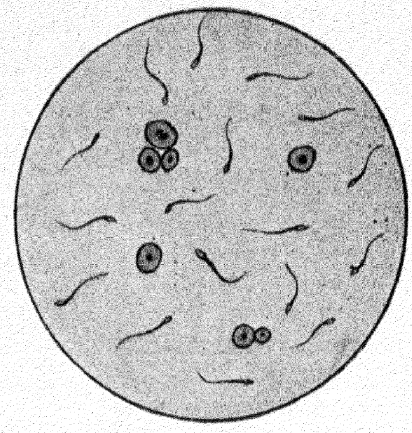


FIG. 2.
Spermatozoa.

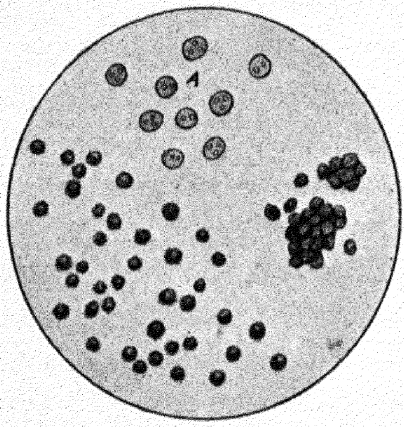


FIG. 3. — Pus.
A, After addition of Acetic Acid.

FIG. 4.
Renal Casts.

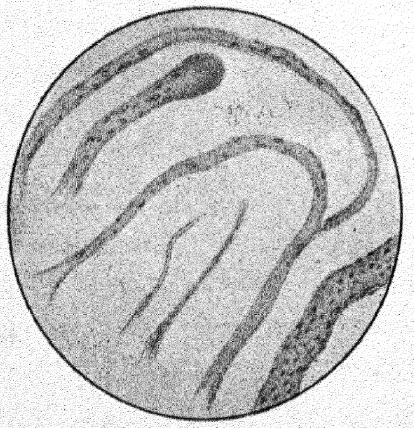


FIG. 5.
False Casts and Mucin.

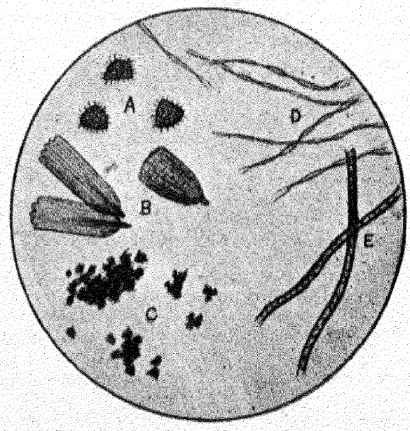


FIG. 6.
A, Lycopodium; *B*, Moth-scales; *C*, Cork; *D*, Cotton-fibres; *E*, Wool-fibres.

tube. A drop or two of formalin may be used to preserve urinary sediment, as suggested on page 187, but if too much of this substance is used, especially in urines containing high percentages of urea, a compound is liable to be formed which has been called formaldehydurea (Plate IX, Fig. 5), which settles with the sediment and seriously interferes with the microscopical examination. This compound may form sheaf-like crystals similar to tyrosin and may be mistaken for crystals of sodium oxalate, especially when examined with a low-power objective.

Uric Acid. — Uric acid is deposited from normal urine, upon standing, with an excess of free acid (HCl). Urines that have a high degree of acidity will also produce a like deposit, and the finding of uric-acid crystals does not necessarily signify that the crystallization took place within the body, unless special care has been taken that the sample examined was perfectly fresh, although the *tendency* to deposit uric acid is, of course, indicated. The urine from which uric acid separates, as such, is usually rather concentrated and of strong acid reaction. These crystals vary in appearance (Plate IX, Figs. 1 and 2), but are almost always colored yellow to red. Colorless crystals are sometimes observed. They are usually quite small, but of the peculiar whetstone shape in which this acid usually crystallizes. The presence of uric acid has practically no effect upon the acidity of the sample; for, if the acid separates in a crystalline form, it is insoluble, and if it does not separate it is in combination as urates, possibly, of course, as acid urates. Uric acid exists normally in proportion to urea as about 1 to 50, but there is no necessary relationship between the quantities of the two substances, and the one may be diminished while the other is increased.

Urates. — Urates may occur as crystalline or amorphous precipitates. The crystalline urates are urate of sodium rarely, acid urate of sodium (Plate IX, Fig. 3), and acid ammonium urate (Plate VIII, Fig. 1, page 213). The amorphous urates are of the alkaline bases, usually sodium, and are frequently precipitated by lowering of the temperature after the sample has been passed; in such cases the urine assumes a cloudy appearance

which is cleared up by the application of heat. A sediment consisting of urates is usually of a pinkish color.

Phosphates. — Phosphates in the urinary sediment may be amorphous or crystalline. They are of the alkaline earths rather than of the alkaline metals, as the latter are soluble in both the acid and neutral forms. The amorphous phosphates deposit with the change of reaction from acid to alkaline, and usually in the form of a so-called triple phosphate of ammonia and magnesia (Plate IX, Fig. 6, page 214). This salt crystallizes in two forms. The prismatic form is the ultimate form; that is, if the crystallization takes place very slowly, the prismatic form is the one in which the salt is thrown out. If it takes place rapidly it may be precipitated in the feathery form, but this slowly changes over to the prismatic form. The acid phosphates may be precipitated in a form closely resembling in appearance the acid urates (Plate IX, Fig. 3), but may be distinguished from them by their ready solubility in acetic acid and failure to produce, after solution in acetic acid, any crystals of uric acid such as are obtained from the urates.

Acid Lactates. — These are soluble salts, and are found in urine only by evaporation of a drop of the clear fluid and an examination of the residue by polarized light. When found in the urine, they have not the same significance as when found in the saliva, as in the urine they may possibly be formed from lactates, which indicate a faulty action of the liver, and of course they have no connection with tooth erosion. The lactates furnish evidence of similar character.

Oxalates. — Oxalates, if found in the sediment, usually occur as calcium oxalates. These crystals assume a variety of forms, as shown in Plate II, Fig. 1, page 77. Sodium oxalate (Plate II, Fig. 4) may occur in the urine (not, however, in the sediment), and is detected only by evaporating a drop of the clear liquid and examining with polarized light. Dr. Kirk claims that a tendency to oxaluria may be detected in this way for a considerable time before the appearance of the oxalate of lime crystals, and hence such examination becomes a valuable aid to diagnosis.

PLATE IX. — URINE.

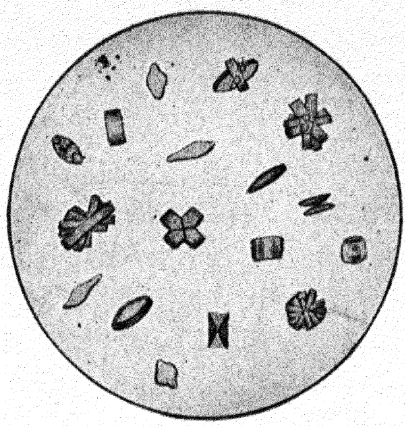


FIG. 1.
Uric Acid.

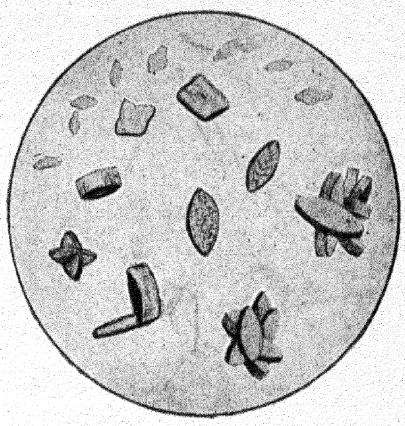


FIG. 2.
Uric Acid.

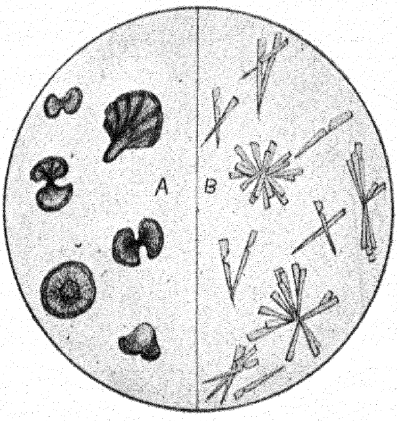


FIG. 3.
A, Sodium Urate; *B*, Sodium Acid Urate.

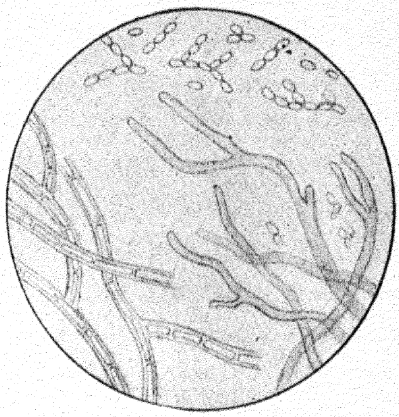


FIG. 4.
Yeast Cells and Molds.

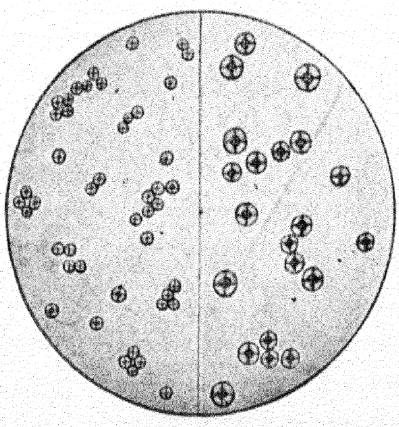


FIG. 5.
Formaldehyd Urea (P. L.).

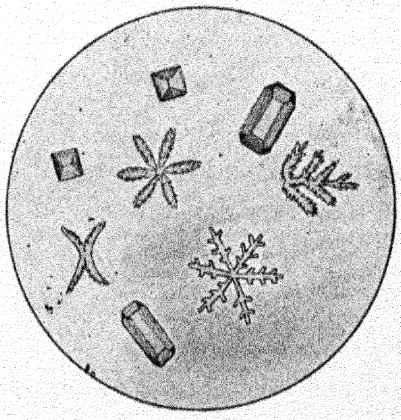


FIG. 6.
Magnesium Ammonium Phosphate.

Cystin. — Cystin occurs as six-sided plates. It is a comparatively rare crystal, and indicates insufficient oxidation, particularly of the organic sulphur compounds.

Epithelium. — Epithelium from any part of the urinary tract may occur in the urinary sediment. In the male urine it is much easier to determine the character of the epithelium than in the female, as in the latter the comparatively large amount of mucous surface, from which epithelium may be gathered, furnishes a great variety of forms which are, of course, without clinical significance. The epithelium from the vagina may be quite readily distinguished as very large cells with small nuclei, lying usually in masses overlapping one another but with comparatively slight density. Renal epithelium may be found as small, round cells, differing but slightly in size from a leucocyte. They may be a little larger, a little smaller, or about the same size. They are round and more or less granular in appearance.

Epithelium from the bladder varies considerably, but the majority of cells would properly come under the general head of squamous epithelium, rather large and flat with a distinct nucleus of medium size. Epithelial cells from the neck of the bladder in male urine are quite typical, being round and comparatively dense with a prominent nucleus. They are four or five times the size of a leucocyte and, in case of irritation at the neck of the bladder, are usually present in considerable numbers and of quite uniform appearance.

Renal casts consist of molds which are formed within the tubules of the kidneys and retain the form of the tubules after expulsion into the bladder. According to Ogden the most probable theory of their formation is " that they are composed of coagulable elements of blood that have transuded into the renal tubules, through pathologic lesions of the latter, and have there solidified to be later voided with the urine, as molds of the tubules." Casts are termed blood casts, pus casts, epithelial or fat casts, as any of these elements may adhere with more or less profusion to the cast itself. Pure hyaline casts are pale, perfectly transparent cylinders, with at least one rounded end which can be plainly seen, and may occur occasionally in urine

from perfectly healthy individuals. Fibrinous casts are highly refractive and when seen by white light are of a yellowish color and indicate acute renal disturbance. Waxy casts resemble the fibrinous casts as regards density, but they have no color, and usually indicate advanced and serious stages of kidney disease, while the presence of fibrinous casts has no necessarily serious significance.

Blood and Pus are readily recognized under the microscope after a very little practice. The blood disks are circular and show a characteristic biconcavity in the alternate shading of the edge and center by slight changes of focus. The red corpuscles usually show a shade of color by white light. The pus corpuscles or leucocytes are larger than the red corpuscles, and are granular in appearance. Treatment with acetic acid destroys the granular matter and brings into prominence the cell nuclei, two or three in number. If the leucocytes are free and scattered they should not be regarded as pus but should be reported simply as an excess of leucocytes; if they are very numerous and occur in clumps they constitute pus.

Spermatozoa. — Occasional spermatozoa may be found in sediment from either male or female urine and are without clinical significance. If persistent and in considerable numbers, seminal weakness is indicated (Plate VIII, Fig. 2, page 213).

Fat occurs in urinary sediment as small globules, highly refractive and varying greatly in size. They are frequently adherent to cells or to casts. Fatty casts indicate a fatty degeneration, which may or may not result from chronic disease. Fat may be demonstrated by staining with osmic acid, which is reduced by the double-bonded fatty constituent (olein), leaving a black deposit which stains the globule.

Mucin appears in the sediment as long and more or less indistinct threads. An excessive amount usually indicates irritation of some mucous surface. The source would have to be determined by other more characteristic elements (Plate VIII, Fig. 5).

The salts which may be obtained by evaporation of a drop of clear urine and detected by the micropolariscope are similar

to those occurring in the saliva; sodium oxalate is probably most frequently found. If the gravity is above normal the urea often crystallizes, making it somewhat difficult to pick out the abnormal crystalline constituents. Phosphates are also usually observed, but these crystals are large and as a rule prismatic, not easily mistaken for anything else.

Recording Results.

As stated at the beginning of the chapter on urine, our object has been the study of this secretion from the standpoint of general metabolism, rather than with a view to differentiating various forms of renal disease; and while it is important that the *presence* of renal disease should be recognized, its further investigation constitutes a proper study for the physician rather than for the dentist. When such conditions are found to exist a patient's physician should be apprised of the fact.

Uniformity of method in making out report cards is desirable although not absolutely necessary for the best class work; hence a few suggestions as to the use of the following blank. If no test is made, make no entry whatever on the blank. This permits the use of a dash, "$-$," to indicate a diminished (less than normal) quantity. If a substance is present in normal quantity use a capital " N," if increased above normal amount use "$+$." If absent use abbreviation " abs.," never the dash or minus sign. Observance of this method greatly facilitates correction of the report slips.

Urine Analysis.

NO.		NAME		
DATE		PHYS.		
			Bracketed numbers are average normal	
24 hr. Amt.		Sp. Gr.	%	Grams
React		Urea	(2.0	(30.
Color	E. Phos	Uric Ac	(.033	(0.6
Ind	A. Phos	Ammonia	(.050	(0.75
		Chlor	(0.67	(10.
Albumin		Phos. Ac.	(0.18	(2.7
Diac. Ac.	Acetone	Sugar		
			Uric Ac. to Urea-1 to	
Sediment				

CHAPTER XX.

METABOLISM.

Metabolism is the building-up and tearing-down process taking place constantly in the living organism. Food is ingested, digested, absorbed, and by various complicated means eventually transformed into the living tissue of the organism or burnt up as fuel by the system. The term anabolism is applied to the constructive metabolic process, while the destructive process and formation of waste is termed catabolism.

Carbohydrate Metabolism.

We have seen that the digestion of carbohydrates is accomplished for the most part by means of five enzymes: first, the ptyalin of the saliva, which reduces starch to maltose; then the amylase of the pancreatic juice, which continues the action started by the saliva; and finally maltase, sucrase, and lactase of the intestinal juice, which convert the disaccharides to the simple sugars. Carbohydrates, then, are absorbed usually in the small intestine as glucose, levulose and galactose.* The blood circulating through the tissues carries these simple sugars to the cells where they are needed.

We may believe that part of the sugar absorbed is almost immediately oxidized, while the remainder is stored by the system until it is required to satisfy the energy demands of the body. The oxidation of the glucose takes place in the muscle cells where, as was indicated under muscle metabolism, page 141, various substances are produced which may be considered as related directly to the oxidation. Lactic acid and alcohol are undoubtedly intermediate products finally resulting in the production of carbon dioxide and water.

The excess of glucose is converted by an endocellular enzyme,

* There is considerable evidence for the belief that *maltose* is capable of absorption in slight amounts.

glycogenase, into glycogen, and stored by the system as such. The liver seems to have a capacity for forming glycogen and it has been believed that this change, from glucose to glycogen, takes place mainly in this organ. At present, however, some authorities are inclined to believe that the conversion of glucose to glycogen is not localized and that the liver may act merely mechanically in holding back the sugar, since other substances seem to be held back also. The size of the liver and the natural relationship of its blood supply to the blood loaded with carbohydrate material from the intestine may also be factors accounting for the large quantities of glycogen found in the liver.

Although glycogen is the storage form for carbohydrates, it is important to remember that during transportation, that is, while the carbohydrate is in the blood, and when oxidation takes place, it is in the form of glucose. Glycogenases seem to be abundant, and the conversion of the carbohydrate from one form to the other apparently takes place with the greatest of ease.

Directly after absorption, and perhaps under some other conditions, levulose and galactose may be present in the blood in detectable quantities, but the *normal blood sugar* is always *glucose.* It is present to the extent of 90–110 mg. per 100 c.c., and these figures are given as the *normal blood sugar level.* Folin has stated that if the sugar content of the blood rises to 160 mg. per 100 c.c. there is a similar rise in all the body tissues; if it rises to between 160 and 170 mg. per 100 c.c. glucose will appear in the urine.

The amount of carbohydrate which can be ingested in twenty-four hours without an appearance of sugar in the urine varies considerably with the individual; that is, each person seems to have what is termed a *carbohydrate tolerance.* These facts have been represented diagrammatically in the accompanying illustration, Fig. 16.

If the individual ingests more carbohydrate material than can be stored as glycogen, the system will convert the excess into fat; if there is a further excess, that is, if the carbohydrate tolerance is exceeded, the sugar is excreted by the kidney and a condition of glycosuria results.

Glycosurias may be divided into two main classes:* namely, those caused by a diseased pancreas, diabetes mellitus; and those caused merely by an over-ingestion of carbohydrate material, producing a renal glycosuria or an alimentary glycosuria.

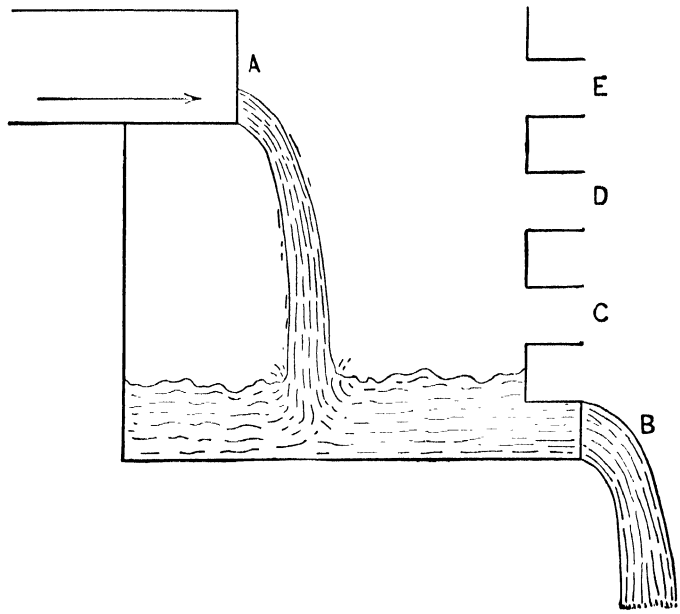


FIG. 16.

The tank represents the blood sugar level. Outlet B represents the minimum requirement; when the blood sugar rises to C it is stored by the system as glycogen, when it rises to D it is stored as fat, while if it rises to outlet E the carbohydrate tolerance is exceeded and sugar will appear in the urine.

Concerning the rôle of the pancreas in carbohydrate metabolism, Stiles says, " A function of this organ even more necessary than its digestive contribution is the delivery to the blood of the hormone which makes it possible for the muscles, including the heart, to oxidize sugar. Abundance of this hormone insures a high tolerance for sugar; want of it produces, according to the degree of the lack, a low tolerance or substantial inability to make use of carbohydrate."

In cases of true diabetes mellitus and in cases of over-ingestion

* Another relatively unimportant but interesting class is the so-called *emotional* glycosuria. A glycosuria is produced in nearly all cases of intense emotion, probably because of some relationship between the secretion of adrenalin and the utilization of carbohydrate in the system.

of carbohydrate, the blood sugar and the urine have been carefully studied and the following facts have been observed.

After a large intake of glucose the normal individual will show a decided rise of blood sugar, 140 mg. per 100 c.c. perhaps, a condition of hyperglycemia. Shortly, however, the blood sugar will drop to the normal level, then become subnormal, and usually two or three hours after ingestion will be perfectly normal again. The subnormal level, hypoglycemia, may be explained on the ground that after a large intake of glucose there is the least possible need for transportation of glucose in the tissue. In the diabetic individual, on the contrary, there will be a decided rise but the return to normal will be very, very slow. These results, plotted, give the following curves (Folin).

FIG. 17.

Further, if the normal individual ingests starch instead of glucose, a slight increase only is observed in the blood-sugar level; while with the diabetic, starch absorption gives a similar rise to the sugar.

The urine of a diabetic patient will contain sugar in appreciable amounts either before or after eating. In cases of renal glycosuria, sugar is always found after eating but just before meals it will frequently disappear entirely. Renal glycosuria may be said to exist in those individuals who naturally have a low *renal threshold* for glucose, the renal threshold being the ability of the kidney to retain sugar. It is obvious that the carbohydrate tolerance of the individual is more or less dependent on this so-called renal threshold. Whether or not prolonged renal or alimentary glycosuria, produced by ingesting more carbohydrate than the system can take care of, will result in pancreatic diabetes is an open question. From a conservative

viewpoint, however, the author would advise a lessened intake of carbohydrate food for individuals who show a tendency toward any kind of glycosuria.

When the system is unable to utilize carbohydrates properly a perverted fat metabolism is nearly always sure to follow, with the production of acetone bodies formed by the insufficient oxidation of butyric acid (page 230). This, of course, accounts for the frequent presence of these substances in diabetic urine.

The treatment* for diabetes has been modified recently. At present the diabetic specialist tries to discover just how much carbohydrate the individual can properly assimilate (it has been found that even in the most diseased condition of the pancreas a small amount of carbohydrate can be utilized) and then sees that the diet of protein and fat is in the right proportion to that amount of carbohydrate. The individual will usually lose weight, but this method of treatment has been found more beneficial than the former increase of protein and fat and the withdrawal of practically all carbohydrate food.

This diet modification has been the result of a study of the relationship found to exist between the amounts of nitrogen and the amounts of sugar excreted. There has been found to be a direct ratio between them, the amount of sugar increasing if the nitrogen is increased, even though no carbohydrate is being fed. It has been determined that if the proportion of sugar to nitrogen excreted bears the ratio 3.65–1, the maximum amount of sugar is being excreted by the kidney. If this proportion is obtained with a patient eating no carbohydrate food it is usually called the *fatal ratio*, for it shows that the system is capable of utilizing almost no carbohydrate and is forming sugar from protein to an extent of 58 per cent.

Protein Metabolism.

It will be remembered that in the study of acids, amino acids were considered important because of their relation to proteins.

* Very recently a new preparation "insulin," obtained from the islands of Langerhan, has been found to be of great value in the control of the blood sugar level and hence in the treatment of diabetic.

The conversion of the complex protein molecule to amino acids takes place during digestion and may be briefly represented as follows:

Substance.	Enzyme.	Place of digestion.	Products.
NATIVE PROTEIN Milk	Pepsin Rennin*	Gastric juice " "	Metaproteins Proteoses Peptones Coagulated protein
Products of peptic digestion Milk	Trypsin Chymosin*	Pancreatic juice	Proteoses Peptones Coagulated protein
Products of tryptic digestion	Erepsin	Intestinal juice	Peptones Peptides AMINO ACIDS

* Rennin and chymosin both act on milk by precipitating the casein. The precipitated casein is then split by the other proteolytic enzymes.

These amino acids are absorbed directly into the blood stream, and as the blood circulates certain of them are taken up by the body cells and are resynthesized into the living protoplasm.

How this resynthesis takes place is entirely unknown, but it has been shown that while some amino acids are absolutely essential to the anabolic process others act as foreign substances. The essential amino acids undoubtedly differ with different species of animals, and probably the demands of one kind of tissue vary with those of another in the same animal. As a rule, the amino acids derived from the protein obtained from animal sources are utilized to a larger extent by man than those derived from vegetable protein. This may be due to the fact that animal protein yields a much larger *variety* of amino acids than does vegetable protein.

In contrast to carbohydrates, proteins cannot be stored as such by the system, unless possibly in very small quantities. They may be converted into carbohydrate and fat, but this process is a difficult one and, as a rule, under normal conditions that which is not needed for actual repair of tissue is immediately converted into substances which are excreted. It follows, then,

that the protein intake should not be greatly in excess of that necessary for the formative metabolism of the body cells.

The excess of amino acids is in large part de-aminized by means of de-aminizing enzymes, and is converted chiefly into ammonia and subsequently urea.

This conversion into urea takes place largely in the liver, although probably the action is not localized. Various theories as to how the change takes place have been suggested, and a few of them will be mentioned. Urea is probably formed in a number of ways, more or less dependent on other conditions present.

After de-aminization of the amino acids takes place, some of the ammonia produced may combine with carbon dioxide

$$2\,NH_3 + CO_2 \rightarrow O{=}C\begin{matrix}\diagup NH_2 \\ \diagdown NH_2\end{matrix}$$

Ammonium carbamate may be formed, and, by oxidation, followed by reduction, may yield urea (Dreschol)

$$NH_2COONH_4 + O \rightarrow NH_2{-}O{-}\underset{\displaystyle NH_2}{\underset{|}{C}}{=}O + H_2O$$

$$NH_2{-}O{-}\underset{\displaystyle NH_2}{\underset{|}{C}}{=}O + H_2 \rightarrow \begin{matrix}NH_2 \\ \diagdown \\ \quad C{=}O + H_2O \\ \diagup \\ NH_2\end{matrix}$$

Cyanamide may be first produced, and by hydrolysis may yield urea (Salkowski)

$$NH_2C{\equiv}N + H_2O \rightarrow \begin{matrix}NH_2 \\ \diagdown \\ \quad C{=}O \\ \diagup \\ NH_2\end{matrix}$$

Excess of ingested protein means an excess of amino acids, urea, ammonia, and other products of nitrogenous metabolism, which must be eliminated. Young people, or older ones in robust health, can usually take care of this excess easily, but too often the system becomes overtaxed with the excess and harmful results follow. If these end-products are not properly eliminated,

more ammonia may combine to form ammonium salts, resulting in a rise of ammonia nitrogen in urine and saliva and a lessened amount of urea nitrogen. The carbon dioxide may then convert neutral salts to acid salts, increasing the acidity of both urine and saliva. The amino acids not properly absorbed or eliminated are liable to undergo putrefaction in the intestine, giving rise to various toxic substances which aggravate, if they do not cause, skin disease, nephritis, etc. As tryptophane is a constituent of nearly all protein foods, the amount of indoxyl obtained from tryptophane in the urine is an indication of this intestinal putrefaction. See page 200.

In addition to the protein metabolism already discussed, the conversion of the purine base derivatives into uric acid is also taking place. This type of metabolism occurs both in the metabolism of the cell itself (endogenous uric acid) and as a result of the ingestion of foods containing the purine nucleus (exogenous uric acid). The uric acid found in the system is chiefly derived from nucleic acid, and a few facts regarding the chemistry of nucleic acid may be worthy of mention.

Nucleic acid, as the name implies, comes from the cell nuclei of plant and animal material. There is a slight difference in the composition of that obtained from plant tissue and that from animal sources, but in general they both contain a molecule of phosphoric acid, a carbohydrate group, purine bases, and pyramidine bases. During metabolism the purine bases are converted into uric acid.

Both plant and animal nucleic acids are insoluble in cold water and soluble in warm water, and with an acid mixture are precipitated with proteins. A precipitate formed in this way may be a salt of the acid, but in general the term nucleo-proteins has been used to designate this class of substances. See page 114.

These nucleo-proteins, which are present in many protein foods, particularly glandular meat, tongue, and beef, are hydrolyzed during the process of gastric digestion into protein and nuclein. The latter, which may be considered as a simpler nucleo-protein, splits again during pancreatic digestion into protein and nucleic

acid. During intestinal digestion the nucleic acid is further broken up into its constituent parts referred to above.

In discussing muscle metabolism, creatinine was referred to as a product of the endogenous metabolism of the living cell. Its production is similar to that of the endogenous uric acid, but in direct contrast to that of the exogenous uric acid, urea, ammonia and amino acids, which are products of food metabolism. This fact, demonstrated by Folin, accounts for the inverse ratios found to exist between the excreted creatinine nitrogen and other protein nitrogen — that is, on a low protein diet Folin has shown that creatinine nitrogen increases in proportion to the total nitrogen eliminated, while other protein nitrogen decreases. In consequence, the creatinine nitrogen in the body fluids is remarkably constant for the individual in normal conditions and quite independent of protein intake. Although it has been shown that an increase of creatinine nitrogen in the urine does not follow from a high protein diet, an increase has been observed in various diseases, as typhoid fever, tetanus, pneumonia. In cases of anemia, advanced nephritis, and paralysis, the creatinine has been found to decrease.

Protein metabolism, as we have considered it, has been wholly from the angle of *nitrogen* metabolism. A study of nitrogenous metabolism is the best index of protein metabolism, because proteins contain a fairly uniform amount of nitrogen while there is no uniformity in the amounts of other elements present, such as sulphur. Also, simpler and more accurate methods for nitrogen determinations are available. However, since sulphur is a common constituent of a large group of proteins, we shall add a few facts regarding its metabolism.

In general, the sulphur is split off from the protein and becomes hydrogen sulphide which is oxidized to sulphuric acid. The sulphuric acid eliminated may be divided into that which combines with inorganic elements present and is thrown off as inorganic sulphates, and that which unites with organic compounds as phenol and indol and is classed as ethereal sulphates. Then, as a third way in which sulphur is eliminated, may be added unoxidized or neutral sulphur, which corresponds closely

to the creatinine of nitrogen metabolism. It seems to come from cell metabolism.

The inorganic sulphates compose the largest part, while the ethereal sulphates are of particular interest as they are the products of the excess amino acids and putrefying proteins in the intestine combined with sulphuric acid. (Page 201.)

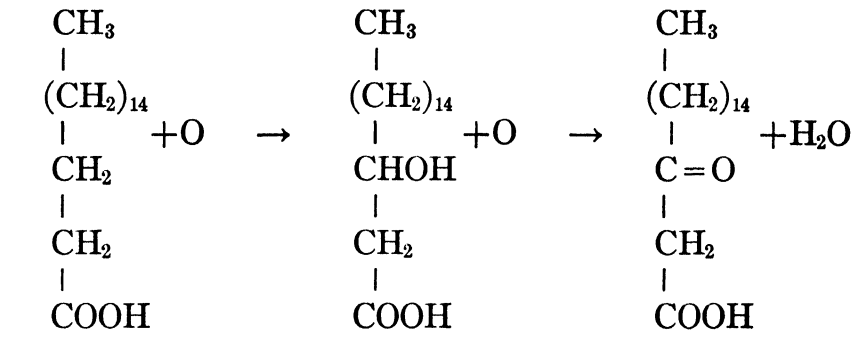
Metabolism of Fats.

In the intestine, by action of the pancreatic lipase and the intestinal lipase, the fats are split into fatty acids and glycerol, and as such they are absorbed. The absorption is greatly helped by the presence of the bile salts. In part at least they are resorbed with the fatty acids. After the absorption, a resynthesis between the glycerol and fatty acid takes place in the cells of the intestinal wall, forming neutral fat. What happens to part of this neutral fat seems to be unknown, but about 60 per cent is carried, in a state of fine emulsion, in the lymphatics up to the jugular vein, where it enters the blood stream. According to Taylor, the neutral fat formed in this way is not the fat characteristic of the species, but is the same as that ingested. This statement he demonstrates by feeding a starving dog mutton fat, whereupon the dog lays on the fat peculiar to sheep, instead of making dog fat. As the neutral fat passes into the blood stream it apparently enters into a complex combination, in which state it is soluble and diffusible. In this condition it is carried to the tissues which need it, and any excess is stored in the fat reservoirs of the body. If it is stored directly, as in the case of the dog cited above, it will be of the same kind as that ingested; if further splitting takes place and the fat is resynthesized from its elemental constituents derived either from fat or from sugar, human fat or fat characteristic of the species is produced. This in turn may combine in various ways, forming some of the complex lipoids present in the body tissues.

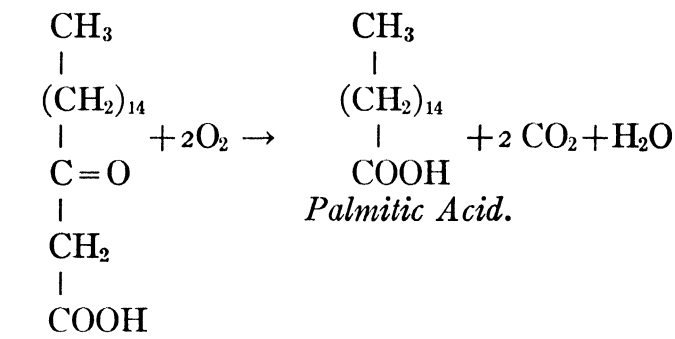
Fat is the fuel of the human machine, and by its oxidation heat is produced. The combustion of fat seems to take place first by hydrolysis of the fat into fatty acids and glycerol, and

then by oxidation of the fatty acids and conversion of the glycerol into glucose.

In the process of oxidation the acid seems to split each time at the β-carbon atom:

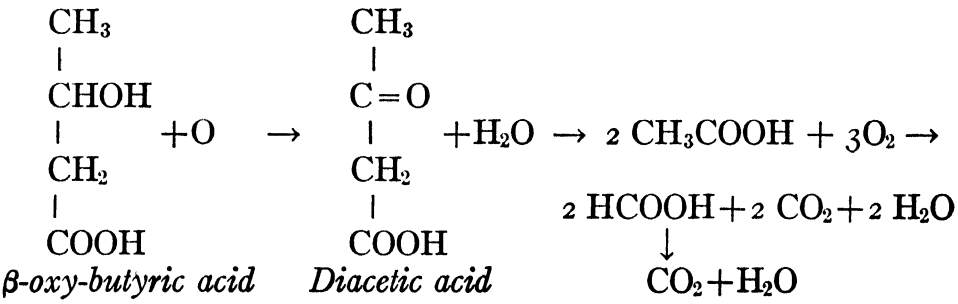


Stearic Acid



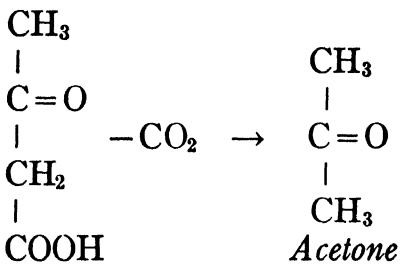
Palmitic Acid.

The oxidation continues as shown above, yielding carbon dioxide and water as end-products until *butyric* acid is formed. This by oxidation gives diacetic (aceto-acetic) acid, which in turn is oxidized to formic acid and finally to carbon dioxide and water. At the same time a small part of the diacetic acid, through loss of carbon dioxide, is converted into acetone:



β-oxy-butyric acid *Diacetic acid*

Then to a limited extent



Acetone

Under normal conditions by far the greater portion of the diacetic acid is oxidized, as shown by the first reaction. When there is insufficient oxidation in the system, or when the metabolism is perverted as in diabetes, a greater proportion of diacetic acid forms acetone, and acetone is excreted in the urine. The normal oxidation of diacetic acid may be considered as in some way interdependent upon the normal oxidation of glucose.

FOODS.

We have discussed in considerable detail the composition of body fluids and tissues, and have found them to contain protein, carbohydrate and fat, salts and water. The food must of necessity, then, supply at least these substances; and as a matter of fact we know that two additional food substances are essential to the proper utilization of those mentioned, the vitamines to produce growth and maintain health, and a certain amount of more or less indigestible material classed as bulk, which serves to prevent undue concentration of the nutritive portion. We may then classify food substances according to the purposes they serve as follows:

Proteins — Tissue Repair
Carbohydrates — Energy
Fats — Heat
Water — Distribution during Metabolism
Mineral Salts — Regulation and Activation
Vitamines — Growth
Bulk — Distribution during Digestion.

We have suggested the dual nature of metabolism, resulting in the maintenance of heat and the repair of tissue, but we have come to accept the measure of food value as expressed in terms of heat production alone. This method may not be ideal, but as yet we have no unit of value which will measure the usefulness of all kinds of food material. The unit generally used is the calorie, which may be defined as the degree of heat necessary to raise one kilo of water one degree centigrade, and is a thousand times as great as the small calorie (seldom used).

Of the above-named groups the first three contain the foods which furnish calories. The caloric value of fats is higher than that of the other two, the combustion of 1 gram yielding a heat equivalent of 9.3 calories, while a gram of either pure protein or pure carbohydrate will furnish only 4.1 calories. These figures are not absolutely accurate, because of slight discrepancies be-

tween the combustion of metabolism and the combustion of the calorimeter, but they are accepted as the basis for computation.

The amount of these foods required differs with the individual, depending primarily on the age, sex, weight and work of the person. An average adult male doing average work, neither wholly sedentary nor wholly muscular, will require perhaps 2500 calories per day. This should be made up of a "balanced" diet consisting approximately of 80 grams of protein, 120 grams of fat, and 300 grams of carbohydrates. The digestibility and adaptability of food should also receive careful attention, but as this is largely a matter of individual peculiarities tables and rules are impractical. During the period of growth the body naturally demands a somewhat greater proportion of protein, as new tissue is being formed constantly, in addition to the waste which is being repaired.

In considering the caloric value of food, an essential point, and one frequently overlooked, is that some foods furnishing an equal number of calories vary widely in their real nutritional value to the individual. This is particularly true of the protein content of certain foods. For example, 63 per cent of the protein of milk is available, while with cereal protein only 20 to 26 per cent is available.

Although not yielding many actual calories, the other constituents of the diet as grouped under the remaining four headings are hardly less important than those having caloric value.

As just suggested, among the sources of protein food, which is essential for tissue building, we may consider milk as one of the most important. During the first few months of life it is an amply sufficient source not only of protein but of all other foods. In adult life a milk diet is hardly sufficient for the normal individual, and proteins from other sources must be used.

Food proteins may be classed as being derived from two sources, animal and vegetable. As already suggested above those derived from animal sources are much better adapted to human needs than those from vegetable sources, because of the incomplete character of the amino acid content of many of the vegetable proteins. To-day we believe that the human organism

cannot synthesize many of the most important amino acids, (page 111), and if the protein is lacking in these it follows that the system will suffer in consequence. Therefore, contrary to the vegetarian's ideas, a certain amount of animal protein is very desirable for the adult. However, an excess of protein is not desirable, and as has already been shown on page 226, when protein metabolism was discussed, such excess may give rise to conditions of high acidity and high uric acid and the production of toxins.

Little need be said about the sources of our carbohydrate food. The cereal grains and vegetable tubers furnish the largest quantity; cow's milk contains about 4 per cent, human milk about 6 per cent of lactose.

Fats are obtained from both animal and vegetable sources and, as in the case of proteins, the fat from milk, eggs, and other animal sources is rather more desirable for the organism. With fats, however, the advantages lie not in the actual composition of the fat but in the fact that those fats mentioned above contain the fat-soluble vitamines.

Mineral Salts. — A well-balanced diet will furnish the proper amounts of mineral solids (excepting perhaps sodium chloride); but all diets are not balanced and it is well to know what part the various salts have in maintaining the health of the individual.

Sodium chloride is essential to digestion. It has been repeatedly demonstrated that if sodium chloride is withheld hydrochloric acid will not enter the stomach. Excess of sodium chloride may cause irritation or place an undue strain upon weak or diseased kidneys and in such cases should be avoided; on the other hand, acidosis usually results from a salt-free diet.

Potassium salts are said to keep the tissues soft and pliable, to prevent hardening of the arteries, etc., but potassium salts may cause a diminution of necessary sodium according to Bunge (Physiologic and Pathologic Chemistry, 2nd Edition), who says that potassium salts will react with sodium chloride in the system, forming potassium chloride and undesirable organic sodium salts, both of which are eliminated by the kidneys, and thus cause loss of sodium.

Tibbles quotes Cahn in *Zeit. f. Physiol. Chem.*, in practically the same statement.

Calcium salts. — *See Vitamines.*

Magnesium occurs generally distributed in the system, the bones containing about one per cent. By increasing the amount of magnesium ingested, the percentage in the bone may be increased but it does not take the place of calcium. The compounds of magnesium are generally more soluble than those of calcium. Magnesium oxide, as milk of magnesia, is used extensively as an antacid. An excessive amount, however, may act in removing necessary calcium in just the same way that potassium acts in removing sodium, as indicated by the following from Pickerills' 'Prevention of Dental Caries and Oral Sepsis,' page 144.

"Weiske's experiments also support these findings. For instance, of two rabbits, one received one gram $CaCO_3$ daily in addition to its food; the other one gram of $MgCO_3$ for three months. The rabbits were then killed, and it was found that, although they were of equal body-weight, the total weight of the bones (dried and fat-free) in the first rabbit exceeded that of the second rabbit (77.45 grams: 69.52 grams); and, further, that the amount of organic matter in the bones of the $MgCO_3$ rabbit was in excess of that in the $CaCO_3$ rabbit."

Iron is an essential constituent of blood, derived from food, and perhaps more than in the case of any other mineral constituent, it is necessary for iron to be taken in natural organic combination.

Phosphates are essential for the development of all cellular tissue. Phosphates are credited with preventing the deposition of uric acid by the reaction given on page 59, also with keeping calcium oxalate in solution. Phosphate acts beneficially in the bowels by slightly stimulating the peristaltic action.

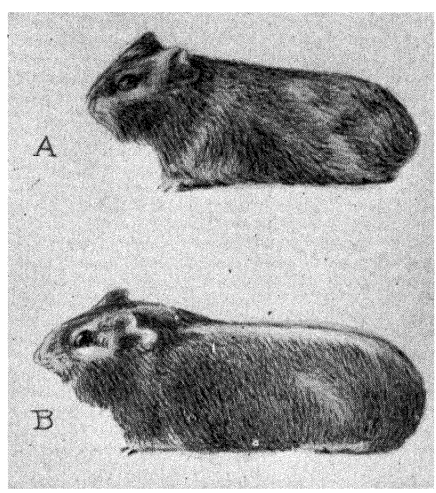
Iodine occurs in the ductless glands, and is apparently necessary for their best development, although this fact has been seriously questioned.

It is impracticable to give tables of food composition, but the following may be noted:

Strawberries, beans, and potatoes are rich in potassium

compounds; beets, spinach, turnips, and cherries are rich in sodium salts; milk, oranges, turnips, and parsnips are rich in calcium oxide; almonds and walnuts are rich in magnesium oxide; carrots and rice are rich in iron; meat, cheese, beans, eggs, and wheat are rich in phosphates; cocoa powders, rhubarb, and spinach, are rich in oxalates.

Vitamines. — There are recognized to-day four very important food constituents, which are called vitamines for the want of a better name. The substances are vital in their importance, but it is very doubtful if they contain nitrogen in any form to warrant the use of "amine" as part of the name. The term "food accessories" has been sometimes used. Three of these substances are specifically known as fat-soluble A, water-soluble B, and water-soluble C, and a fourth vitamine has recently been discovered by E. V. McCollum. The fat-soluble A vitamine, first recognized by McCollum and Davis, occurs in cod-liver oil, yolk of egg, milk-fat (butter), to a less extent in other animal fats, and in slight amounts in vegetables and cereals. It is anti-rachitic and essentially growth-promoting in its action.



Permission Dr. P. R. Howe.

FIG. 18.

A and B were twin-sister guinea-pigs. Water soluble C was withheld from the diet of A while B had an unrestricted diet. The illustration shows the stunted growth of A.

The water-soluble B vitamine is antineuritic and occurs in fresh green vegetables, yeast, milk, whole cereal grains, and to some extent in lean meat.

The water-soluble C is the antiscorbutic vitamine and occurs in the juice of citrus fruits, particularly oranges, lemons, and limes. It is also found in considerable quantity in fresh cabbage and tomatoes.

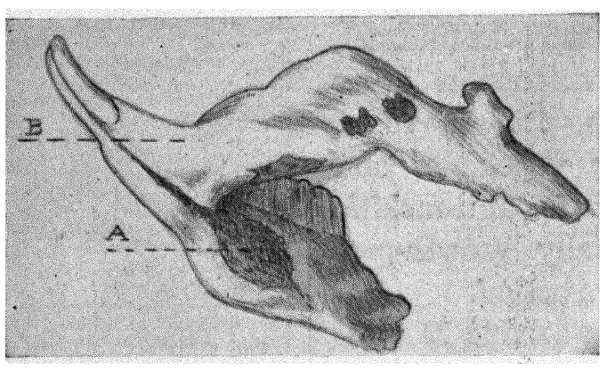
Water-soluble C is much more easily destroyed by heat than

either of the other two. Canned tomatoes, however, contain the vitamine in such proportion that they are usually recommended, in absence of fresh orange juice, for infant feeding.

Dr. Percy R. Howe has shown the intimate relationship between this antiscorbutic vitamine and the proper utilization of calcium in the formation of teeth and bone and its consequent importance in the prevention of caries.

Dr. Howe found that he could produce, at will, decalcification or recalcification of the bones of guinea pigs, by simply withdrawing or restoring vitamines in the diet, calcium salts always being given in abundance. Fig. 19.

Hart-Steenbock and Hoppert* have shown (*J. Biol. Chem.*, September, 1921, page 33) that the antiscorbutic vitamine, as contained in orange juice, fresh green vegetables, etc., is without effect on calcium metabolism. We must therefore regard Dr. Howe's experiments as showing that lack of antiscorbutic vi-



Permission Dr. P. R. Howe.

FIG. 19.

A indicates softened, decaying bone produced on a diet lacking in water soluble C. B indicates the new calcification formed on top of the softened bone when the water-soluble C vitamine is included in the diet.

tamine produces non-calcification only as it allows the development of scurvy.

Hart-Steenbock and Hoppert have also shown in the above article that fresh green oats, or oat-hay dried out of direct sunlight, and cod liver oil will increase calcium metabolism.

* These experiments were performed with goats, animals naturally immune to scurvy, and the effect of the vitamine was thus studied independently of the disease.

While the water-soluble C vitamine has a marked influence on calcium metabolism of animals (including human) subject to scurvy, it has also been shown (Bogart and Trail, *J. Biol. Chem.* LIV, October, 1922, page 39) that yeast or pure butter-fat (water-soluble B and fat-soluble A) added to basal diet would decrease calcium excretion, i.e., aid in retaining calcium in the system. On page 386 of the above Journal, Bogart and Kirkpatrick conclude that calcium is more apt to be retained on a base-forming diet than on either a balanced or an acid-forming diet.

In connection with calcium metabolism it is worthy of note that " children do not seem to utilize the calcium of vegetables as efficiently as they do that of milk. Calcium balances were more variable and always less favorable when vegetables replaced about half the milk as sources of calcium." This paragraph is from " Calcium and Phosphorus Metabolism in Childhood," by H. C. Sherman and E. Hawley (*J. Biol. Chem.*, August, 1922, page 398).

Dr. E. V. McCollum has recently shown that the vitamine most essential to the proper utilization of calcium is neither A, B, nor C, but a fourth vitamine, D, occurring for the most part with the fat-soluble A but not identical with it. This, of course, raises a question as to whether the fat-soluble A vitamine possesses antirachitic properties. Dr. McCollum found this fourth vitamine to be present in considerable quantities in cod-liver oil, in lesser quantities in butter-fat and in small quantities in cocoanut oil, differing in this last source from fat-soluble A, which does not occur in cocoanut oil.

Bulk. — Unless the diet includes considerable bulk — undigested cellulose — the material in the intestine becomes too concentrated, peristaltic action is decreased, and the action of enzymes is retarded. This condition favors the production of toxic substances in the intestine and gives rise to all the evils resulting from constipation.

To furnish bulk, fruit, vegetables as lettuce, spinach, carrots, celery, etc., are advised and such cereals as contain considerable bran.

A well-balanced diet and sufficient out-door exercise are two of the greatest factors contributing to health. The diet correct for one individual may not be correct for another, but in general the diet most beneficial to the average person is one low in protein and rich in carbohydrate foods, with an abundance of vegetables, fruits and milk.

Acid-Forming and Base-forming Foods.

One of the reasons for the above recommendations is the desirability of producing a tendency toward alkalinity in the system rather than a condition favoring acidosis. Most vegetables and fruits are alkaline-forming foods; that is, the final products of metabolism are alkaline. On the other hand, meats, bread and pastries are acid-forming. Prunes and cranberries are two fruits which do not follow the general rule but are acid-forming. This is because they contain benzoates which give rise in the system to hippuric acid. One of the most strongly alkaline-forming foods is potatoes.

CHAPTER XXI.

RELATION OF SYSTEMIC TO ORAL CONDITIONS AND THEORIES OF TOOTH DECAY.

The establishment of relations between oral and systemic conditions is, of course, the goal toward which the dentist's study of physiological chemistry should be directed.

It has been too much the practice to study a single relation and jump at conclusions without regard to co-relation of factors which may not appear to be closely allied but which nevertheless exert important influences. Witness the effort to establish the relationship of tartar deposition to calcium content of the saliva without considering the quantity of carbon dioxide present or the fact that certain colloidal substances (such as occur in saliva) may prevent precipitation of calcium salts.

The relations of potassium sulphocyanate to dental caries, and other problems, have been studied in much the same way, and the object of this chapter is to emphasize the necessity of getting all possible viewpoints on a given question before attempting to draw positive conclusions regarding it.

History has repeatedly shown us that early, and frequently discarded, theories have ultimately been proven to be of more or less value; hence, a brief review of theories bearing on our subject will form a part of this chapter. The *history* of these theories is also valuable for its own sake.

Dr. J. P. Michaels, in a review of this subject, tells us that "in 1835 Dr. A. Donne published in French a study on the chemical characteristics of saliva considered as a means of diagnosing certain diseases of the stomach. This was a very interesting special study, but chemistry was not sufficiently advanced to throw enough light on these questions."

In 1884 Dr. Binet published in Paris a thesis on saliva, in which he noted "a correlation of biochemical principles between

the sweat and the saliva." The importance of such "correlation" does not to-day appeal to us as great, compared with some other facts which Dr. Binet presented, as for example the determination of the *density* of saliva or an idea as to the twenty-four-hour amount. Dr. Binet also confirmed the previously demonstrated fact of the presence of urea as an organic salivary constituent.

In 1900 Dr. J. P. Michaels presented to the Third International Dental Congress in Paris a paper on the "Application of the Analysis of Saliva to the Diagnosis of Oral Disease," and gave us what is perhaps the first "relationship" worthy of serious consideration. Dr. Michaels classified people in general as (a) "Adiathetic" i.e., without indication as to future conditions of oral disease, (b) "Hypoacid" individuals who he said were liable to have trouble from carious teeth, and (c) "Hyperacid" individuals with a tendency to immunity from caries. His work led him to believe that the hyperacid condition was characterized by a high thiocyanate content of the saliva and a correspondingly low ammonia, while in cases with hypoacid tendencies the opposite condition prevailed.

A considerable number of the leading dentists of this country took up this single test of high or low sulphocyanates, gave it a place of first importance in the prognosis of dental disease, made hundreds, probably thousands of tests and experiments, including the administration of sulpho-cyanate in tablet form to *overcome* the carious tendency, and apparently forgot all about the more important question of hypoacidity and hyperacidity, of which the thiocyanate was only supposed to be indicative. We know now that there are much better methods of determining the systemic acidities than a qualitative sulphocyanate test, but let us keep in sight the original divisions made by Dr. Michaels, because the markedly hypoacid and hyperacid salivas are without doubt indicative of systemic conditions.

Whether or not hyperacidity is accompanied by immunity to dental decay has not been demonstrated, but the condition is consistent with pyorrhea and in many cases with erosion. As a rule, the more advanced the pyorrhea the more pronounced the

hyperacidity. This does not argue for any etiological relationship between the two conditions; in fact, it is *very* improbable that hyperacidity ever caused pyorrhea and more improbable that pyorrhea ever caused hyperacidity; but the fact is that the hyperacid condition is the one favorable to the *development* of pyorrhea. Dr. Michaels described hyperacidity as a condition of insufficient oxidation, and therefore one that is often accompanied by the appearance of oxalates, acid lactates and urates (so called "acidic principles") in the saliva. The discussion of soluble salts of the saliva is taken up later; and pyorrhea, as usually accompanying a condition of slight acidosis and consequent poor oxidation, has now been conceded by nearly everyone. These facts are of value to the dentist because from a few saliva and urine tests he can easily get some idea of the systemic condition of the patient. A patient of this type is very liable to show a considerable salivary acidity together with a high urinary acidity. Increased indoxyl, high ammonia and frequently high uric acid in both saliva and urine are usually found.

As a rule, the insufficient oxidation indicated by such analyses is benefited not by more oxygen necessarily, but by modifications of diet and improved habits of living which will tend toward the correction of the systemic acidosis. This treatment combined with the local treatment is of far more permanent value than local treatment alone.

Glycosuria and Pyorrhea.

A condition of glycosuria is an indication of low oxidation; therefore we should expect, perhaps, to find conditions of this kind more or less associated with pyorrhea. Let us bear clearly in mind, first, that glycosuria and diabetes are not synonymous terms, and while diabetic pyorrhea has upon occasion been suggested as one of the types of the disease, it is doubtful if there is any direct relationship between the two disorders.

From the examination of urine from more than sixty patients attending the pyorrhea clinic at the Harvard Dental School we obtained only one case of undoubted diabetes, while about

fifteen of the patients had alimentary or renal glycosuria. That this proportion is larger than normal is shown by some figures obtained from the Life Extension Bureau of New York. These and also some Community Hygiene Experiments conducted at Framingham, Mass., by the National Antituberculosis Association, showed less than 3 per cent of renal glycosuria among supposedly healthy individuals. We conclude, therefore, that in this 3 per cent the sugar in the urine is incidental while in the pyorrhea patients it is an indication of *systemic acidosis.*

Pyorrhea is a disease of lowered resistance and reduced vitality, whatever the initial cause may be (malocclusion if you choose). The gums are " end organs," physiologically considered, and as such are among the first to suffer from diminished blood supply. Dr. Walter Cannon has demonstrated that where there is diminished blood supply the fatigue products of metabolism are likely to be found. These, as we know from our study of physiological chemistry, include lactic acid and di-hydric-phosphate. This is only a statement of two and two; whether or not they make four is yet to be proven.

Sugar and Tooth Decay.

Caries is a disease of childhood, and there is an undoubted relation between the decaying teeth of children and *excessive* use of carbohydrates; but the relation is an indirect one and often resolves itself into a case of malnutrition, which might be produced by fine white bread as well as by sweets, provided the bread was taken in the same way i.e., between meals and whenever opportunity offers. Any food taken in this way " spoils the appetite " for regular meals, with the result that less vegetables are eaten. The consequences are disorders of digestion, intestinal stasis, lack of vitamines, and consequent lowered resistance to bacterial activity or other factors producing tooth decay.

Miller's Lactic Acid Theory.

Miller's theory that all tooth decay was a matter of decalcification of tooth substance, caused by lactic acid produced from acid-forming bacteria growing on carbohydrate débris,

furnishes the starting point for our present theories. Granting that decay is due to bacterial activity of some sort, we have to include a study of conditions favorable for bacterial growth and a study of conditions favorable to immunity, i.e., resistance.

Among the conditions favorable for growth of acid-forming bacteria, the hydrogen-ion concentration is of prime importance, but must be considered in connection with the carbohydrate content and the presence of so-called " buffer substances," e.g., alkaline phosphates.

Dr. Lawrence F. Foster has shown in the *Journal of Bacteriology*, Vol. 6, 1921, that for the growth of streptococcus hemolyticus (which will produce lactic acid from glucose) a P_H of 7.6–8.1 is optimum concentration, and that a medium containing 1 per cent glucose and 1 per cent potassium phosphate (K_2HPO_4) is best suited for acid production. Figures also quoted as optimum P_H for streptococcus viridans, which has been repeatedly found in cavities of decayed teeth, are 7.6, 7.8, and 6.8, and we also know that these acidities are within the range of ordinary salivas.

It is safe to say that a number of acid-producing bacteria would be influenced in similar ways and by similar conditions. We know that in many mouths, and in restricted areas of many more, carbohydrates occur in various concentrations aside from the carbohydrate constituents of the saliva, glycogen (?). Also, we know, as Dr. Howe has shown in an article to which previous reference has been made, that phosphates increase the growth of bacteria in the mouth. Considering, then, the probable carbohydrate content of the saliva together with the fact that the presence of phosphates tends to increase the alkalinity of the saliva, thus making a more favorable medium for bacterial growth, we must at least admit a possible relationship between these factors and tooth decay. We know that in our own experience cases of marked decay have usually been accompanied by a P_H higher than normal, while as a rule the lower P_H has been found in cases of pyorrhea and erosion.

Dr. Percy R. Howe's Theory of Tooth Decay.

Dr. Howe, after years of experiments at the Forsyth Infirmary, concludes that the lactic acid theory of Dr. W. D. Miller is erroneous. He claims that sugar and local fermentations are of secondary importance and that the prime cause of tooth decay is malnutrition, and lack of proper mastication. He has shown that the lines of lowered resistance can be plainly traced in many cases and that they extend from the inside, the pulp chamber, outward toward the enamel. As the author understands Dr. Howe's theory (after intimate discussion with him on the subject) he does not deny the part played by bacteria in breaking down the tooth substance but does maintain that the *initial* cause is as above stated, and bacterial action is incidental.

The author would differ with Dr. Howe in the use of terms, considering the lack of resistance a predisposing condition and the action of bacteria the immediate or exciting cause of dental caries.

Saliva and Blood.

That the constituents of saliva follow closely the constituents of the blood has long been a recognized fact, but that there is any relation existing between the quantities of the various constituents which are common to both has only recently been asserted. In the author's experience, however, there is a very direct relationship which may frequently prove of value to the dentist in detecting pathological conditions. The percentages of the constituents present, of course, vary, the blood containing a much higher concentration of most substances than the saliva, but the rise and fall of many substances in the blood is accompanied by a similar change in the saliva. This has been found to be particularly true of urea nitrogen, creatinine, and uric acid. An analysis of nephritic saliva will invariably show these substances high, following the same curve as the blood analysis.

Aside from the point of view of nephritis, which may but seldom come to the dentist's attention, the uric acid content of the saliva, although of very recent investigation, seems now at least to be one of the most valuable from a diagnostic viewpoint.

In every case of apical infection or pus absorption which has come under the author's observation recently (about fifty cases), the uric acid content of the saliva has been double or more than double what it seems to be in people with perfectly healthy mouths. The determination is so simple and the results so far have been so consistent that it would certainly seem to be one of the most promising new suggestions in regard to salivary analysis. In an article by R. N. De Niord and B. J. Bixby on focal infection,* the following conclusions are drawn in regard to blood uric acid, and since the saliva curve seems to follow the blood curve as a rule, it is reasonable to suppose that these same conclusions may be applicable to saliva.

" 1. High uric acid value in the blood is indicative of nuclear degeneration which in turn may mean focal infection.

" 2. Other factors productive of high uric acid, aside from nuclear degeneration, are comparatively easy to determine, i.e., leukemia, primary anemias, cachexias† from whatever cause and massive doses of x-ray or radium.

" 3. Elimination of all foci of infection invariably is followed by a return of the uric acid to normal.

" 4. Failure to eliminate all foci will prevent the return to a normal uric acid, and this substance therefore furnishes a reliable index to the complete elimination of foci of infection."

In another paragraph of this paper we read

" The presence of a high blood uric acid does not of course point to any particular part of the body as its source. Hence the diagnosis of focal infection through this medium does not indicate that the teeth or tonsils or any other organ is at the bottom of the

* Studies in Focal Infection. The Journal of Laboratory and Clinical Medicine, Vol. VII, No. 10, July, 1922.

† Cachexia means any severe diseased condition — nephritis and gout are perhaps the two most common ones to be eliminated.

disturbance. But it does point to the necessity for a thorough search for all possible primary foci, and it gives definite authority for the removal of such foci when found."

In the author's experience, cases of pyorrhea, where there is pus absorption, invariably show high salivary uric acid, and cases of abscessed teeth and sinus infection have been diagnosed and verified by the high uric acid content of the saliva.

The value to the dentist of being able to determine from the saliva the approximate normality or abnormality of the blood, as regards these three constituents, and of thus being able to detect pathological systemic conditions, is self-evident. Furthermore, the determination of the possible direct relationship between high uric acid in the blood, as well as in the saliva, and pus absorption is of unquestionable value.

Polarized Light Pictures and Oral Conditions.

Of the soluble salts which may be found by evaporation of a drop of clear urine or of dialyzed saliva, the majority have not been identified with sufficient certainty, or else their relation to definite conditions of any sort has not been sufficiently established to make the examination particularly valuable as yet. However, a few statements regarding our findings may help as a basis for further investigation.

The test is very simple and in the author's experience it has seemed worth while to make it, and some interesting results have been obtained. The alkaline chlorides, sodium, potassium, and ammonium, are usually present in saliva; various forms of phosphates and frequently soluble oxalates are found in the urine. Crystals consistent with lactates are more rarely observed, and these have sometimes led to the detection of lactates by chemical means. Dr. E. C. Kirk suggested that the soluble oxalates in the urine may antedate the appearance of calcium oxalate and thus give warning of an undesirable metabolic process.

The presence of oxalates, sometimes in the saliva and more frequently in the urine, has in our experience gone hand in hand

with a condition of nervousness of the patient and of an acidosis more or less pronounced.

Lactates in the urine are indicative of insufficient oxidation by endocellular enzymes and have occasionally given evidence

FIG. 20.

The long needle-shaped crystals resemble lactates and the round ones are consistent with sodium oxalates.

of faulty liver action. The accompanying photomicrograph (Fig. 20), obtained from a drop of urine, indicated the presence of lactic acid, which was subsequently proven by careful chemical tests.

EXPERIMENTS.

Organic Chemistry.

Experiments with Carbon and Hydrocarbons.

Exp. 1. *Carbon as a decolorizing agent.* To 25 or 30 c.c. of a dilute solution of aniline color, contained in a small beaker, add a teaspoonful of bone charcoal. Heat to the boiling-point, rotate or stir thoroughly for a few minutes, and filter.

Exp. 2. *Absorption of metallic salts.* To 25 c.c. of solution of lead acetate of such strength that H_2S water gives marked color but no precipitate, add a teaspoonful of bone charcoal and treat as in preceding experiment. Test the filtrate with H_2S water and note whether lead has been removed.

Exp. 3. Perform an experiment with a view to determining whether bone charcoal will absorb H_2S from H_2S water.

Exp. 4. Repeat any one of the three preceding experiments, using wood charcoal in place of bone charcoal. Does the wood charcoal work as well as the bone charcoal in the absorption of color or other substances? How does bone charcoal differ in composition from wood charcoal?

Exp. 5. Arrange apparatus as shown in Fig. 21. To the boiling flask (*B*) provided with a thermometer registering 200° C. connect a beaker condenser, *C*, immersed in ice water. In this apparatus distil slowly 25 c.c. of crude petroleum until at least four fractional products are obtained, with boiling points differing by at least 15°. Compare the physical properties of the distillates thus obtained.

Exp. 6. Charge an ignition-tube with dry "marsh-gas mixture," found on side shelf (consisting of $NaC_2H_3O_2$, NaOH, and CaO_2H_2). Fit with a delivery tube and collect two small bottles of the gas over water.

$$NaC_2H_3O_2+NaOH=CH_4+Na_2CO_3.$$

Test the inflammability of this gas. Notice the odor.

Exp. 7. Mix carefully in a test-tube 2 c.c. of alcohol and 8 c.c. of strong sulphuric acid. Heat gently and notice odor of gas. Fit a bent glass tube to the test-tube and collect over water a test-tube full of the gas. To this apply a flame. Note the color of the burning gas.

$$C_2H_5OH-H_2O=C_2H_4.$$

Exp. 8. Collect a test-tube full of ethylene (Exp. 7), add a few c.c. of dilute permanganate solution and shake. Then

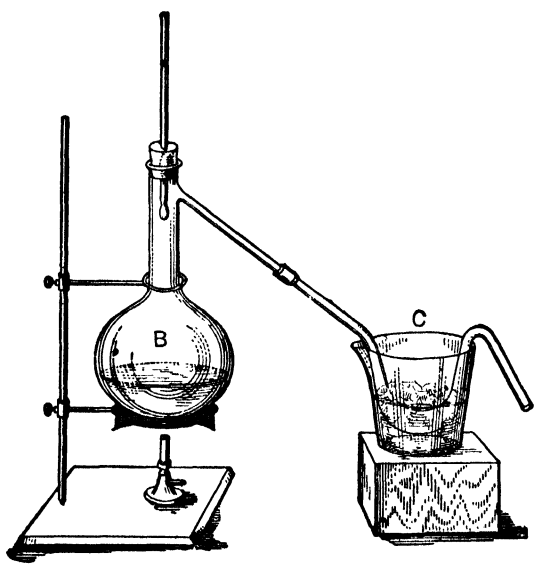


FIG. 21.

repeat, using marsh gas in place of the ethylene (test for unsaturated hydrocarbons).

Exp. 9. Shake together, in separate test-tubes, small quantities of petroleum and sulphuric acid in one tube, and petroleum and nitric acid in the other. If no action results, mix contents of the two tubes and shake again. Explain any change or absence of change which may be apparent.

Exp. 10. In a small generator (see model) place a few small pieces of calcium carbide (CaC_2), add strong alcohol through the funnel tube till the lower end of the tube is "sealed." Now

add very slowly a little water till a brisk evolution of gas is obtained. Collect over water two or three test-tubes full of the gas. (Acetylene.)

Test with a lighted splinter. Note odor of gas cautiously, as it is poisonous when inhaled in quantity.

$$CaC_2 + 2\ H_2O = Ca(OH)_2 + C_2H_2.$$

Exp. 11. Conduct a little of the acetylene gas into an ammoniacal cuprous chloride solution.* What is the red precipitate?

Exp. 12. If the evolution of gas (Exp. 11) has not been interrupted the delivery tube may be replaced by a short tube drawn out to a fine point, and the gas ignited. Note color of flame. If it smokes badly, explain the reason for it.

Experiments with the Halogen Derivatives of the Hydrocarbons.

Exp. 13. Place in a test-tube a little bleaching-powder, cover with strong alcohol and heat the mixture to boiling. Notice carefully the odor of the vapor produced and compare with a little chloroform ($CHCl_3$) from side shelf.

$$4\ C_2H_5OH + 8\ Ca(ClO)_2 = 2\ CHCl_3 + 3\ \underset{\text{(Formate of Ca)}}{Ca(CHO_2)_2} + 5\ CaCl_2 + 8\ H_2O.$$

Exp. 14. Heat 1 c.c. of chloroform with about 5 c.c. of one per cent NaOH. Test a portion of the resulting solution for inorganic chlorides. Distil the remainder of the solution and test the distillate, collected in a test-tube, with litmus paper.

Exp. 15. Place in a test-tube about 1 gram of crystallized carbonate of sodium, about half as much iodine and 1 or 2 c.c. of alcohol. Now add 10 or 15 c.c. of H_2O and keep the mixture at moderate heat (not boiling) till the color of the iodine is discharged. Allow to cool; collect on a small filter paper some of the yellow crystals which have been formed and examine under the microscope. What are the crystals? (See Plate III, Fig. 6.) Explain their relation to marsh gas.

* See Appendix for preparation of reagent. This test is characteristic of the triple-bonded hydrocarbons.

Exp. 16. Cover one or two small pieces of calcium carbide, in a small porcelain dish, with a mixture of three parts water and one part alcohol. While the gas is being evolved hold over the mixture a test-tube full of chlorine.

Exp. 17. *Oxidation of chloroform.* If chloroform is oxidized by heating a few drops with a crystal of potassium dichromate and 2 or 3 c.c. of concentrated sulphuric acid, phosgene gas is produced, $COCl_2$. Note the odor *very cautiously* and write reaction.

Exp. 18. *Ethyl iodide from ethyl alcohol.* Place 2 1/2 grams of red phosphorus, and 20 grams of absolute alcohol in a small flask of 100 c.c. capacity. To this mixture add gradually 25 grams of finely powdered iodine, shaking the flask frequently and keeping it cool by allowing cold water to *run* over it. Connect the flask with an upright air-condenser and allow the reaction to continue for at least four hours. It may continue overnight without detracting from the value of the experiment. Then heat the flask over a water-bath for two hours, the air-condenser acting as a reflux condenser. After the reaction is completed, distil off the ethyl iodide. To facilitate distillation a rapidly boiling water-bath should be used, or a naked flame if used cautiously.

To obtain the distillate free from alcohol and the iodine which gives it its brown color, wash several times with water and then with a dilute solution of sodium hydroxide. Separate the colorless oil in a separating funnel, dry with a small piece of granular calcium chloride and redistil. The boiling-point of ethyl iodide is 72° and the yield should be about 25 grams.

Exp. 19. Prepare ethyl bromide from alcohol, potassium bromide and sulphuric acid as follows: Using the apparatus suggested for Experiment 62, place in the distilling flask about 30 c.c. of 50 per cent alcohol. Add slowly with constant agitation 30 c.c. of strong sulphuric acid. Cool thoroughly, then add 30 grams of powdered potassium bromide. Distil carefully until condenser is nearly full of distillate. Pour about a quarter of the product into excess of water. Shake well to wash the ethyl bromide. Remove from the wash water by means of a pipette

and dissolve in a little alcohol. Test this alcoholic solution for bromine with alcoholic silver nitrate.

To another portion of the ethyl bromide add 5 to 10 c.c. of alcoholic potassium hydroxide (5 per cent in absolute alcohol). Boil for a minute or two, dilute with water and make the usual qualitative test for bromides.

Write reactions.

Ethyl bromide may also be prepared by distilling a mixture of 1 part of alcohol and 5 parts of strong hydrobromic acid.

Exp. 20. *Preparation of dithymol-di-iodide* $(C_6H_2CH_3C_3H_7OI)_2$. Dissolve 1.5 grams of thymol in 30 c.c. of 6 per cent NaOH. It will take considerable time to dissolve the thymol and it may be necessary to add some stronger alkali. In a separate beaker dissolve 6 grams of iodine in 30 c.c. of water, using sufficient potassium iodide to put the iodine into solution easily. Then slowly and with constant stirring add the iodine solution to the thymol solution. A brown precipitate of thymol iodide results; this should be washed until there is no trace of alkali in the wash water, and dried at a temperature not exceeding 50° C.

Experiments with Alcohols.

Exp. 21. *The detection of water in alcohol.* Prepare a little anhydrous copper sulphate by heating a few crystals of $CuSO_4$ on a crucible cover until the water is driven off and a nearly white powder results. If this white powder is added to half a test-tube full of alcohol, the absorption of water, if present, will result in the reforming of the crystallized salt and a consequent production of blue color.

Exp. 22. Water may be separated from alcohol by saturating with potassium carbonate. To demonstrate this, take a mixture of alcohol and water, containing 15 or 20 per cent of alcohol, and add solid potassium carbonate until the salt will no longer dissolve. Agitate and allow to stand. Two layers will form, one consisting of alcohol, the other of the water solution of K_2CO_3.

Exp. 23. To about 75 c.c. of a 10 per cent glucose solution add a little yeast and allow to stand for twenty-four hours at a tem-

perature of about 37° C.; then distil by means of gentle heat, 10 or 15 c.c., and test distillate for alcohol by iodoform test, as given on page 250, Exp. 15. The production of CO_2 may also be demonstrated if the gases evolved during the fermentation are passed into clear lime water:

$$C_6H_{12}O_6 = 2\ C_2H_5OH + 2\ CO_2.$$

Exp. 24. *A test for methyl alcohol.* This test is applicable only to slight traces of methyl alcohol and may be made with a 1 to 2 per cent solution or with the first cubic centimeter of distillate from the substance suspected of containing methyl alcohol. Place 2 or 3 c.c. of very dilute methyl alcohol in a test-tube, heat a spiral of copper wire to white heat in a Bunsen flame and plunge immediately into the solution to be tested. Cool the contents of the tube by immersion in freezing mixture or ice water, and repeat the treatment with the hot copper wire. Cool again, and a third time introduce the hot copper wire. The copper spiral can be made by winding copper wire around a lead pencil, and should be of such a length that it is not wholly covered by the liquid in the tube.

This process serves to oxidize a portion of the alcohol to aldehyde. Now add to the solution which is being tested a few drops of a 1/2 per cent water solution of resorcinol and underlay the mixture with strong sulphuric acid. A pink ring will indicate the presence of methyl alcohol. The higher alcohols will give red or brown rings when similarly treated.

Exp. 25. Repeat Experiment 24, using ethyl alcohol in place of methyl alcohol.

Exp. 26. In 5 or 10 c.c. of absolute alcohol, dissolve 1/4 to 1/2 gram of metallic sodium. Test the gas given off.

Write reaction. Save the product.

Experiments with Aldehydes and Ketones.

Exp. 27. Mix about 1 c.c. of a very dilute solution of formaldehyde with four or five times its volume of milk in a test-tube. Keep at a temperature of 40 to 50° C. for half an hour, then carefully underlay the mixture with commercial sulphuric

acid of a specific gravity of 1.80. At the point of contact of the two layers of liquid, a violet-colored ring indicates the presence of formaldehyde. It is necessary that time be allowed for the casein of the milk to unite with the formaldehyde, also that the sulphuric acid should contain a trace of iron; this the *commercial* acid usually does. It is undesirable that the acid should be stronger than of 1.80 specific gravity; for, if it is, a *reddish-brown* ring may be formed, due to partial carbonization of the casein.

Exp. 28. To a very dilute solution of formaldehyde add a few drops of 1/2 per cent resorcinol solution and underlay the mixture with H_2SO_4 as in Exp. 24. The appearance of a violet ring will constitute a test for formaldehyde.

Exp. 29. To about 5 c.c. of a *strong* aqueous solution of potassium dichromate add a little sulphuric acid, then a few cubic centimeters of alcohol, and notice the odor of acetaldehyde produced by oxidation of the alcohol. Note also the reduction of the dichromate to $Cr_2(SO_4)_3$, as follows:

$$K_2Cr_2O_7 + 4\ H_2SO_4 + 3\ C_2H_5OH = K_2SO_4 + Cr_2(SO_4)_3 + 3\ C_2H_4O + 7\ H_2O.$$

Exp. 30. Test dilute solutions of acetone, formic and acetic aldehydes by Tollen's test for aldehyde as follows: Into a clean test-tube which has been rinsed with NaOH solution, place 5 c.c. of Tollen's reagent, add 10 c.c. of solution to be tested, shake; the silver is reduced, forming a metallic mirror on the inner surface of the tube.

To make Tollen's reagent, dissolve 3 grams of silver nitrate in 30 c.c. of ammonia water and add 3 c.c. of solution of sodium hydroxide.

Exp. 31. Prepare acrolein in each of the following ways:

1st: From glycerol according to the test given on page 84.

2nd: Oxidize one or two drops of allyl alcohol with potassium bichromate and H_2SO_4, as in the oxidation of ethyl alcohol in Exp. 29.

Exp. 32. To about 5 c.c. of an aqueous solution of chloral hydrate add a few cubic centimeters of strong NaOH solution and boil. Note odor of chloroform.

Exp. 33. *Isobenzonitril test for chloral or chloroform:* Place a few drops of a dilute chloral hydrate solution (or a small drop of chloroform) in a test-tube, add 5 c.c. of an alcoholic solution of alkali hydrate* (NaOH or KOH) and one drop only of fresh aniline oil. Heat till the mixture just begins to boil and note the odor of the nitril. — For reaction see test for chloroform, page 13.

Exp. 34. Test 2 or 3 c.c. of an aqueous solution of aldehyde with an equal volume of Schiff's reagent.

Exp. 35. *Preparation of Schiff's Reagent.* Into a dilute solution of fuchsin, pass sulphur dioxide gas until color is entirely discharged.

Experiments with Acetone.

Exp. 36. *Preparation of acetone.* Heat a few grams of dried calcium acetate in an ignition-tube, collect the distillate, which consists of an impure acetone. If this is mixed with a little water and filtered, part of the impurities may be removed, and the filtrate tested for acetone by the following experiment.

Exp. 37. Dilute the filtrate from the last experiment with distilled water; add a crystal of sodium nitroprusside. After the crystal is dissolved, add a few drops of acetic acid, and then an excess of ammonia water. A violet or purple color indicates the presence of acetone. Using a dilute solution of acetone in place of the alcohol in Experiment 15 on page 250, produce iodoform crystals by similar reaction with iodine and sodium or potassium carbonate.

Exp. 38. Acetone may be dissolved or mixed with water in all proportions; but, upon saturating the water with KOH, the acetone will form a separate layer which may be drawn off as in the separation of alcohol in Experiment 22, page 252.

Experiments with Ethers.

Exp. 39. Into a large test-tube put a little alcohol and about half its volume of strong H_2SO_4. Warm gently and notice the odor.

* If alcoholic potash or soda is not at hand, the test may be performed with 5 c.c. of alcohol and 1 or 2 c.c. of a 40 per cent aqueous solution of NaOH.

Ether is formed by two reactions. First, $C_2H_5OH+H_2SO_4 = C_2H_5HSO_4+H_2O$. Then the ethyl-hydrogen sulphate ($C_2H_5HSO_4$) is acted upon by a second molecule of C_2H_5OH, as follows: $C_2H_5HSO_4+C_2H_5OH=(C_2H_5)_2O+H_2SO_4$.

Exp. 40. Test the inflammability of ether by applying a lighted match to a few drops on a watch-crystal.

Exp. 41. Determine the solubility of water in ether by shaking 10 c.c. of ether with an equal quantity of water. Then turn the ether off into a dry test-tube, preferably allowing it to pass through a dry filter-paper first. The filtered ether may be tested for water by treating it with some anhydrous copper sulphate.

Exp. 42. Into a 10 c.c. graduate introduce exactly 6 c.c. of distilled water and 4 c.c. of ether. Close tightly (thumb may be used) and shake gently for a minute. Without uncovering tube, allow liquids to separate and read volume of water. Conclusion?

Exp. 43. Try the solubility of ether in each of the following: alcohol, benzene, petroleum ether, dilute hydrochloric acid, dilute sodium hydroxide.

Experiments with Organic Acids ($C_nH_{2n}O_2$).

Exp. 44. Introduce into a small flask (250 c.c. capacity) about 30 c.c. of anhydrous glycerin and an equal weight of oxalic acid crystals. Boil for several minutes; CO_2 is given off and a compound formed between the acid and glycerin; then, upon addition of more acid and continued heating, formic acid may be distilled. Collect about 10 c.c. of distillate; test reaction with litmus paper. Make silver-mirror test, described on page 254, Exp. 30. The silver solution will be reduced, but difficulty will be experienced in obtaining the mirror.

Exp. 45. To 5 c.c. of formic acid solution add 2 or 3 c.c. of dilute H_2SO_4 (1–5) and a little potassium permanganate solution; heat the mixture and conduct the gas evolved into a tube containing lime-water.

Exp. 46. From a mixture of formic acid, alcohol, and sulphuric acid, ethyl formate may be evolved in a manner similar

to that recommended in the production of ethyl acetate or butyrate (page 259). Compare the odors of these two ethers.

Exp. 47. To a dilute aqueous solution of acetone add potassium permanganate slowly until the mixture is permanently colored pink; filter, add dilute sulphuric acid and distil until 1 or 2 c.c. of distillate is obtained. This may be tested for acetic acid by litmus paper and ferric chloride.

Exp. 48. To a dilute solution of ferric chloride add a little acetic acid; divide the solution into two parts; to one add mercuric chloride and to the other HCl, and note results.

Exp. 49. Repeat Exp. 48, using diacetic acid in place of acetic.

Exp. 50. Repeat Exp. 48, using meconic acid* in place of acetic.

Compare results of these three experiments and save record for future use in the study of saliva.

Exp. 51. In a small flask saponify a little butter by heating with alcoholic potash over a steam-bath till mixture is dry. Dissolve in water, add dilute H_2SO_4, and distil off a portion of the butyric acid. Record whatever can be learned from this experiment regarding the physical properties of the butyric acid.

Exp. 52. In separate test-tubes take about 5 c.c. of solutions of stearic and oleic acids in carbon tetrachloride. Add to each about 1 c.c. of a one-tenth per cent solution of iodine, also in carbon tetrachloride, allow to stand for some time, and explain *fully* the difference in action exhibited by the two fatty acids.

Experiments with Organic Acids not of the $C_nH_{2n}O_2$ *Series.*

Exp. 53. To a dilute solution of permanganate of potassium add a few drops of sulphuric acid and heat nearly to boiling. Note if any change takes place. Now add a few crystals of oxalic acid and watch carefully. Explain the use of sulphuric acid.

Exp. 54. In separate test-tubes, insoluble oxalates may be produced by adding a solution of ammonium oxalate to a solu-

* Laudanum diluted with water till color is light brown may be used

tion of (*a*) calcium chloride, (*b*) silver nitrate, (*c*) zinc sulphate, (*d*) copper sulphate, (*e*) lead nitrate.

Exp. 55. Place in an ignition-tube, fitted with delivery tube to collect evolved gas in test-tube, about 3 grams of dry calcium oxalate. Heat strongly and test gas evolved with lighted match or splinter. After ignition tube has become *cold* add dilute H_2SO_4 and pass gas evolved into lime-water.

Exp. 56. Dissolve about 3 grams of dry oxalic acid (100° C.) in a test-tube half full of methyl alcohol. It will probably be necessary to boil the mixture before solution is complete, and great care must be used to avoid burning of the alcohol. The use of a water-bath is recommended. As the hot mixture cools, dimethyl oxalate will crystallize out.

Separate sufficient of the crystals to obtain melting-point, which should be about 54° C.

Exp. 57. The ester prepared in above experiment may be dissolved in alcohol and upon addition of NH_4OH will give a precipitate of oxamide.

Exp. 58. Take a test-tube half full of calcium chloride (10 per cent), make strongly alkaline with NH_4OH and pass CO_2 into the mixture for several minutes. A solution of calcium carbonate will result.

Write reaction, $CaCl_2 + 2\ CO_2 + 4\ NH_4OH = ?$. Heat the solution of calcium carbonate just produced till a precipitate of $CaCO_3$ is produced.

Write reactions showing the formation of $CaH_2(CO_3)_2$ and the precipitation of $CaCO_3$ from the acid salt.

Exp. 59. To one-third of a test-tube of cider vinegar add a few cubic centimeters of basic acetate of lead solution; a bulky precipitate of lead malate separates.

Exp. 60. Dilute a few drops of neutral ferric chloride solution until no color is discernible, then to 10 c.c. of this dilution add 4 to 5 drops of 1/2 per cent solution of lactic acid. A greenish-yellow color constitutes a positive test.

In practical application of this test, it needs further confirmation by boiling the unknown solution with a drop or two of HCl and then extracting with ether. Evaporate the ether,

take up the residue in 2 or 3 c.c. of water and repeat the test as given above. If the yellow color persists, it is due to lactic acid.

Exp. 61. The production of esters may be demonstrated by the test for acetic acid, forming ethyl acetate, or by the following experiment used to detect butyric acid in gastric contents:

Exp. 62. Mix in a test-tube 5 c.c. of a dilute 1/2 per cent solution of butyric acid with an equal volume of strong H_2SO_4 and as much strong alcohol. Heat gently and note the odor of ethyl butyrate (pineapples).

Exp. 63. The action of fixed alkalies on esters is known as "saponification." It may be illustrated by heating 10 c.c. of ethyl acetate with 80 c.c. of a 10 per cent NaOH solution for 30 to 40 minutes, when the odor of ethyl acetate should be destroyed. The flask should be connected with a reflux condenser and the heat applied by immersing the flask in boiling water. Write reaction.

Exp. 64. *Preparation of ethyl nitrite.* Take 8 c.c. of strong H_2SO_4 and add 25 c.c. of water. Cool the liquid and add 17 c.c. of alcohol previously diluted with an equal volume of water. Place the whole in a 200 c.c. flask surrounded with ice-water. Dissolve 20 grams of $NaNO_2$ in 56 c.c. of water. Filter, and from a separatory funnel allow the sodium nitrite solution to drop into the cold acid solution previously prepared.

Allow the crystals which may have been formed to settle, and decant the liquid into a clean separatory funnel. The ethyl nitrite will rise to the top and the water may be drawn off. Wash the ethyl nitrite with 10 c.c. of ice-water to remove acid, and then with 10 c.c. of ice-water to which Na_2CO_3 has been added.

Exp. 65. *Test for ethyl nitrite.* Fill the large tube of a Doremus Hind's ureometer with a mixture containing 20 c.c. of saturated NaCl, 5 c.c. of 20 per cent KI, 5 c.c. of 20 per cent H_2SO_4. In the small arm place 2 c.c. of a saturated solution of NaCl and 2 c.c. of the prepared ethyl nitrite. Add cautiously *1 c.c.* of the mixture in the small arm. Nitric oxide is evolved according to the following equation:

$$C_2H_5NO_2+KI+H_2SO_4\rightarrow C_2H_5OH+KHSO_4+I+NO.$$

Experiments with Cyanogen Compounds.

Exp. 66. In a large test-tube dissolve 1/2 gram or *less* of potassium ferrocyanide in about 4 c.c. of water. Add a little H_2SO_4 and boil, conducting the gas evolved into a beaker condenser (Fig. 23) by means of a bent glass tube. Note the odor of this dilute solution. (Do not smell of the contents of generating tube, as the strong acid is intensely poisonous.) Write reaction.

Exp. 67. To one half of the dilute hydrocyanic acid prepared in the previous experiment add a drop or two of $AgNO_3$ solution with a little HNO_3. After the precipitate has settled, decant the fluid, then add an excess of ammonia water.

Exp. 68. To the other half of the HCN from Exp. 66 add a little solution of ferrous sulphate; also a few drops of ferric chloride solution; then a little KOH solution; mix thoroughly and acidify with HCl. A blue precipitate, $Fe_4[Fe(CN)_6]_3$, is a test for HCN or any soluble cyanide.

Exp. 69. To a few drops of KCN solution add a little yellow ammonium sulphide, $(NH_4)_2S$, and evaporate to dryness. Dissolve in water; acidify with HCl and add Fe_2Cl_6 solution.

Exp. 70. In a small flask boil a solution of KCN. While boiling, test the vapors for ammonia gas. Solution of potassium formate remains in the flask.

Complete reaction, $KCN + 2\ H_2O = ?$

Exp. 71. To a little dilute (2 per cent) solution of $K_4Fe(CN)_6$ add a little bromine water and boil. Prove the formation of $K_3Fe(CN)_6$ by use of $FeCl_3$.

Judging from this experiment, what is the relative valence of iron in the two compounds? Why?

Exp. 72. To a fresh solution of $K_3Fe(CN)_6$ add a little 10 per cent KOH solution and some PbO, shake and filter. To the clear filtrate add $FeCl_3$.

Give reason for the statement that the PbO has acted as a reducing agent.

Exp. 73. Dissolve a piece of potassium ferricyanide, as large as a pea, in 5 c.c. of water, add 2 c.c. of a solution of potas-

sium ferrocyanide. Dilute to the capacity of a test-tube with distilled water and put equal amounts of this solution into 2 shell tubes. Examine the color through the length of tube, then add to one tube 2 or 3 drops of strong HCl. Examine again and notice that a trace of prussian blue has been produced. Explain.

Experiments with Amines and Amides.

Exp. 74. Distil 60 c.c. of ammonium acetate in a glass retort fitted with a thermometer, as in Fig. 22. Acetamide

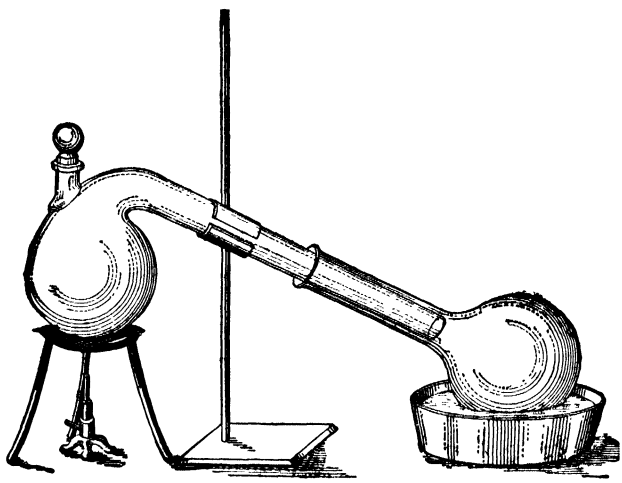


FIG. 22.

should distil at about 222° C. and condense as a white solid in the receiver.

Exp. 75. In a 500-c.c. flask place 10 grams of strong, fresh, bleaching powder; add 3 grams of acetamide dissolved in about 10 c.c. of water. Mix as thoroughly as possible and add slowly 25 c.c. of a 20 per cent solution of NaOH. Distil with steam, collecting distillate in 15 c.c. of cold water.

Exp. 76. To a little of the water solution of methyl amine prepared in the last experiment, add 2 or 3 drops of chloroform and a little alcoholic potash. This mixture upon warming will give carbylamine. Note the odor. Warm a little of the solution with a little 5 per cent NaOH. Test the vapor given off with litmus paper and compare with ordinary qualitative test for ammonia.

Exp. 77. Prepare acetanilide (phenyl acet-amide) by mixing intimately 10 c.c. each of acetic anhydride and aniline oil. Cool and recrystallize from repeated portions of hot water.

Exp. 78. *Preparation of camphor sulphonic acid.* In a small beaker place 10 to 12 c.c. of acetic anhydride. Place the beaker in an ice-salt bath and keep the temperature *below 10° C.* When the acetic anhydride is thoroughly cold, add slowly 3.5 c.c. of concentrated H_2SO_4. Then add slowly about 7 grams of camphor. Stir until all the camphor is dissolved. Let stand at room temperature for two days, when crystals of camphor sulphonic acid will appear. Wash the crystals with ether, and dry. During the addition of the camphor it is necessary to *keep the temperature low* or the sulphuric acid is apt to char it.

Urea and Uric Acid.

Exp. 79. Make separate solutions of 10 grams of potassium cyanate* and 8.25 grams of ammonium sulphate. Mix and evaporate on a water-bath in a shallow dish. Separate the potassium sulphate as the evaporation proceeds; finally, evaporate to dryness and extract with absolute alcohol. Evaporate alcohol and reserve the urea for subsequent experiments. (See Urea, page 54.)

Exp. 80. Heat a few crystals of urea in a test-tube until they fuse and no more gas is given off; cool, and dissolve the fused mass in water; add 1 or 2 c.c. of strong NaOH solution, then not more than 1 or 2 drops of a 1 per cent $CuSO_4$ solution. Note the pink to violet color produced. This constitutes the biuret reaction used in physiological chemistry as a test for albumoses and peptones. Biuret is formed from urea as follows:

$$2\,O{=}C\begin{matrix}NH_2\\NH_2\end{matrix} = \begin{matrix}O{=}C\begin{matrix}NH_2\\NH\end{matrix}\\O{=}C\begin{matrix}\\NH_2\end{matrix}\end{matrix} + NH_3$$

Exp. 81. Produce crystals of urea nitrate and oxalate (page

* For method of making potassium cyanate, see Preparation of Reagents and Organic Compounds, in the Appendix.

77) and examine under the microscope. (*Repeat* with urea obtained from urine).

This experiment may be performed by concentrating a little urine to about one-fifth of its bulk and using the concentrated solution as a solution of urea.

Exp. 82. Treat 5 c.c. of urea solution (urine may be used) with a little sodium hypochlorite or hypobromite; note results and study reaction given on page 55.

Exp. 83. Heat one-third of a test-tube of urine with barium hydroxide (baryta-water); test vapor with red litmus for NH_3.

Exp. 84. *Murexide test for uric acid*: Place a very small quantity of uric acid on a porcelain crucible cover, or in a small evaporating dish. Add 2 or 3 drops of strong nitric acid and evaporate to dryness over a water-bath. A yellowish-red residue remains, which changes to a purplish red upon addition of a drop of strong NH_4OH, and to purple-violet upon further addition of a drop of KOH solution, the color disappearing upon standing or upon the application of heat. (Difference from xanthine, which also gives a deeper red color.)

Exp. 85. Repeat No. 84, using caffein in place of uric acid.

Exp. 86. Heat a little sodium acid urate in a dilute solution of NaH_2PO_4. Allow to cool, and examine any deposit for uric acid crystals. Test reaction of solution both hot and cold (page 59).

Exp. 87. Mix, and allow to stand for some time at reduced temperature, 30 c.c. of urine (a 2 per cent urea solution), 2 or 3 c.c. of strong Na_2CO_3 solution, and 5 c.c. of saturated NH_4Cl solution.

A precipitate consists of ammonium urate.

Examine under the microscope and make murexide test.

Exp. 88. To a mixture of sodium urate, sodium chloride, and sodium phosphate, add a little magnesium mixture. Filter. Make filtrate quite strongly alkaline with NH_4OH and add ammoniacal silver nitrate. Precipitate is AgMg urate. The three salts used in this experiment are all found in urine, and the uric acid is precipitated and may be roughly determined by use

of ammoniacal $AgNO_3$ (page 193). Use of mixture? Use of excess of ammonia?

Exp. 89. Separate caffein from a spoonful of coffee as follows: Boil with about 200 c.c. of water for twenty minutes. Filter, remove coloring matter and tannic acid by careful addition of subacetate of lead solution. Filter and concentrate filtrate to 100 c.c. or less and extract with 30 c.c. of chloroform. Allow chloroform to evaporate without heat, and dissolve residue in about 5 c.c. of water. Test this solution according to Exp. 90.

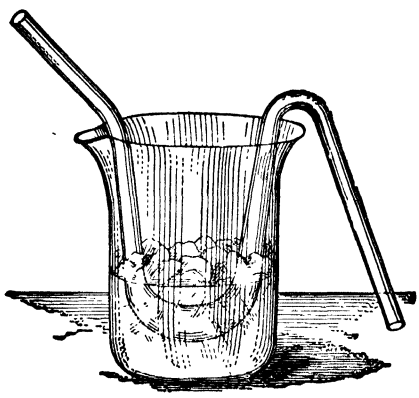


FIG. 23.

Exp. 90. To a few cubic centimeters of uric acid solution add 1 c.c. of phospho-tungstic acid and 2 c.c. of sodium cyanide ($7\frac{1}{2}$ per cent). Compare the color obtained with a blank test in which distilled water has been used in place of the uric acid.

Exp. 91. *Schiff's test for uric acid.* To a solution of sodium carbonate add a trace of uric acid. Pour the solution upon a piece of paper wet with silver nitrate, and note the reduction of the silver salt.

Experiments with Aromatic Hydrocarbons.

Exp. 92. Into a small and thoroughly dry flask (250 c.c.) introduce about 50 grams of a mixture consisting of 1 part of benzoic acid and 2 parts of quicklime; connect with a beaker condenser (Fig. 23) and heat. Benzene (benzol) distils over:

$$CaO + C_6H_5COOH = CaCO_3 + C_6H_6.$$

Exp. 93. Turn a little of the benzene prepared in the last experiment on to some water contained in a porcelain capsule. Set fire to it and note that it burns with a *smoky* flame. Cool a few cubic centimeters of pure benzene, contained in a narrow test-tube, by immersion in a freezing mixture of ice and salt.

Exp. 94. Test benzene for double bond. Exp. 8.

Exp. 95. Test benzene samples A and B for CS_2. Use 10 c.c. of sample and add to each 2 drops of phenylhydrazine. Presence of CS_2 will be indicated by formation of crystalline precipitate. $(C_6H_5NH.NH_2)_2CS_2$.

Exp. 96. Test solubility of naphthalene in alcohol, water, and gasolene.

Exp. 97. Determine melting-point of naphthalene.

Exp. 98. In a wide test-tube mix 5 c.c. of concentrated H_2SO_4 with about half its volume of *strong* HNO_3; cool in ice-water or cold running water, and add *very slowly* about 2 c.c. of benzene. Nitrobenzene is formed and may be separated as a heavy oily liquid by pouring the mixture into an excess of water. Notice the odor of oil of bitter almonds.

Exp. 99. Observing the same precaution against overheating as given in Exp. 98 reduce nitrobenzene to amino-benzene as follows: In a large test-tube or small flask place 1 or 2 c.c. of nitrobenzene with three times its weight of tin powder. To this add 10 or 15 c.c. of strong HCl in successive small portions, keeping cool as indicated. The odor of nitrobenzene should be replaced by that of aniline.

Exp. 100. Heat a mixture of 2 c.c. of aniline, 5 c.c. of water and 1 c.c. of strong sulphuric acid to the boiling point; then set aside where it may cool slowly. Crystals of aniline sulphate will separate.

Exp. 101. Repeat preceding experiment, using 5 c.c. of aniline, 5 c.c. of water and 10 c.c. of strong hydrochloric acid. When the mixture has become thoroughly cold, filter off the crystals of aniline hydrochloride and dry in a current of air. Test solubility in water, using only a very little of the crystallized salt.

Exp. 102. Place 5 c.c. of an aqueous solution of aniline in each of three test-tubes. Add to the first a few drops of bromine water; to the second a few drops of dilute ferric chloride; and to the third a solution of hypochlorite of calcium or sodium.

Exp. 103. Shake together in a test-tube 1 part of aniline oil and 5 parts of water. Is the oil soluble in water?

Agitate with HCl added in small portions till liquid becomes clear. Explain.

Exp. 104. Mix in a large test-tube or small flask a little dry slaked lime and salicylic acid, connect with a beaker condenser (see cut on page 264) and distil. Test distillate for phenol. Write reaction.

Note. — After the first heating, the tube containing the lime and acid may be inclined so that any moisture in distillate will run into collecting tube rather than back on to the mixture.

Exp. 105. To a few cubic centimeters of a 3 per cent phenol solution add dilute bromine water. A yellowish-white, crystalline precipitate of tribromphenol is produced (see page 77).

Exp. 106. To an aqueous solution of phenol add a few drops of solution of ferric chloride.

Exp. 107. To 5 c.c. of an aqueous solution of phenol add one quarter of its volume of ammonia water and then a few drops of sodium hypochlorite solution. Mix and warm. A blue-green color develops, turning red upon addition of hydrochloric acid to slight acid reaction.

Exp. 108. Repeat Exps. 105 and 106, using an aqueous solution of cresol in place of phenol.

Exp. 109. To a test-tube one-third full of nitric acid (50 per cent absolute HNO_3), add, 1 drop at a time, about 1 c.c. of phenol with constant agitation. When all the phenol has been added, heat carefully to boiling. Allow to cool slowly, when trinitrophenol will be precipitated.

Exp. 110. Evaporate a few drops of a 1 per cent solution of potassium nitrate to dryness in a small porcelain capsule. Add 2 c.c. of phenoldisulphonic acid;* stir thoroughly, and keep hot for three to five minutes; dilute with water, make strongly alkaline with ammonia, and note the intense yellow color of ammonium picrate. The reaction is used as a test for nitrates in drinking water.

Exp. 111. Determine melting-point of benzoic acid.

Exp. 112. Arrange two watch glasses of equal size with the concave surfaces together and a piece of filter-paper stretched between them. The glasses may be held together with a small brass clamp.

* For method of preparation see Appendix.

A little benzoic acid placed in the lower glass may be sublimed by means of a gentle heat through the paper, and collected upon the upper glass. Examine the sublimate by polarized light. See Plate V, Fig. 5, opposite page 99.

Exp. 113. *Preparation of benzoic acid from toluene* (oxidation of side chain). Place 5 c.c. of toluene in a 250 c.c. boiling-flask containing 125 c.c. of distilled water and 7 grams of $KMnO_4$. Shake mixture well. Connect flask with a reflux condenser and boil for at least thirty minutes. If the permanganate color disappears during this time add 3 grams more of $KMnO_4$. Remove condenser and filter solution. The filtrate will contain excess permanganate and potassium benzoate. Let cool slightly and then add 20 per cent H_2SO_4. Heat to boiling and add sufficient oxalic acid to cause the solution to become colorless.

When the solution has been cooled by immersion in ice-water, crystals of benzoic acid will separate out, and may easily be filtered off and dried.

Exp. 114. With an aqueous solution of benzaldehyde, determine whether Tollen's test for aldehydes (Exp. 30) is applicable to aromatic compounds.

Exp. 115. Boil 10 c.c. of oil of wintergreen with a little 20 per cent NaOH; keep the volume constant by frequent addition of water. When the oil has entirely disappeared, cool and add HCl to acid reaction. Salicylic acid will separate, white and crystalline.

Exp. 116. To a dilute solution of sodium salicylate, or saturated aqueous solution of salicylic acid, add a few drops of Fe_2Cl_6. A slight amount of salicylates in the urine will produce this color when a test is being made for diacetic acid (q.v.).

Exp. 117. *Preparation of methyl salicylate* (synthetic oil of wintergreen). Dissolve 15 grams of salicylic acid in 60 c.c. of absolute methyl alcohol. Very gradually add 30 c.c. strong H_2SO_4 and let stand in a warm place for twenty-four hours, covered. Add half its volume of water and distil slowly.

Experiments for Physiological Chemistry.

Preparation of Oxidase.

Exp. 118. Clean thoroughly a small potato and grate the skin into a small beaker; cover with water and allow to stand in a cool place for an hour. Filter through coarse paper. Turn

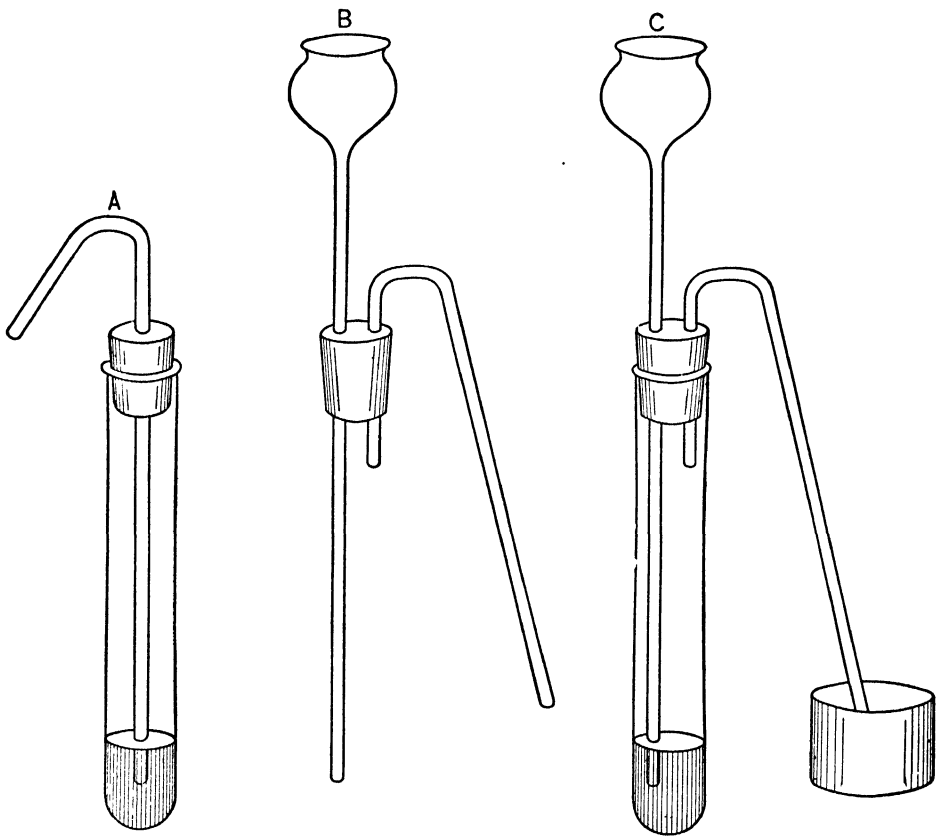


Fig. 24.

about 5 c.c. of the filtrate slowly into 25 c.c. of strong alcohol. The enzyme will be precipitated. Filter and test as follows:

Exp. 119. Transfer the moist precipitate from the above experiment into a test-tube half-filled with distilled water. Shake frequently for about ten minutes and filter. The filtrate will contain oxidizing enzymes in solution. Divide the solution into two parts; to one add a few drops of tincture of guaiacum, and to the other a little of a 1 per cent solution of pyrocatechol. The guaiacum gives a blue color, and the pyrocatechol a red-brown color in the presence of oxidizing enzymes.

Experiments with Enzymes.

Hydrolytic enzymes produce cleavage of the molecule.

Exp. 120. Take four test-tubes *a–b–c–d*. Make a thin paste by rubbing one-sixth of a yeast cake with water, and place a little in each of the four tubes; then fill *a* with a dilute glucose solution; *b* with a dilute solution of milk-sugar; *c* with dilute solution of cane-sugar; prepare *d* in the same manner as *c*, but before adding the sugar solution, boil the enzyme (yeast) for at least one minute. Have each tube full and fit with a delivery tube (Fig. 24, page 268), making provision to collect any liquid which may be forced out of the tube. Allow the four tubes to stand overnight and then test the liquid which has been forced out of the tubes during the night for alcohol (Exp. 15), and the gas for CO_2 by means of forcing it into baryta-water. (This may be done by use of a special stopper fitted with thistle tube and delivery tube.)

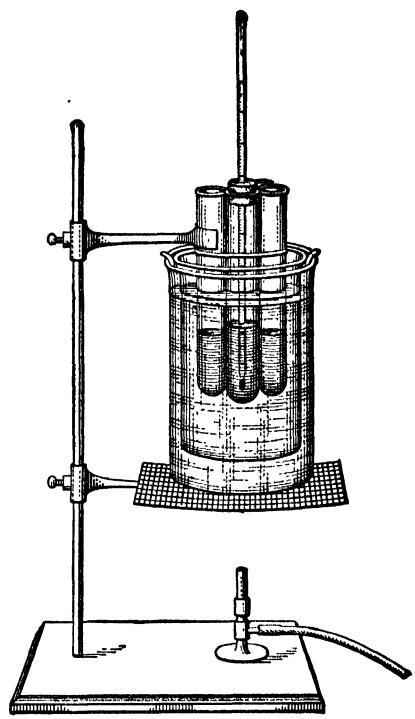


FIG. 25.

Exp. 121. Take four test-tubes, *a–b–c–d*, arrange as indicated in Fig. 25, and half fill each with some thin starch paste (see page 301 of Appendix). Into *a* put a little of the yeast from last experiment; into *b* a little pepsin solution; into *c* a little saliva (the enzyme of the saliva is ptyalin); into *d* a little invertase, as used in preceding experiment. Warm all the tubes to about 37 or 38° C., and allow to stand overnight; then test contents of each tube for a reducing sugar which may have been produced from the starch. (Use Exp. 129.)

Exp. 122. The student may prepare a fat-splitting enzyme (lipase) from an animal source, pig's pancreas, or from a

vegetable source, castor beans, according to direction in the Appendix.

Fat Digestion with Lipase (Castor Bean). — Grind with the powder,* in the order named, 5 c.c. N/10 sulphuric acid, 5 c.c. of neutral cotton oil (sp. gr. 0.92) and 5 c.c. lukewarm water. The water should be added a little at a time and thoroughly worked into the mixture so that at the end of the operation a good emulsion is secured. Cover the evaporating dish and let stand in a warm place overnight.

Add 50 c.c. of alcohol, 10 c.c.of ether, and a few drops of phenolphthalein, and titrate with N/1 sodium hydrate. Calculate the amount of fatty acid and the per cent of fat digestion.

Exp. 123. In a test-tube one-third full of milk, colored slightly blue with nearly neutral litmus solution, place half as much solution of lipase (fresh pancreatic extract) and keep at about 40° C. for twenty to thirty minutes. Sufficient fat acid should be separated to change the blue litmus to red. Write reaction.

Experiments with Sugars.

Exp. 124. Fill a test-tube about one-third full of dry straw. Cover with 10 per cent hydrochloric acid; boil, collecting the distillate in a dry tube. Divide the distillate into two parts, and make the following tests for furfuraldehyde which has been produced from the pentose contained in the straw. Treat the contents of one tube with a little aniline and hydrochloric acid. Red coloration indicates the presence of furfuraldehyde. To the contents of the other tube add a little solution of casein (skimmed milk) and underlay with strong sulphuric acid. Furfurol will give a blue or purple line at the point of contact of the two liquids.

Monosaccharides. — Exp. 125. Test for C and H, using cane-sugar. Make closed-tube test for H, which is given off as H_2O, and for C, which remains as such in tube. (See Vol. I.) Write reactions.

Exp. 126. *Molisch's test for Carbohydrates.* — To a few cubic centimeters of a 3 per cent glucose solution add a few drops of

* For preparation of powder, see page 299.

an alcoholic solution of α-naphthol, and carefully underlay the mixture with strong H_2SO_4.

Exp. 127. To a few cubic centimeters of $CuSO_4$ solution in a test-tube add a little NaOH. Boil and write reaction.

Exp. 128. Repeat Exp. 127 with the addition of Rochelle salt; if solution remains clear on boiling, add a few drops of a glucose solution.

Exp. 129. *Fehling's Test for Sugars.* — Take about 5 c.c. of Fehling's solution, made by mixing equal parts of the $CuSO_4$ solution and the alkaline tartrate on side shelf. Boil and add immediately a few drops of glucose solution. Set aside for a few minutes, watching the results.

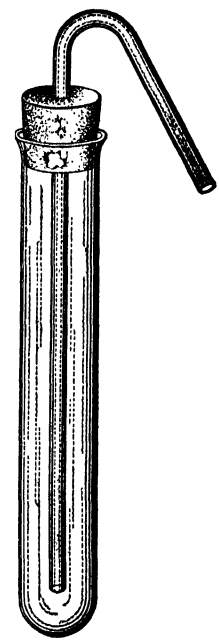


FIG. 26.

Exp. 130. Repeat Exp. 129, using diabetic urine instead of glucose.

Exp. 131. Repeat Exp. 129 without heat and allow to stand for twenty-four hours.

Exp. 132. To 5 c.c. of Benedict's solution (for preparation see Appendix) add 8 or 10 drops of a 2 per cent glucose solution. Heat the mixture to boiling; keep at this temperature for one or two minutes.

Exp. 133. *Barfoed's Test.* — To about 5 c.c. of Barfoed's reagent add a few drops of glucose solution; boil and set aside for a few minutes, watching results.

Exp. 134. *Fermentation Test.* — Fill the "fermentation tube" (Fig. 26) found in the desk with glucose solution; add a little yeast; insert stopper, with long arm of tube extending into glucose mixture nearly to bottom of tube, and allow it to stand upright, in a *warm* place, overnight. On the next day, test the gas, with which the tube is filled, with lime-water.

Exp. 135. *Phenylhydrazine Test.* — Place about 5 c.c. of glucose solution in a test-tube; add an equal volume of phenylhydrazine solution; keep the tube in boiling water for thirty minutes. Allow to cool gradually. Examine the precipitate microscopically and sketch the crystals.

Disaccharides. — Exp. 136. Use dilute solutions of cane-sugar, milk-sugar, and maltose, and make on each Fehling's test (Exp. 129), Barfoed's test (Exp. 133), and the phenylhydrazine test (Exp. 135). Sketch the different osazone crystals obtained.

Exp. 137. To a dilute solution of cane-sugar add a few drops of dilute H_2SO_4 and boil for five minutes. Cool the mixture and make slightly alkaline with NaOH. With this solution perform Exps. 129, 133, and 135. Explain results. Compare with Exp. 136.

Experiments with Starches and Cellulose.

Polysaccharides. — Exp. 138. Examine potato, corn, and wheat starch under the microscope, use a drop of water and a cover glass. Sketch the granules of each in notebook, and, while still on the slide, treat with a dilute iodine solution. Note changes in appearance of granules.

Exp. 139. *Preparation of starch.* — Grate a little raw potato. Mix thoroughly with water and strain through " bolting " cloth or stout, coarse muslin. After the liquid has run through, compress the cloth by twisting till no more liquid can be squeezed out. The starch has passed through the cloth and may be washed by decantation, dried on filter paper, examined, and used for the following experiments:

Exp. 140. Make some starch paste by rubbing 1 gram of starch to a smooth, *thin* paste with water; then slowly pour it into 100 c.c. of boiling water, stirring constantly. With this solution compare a 1 per cent solution of dextrine and a solution of glycogen* as follows:

(*a*) Treat each by boiling with Fehling's solution.

(*b*) Add to 5 c.c. of each a few drops of tannic-acid solution.

(*c*) To each solution add a drop of iodine solution. Note color of mixture while cold. Heat nearly to boiling and allow to cool again, watching the color during process.

(*d*) To 5 c.c. of each solution add twice its volume of 66 per cent alcohol.

* For the isolation of glycogen, see Appendix.

(*e*) Tabulate results of the tests and formulate method of distinguishing these three substances from one another.

Experiments with Fats and Oils.

Exp. 141. Test solubility of olive oil in water, ether, chloroform, and alcohol, carefully avoiding the vicinity of a flame.

Exp. 142. Let one or two drops of an ether solution of the oil drop on a plain white paper, also an ether solution of a volatile oil found on side shelf. Watch behavior of the two oils, and report differences, if any.

Exp. 143. Dissolve a little butter in warm alcohol, examine, with the microscope and micropolariscope, the crystals which separate on cooling.

Note. — If possible perform the next experiment in triplicate, i.e., carry three experiments along at the same time, using for "fat" the glyceryl ester of the three most common fat acids: olein (lard oil or olive oil), stearin (beef fat or tallow), palmatin (bayberry wax or tallow, which contains a large amount of free palmitic acid).

Exp. 144. *Saponification.* — To about 2 grams of solid fat placed in a narrow beaker, or 150-c.c. Erlenmeyer flask, add 10 or 15 c.c. of alcoholic solution of potassium hydroxide. Allow the beaker to stand on the water-bath till the alcohol is entirely evaporated, then dissolve the resulting soap in water; filter, if necessary, to obtain a clear solution, and make the following tests:

(*a*) Add to a portion of solution a saturated solution of sodium chloride. What takes place?

(*b*) To another portion add a few cubic centimeters of a solution of calcium or magnesium chloride. Explain the results.

(*c*) Pour the remainder slowly, and with constant stirring, into warm dilute H_2SO_4, and heat on the water-bath. What is the result? Write the equation. Transfer the mixture to a filter-paper which has been moistened with hot water, and wash with hot water till all H_2SO_4 is removed. Reserve the filtrates.

Exp. 145. *Fatty acids.*

(*a*) Dissolve a portion of the above precipitates (144 *c*) by warming with strong alcohol. Test the reaction of the solution. Examine the crystals, which separate upon standing, with microscope and micropolariscope. (Plate VII, Fig. 3, page 185.)

(*b*) Add to a portion a few cubic centimeters of a strong Na_2CO_3 solution, and heat till the fatty acids dissolve. Cool. What takes place? Explain the reaction. Reserve the jelly.

Exp. 146. Neutralize the filtrates of Exp. 144 *c* and evaporate almost to dryness on the water-bath. Extract with alcohol and evaporate. Note the taste. Heat another portion of the residue with a little powdered dry $KHSO_4$ in a dry test-tube, and note the odor, which is due to acrolein, $CH_2=CH-CHO$. Fuse some borax and glycerin on a platinum loop: green color.

Exp. 147. *Emulsification.* — (*a*) Put 1 to 2 c.c. of a solution of sodium carbonate (0.25 per cent on a watch glass, and place in the center a drop of rancid oil. The oil-drop soon shows a white rim, and a white milky opacity extends over the solution. Note with the microscope the active movements in the vicinity of the fat-drop, due to the separation of minute particles of oil (Gad's experiment).

(*b*) Take six test-tubes and arrange as follows:

1. 10 c.c. of a 0.2 per cent Na_2CO_3 solution + 2 drops of neutral oil.
2. 10 c.c. of a 0.2 per cent Na_2CO_3 solution + 2 drops of rancid oil.
3. 10 c.c. of soap-jelly, warm, + 2 drops of neutral oil.
4. 10 c.c. of albumin solution + 2 drops of neutral oil.
5. 10 c.c. of gum-arabic solution + 2 drops of neutral oil.
6. 10 c.c. of water + 2 drops of neutral oil.

Shake all the mixtures thoroughly and note the results. What conclusions do you form relative to the influence of conditions upon emulsification?

(*c*) Examine a drop of an emulsion under the microscope.

Identification of Fats.

Exp. 148. *Determination of the Reichert-Meissel number for volatile fatty acids.* — In an Erlenmeyer flask of 250 c.c. capacity, saponify 5 grams of fat with N/2 alcoholic NaOH until solution is free from fat globules. Evaporate on a water-bath and dissolve the dry soap in 100 c.c. of warm water. Cool and add

a few cubic centimeters of 10 per cent H_2SO_4. Connect flask with a condenser, and distil. After the distillation has continued for thirty minutes, disconnect apparatus and titrate the entire distillate with N/10 NaOH.

The number of cubic centimeters of N/10 alkali necessary to neutralize the volatile fatty acids obtained from 5 grams of fat is the Reichert-Meissel number of the fat.

Exp. 149. *Determination of the iodine absorption number for fat.* — Place 1 gram of fat or .2 to .4 gram of oil in a 250 c.c. flask. Dissolve in 10 c.c. of chloroform and then add 30 c.c. of prepared iodine solution (iodine and mercuric chloride). Shake and place flask in a dark place for three hours, shaking frequently. Add 20 c.c. of KI solution and 100 c.c. of water. Titrate excess iodine solution with standard thiosulphate solution, using starch paste as an indicator.

Calculate the amount of iodine absorbed by 1 gram of fat.

Lipoid Experiments.

Exp. 150. Boil 75 grams of brain tissue with 150 c.c. strong alcohol for thirty or forty minutes, using a reflux condenser and a water-bath. Filter hot, and allow to cool overnight. A cloudy precipitate will consist of a mixture of the so-called lipins or lipoids (cerebrin, cholesterol, lecithin, cephalin.) If the precipitate is very slight, concentrate the solution by evaporating and cool again. Filter on black paper and make following tests.

(1) A little of the precipitate mixed thoroughly with water and examined under the microscope will show myelin movements (due to cerebrin).

(2) With another portion of the precipitate, make microchemical test for phosphorus (showing presence of phosphorized fat).

(3) In a small test-tube, heat a portion with fixed alkali and if possible detect fishy odor of trimethylamine (chlolein).

(4) Warm (on a microscope slide) a little precipitate with a few drops of alcohol. Look for crystals of cholesterol. See also Exp. 239.

(5) If sufficient precipitate remains, hydrolyze for one hour with dilute hydrochloric acid, neutralize and test for a reducing sugar (galactose).

Exp. 151. In a thoroughly dry test-tube place 2 c.c. of chloroform, in which dissolve a little cholesterol. Add about 10 drops of acetic anhydride and two drops of concentrated sulphuric acid. The mixture produces a violet color quickly changing to blue-green. (Liebermann-Burchard acetic anhydride test).

General Protein Reactions.

Exp. 152. Test dried egg-albumin for C, H, S, and N, according to the methods described on pages 2, 3 and 4. Test casein for phosphorus, and dried blood for iron.

There are several reactions which are common to nearly all proteins. For the following tests use a solution of egg-albumin (1/50) in water, as a general type of a protein.

1. *Color Reactions.*

Exp. 153. *Xanthoproteic test.* — To 10 c.c. of the albumin solution add one-third as much concentrated HNO_3; there may or may not be a white precipitate produced (according to the nature of the protein and the concentration). Boil; the precipitate or liquid turns yellow. When the solution becomes cool add an excess of NH_4OH, which gives an orange color. (This color constitutes the essential part of the test.)

Exp. 154. *Millon's test.* — Add a few drops of Millon's reagent* to a part of the albumin solution. A precipitate, which becomes brick-red upon heating, forms. The liquid is colored red in the presence of non-coagulable protein or minute traces of albumin.

Exp. 155. *Piotrowski's test.* — To a third portion add 2 drops of a *very* dilute solution of $CuSO_4$, and then 5 to 10 c.c. of a 40 per cent solution of NaOH. The solution becomes blue or violet. Proteoses and peptones give a rose-red color (biuret

* Mercuric nitrate in nitric acid. For the preparation of this and other reagents, see Appendix.

reaction) if only a trace of copper sulphate is used; an excess of $CuSO_4$ gives a reddish-violet color, somewhat similar to that obtained in the presence of other proteins. All proteins respond to this test.

Exp. 156. *Hopkins-Cole reaction.* — Mix 2 or 3 c.c. of the unknown protein solution with 3 or 4 c.c. of the reagent (glyoxylic acid). Then carefully superimpose upon 5 c.c. of strong sulphuric acid in another test-tube.

The glyoxylic acid is made by the reduction of oxalic acid with nascent hydrogen produced by the action of sodium amalgam and water. Formula is CHO.COOH.

2. *General Precipitants.*

Proteins are precipitated from solution by the following reagents (peptones are exceptions in some cases):

Exp. 157. *Acetic acid and potassic ferrocyanide.* — Make part of the solution to be tested strongly acid with acetic acid, and add a few drops of potassic ferrocyanide solution. A white flocculent precipitate is formed (not with peptones).

Exp. 158. *Alcohol.* — To another part add one or two volumes of alcohol.

Exp. 159. *Tannic acid.* — Make the solution acid with acetic acid, and add a few drops of tannic-acid solution.

Exp. 160. *Potassio-mercuric iodide.* — Make acid another portion with HCl, and add a few drops of the reagent.

Exp. 161. *Neutral salts.* — Certain neutral salts precipitate most proteins. $(NH_4)_2SO_4$, added to complete saturation to protein solutions, faintly acid with acetic acid, precipitates all proteins, with the exception of peptones.

Experiments with Albumin and Globulin.

The albumins and globulins respond to all the general protein reactions. Experiments 153 to 161.

Exp. 162. A specimen of solid egg-albumin, prepared by evaporating a solution to dryness at 40° C., is provided. Test its solubility in water, alcohol, acetic acid, KOH solution, and concentrated HCl. Report results.

Perform the following additional experiments, using a dilute (1/50) solution of egg-albumin.

Exp. 163. *Nitric-acid test.* — Take 15 c.c. of the solution in a wine-glass, incline the glass, and allow 5 c.c. of concentrated HNO_3 to run slowly down the side to form an under layer. What other proteins respond to this test?

Exp. 164. *Picric-acid test.* — Take a portion of the albumin solution and add a few drops of a solution of picric acid acidified with citric acid (Esbach's reagent). What other proteins respond to this test?

Exp. 165. *Action of* $(NH_4)_2SO_4$. — To 10 c.c. of the albumin solution in a test-tube add some solid $(NH_4)_2SO_4$, shaking until solution is thoroughly saturated. Allow to stand a little while, shaking occasionally, then filter, saving the filtrate to test for albumin by the heat test. Report result. Test the solubility of the precipitate on the filter-paper.

Exp. 166. *Action of* $MgSO_4$. — Perform an experiment similar to Exp. 165 using solid $MgSO_4$ instead of $(NH_4)_2SO_4$. With what results?

Exp. 167. *Salts of the heavy metals.* — Note the action of the following: $AgNO_3$, $HgCl_2$, $CuSO_4$, $Pb(C_2H_3O_2)_2$. Use solutions of the salts and of albumin.

Why is white of egg an antidote in cases of metallic poisoning?

The following tests serve to distinguish the globulins from other proteins.

The tests may be made upon blood serum, or upon a globulin (edestin) which may be separated from hemp seed according to preparation in Appendix, page 306.

Globulins.

Exp. 168. *Action of* CO_2. — To 5 c.c. of blood serum add 45 c.c. of ice-cold water. Place the mixture in a large test-tube or cylinder, surround it with ice-water, and pass through it a stream of CO_2. A flocculent precipitate (paraglobulin) will be formed.

Exp. 169. *Precipitation by dialysis.* — Into a parchment dialyzing tube, previously soaked in distilled water, pour 20 c.c.

of serum; swing the tube, with its contents, into a large vessel of distilled water, which is to be changed at intervals. Let stand twenty-four hours, then examine the serum in the dialyzing tube; it will contain a flocculent precipitate of paraglobulin. Give explanation of cause of precipitation.

Exp. 170. Pour a solution of globulin, drop by drop, into a large volume of distilled water (in a beaker). What takes place? Explain.

Exp. 171. *Precipitation by magnesium sulphate.* — Saturate about 5 c.c. of globulin solution with solid magnesium sulphate. A heavy precipitate will be formed. Compare this with the action of the same salt on the egg-albumin solution. Paraglobulin is so completely precipitated by this salt that the method is used for its quantitative estimation.

Experiments with Keratin and Gelatin.

Keratins are characterized by their insolubility, and by their high content of loosely combined sulphur.

Exp. 172. Test solubility of keratin (nail or horn) in water, acids, alkalies, gastric and pancreatic juices.

Exp. 173. Warm a bit of keratin with 5 c.c. strong NaOH solution for a few minutes, and add a few drops of a lead acetate solution. What is the result?

Exp. 174. With a solution of gelatin make the usual tests for protein.

Exp. 175. Precipitate gelatin from dilute solution with the following reagents:

(*a*) Tannic acid.
(*b*) Alcohol.
(*c*) Acetic acid and potassium ferrocyanide.
(*d*) Mercuric chloride.
(*e*) Picric acid.

Experiments with Milk.

Exp. 176. Examine microscopically whole milk, skim-milk, and cream. Note the relative amounts of fat in the three varieties.

Exp. 177. Shake a little cream with chloroform in a test-tube; separate the chloroform, evaporate, and melt the fat residue obtained; allow it to cool slowly, when fat crystals will be obtained, which may be examined under the microscope and micropolariscope.

Exp. 178. With a lactometer take the specific gravity of whole milk and skim-milk and explain the difference in results.

Exp. 179. Test the reaction of milk with litmus.

Exp. 180. Dilute some milk with six or seven times its volume of water, and add acetic acid, drop by drop, till the paracasein is precipitated. Filter and reserve the precipitate. Test the filtrate for proteins, if any remain; determine their character if possible.

Exp. 181. Test another portion of the filtrate for carbohydrates, determining the variety present.

Exp. 182. To 50 c.c. of milk add a few drops of rennin solution; keep at a temperature of 40° C. for a few minutes, and explain results.

Exp. 183. *Determination of total nitrogen-Kjedahl method.* — Take 5 c.c. of milk (dilute 1–10) in a 300 c.c. long-neck Kjehdahl flask. Add 15 c.c. of strong H_2SO_4 and .1 to .2 gram of $CuSO_4$. Heat, cautiously at first, gradually raising the temperature until white fumes appear in the flask. Cover with a watch-crystal. Reduce the heat somewhat and digest until clear. It may be necessary to add more sulphuric acid during the process. Allow to cool, then dilute with 100 c.c. of ammonia-free water. Add a slight excess of strong NaOH solution and introduce into the flask a few glass beads or granular zinc to prevent bumping during distillation. Connect with a condenser and distil off about half of the solution, conducting the distillate into 30 c.c. of N/10 HCl.

Titrate the excess acid with N/10 NaOH and calculate the number of cubic centimeters of N/10 acid used in neutralizing the ammonia produced from the nitrogen of the protein. Calculate as nitrogen.

Exp. 184. Take a portion of the precipitated paracasein from Exp. 180, digest at 40° C. with pepsin HCl for twenty minutes

or half an hour. While digesting, test other portions of paracasein, for solubility in water, in dilute acid and dilute alkali. Also test a portion for phosphorus by boiling in a test-tube with dilute nitric acid, cooling to at least 50° C., and adding ammonium molybdate solution.

Exp. 185. To a little skim-milk contained in a test-tube add a saturated solution of ammonium sulphate.

Experiments with Mucin.

Exp. 186. To a solution of mucin* found on the side shelf add acetic acid till precipitation takes place. Settle, filter, wash, and test solubility in water, dilute alkali solution and 5 per cent HCl.

Exp. 187. Make color-tests for proteins.

Exp. 188. Boil a little mucin solution with dilute HCl for several minutes. Cool, neutralize, and test for sugar.

Experiments with Protein Derivatives.

Exp. 189. *Preparation of meta-protein.* — To a solution of egg-albumin add a few drops of a 0.5 per cent solution of NaOH, and warm gently for a few minutes. With the solution thus obtained make the following tests:

Exp. 190. (*a*) *Effect of heating.* — Boil some of the solution and report result.

(*b*) *Effect of neutralizing.* — Add a drop of litmus solution, and cautiously neutralize.

Acid Meta-protein.

Exp. 191. Add a small quantity of a 0.2 per cent HCl solution to a solution of egg-albumin, and warm at 40° C. for one-half to one hour. Or cover with an excess of 0.2 per cent HCl some meat cut into fine pieces, and expose for a while to a temperature of 40° C. Filter. With either of the solutions thus obtained make same tests as on alkali meta-protein, and compare results. How distinguish between them?

Exp. 192. Determine whether cheese forms a meta-protein,

* For preparation of mucin solution from navel cord, see Appendix.

and if so whether the acid or alkali is most easily produced. Write detail of the experiment in laboratory notebook.

Experiments with Proteoses.

Albumoses (*hemialbumose*). — This name includes four closely allied forms of albumose, namely: (1) protoalbumose, (2) deuteroalbumose; (3) heteroalbumose; (4) dysalbumose, an insoluble modification of heteroalbumose. Commercial peptone, which is substantially a mixture of albumoses and peptones, will be given out for use.

Exp. 193. Make a solution of the peptone in water, filter if necessary, and saturate with solid $(NH_4)_2SO_4$. Filter. The precipitate contains the albumoses, the filtrate the peptones. Reserve the filtrate for subsequent tests for peptone. Wash the precipitate with a saturated solution of ammonium sulphate; dissolve in water, and, with the solution obtained, perform the following tests, noting especially the tendency of albumose precipitates to dissolve upon the application of heat and to reappear upon cooling.

Using this solution of albumose, repeat Exps. 153, 154, 155, 163, 164. If no precipitate forms with HNO_3 in Exp. 153, add a drop or two of a saturated solution of common salt. (Deuteroalbumose gives this reaction only in the presence of HCl.)

Exp. 194. Saturate some of the solution with $(NH_4)_2SO_4$. Report the result.

Exp. 195. To some of the solution add 2 or 3 drops of acetic acid and then a saturated solution of NaCl. A precipitate forms, which dissolves on heating, and reappears on cooling.

Experiments with Peptones.

Exp. 196. Using the peptone solution prepared, in the manner above described, from commercial peptone, repeat the experiments indicated in Exp. 193.

Exp. 197. *Effect of heating.* — Boil some of the peptone solution. Report the result.

Exp. 198. *Power of dialyzing.* — Dialyze some of the peptone solution. Use 10 c.c. of the peptone solution, and in the outside

vessel about 100 c.c. of water, which in this case is not to be changed. After twenty-four hours test the outside water for peptone, employing the biuret test.

Exp. 199. *Action of ammonium sulphate.* — Saturate some of the peptone solution with solid $(NH_4)_2SO_4$. Report the result.

A number of unknown solutions will be given out to be tested for carbohydrates and proteins. A report of the results, together with the methods employed, is to be made.

Experiments on Blood.

Exp. 200. Test the reaction of blood with a piece of litmus paper which has been previously soaked in a concentrated NaCl solution. To what is reaction due?

Exp. 201. *Blood-corpuscles.* — (*a*) Examine a drop of blood under the microscope. Sketch the red and white corpuscles.

(*b*) Note the difference between the corpuscles of mammals and those of birds and reptiles.

(*c*) Note the effect upon the red corpuscles produced by the addition of (1) water, (2) a concentrated solution of salt.

Exp. 202. *Hemoglobin crystals.* — Place a drop of defibrinated rat's blood on a slide; add a drop or two of water; mix, and cover with a cover-glass. Sketch the crystals which separate after a few minutes. Or instead of above, add a few drops of ether to some blood in a test-tube; shake thoroughly until the blood becomes " laky," and then place the tube on ice till crystals appear.

Exp. 203. A spectroscope will be found ready for use in the laboratory, and the absorption-bands given by oxyhemoglobin and hemoglobin will be demonstrated. The student may prepare solutions for examination as follows:

(*a*) *Oxyhemoglobin.* — Use dilute blood (1 part of defibrinated blood in 50 parts of distilled water).

(*b*) *Hemoglobin* (reduced hemoglobin). — Add to blood a few drops of strong ammonium sulphide, or 1 or 2 drops of freshly prepared Stoke's reagent.* Note the change in color produced

* Stoke's reagent consists of 2 parts of ferrous sulphate and 3 parts of tartaric acid dissolved in water, with ammonia added to distinct alkaline reaction. There should be no permanent precipitate.

by the addition of the reducing agent. Shake with air and note the rapid change to oxyhemoglobin.

(*c*) *Hemochromogen.* — To a little of the hemoglobin, reduced with ammonium sulphide, add a few drops of concentrated NaCl, and note the spectrum of reduced hematin or hemochromogen.

(*d*) *Carbon-monoxide hemoglobin.* — Pass a current of illuminating gas through a dilute oxyhemoglobin solution for a few minutes and filter. Note the change of color. Try the effect on the solution of (1) ammonium sulphide; (2) Stoke's reagent; (3) shaking with air. Note the stability of the compound.

Exp. 204. Take the specific gravity of blood by filling a test-tube one-half full of benzene; add one drop of blood, and then add chloroform, a drop at a time, with careful but thorough mixing, until the drop of blood floats at about the middle of the mixture, indicating that the gravity of the mixture and of the blood are the same. The specific gravity of the benzene and chloroform may be taken in any convenient way.

Exp. 205. Make the guaiacum test for blood on a sample of dried blood; also on potato scrapings. The method is as follows:

Boil a little clear solution of blood for twenty seconds. Add one drop tincture of guaiacum and then a few drops of an ethereal solution of hydrogen peroxide; shake the mixture and note the blue color obtained.

Try the same test with material obtained from potato scrapings *with and without boiling.*

Exp. 206. *The benzidine reaction* consists in adding to a few cubic centimeters of a saturated benzidine solution in glacial acetic acid or alcohol acidified with acetic acid an equal volume of commercial H_2O_2 and 1 c.c. of the suspected solution. If blood is present a green or blue color will develop. It is better to make a blank test to insure purity of reagents.

Exp. 207. *Hemin crystals* (*Teichmann's test*). — Place a bit of powdered dried blood on a glass slide; add a minute crystal of NaCl (fresh blood contains sufficient NaCl) and two drops of *glacial* acetic acid. Cover with a cover-glass and warm

gently over a flame until bubbles appear. On cooling, dark-brown rhombic crystals, often crossed, separate (chloride of hematin). Similar crystals can be obtained by using an alkaline iodide or bromide in place of NaCl.

Exp. 208. *Coagulation of blood.* — Observe the phenomenon of coagulation as it takes place (*a*) in a test-tube; (*b*) in a drop of blood examined under the microscope. Explain fully.

Exp. 209. *Proteins of blood-plasma.* — (*a*) Serum-albumin. (*b*) Serum-globulin. Using blood-serum, separate and identify these two proteins.

(*c*) *Fibrinogen.* — Fibrinogen is a globulin found in blood-plasma, lymph, etc., together with paraglobulin. Like paraglobulin it responds to all the general precipitants and tests, and in addition gives the reactions with CO_2, dialysis, and $MgSO_4$. It is easily distinguished from paraglobulin by two reactions, viz., its power to coagulate, i.e., to form fibrin when acted on by fibrin ferment, and its temperature of heat coagulation, which will be found to be from 56° to 60° C.

Exp. 210. *Fibrin.* — (*a*) Note its physical properties.

(*b*) Note action of 0.2 per cent pepsin hydrochloric acid.

(*c*) Apply the protein color tests.

Experiments with Muscle.

Exp. 211. Place 25 grams of fresh, finely chopped muscle in a beaker with 75 c.c. of 5 per cent solution of common salt, and allow to stand for about one hour, with frequent stirring. (In the meanwhile perform Exp. 212.) Then filter off the liquid and make the following tests with the filtrate.

(*a*) Test for proteins.

(*b*) Having found proteins, pour a little of the solution into a beaker of water. Result. Inference (myosin).

(*c*) Make a fractional heat coagulation in the following manner (upon the care with which the temperatures given are adhered to, depends the success of the separation): Warm to from 44° to 50° C., and keep at that temperature for a few minutes. The coagulum is myosin [synonyms: paramyosinogen (Halliburton), musculin (older authors)]. In solutions the myosin,

which has the properties of a globulin, becomes insoluble after a time, because it changes to myosinfibrin. In heating the solution as above, a slight cloud may appear at from 30° to 40° C. This is due to coagulation of soluble myogenfibrin. Now filter off the coagulated myosin.

Heat filtrate to from 55° to 65° C. The coagulum is myogen (synonym: myosinogen). In spontaneous coagulation of its solutions it forms, first, soluble myogenfibrin, and, finally, insoluble myogenfibrin. Filter.

Heat to from 70° to 90° C. Coagulum is serum-albumin from the blood within the muscle, and is not a constituent of the muscle plasma. Filter.

Test filtrate for proteins. If it shows a slight biuret test, this is due either to incomplete precipitation by coagulation or to the post-mortem formation of albumose or peptone by auto-digestion (autolysis).

Exp. 212. Make an aqueous extract of muscle, and test for lactic acid by acidulating with H_2SO_4, extracting with ether, and testing the ethereal extract with *very* dilute ferric chloride solution. The presence of lactic acid is shown by a bright-yellow color. (See Exp. 60.)

Digestion Experiments.

Experiments with Saliva.

Exp. 213. *Action of saliva upon starch.* — Take some filtered saliva in a test-tube and place in the water-bath at 40° C., for five or ten minutes. Put some starch paste into a second test-tube and place this also in the water-bath for a while, then mix the two (10 c.c. of starch paste to 3 c.c. of undiluted saliva) and return to the water-bath. The starch is changed first to soluble starch (if originally a thick paste, it becomes fluid and loses its opalescence), then to erythrodextrin, which gives a red color with iodine, and finally to achroodextrin, which gives no reaction with iodine, and to maltose. Prove these changes as follows: Every minute or two take out a drop of the mixture, place it on a porcelain plate, and add a drop of iodine solution.

This gives first a blue color, showing the presence of starch; later a violet color, due to the mixture of the blue of the starch reaction with the red caused by the dextrin; next a reddish-brown color, due to erythrodextrin alone (starch being absent), and finally no reaction at all with iodine, proving the absence of starch and erythrodextrin. The fluid now contains achroodextrin and maltose. Test for the latter with Fehling's solution and with Barfoed's reagent.

Exp. 214. *Influence of conditions on ptyalin and its amylolytic action.* — Report and explain the results of the following experiments:

(*a*) Boil a few cubic centimeters of the saliva, then add some starch paste, and place in the water-bath at 40° C. After five minutes test for sugar.

(*b*) Take two test-tubes: put some starch paste in one, and saliva in the other, and cool them to 0° C., in a freezing mixture. Mix the two solutions, and keep the mixture surrounded by ice for several minutes, then test a portion for sugar. Now place the remainder in the water-bath at 40° C., and after a time test for sugar.

(*c*) Carefully neutralize 20 c.c. of saliva with very dilute HCl (the 0.2 per cent diluted), and dilute the whole to 100 c.c. Test the action of this neutralized saliva on starch.

(*d*) To 5 c.c. of starch paste add 10 c.c. of 0.2 per cent HCl and 5 c.c. of neutral saliva, and expose the mixture for a while at 40° C., and test for sugar.

(*e*) To 5 c.c. of starch paste add 10 c.c. of a 0.5 per cent solution of Na_2CO_3 and 5 c.c. of neutral saliva, and expose the mixture for a while at 40° C., and test for sugar.

(*f*) Carefully neutralize (*d*) and (*e*), and again test the action of the two on starch.

(*g*) Mix a little uncooked starch with saliva, expose to a temperature of 40° C. for a while, and test for sugar.

Exp. 215. In three separate test-tubes place a few cubic centimeters of dilute solutions of KCNS or NH_4CNS, of meconic acid, and of acetic acid; add to each a few drops of ferric chloride, and notice that a similar color is obtained in each case. Divide

the contents of each tube into two portions, and to one set add HCl; to the other add mercuric-chloride solution. Formulate a method of distinguishing from the sulphocyanates, meconates, and acetates.

Analysis of Gastric Contents and Experiments with Pepsin.

The following solutions will be found in the laboratory:

A. *A 0.2 per cent Solution of HCl.* — This is prepared by diluting 6.5 c.c. of concentrated HCl (sp. gr. 1.19) with distilled water to 1 liter.

B. *A Solution of Pepsin.* — Prepared by dissolving 2 grams of pepsin in 1000 c.c. of water.

C. *A Pepsin-hydrochloric-acid Solution.* — Prepared by dissolving 2 grams of pepsin in 1000 c.c. of solution A.

Or, add to 150 c.c. of solution A about 10 c.c. of the glycerol extract of the mucous membrane of the stomach.

Exp. 216. Take five test-tubes and label *a*, *b*, *c*, *d*, *e*. Fill as indicated below. Place in a water-bath at 40° C., and examine an hour later, and again the next day.

(*a*) 3 c.c. pepsin solution + 10 c.c. water + a few shreds of fibrin.

(*b*) 10 c.c. 0.2 per cent HCl + a few shreds of fibrin.

(*c*) 3 c.c. pepsin solution + 10 c.c. 0.2 per cent HCl, and a few shreds of fibrin.

(*d*) 3 c.c. pepsin solution + 10 c.c. 0.2 per cent HCl, boil, and then add a few shreds of fibrin.

(*e*) 3 c.c. pepsin solution + 10 c.c. 0.2 per cent HCl, and a few shreds of fibrin which have been tied firmly together into a ball with a thread.

Make a note of all changes.

Exp. 217. Filter *c*. Neutralize with dilute Na_2CO_3. Filter again. Why? Test the filtrate for the biuret reaction.

Exp. 218. To 5 grams fibrin add 30 c.c. of the pepsin solution and 100 c.c. 0.2 per cent HCl. Set in the water-bath at 40° C., stirring frequently, and leave in the water-bath overnight. Observe the undigested residue, on the following day, and also a slight flocculent precipitate. What is this precipitate?

Filter and carefully neutralize the filtrate. A precipitate varying with the progress of the digestion will form. What is it?

Remove this by filtration, and saturate this filtrate with $(NH_4)_2SO_4$. Filter. Save precipitate and filtrate. Of what does each consist?

Exp. 219. Dissolve the last precipitate of Exp. 218 in water, and try the following tests:

(*a*) Biuret reaction.

(*b*) Effect of boiling.

(*c*) Test with HNO_3, as in performing test for albumin in the urine, page 203.

Exp. 220. To the last filtrate of Exp. 218 add an equal volume of 95 per cent alcohol, and stir thoroughly. The peptones will collect in a gummy mass about the stirring-rod.

(*a*) Determine the solubility of peptones in water.

(*b*) What is the effect of heat when they are so dissolved?

(*c*) Try the biuret reaction.

Exp. 221. *Demonstration of rennet enzyme.* — Place 10 c.c. of milk in each of three test-tubes. Label the test-tubes 1, 2, 3.

To 1 add a drop of neutralized glycerol extract of the mucous membrane of the stomach (made from the stomach of the calf).

To 2 add a drop of neutralized glycerol extract, and boil at once.

To 3 add a few cubic centimeters of $(NH_4)_2C_2O_4$ solution, and then a drop of a glycerol extract.

Place these tubes in the water-bath at 40° C., and examine after five to ten minutes. Explain results in each case.

Continue heating tube 3 for half an hour, then add 2 or 3 drops $CaCl_2$ solution. The liquid instantly solidifies. Why?

Exp. 222. *Digestion of paracasein.* — Determine the products of the digestion of the curd from the last experiment.

Exp. 223. *Tests for free hydrochloric acid.* — Try each of the following tests with (*a*) HCl (0.2 per cent, 0.05 per cent, and 0.01 per cent successively); (*b*) lactic acid (1 per cent); (*c*) mixtures containing equal volumes of (*a*) and (*b*). Tabulate the results.

(*a*) *Dimethylaminoazobenzene.* — Use 1 or 2 drops of a 0.5

per cent alcoholic solution. In the presence of free mineral acids a carmine-red color is obtained.

(*b*) *Gunzburg's reagent.* — Phloroglucin, 2 grams; vanillin, 1 gram; alcohol, 100 c.c. Place 2 or 3 drops of the solution to be tested in a porcelain dish, add 1 or 2 drops of the reagent, and evaporate on a water-bath. In the presence of free hydrochloric acid a rose-red color develops.

(*c*) *Boas' reagent.* — This is prepared by dissolving 5 grams of resublimed resorcinol and a gram of cane-sugar in 100 grams of 94 per cent alcohol. Take three or four drops each of the reagent and the solution to be tested, and cautiously evaporate to dryness. In the presence of a free mineral acid a rose or vermilion red color is obtained. This gradually fades on cooling.

(*d*) *Tropœolin OO.* — Use 1 or 2 drops of a saturated alcoholic solution.

(*e*) *Congo-red.* — Use filter-paper which has been dipped into a solution of the reagent and then dried.

Exp. 224. To 5 c.c. egg-albumin in solution add 1 c.c. of 0.2 per cent HCl. Mix thoroughly, and test for the presence of free HCl. What is the result? How do you explain it? Repeat the test, using a solution of peptone in place of the egg-albumin.

Exp. 225. *Tests for lactic acid.* — *Uffelmann's reagent.* Mix 10 c.c. of a 4 per cent solution of carbolic acid with 20 c.c. of water, and add a drop or two of ferric chloride.

To 5 c.c. of the reagent add a few drops of the lactic-acid solution. Note the canary-yellow color.

Does the presence of free HCl interfere with this reaction?

A more delicate reagent is obtained by adding 3 or 4 drops of a 10 per cent ferric-chloride solution to 50 c.c. of water. Such a solution has a *very faint* yellow color, which is distinctly intensified by lactic acid. (See Exp. 60.)

Using 5 c.c. of this nearly colorless solution for each experiment, note the effect of (*a*) 0.2 per cent HCl; (*b*) acid phosphate of sodium; (*c*) alcohol; (*d*) glucose; (*e*) cane-sugar. What conclusions do you reach concerning the value of this test, when applied directly to the gastric contents?

The test is best applied to an aqueous solution of the ethereal

extract of the gastric contents. Add to the contents 2 drops of HCl, boil to a syrup, and extract with ether. Dissolve the residue obtained upon evaporation of the ether in a little water, and test for lactic acid.

Exp. 226. Test for butyric acid; see ethyl butyrate, page 259.

Exp. 227. Test for acetic acid similarlarly forming ethyl acetate.

Exp. 228. The acidity of the gastric contents may be determined as follows: To 5 c.c. of the filtered contents, diluted with 25 to 30 c.c. of water in an Erlenmeyer flask, add 2 or 3 drops of a solution of dimethylaminoazobenzene. Titrate with N/10 alkali till the color changes to a yellow which fairly matches the indicator; this represents the free HCl. To this mixture add a few drops of phenolphthalein solution, and continue the titration until a permanent pink color is obtained. The N/10 alkali used will represent the total acidity, combined HCl, and organic acids. The organic acids will not be present in gastric contents in the presence of any appreciable amount of free HCl, as they are derived almost entirely from fermentations which are inhibited by the hydrochloric acid.

Experiments with Pancreatic Juice.

Exp. 229. *Proteolytic action.* — To 25 c.c. of a 1 per cent solution of Na_2CO_3 add a few drops of the pancreatic extract. Place some pieces of fibrin in this liquid, and keep in the water-bath at 40° C. till the fibrin has disappeared (one or two hours probably). Observe the digestion from time to time. Note that the fibrin does not swell and dissolve as in gastric digestion, but that it is eaten away from the edges.

Filter. What is the precipitate? Carefully neutralize the filtrate with 0.2 per cent HCl. Another precipitate may appear. What is this?

Again filter, if necessary, and test the filtrate for proteoses and peptones as directed under gastric digestion.

Exp. 230. *Amylolytic action.* — To some starch paste in a test-tube add a drop or two of the pancreatic extract and place in the water-bath at 40° C. After a few minutes test for sugar and report the result.

Exp. 231. *The Piolytic (fat-splitting) action.* — For the demonstration of this action use natural pancreatic juice, or finely divided fresh pancreas, or a *recently prepared* extract.

To some perfectly neutral olive oil, colored faintly blue with litmus, add half its volume of the pancreatic extract, shake thoroughly, and keep at 40° C. for twenty minutes. Record the result. Reserve for next experiment.

Exp. 232. *Emulsifying Action.* — To 10 c.c. of a 0.2 per cent solution of Na_2CO_3 add a few drops of the mixture used in Exp. 231. Shake thoroughly, and report the result. Referring to the earlier experiments on emulsification (see Fats), explain the efficacy of the pancreatic juice in emulsifying fats.

Experiments with Bile.

Exp. 233. *Color.* — Note the difference in color between human bile and ox bile. Explain.

Exp. 234. *Reaction.* — Dilute some bile with 4 parts of water. Immerse a strip of red litmus paper, then remove and wash with water. Note the reaction.

Exp. 235. *Nucleo-albumin.* — Dilute bile with twice its volume of water, filter if necessary, and add acetic acid. What is the precipitate? How distinguished from mucin?

Exp. 236. Filter 235 and test the filtrate for proteins. Report the result.

Exp. 237. *Separation of Bile Salts.* — Mix 20 c.c. of bile with animal charcoal to form a thick paste, and evaporate on the water-bath to complete dryness. Pulverize the residue in a mortar, transfer to a flask, add 25 c.c. of absolute alcohol, and heat on the water-bath for half an hour. Filter. To the filtrate add ether till a permanent precipitate forms. Let the mixture stand for a day or two, and then filter off the crystalline deposit of bile salts. Save the filtrate which contains cholesterin. (Plate VI, Fig. 4, page 132.)

Exp. 238. *Bile-pigments.* — (*a*) *Gmelin's test.* — Take some bile in a wine-glass and underlay with *yellow* HNO_3, in the manner described in testing saliva for albumin. Notice the play of colors, beginning with green and passing through blue,

violet, and red, to yellow, at the junction of the two liquids. Explain.

(*b*) *Iodine test.* — Place 10 c.c. of dilute bile in a test-tube, and add slowly 2 or 3 cubic centimeters of dilute tincture of iodine, so that it forms an upper layer. A bright green ring forms at the line of contact.

Exp. 239. *Cholesterol.* — Examine under the microscope the crystals obtained by the cautious evaporation of the alcohol-ether filtrate of Exp. 237.

Concentrated H_2SO_4, containing a little iodine, gives with cholesterol a series of colors passing from violet to blue, then to green, and finally red.

Exp. 240. *Action of bile in digestion.* — (*a*) Take three test-tubes. In one mix 10 c.c. of bile and 2 c.c. of neutral olive oil; in the second, 10 c.c. of bile and 2 c.c. of rancid olive oil; in the third, 10 c.c. of water and 2 c.c. of neutral oil. Shake and place in a water-bath at 40° C. for some time. Note the extent and the permanency of the emulsion in each case.

(*b*) Into each of two funnels fit a filter-paper. Moisten one with water and the other with bile, and into each pour an equal volume of olive oil. Set aside for twelve hours (with a beaker under each funnel). Do you notice any difference in the rate of filtration?

(*c*) Add, drop by drop, a solution of bile salts to (*a*) a solution of egg-albumin; (*b*) a solution of acid-albumin; (*c*) a solution obtained by digesting a bit of fibrin in gastric juice and filtering. Explain the results.

APPENDIX.

REAGENTS.

It is desirable that all reagents be made with reference to the molecular weights of the substances employed. These may be from one to ten times the molecular weight per liter, while the solutions for practice are from one-tenth to one-fourth the molecular weight per liter. Salt solutions used as reagents are conveniently from five to ten per cent; that is, a molar concentration bringing the strength within these limits is selected.

In the following list a few exceptions will be noted.

Ammoniacal Cuprous Chloride. — This may be made by dissolving copper oxide with metallic copper in dilute hydrochloric acid with the aid of heat. To the clear, cool, resulting solution add ammonia to marked alkaline reaction.

Ammoniacal Silver Solution. — Dissolve 10 grams of silver nitrate in 200 c.c. of water and add about 50 c.c. of strong ammonia, or an amount considerably in excess of that required to dissolve the precipitate first formed.

Ammonium Molybdate Solution for Phosphates. — This may be made by dissolving 20 grams of ammonium molybdate in a mixture of 250 c.c. NH_4OH and 250 c.c. of water. Then this solution is added to 1000 c.c. of nitric acid, making 1500 c.c. of reagent. In using this solution as a test for phosphates it is necessary to heat the mixture to about 60° C.

If the reagent is prepared in the following manner it reacts without heating, is more sensitive than that produced by the first formula and is recommended as the better of the two. Dissolve 100 grams of molybdenum trioxide (molybdic acid) in 400 c.c. of dilute NH_4OH (10 per cent). Allow to cool and add all at once 1000 c.c. of dilute HNO_3 (HNO_3 three parts, H_2O two parts). The precipitate first formed is immediately redissolved

and the product should be a perfectly clear, nearly colorless solution.

Ammonium Oxalate M/4, 35.52 grams per liter.

Ammonium Sulphate. (Standard for ammonia determination). — Dissolve .4716 gram of the pure salt in a liter of water. Ten c.c. then contains 1 mg. of nitrogen.

A solution of twice this strength may be used and seems preferable to the author. Ten c.c. then contains 2 mg. of nitrogen and 5 c.c. used in the determination will contain 1 mg.

Ammonium Sulphate (for protein precipitation). — A saturated solution is required — that is, the solution of protein must be saturated with the salt.

Note. In case a half-saturated solution is called for, take equal volumes of the unknown protein solution and saturated ammonium sulphate.

Barfoed's Reagent. — Dissolve 1 part of copper acetate in 15 parts of water; to each 200 c.c. of this solution add 5 c.c. of acetic acid containing 38 per cent of glacial acetic acid.

Benedict's Qualitative Solution has the following composition:

	Grams or c c.
Copper sulphate (pure crystallized)	17.3
Sodium or potassium citrate	173.0
Sodium carbonate (crystallized)	200.0
or one-half the weight of the anhydrous salt	
Distilled water to make	1000.0

The citrate and carbonate are dissolved together (with the aid of heat) in about 700 c.c. of water. The mixture is then poured (through a filter if necessary) into a larger beaker or casserole. The copper sulphate (which should be dissolved separately in about 100 c.c. of water) is then poured slowly into the first solution with constant stirring. The mixture is then cooled and diluted to 1 liter.*

Benedict's Quantitative Solution.

$CuSO_4.5\ H_2O$	18 grams
KCNS	125 "
Na_2CO_3	200 "

* Journal American Medical Association, Oct. 7, 1911, page 1193.

Sodium or potassium citrate 200 grams
$K_4Fe(CN)_6$ 5 c.c. of a 5 per cent solution
Distilled water to make a total volume of 1000 c.c.

The citrate, cyanate, and carbonate are dissolved by aid of heat, and the solution filtered if necessary. The copper sulphate is dissolved in a separate portion of water and added to the *hot* filtered solution, the flask being shaken vigorously during the addition.

Benzidine Solution. — Saturated solution of benzidine in glacial acetic acid with an equal volume of peroxide of hydrogen solution. The two solutions are to be mixed when used as a test for blood.

The following method of making the benzidine solution is suggested by Hawk's 'Physiological Chemistry': 4.33 c.c. of glacial acetic acid is warmed in a small Erlenmeyer flask to about 50° C., a half gram of benzidine added, and the mixture heated eight or ten minutes at 50° C. and then the solution diluted with 19 c.c. of distilled water. If kept in a dark place it is fairly permanent.

Brom-cresol Purple. — See page 297.

Brom-thymol Blue. — See page 297.

Cochineal. — Extract cochineal with 30–50 per cent alcohol and filter.

Congo Red. — Two per cent aqueous solution.

Copper Sulphate. — One per cent solution for Biuret Test.

Creatinine Solution. — Dissolve 1.6106 grams of creatinine zinc chloride in 1 liter of N/10 HCl. This solution contains 1 mg. of creatinine per c.c.

Standard for Saliva and Blood. — Take 6 c.c. of the above standard (i.e., 6 mg. creatinine), and 10 c.c. of N/1 HCl and make up to a liter with distilled water. Add a few drops of toluol and mix. Five c.c. of this solution contains .06 mg. creatinine.

Dimethylaminoazobenzene. — 0.5 per cent alcoholic solution.

Esbach's Reagent. — Dissolve 10 grams of picric acid, and 20 grams of citric acid in sufficient water to make 1 liter of solution.

Fehling's Solution. — The Fehling's solution recommended

for experiments in this book is one-half the strength frequently employed, and is prepared in separate solutions as follows: Dissolve 34.639 grams of pure crystallized copper sulphate in water, and make solution up to 1 liter. This constitutes the first part of the reagent. The second part may be made by dissolving 173 grams of Rochelle salts and 52.7 grams of caustic soda (NaOH) in water and making up to 1 liter. When prepared in this way 10 c.c. of each of these solutions mixed together will be reduced by 0.05 gram of glucose.

Ferric Alum. — For use in silver and chlorine determinations. A saturated aqueous solution to which sufficient strong HNO_3 is added to make about a 10 per cent solution.

Ferric Chloride. — 2.5 per cent solution acidified with HCl.

Goulard's Extract. — A solution of lead subacetate, *q.v.*

Gram's Solution. — See iodine solution.

Gunzburg's Reagent. — Phloroglucin, 2 grams; vanillin, 1 gram; alcohol, 100 c.c.

Hopkins-Cole Reagent (glyoxylic acid, $CHO.COOH.H_2O$). — Prepared by saturating a liter of water with oxalic acid, adding 60 grams of sodium amalgam and allowing to stand until reduction is complete or until hydrogen ceases to be evolved. For use this solution should be filtered and diluted with two or three volumes of water.

Hydrochloric Acid (dilute). — Hydrochloric acid, strong, (sp. gr. 1.20) 1 part; distilled water, 2 parts.

Hypobromite Solution for Urea. — Consists of a mixture of equal parts of the following solutions kept separately and mixed for use.

Bromine Solution for Urea. — 125 grams KBr and 125 grams Br to one liter water.

NaOH Solution for Urea. — A 40 per cent solution, or a ten molar solution.

Indicators for H-Ion Concentration Determination.

Brom-thymol Blue. — A .02 per cent watery solution.

Brom-cresol Purple. — A .02 per cent watery solution.

Methyl Red. — A .02 per cent watery solution.

If a flocculent precipitate occurs in the case of the methyl red it may be cleared away by the addition of .5 c.c. of N/20 NaOH (sterile). This does not interfere with obtaining accurate results.

Instead of being prepared directly, these indicators may be prepared from " stock alcoholic solutions " containing .1 gram of indicator dissolved in 50 c.c. of 95 per cent alcohol.

To prepare the .02 per cent watery solution in this way, take 45 c.c. of distilled water and 5 c.c. of the alcoholic solution. This makes 50 c.c. of the dilute watery solution.

Note. All these solutions should be kept in *amber colored bottles* and should be prepared under as nearly *sterile conditions* as possible.

Iodine Solution. — For determination of iodine absorption number of fats.

Solution I.

26 grams iodine
500 c.c. 95 per cent alcohol

Solution II.

30 grams mercuric chloride
500 c.c. 95 per cent alcohol

Mix the two solutions and allow to stand at least twelve hours before using.

Iodine Tincture. — See Tincture.

Invertase. — Mix 500 grams of " beer yeast," 200 c.c. of water and 10 grams of sugar; allow to stand one hour. Add 50 c.c. of 60 per cent alcohol and a little thymol. Filter, press or allow to dry, put the nearly dry mass in a flask, add 20 grams of sugar and shake till solution is effected. Keep in ice chest.

If " beer yeast " is not available, a solution of invertase, rather less satisfactory than the above, can be made as follows: Take one dozen compressed yeast cakes, grind with sand and mix with 500 c.c. of water, and a little chloroform as preservative. Allow to stand twelve hours and filter.

Iodine Solution. —

Lugol's solution. — Iodine 5 grams, potassium iodide 10 grams, and sufficient distilled water to make 100 grams. (U.S.P.)

Gram's solution. — Iodine 1 gram, potassium iodide 2 grams, and sufficient distilled water to make 200 grams.

Lead Subacetate (basic acetate of lead). — The U. S. P. method of preparation is as follows: lead acetate 180 grams, lead oxide 110 grams, distilled water to make 1000 grams. Rub lead oxide to a paste with 100 c.c. of water, dissolve lead acetate in 700 c.c. of boiling distilled water; add slowly with constant stirring to lead oxide and boil the mixture for half an hour. Cool and filter and make up to 1000 c.c. with water free from carbon dioxide.

Leucin. — See under Cystin, page 303.

Lipase. — From castor bean (see page 270). Remove the shells from 10 grams of fresh beans, break them up as fine as possible and allow to stand overnight in a loosely stoppered test-tube full of alcohol ether mixture. Pour off; grind the beans to a powder in a small mortar, transfer to a test-tube and let stand under ether overnight. Filter with suction and wash two or three times with small amounts of the alcohol ether mixture.

Lipase. — From pancreas. Take a pig's pancreas, remove all fat, grind and allow to stand overnight. Then add four times its weight of 25 per cent alcohol and allow to stand three days. Syphon off clear fluid and neutralize with sodium carbonate. The solution will contain a fat-splitting enzyme.

Lithium Sulphate (for use in uric acid determination). — Twenty per cent aqueous solution.

Lugol's Solution. — See Iodine.

Magnesia Mixture. — Dissolve 125 grams of ammonium chloride, and 125 grams of magnesium sulphate, in sufficient water to make 1 liter of solution; then add 125 c.c. of strong ammonia water.

Marmé's Reagent. — Ten grams of potassium iodide, 5 grams of cadmium iodide, 100 c.c. of water.

Mercuric Chloride Solution. — Five per cent $HgCl_2$ in distilled water.

Millon's Reagent. — To one part of mercury add two parts of nitric acid of specific gravity 1.4, and heat on the water-bath

till the mercury is dissolved. Dilute with two volumes of water. Let the precipitate settle, and decant the clear fluid.

Molisch's Reagent for Carbohydrates. — Fifteen per cent solution of α-naphthol in alcohol.

Nessler's Solution. — An alkaline solution of potassio-mercuric iodide, made as follows: Dissolve 35 grams of potassium iodide in about 200 c.c. of water. Dissolve 17 grams of mercuric chloride in 300 c.c. of hot water. Add the potassium iodide to the mercuric chloride, until the precipitate at first formed is nearly all redissolved. If the precipitate should entirely dissolve, add a few cubic centimeters of a saturated solution of mercuric chloride, until a slight permanent precipitate is obtained. After the mixture is cold, make up to 1 liter with a 20 per cent solution of caustic potash. Allow to settle and use the clear solution.

Nitric Acid (dilute). — Strong HNO_3 (sp. gr., 1.42) one part, and water three parts.

Pancreatic Extract. — Obtain a fresh pancreas and soak in four times its weight of 25 per cent alcohol for two or three days. Filter and make the solution neutral or very slightly alkaline with sodium carbonate. This solution will contain the fat-splitting enzyme.

Phenoldisulphonic Acid. — Phenoldisulphonic acid, for estimation of nitrates in water analysis, may be prepared by heating on a water-bath for several hours a mixture of 555 grams of concentrated sulphuric acid and 45 grams of pure carbolic-acid crystals.

Phenolphthalein. — Make a 1 per cent alcoholic solution and then dilute with an equal volume of water.

Phenyl-hydrazine Solution. — Dissolve 1 gram of phenyl-hydrazine hydrochloride and 2 grams of sodium acetate in 10 c.c. of water.

Phosphoric-Sulphuric Acid Digestion Mixture (Folin). —

50 c.c. 5 per cent $CuSO_4$ (Crystallized)
300 c.c. 85 per cent H_3PO_4
100 c.c. conc. H_2SO_4 (ammonia-free)

Mix and prevent absorption of ammonia from air. This acid

mixture acts on glass and consequently can only be used in microchemical analyses when the digestion takes place in a few minutes.

Phospho-tungstic Acid (Folin-Denis uric acid reagent). —

750 c.c. distilled water
100 grams sodium tungstate — *must be very pure*
80 c.c. 85 per cent H_3PO_4

Let boil slowly for two hours and then dilute to a liter and mix.

Picric Acid (for creatinine determination). — To make a saturated solution of picric acid, dissolve with heat (do not boil) an excess of picric acid in distilled water. Then cool the solution, and as crystallization takes place pour off the supernatant fluid.

Picric-acid Solution (Esbach's Reagent). — Dissolve 10 grams of picric acid and 20 grams of citric acid in sufficient water to make one liter.

Potassium Ferrocyanide Solution. — $K_4Fe(CN)_6$, one-fourth molar solution (9.2 per cent).

Schiff's Reagent. — Into 50 c.c. of a 2 per cent solution of fuchsine or rosaniline pass SO_2 gas until the solution is colorless. Then dilute with an equal volume of water and keep in small full bottles in a dark place.

Silver-nitrate Solution. — Drop solution, 1: 8, used as a qualitative test for chlorine in urine.

Silver-nitrate Solution (for sterilization of root canals). — See Howe's silver-nitrate preparation, page 84.

Sodium Cyanide (for use in uric acid determination). — Make a 7.5 per cent aqueous solution and allow to stand at least one week before using.

Starch Paste (thin). — Rub about one-half gram of starch to a thin paste with cold water. Add sufficient boiling water to dissolve, then dilute to 100 or 150 c.c.

Sulphuric Acid (dilute). — Twenty per cent strong H_2SO_4 in distilled water.

Tincture Iodine for Bile Test. — Dilute the U. S. P. tincture with alcohol until just transparent in test-tube.

Tollen's Reagent. — Make a 10 per cent solution of $AgNO_3$ in

dilute ammonia, and just before using mix an equal volume of this solution with a 10 per cent solution of NaOH.

Tropæolin oo. — Saturated alcoholic solution.

Tungstic Acid (for protein precipitation). —

10 per cent solution sodium tungstate (must be C.P.)
2/3 N sulphuric acid

Equal volumes of each are added for complete protein precipitation.

Urease Solution. — Five per cent water solution of *arlco urease*. This urease dissolves easily, but the solution does not keep well. It should be made fresh each time.

Uric acid Solution (Folin). — Weigh 2 grams of uric acid on watch glass. On another watch glass weigh 1 gram lithium carbonate; dissolve the lithium salt in 300 c.c. of water and heat to 60°. With the lithium solution rinse the uric acid through funnel into a 2-liter flask. Shake to solution and cool. Add 50 c.c. of 40 per cent formalin and shake. Then add 10 c.c. of glacial acetic acid and shake until rid of CO_2. Dilute to volume and mix. Put up in 100-c.c. bottles. This solution keeps in the *dark* indefinitely.

For Use:

Transfer 1 c.c. of the above standard to a 250-c.c. volumetric flask. Half fill with distilled water and add 1 c.c. of formalin. Dilute to volume and mix. This solution keeps at least ten days.

Uffelmann's Reagent. — Mix 10 c.c. of a 4 per cent solution of carbolic acid with 20 c.c. of water, and add a drop or two of ferric chloride.

Uranium Solution (standard for phosphate determination). — See page 197.

PREPARATIONS.

Creatine may be most conveniently prepared from a strong solution of Liebig's extract. Dissolve the extract in 20 parts of water, add basic lead acetate drop by drop, to avoid more than a slight excess, then remove excess of lead; concentrate to a syrup over a water-bath and allow to stand in a cool place,

whereupon creatin crystals will separate out. Two or three days' time may be required before the crystals are obtained. They may be washed with 88 per cent alcohol and purified by recrystallization from water. Hypoxanthin and sarcolactic acid may be obtained from the mother liquor.*

Creatinine may be prepared from creatine by boiling for ten or fifteen minutes with very dilute sulphuric acid. Neutralize the acid with $BaCO_3$, filter, evaporate to dryness on a water-bath, and extract the creatinine with alcohol. Upon evaporation, the creatinine is obtained in the form of crystals.

Cystin. — 1. Clean 200 grams of hair by washing with dilute HCl and then with ether. Boil the clean hair with 600 c.c. of concentrated HCl (specific gravity, 1.19) for four hours (in a 3-liter flask with condenser) on a sand-bath in hood. Then let cool.

2. Add concentrated NaOH solution (750 c.c. H_2O, 500 grams NaOH) till the reaction is only faintly acid.

3. Add to the solution, which has begun to boil on neutralization, plenty of animal charcoal, and boil three-quarters of an hour.

4. Filter hot, being careful to moisten filter and funnel with hot water to prevent funnel from cracking.

5. The filtrate should be faintly yellow. On cooling, a crystalline precipitate forms, mainly cystin, with some tyrosin and leucin. If this is not the case, or if the precipitate is slight, the solution must be concentrated. Save the filtrate, which with the filtrate from 6 is to be worked up later for tyrosin and leucin.

6. Filter off the precipitate after letting it stand overnight.

7. Dissolve this precipitate in 350 c.c. of hot 10 per cent NH_4OH (hood) and let cool. Then continue the cooling with finely chopped ice or with snow. Filter off any tyrosin that may have precipitated, and combine it with the filtrate of 6.

8. Add glacial acetic acid, being careful not to acidify. The precipitate is a mixture of tyrosin and cystin. Filter.

9. Make filtrate from 8 quite acid with glacial acetic acid. The precipitate is almost pure cystin. Let stand twenty-four hours. Then filter, and wash with H_2O and alcohol.

* Lea's 'Chemical Basis of the Animal Body.'

10. Recrystallize by redissolving in the smallest quantity of hot 10 per cent ammonia that will effect solution, cooling and precipitating with glacial acetic acid.

The preparations should be pure and should contain no tyrosin, for which test may be made with Millon's reagent.

Reactions. — Put a trace of cystin into a test-tube with some dilute NaOH and a little lead acetate. Boil. H_2S is formed because S is split off.

Tyrosin. — 1. Concentrate the neutralized filtrate of 6 of cystin preparation till, on cooling, tyrosin crystallizes out.

2. Filter, and save filtrate for the preparation of leucin.

3. Dissolve the tyrosin crystals in a very little hot water.

4. Add amyl alcohol till a heavy precipitate forms.

5. Filter precipitate.

6. Redissolve in a very little hot water, and let crystallize out by cooling.

Examine crystals under the microscope.

Test with Millon's reagent.

Leucin. — 1. Take the filtrate of 2 in the preparation of tyrosin, and evaporate to dryness on the water-bath.

2. Extract with alcohol.

3. On standing, the leucin crystallizes out of the alcoholic extract as it evaporates.

4. Filter, and dry the crystals.

Examine under the microscope.

Gelatin. — Take about 10 grams of bone, preferably small pieces of the shaft of a long bone, clean carefully, and allow to stand for a few days in 60 c.c. of dilute HCl (1/20). The dilute acid dissolves the inorganic portion of the bone, leaving the collagen. Note the effervescence due to the presence of carbonates. The acid solution is poured off and kept for further investigation. The remains of the bone are allowed to stand overnight in a dilute solution (1/10) of Na_2CO_3, and then boiled in 100 c.c. of water for an hour or two. The collagen undergoes hydrolysis and is converted into gelatin, which dissolves. A core of bone untouched by the acid usually remains. Evaporate the solution to 25 c.c. bulk and allow to cool. A firm jelly is

formed if the solution is sufficiently concentrated. If the solution gelatinizes, add an equal bulk of water and heat anew. If the solution thus obtained is sufficient in quantity it may be used for Experiments 208 and 209.

Gelatin may also be prepared from tendons, which consist almost wholly of white fibers. Collagen is the substance of which white fibers are made up.

Glycogen $(C_6H_{10}O_5)_n$. — Use a liver taken from an animal just killed, or, if the season permits, oysters just removed from the shell. Cut an oyster, as rapidly as possible, into small pieces, and throw it into four times its weight of boiling water, slightly acidulated with acetic acid. After boiling the first portion for a short time, remove the pieces, grind in a mortar with some sand, return to the water, and continue the boiling for several minutes. Filter while hot. The opalescent solution thus obtained is an aqueous solution of glycogen and other substances.

If a purer solution is desired, continue as follows: Add to the filtrate alternately a few drops of hydrochloric acid and potassio-mercuric iodide, until a precipitate of protein ceases to form. This may be determined more conveniently by filtering off a small portion of the liquid from time to time, and adding to the clear filtrate the hydrochloric acid and potassio-mercuric iodide. When the precipitation of the proteins is complete, filter, and to the milky filtrate add double its volume of alcohol; the glycogen will precipitate as a white powder. Filter this off, wash with 66 per cent alcohol (one part of water to two of alcohol), and dissolve in water.

Mucin Solution. — Cut a portion of a navel-cord into small pieces. Shake in a flask with water, changing the water several times. This removes salts and albumin. Extract for twenty-four hours with N/100 NaOH in a corked flask. Add N/100 acetic acid, which precipitates the mucin. Let settle, filter, and wash with water.

Mucin may also be prepared from the saliva by precipitation with acetic acid.

Potassium Cyanate (KCNO). — Melt in an iron ladle, of at

least 50 c.c. capacity 5 grams of commercial potassium cyanide, and stir in gradually 20 grams of litharge. When the entire amount has been added, pour the mass out upon an iron plate, and allow to cool. Separate as far as possible the reduced lead from the potassium cyanate that has been formed, powder the latter, and dissolve in 25 c.c. of cold water. Filter if necessary and purify by repeated crystallization.

Tyrosin. — See paragraph under Cystin, page 303.

Urea, Synthesis of. — Add to a filtered solution of KCNO a cold saturated solution of ammonium sulphate, containing at least 6 grams of $(NH)_2SO_4$. Heat the mixture slowly on a water-bath at a temperature of 60° C., and maintain at that point for one hour. By this process ammonium cyanate is formed and then changed to urea, which may be obtained in an impure state by evaporating the solution to dryness on a water bath, and extracting the residue with hot, strong alcohol. The urea will crystallize from the alcohol as it cools.

Vegetable Globulin: e.g. Edestin. Extract about 1 ounce of crushed hemp seed with water containing about 5 per cent sodium chloride. This extraction should take from one-half hour to one hour at a temperature of about 60° C. Filter while hot. Upon cooling, a portion of the globulin (edestin) will probably separate out. Use the clear separated fluid for the general protein reactions and precipitates. Boil the cloudy portion until the precipitated globulin has dissolved. Then set aside for twenty-four hours in order that the edestin may crystallize slowly, whereupon hexagonal plates should be obtained. Examine by the microscope. (See Plate VI, Fig. 1, page 132.)

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