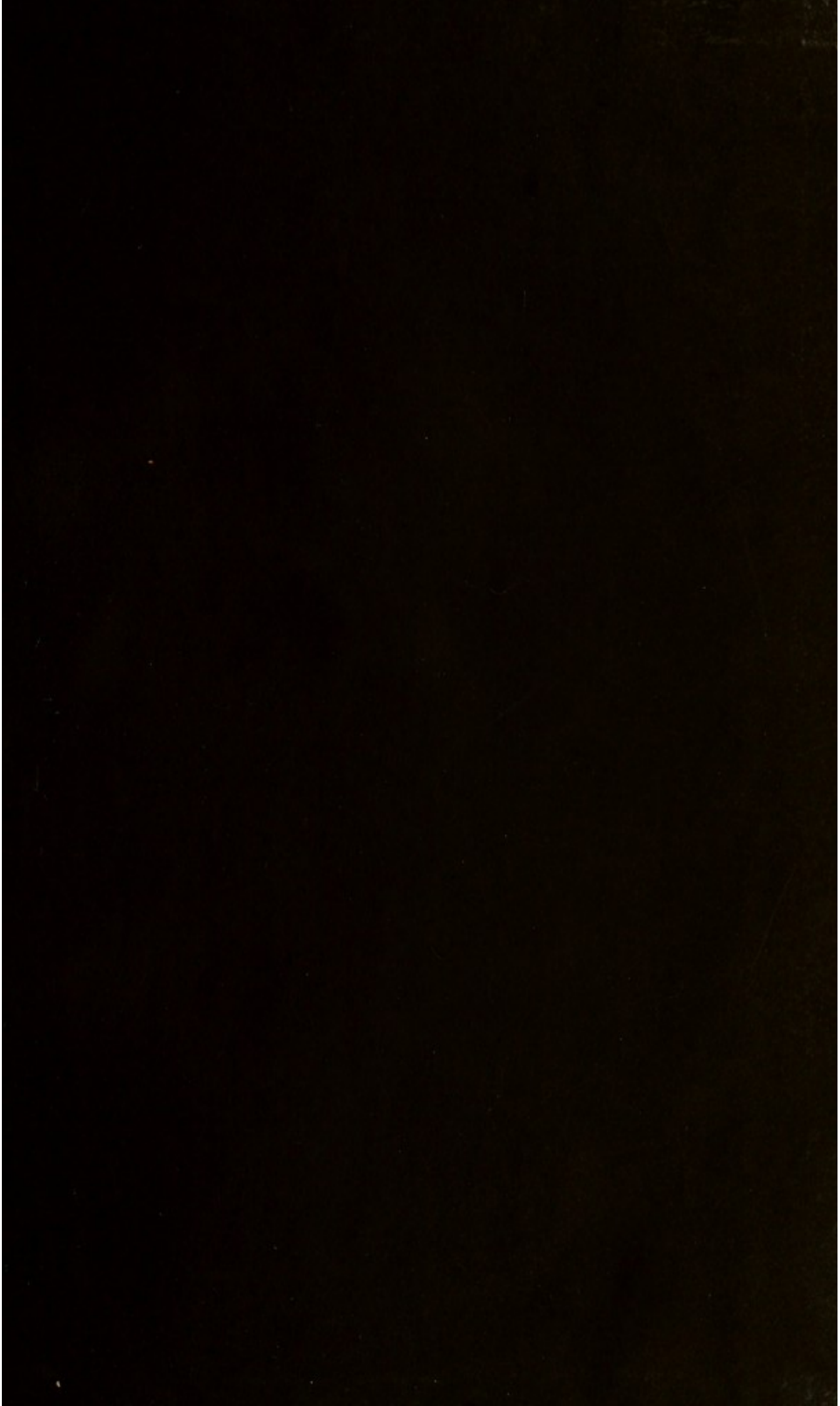
Lecture-notes on chemistry for dental students : including dental chemistry of alloys, amalgams, etc., such portions of organic and physiological chemistry as have practical bearing on the subject of dentistry, an inorganic qualitative analysis with specially adapted blowpipe and microscopical tests, and the chemical examination of urine and saliva / by H. Carlton Smith.



LECTURE-NOTES

ON

CHEMISTRY

FOR

DENTAL STUDENTS

INCLUDING

DENTAL CHEMISTRY OF ALLOYS, AMALGAMS, ETC.

SUCH PORTIONS OF ORGANIC AND PHYSIOLOGICAL CHEMISTRY AS HAVE PRACTICAL BEARING ON THE SUBJECT OF DENTISTRY

AN INORGANIC QUALITATIVE ANALYSIS WITH SPECIALLY ADAPTED BLOWPIPE AND MICROSCOPICAL TESTS, AND THE CHEMICAL EXAMINATION OF URINE AND SALIVA

BY

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PREFACE.

THE arrangement of this book follows rather closely the lecture course in Dental Chemistry as given by the author at the Harvard Dental School. It has been the aim of these lectures to give the student, as concisely as possible, such portions of the various branches of chemistry as are most likely to be of value in practical work.

Simplicity of manipulation has in some cases been considered of greater practical value than extreme accuracy, especially in the chapter on Quantitative Analysis. The volumetric processes being given as a rule, rather than the more exact but more difficult gravimetric methods.

The usual equipment of a dental laboratory has been borne in mind, and considerable prominence given to the simpler analytical tests made in the dry way by means of few reagents.

Recent text-books and current literature have been very generally consulted. New tests have been verified so far as possible—often modified—before being recommended to the student.

The U. S. Dispensatory and the Newer Materia Medica, as given in the Druggists' Circular, have been drawn upon in the sections on Local Anæsthetics; Hall's and Essig's Chemistries in the section on Alloys and Amalgams.

A chapter on Organic Chemistry has been introduced, designed to furnish an understanding of this branch of chemical

science, which will enable the student to better comprehend the physiological chemistry which follows.

The chapter on the Analysis of Saliva is one which is of necessity incomplete and imperfect. The investigations being at present carried on along the lines suggested by Dr. Joseph Michaels of Paris and Dr. Kirk of Philadelphia are opening up fields of research of the greatest magnitude and of utmost importance, and they can only be touched upon in this work.

The atomic weights given are from the international atomic weights for 1905. O = 16.

In the chapter on Physiological Chemistry the author wishes to particularly acknowledge his indebtedness to Professor Wm. B. Hills of the Harvard Medical School, who furnished the majority of the laboratory experiments for this portion of the work.

H. C. S.

TO THE STUDENT.

As the student of dentistry takes up the study of chemistry, it is necessary that he should realize that the course will be of value to him in the ability acquired to draw correct inferences from observed phenomena, and in the attainment of accuracy and delicacy in manipulation, fully as much as in amount of chemical knowledge obtained. In other words, he must do his own thinking, carry out his own processes and experiments, make his own analyses, or the time spent will be little better than wasted, for the chemical facts which may happen to be remembered will be of slight benefit in the work to which every student, worthy of the name, aspires, that of developing, broadening, and elevating the profession which he has chosen as his own.

The course of study outlined in this book is designed to furnish the starting-points in the various branches of chemistry, which will be of practical value in solving the problems constantly presenting themselves for consideration. It is hoped that these starting-points may, in the future, serve as the basis for work along the lines of original research, and that the best interests of dental science may be furthered thereby.

It is supposed that the student has had the advantage of a laboratory training in general chemistry, and is conversant with the properties and methods of preparation of the so-called non-metallic elements, also with the fundamental principles and laws of theoretical and physical chemistry. That he is

familiar with laboratory apparatus, such as test-tubes, beakers, crucibles casseroles, evaporating-dishes, retorts etc., and that he has had some experience in the ordinary processes of precipitation, filtration, evaporation, distillation, sublimation, and crystallization.

If there is any feeling of insufficient preparation it is strongly advised that a short course of preliminary study be taken. Chemistry furnishes the groundwork of all branches of medical science to a much greater extent than we are apt to think, and even in the study of subjects which in times past have been carried on with little reference to chemistry, we now see the desirability if not the necessity of a good general knowledge of chemical science. The physiologist and the bacteri logist are to-day turning to chemistry for the ultimate solution of their most perplexing problems. H. C. S.

TABLE OF CONTENTS.

PART IV.

MICROCHEMICAL ANALYSIS.

PART V.

ORGANIC CHEMISTRY.

PART VI.

PHYSIOLOGICAL CHEMISTRY.

PART VII.

DIGESTION.

PART VIII.

URINE.

DENTAL CHEMISTRY.

PART I.

QUALITATIVE ANALYSIS.

SECTION I.—THE METALS.

The metals, from certain physical properties, have been variously classified. Thus in the older books we read of the *Noble* metals, those unaffected by heat, as gold, silver, and platinum; the *Base* metals, the *Bastard* metals, those easily crystallizable, as antimony and zinc; the *Metalloids*, sodium and potassium.

As the fact became better understood that the properties of metals were to a considerable extent dependent upon conditions of temperature, pressure, etc., the old classifications were less and less used, until now we are very apt to group them according to the chemical behavior of their salts, irrespective of their properties as metals. Thus Ag, Pb, and Hg (Mercurous) form a group of metals whose chlorides are insoluble in water or dilute acids. These metals may consequently be thrown out of solution or precipitated by the addition of HCl to any solution of their salts. We therefore let Ag, Hg′, and Pb constitute the *First Analytical Group*, and HCl is the *First Group Reagent*.

In like manner we find a group of nine metals that are precipitated from dilute acid solution by hydrosulphuric acid (H_2S). These metals are Cu, Cd, Bi, Hg, As, Sb, Sn, Au, and Pt, and constitute the *Second Analytical Group*, and H_2S is the *Second Group Reagent*.

The fact that the sulphids formed by the first four of these metals are *insoluble* in ammonium sulphid, and those formed by the last five are soluble, furnishes a simple method of separating this group into two parts, *a* and *b*:

Pb,* Cu, Cd, Bi, and Hg constituting Group II (*a*) and

As, Sb, Sn, Au, and Pt, Group II (*b*).

Thus the metals are divided into various analytical groups, each with its own peculiar group reagent. Different groupings are possible, and hardly any two analysts will employ exactly the same scheme for identifying all the metals, although the following group divisions are generally used:

Analytical Grouping.

Group I.—Ag, Pb, and Hg′. Metals that form insoluble chlorids and precipitated from aqueous solution by HCl (the group reagent).

Group II (*a*).—Cu, Cd, Bi, Hg″, and Pb. Metals that form sulphids insoluble in dilute HCl solution and also insoluble in ammonium sulphid.

Group II (*b*).—As, Sb, Sn, Au, and Pt. Metals that form sulphids insoluble in dilute HCl but soluble in yellow ammonium sulphid, or alkaline hydrates.

Group III.—Fe, Al, and Cr. In solutions free from H_2S and which do not contain phosphoric, oxalic, tartaric, and certain other organic acids, these three metals may be separated by ammonium hydrate, NH_4OH.

Group IV.—Co, Ni, Mn, and Zn. Metals forming sulphids

* Lead is included in this group because it is not entirely separated as a chlorid in Group I; traces of it remaining in solution even after addition of HCl.

soluble in acid but insoluble in alkaline solution. Ammonium sulphid, $(NH_4)_2S$, is the group reagent.

Group V.—Ba, Sr, Ca, and Mg.* Metals forming carbonates, insoluble in alkaline solutions. The group reagent is ammonium carbonate, $(NH_4)_2CO_3$.

Group VI.—K, Na, Li NH_4. Metals which cannot be precipitated by any single reagent and for which it is necessary to make individual tests.

It is our purpose to take up the study of the metals according to their analytical grouping: first, the deportment of their salts in solution; later, the metals themselves and their specific application to dentistry.

REACTIONS OF GROUP I.

Silver, Ag (Argentum).

Atomic weight 107.93. Occurs free also as various sulphids, silver glance, Ag_2S, and in combination with the sulphids of antimony, lead, and copper.

Silver is readily soluble in nitric acid with formation of $AgNO_3$, colorless crystals, without water of crystallization.

Make the following tests with a weak solution of $AgNO_3$ (about 2%). Write the reactions and enter color and solubility of each precipitate formed in laboratory note-book.†

$AgNO_3$ with HCl gives a white *curdy* precipitate of AgCl which darkens by action of sunlight. If Ag solution is very dilute, the precipitate will assume the curdy appearance and filter more easily if it is heated and rotated quite rapidly in the test-

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* In the process of analysis, magnesium is held in solution by the presence of NH_4Cl and is not thrown out as a carbonate with the other three members of the group.

† The author uses mimeograph copies of these experiments with spaces for the reactions and colors of precipitates, which are filled out without reference to the book and handed in by the student at the close of the laboratory exercise.

These reactions have purposely been confined to such as may be applied to the processes of analysis.

tube. Allow the precipitate to settle. Decant the liquid carefully, divide precipitate into two parts and test its solubility in dilute nitric acid, also in ammonia-water.

$AgNO_3$ with KBr gives a white precipitate of AgBr, less easily soluble in ammonia than the AgCl.

$AgNO_3$ with KI gives a pale yellow precipitate of AgI, insoluble in ammonia.

$AgNO_3$ with H_2S gives a black precipitate of Ag_2S.

$AgNO_3$ with K_2CrO_4 gives a red precipitate of $AgCrO_4$ in neutral solution. Test the solubility of $AgCrO_4$ in NH_4OH, HCl, and HNO_3.

Mercury, Hg (Hydrargyrum).

Atomic weight 200. Occurs as red sulphid, cinnabar, and in small quantities amalgamated with silver or gold or combined with chlorin or iodin. It is the only metal which is liquid at ordinary temperatures, solidifying at $-39°$. In studying the reaction of mercurous salts it is convenient to use a solution of the nitrate, made by treating mercury with cold dilute HNO_3. (This solution of mercurous nitrate, upon standing, will be found to contain more or less mercuric nitrate, unless care is taken to keep excess of mercury in the bottom of the bottle.)

$HgNO_3$ with HCl gives a white precipitate of HgCl (calomel). After the precipitate has settled decant the liquid and test the solubility of the HgCl in ammonia water. Does it dissolve? How does its behavior differ from that of AgCl?

Alkaline hydroxids form with mercurous salts the black oxid Hg_2O; a preparation of which, made with lime-water and calomel, is known as "blackwash."

Lead, Pb (Plumbum).

Atomic weight 206.9. Occurs as sulphid (galena), PbS, also in lesser quantities as native carbonate and sulphate. Lead is soluble in nitric or acetic acid, forming $Pb(NO_3)_2$ or

$Pb(C_2H_3O_2)_2$. A 2% solution of the nitrate may be used in the following tests:

$Pb(NO_3)_2$ with 2HCl gives white precipitate of $PbCl_2$. Test its solubility in hot water and in NH_4OH.

$Pb(NO_3)_2$ with NH_4OH gives white precipitate of $Pb(OH)_2$ insoluble in hot water.

$Pb(NO_3)_2$ with H_2S gives black PbS. Test solublility of precipitate in warm dilute HNO_3.

$Pb(NO_3)_2$ with H_2SO_4 gives white precipitate of $PbSO_4$, forming slowly in dilute solutions.

$Pb(NO_3)_2$ with K_2CrO_4 (or $K_2Cr_2O_7$) gives a yellow precipitate of $PbCrO_4$.

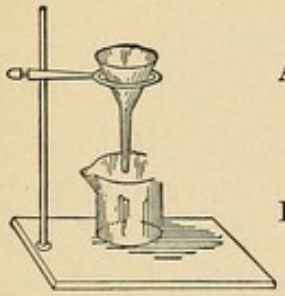
$Pb(NO_3)_2$ gives with KI a yellow recipitate, PbI_2. Avoid excess of the potassium iodid.

By the application of the foregoing reactions we may formulate a scheme for the separation and identification of the metals of Group I as follows:

Analysis of Group I.

(Ag, Pb, Hg′.)

To the clear solution to be tested add slowly dilute HCl as long as any precipitation occurs. Filter and wash the precipitate *once* with cold water, add this washing to filtrate to be tested for remaining groups; then wash precipitate on the paper with several small portions of *hot* water.



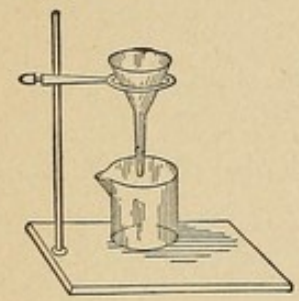
AgCl and HgCl remain undissolved.

$PbCl_2$ is in the hot-water solution.

Divide this hot-water solution into three parts and make three of the following tests for lead: First, with $K_2Cr_2O_7$, which gives yellow precipitate of $PbCrO_4$. Second, with H_2SO_4,

giving a white precipitate of $PbSO_4$. Third, with H_2S water, giving black precipitate of PbS. Fourth, with KI, which forms a yellow precipitate of PbI_2. Write these reactions.

To undissolved residues of Hg and Ag chlorids add warm NH_4OH.



Hg remains on the paper, black, as $HgNH_2HgCl$.

Ag is dissolved by the NH_4OH and may be precipitated as AgCl by adding HNO_3 to acid reaction. Presence of Hg in the black residue may be confirmed as in Group II (page 17).

QUESTIONS ON GROUP I.

Why wash the precipitated chlorids only *once* with cold water?

Why is it necessary to wash the $PbCl_2$ out with hot water *before* using ammonia?

Why is the ammonia used?

How does HNO_3 reprecipitate silver *chlorid?*

REACTIONS OF GROUP II.

Copper, Cu (Cuprum).

Atomic weight 63.3. Occurs free in vicinity of Lake Superior, also in western United States, Chili, and Spain as sulphids, copper pyrites, $CuFeS_2$, and copper glance, Cu_2S. Malachite green and malachite blue are native basic carbonates of Cu. Copper dissolves easily in nitric and acetic acid with difficulty in HCl, and heated with H_2SO_4 forms $CuSO_4$ with the evolution of SO_2. It forms two series of salt, cuprous (comparatively unimportant) and cupric, the common salts of which are the sulphate, acetate, and nitrate, while the chlorid and carbonate are frequently used.

Salts and solutions of Cu are usually blue or green.

A 1% solution of $CuSO_4$ will give the following reactions:

$CuSO_4$ with H_2S gives CuS, brownish-black sulphid; test its solubility in $(NH_4)_2S$ and in warm dilute HNO_3.

$CuSO_4$ with NH_4OH (one or two drops of reagent) will precipitate $Cu(OH)_2$ bluish white. Add more NH_4OH to same test-tube and note the result. To this clear solution add a sufficient amount of dry KCN to completely decolorize the liquid. Then add to the mixture some H_2S water. Is the black CuS thrown out? The behavior of Cu solutions thus treated is due to the formation of double salts; the solution in ammonia being due to a compound of $CuSO_4$ and NH_3, and the decolorization of the blue solution to one of $Cu(CN)_2$ and KCN.

$CuSO_4$ with K_4FeCy_6 (potassium ferrocyanid gives in acetic acid solution a red-brown precipitate of Cu_2FeCy_6.

Metallic zinc or iron will precipitate copper from solution. Hold a knife-blade in a solution of $CuSO_4$ for a few seconds.

MERCURY in *mercuric* combination.

A 2% solution of corrosive sublimate ($HgCl_2$) may be used in demonstrating the reactions of dyad mercury.

$HgCl_2$ with H_2S gives first a white precipitate, turning yellow, brown, and finally black as proportion of H_2S increases. The black precipitate *only* is mercuric sulphid, and care must be taken to add H_2S till this compound is produced.

Test the solubility of HgS in $(NH_4)_2S$ and HNO_3.

To $HgCl_2$ solution add $SnCl_2$. The mercuric chlorid is reduced to mercurous chlorid (HgCl, white) or metallic mercury (Hg, gray), according to proportion of the tin salt used: $2HgCl_2 + SnCl_2 = 2HgCl + SnCl_4$ or $HgCl_2 + SnCl_2 = Hg + SnCl_4$.

$HgCl_2$ with KI gives red HgI_2, easily soluble in excess of either of the reagents.

Alkaline hydroxids or carbonates precipitate from soluble mercuric compounds the yellow oxid, HgO. A preparation made from mercuric chlorid and lime-water is known in pharmacy as "yellow-wash."

$HgCl_2$ with NH_4OH gives white precipitate of $(NH_2Hg)Cl$,

known as "white precipitate." "Red precipitate" is a term sometimes used to designate the red oxid of mercury, HgO, made in the dry way.

Bismuth, Bi.

Atomic weight 208.5. Bismuth does not occur in large quantities, but is usually found in the free state. Small amounts are obtained from the oxid, Bi_2O_3, bismuth ochre, and from the sulphid, Bi_2S_3.

It is easily identified by means of blowpipe test on plaster with S and KI.

The most available salt is the nitrate, insoluble in water unless strongly acidulated.

Use a 2% solution of $Bi(NO_3)_3$ in the following tests:

$Bi(NO_3)_3$ with NH_4OH gives white precipitate of bismuth hydroxid, $(BiOH)_3$.

$Bi(NO_3)_3$ with H_2S precipitates Bi_2S_3, brownish black, insoluble in $(NH_4)_2S$, but soluble in warm dilute HNO_3.

Bismuth forms with water insoluble basic salts, according to the following equation:

$$Bi(NO_3)_3 + H_2O = BiONO_3 + 2HNO_3.$$

This may be demonstrated by allowing a few drops of bismuth solution to fall into a comparatively large amount of H_2O (two to six ounces). A white cloud of insoluble oxy-salt may be observed settling through the clear water. This may be employed as a final test for Bi in the course of systematic analysis.

Cadmium, Cd.

Atomic weight 112.4. Occurs associated with Zn in zinc blend. It is much more easily volatile than zinc, and advantage is taken of this fact in effecting its separation from that metal.

A 2% solution of the sulphate or nitrate may be used in studying the deportment of cadmium salts.

$CdSO_4$ with H_2S gives a bright yellow sulphid, CdS, soluble in dilute nitric acid.

$CdSO_4$ with $(NH_4)_2S$ also precipitates the yellow sulphid.

Cadmium sulphid forms slowly and, in presence of Cu or other second-group metals, may escape precipitation if the reagent is added in insufficient quantity.

Arsenic, As.

Atomic weight 75.0. Occurs associated with copper and iron sulphids, as arsenical pyrites, $FeAs.FeS_2$; as native sulphids, orpiment, As_2S_3, and realgar, As_2S_2; also to some extent as the trioxid, As_2O_3.

Arsenic forms two series of salts, the arsenious, As‴, and arsenic, As^v, and it also acts as an acid radicle forming arsenious and arsenic acids. In the process of analysis, arsenic compounds whether acid or basic are reduced to arsenious by action of H_2S. It is most easily obtained in the form of the trioxid, As_2O_3, also known as arsenious acid or white arsenic.

A solution for studying the reactions of arsenic (As‴) is conveniently made by dissolving about 15 grams of white arsenic in dilute NaOH solution by aid of heat, then diluting to one liter and acidifying slightly with HCl.

To an arsenious solution, which may be represented by $AsCl_3$, add H_2S water. A lemon-yellow precipitate of As_2S_3 will be thrown down. Test the solubility of this precipitate in yellow ammonium sulphid and ammonium carbonate.

To the alkaline solution of the sulphid add excess of HCl: As_2S_3 is precipitated.

To an arsenious solution add $(NH_4)_2S$ in repeated small portions.

In neutral solution, as of sodium arsenite, Na_3AsO_3 silver nitrate will throw down yellow silver arsenite, soluble in excess of nitric acid or ammonia.

SPECIAL TESTS FOR ARSENIC.

REINSCH'S TEST for arsenic, applicable to any solution whether organic or not, and very valuable for a preliminary test, is made as follows: place the solution or mixture to be tested in a porcelain dish, acidify strongly with HCl, and add a small strip of bright copper foil (cleaned in dilute HNO_3 and thoroughly washed in distilled H_2O) and boil for ten or twenty minutes, adding sufficient water to replace loss by evaporation. Remove the copper foil: a dark gray to black coating is an indication of arsenic but not conclusive, as some other substances give similar deposits, mercury and antimony in particular.

To prove the presence of As, roll the foil as tightly as possible and introduce into the bulb of a small glass matrass. (Fig. 1.)

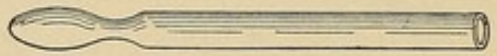


FIG. 1.

Heat the bulb over a very small luminous flame, when crystals of As_2O_3 (tetrahedral or octahedral) will deposit in the constricted portion of the tube, and may be identified by microscopical examination. If the test is carried out in this way, there will be sufficient air in the matrass for the formation of the oxid and it becomes much more delicate than if heated in the ordinary open tube as often recommended.

GUTZEIT'S TEST is made by placing the suspected solution in a test-tube, acidifying with H_2SO_4, adding a few pieces of arsenic-free zinc, and, as hydrogen begins to be given off, placing over the mouth of the tube a piece of filter-paper carrying a drop of a strong solution of $AgNO_3$. The presence of arsenic is indicated by the darkening of the moistened filter-paper in accordance with the following reactions:

The nascent H liberated by action of the Zn upon the acid forms with any As present the gaseous AsH_3 which, in contact with the filter-paper wet with $AgNO_3$ solution, produces a brown

or black stain of metallic Ag, while the As becomes arsenious acid, H_3AsO_3. The stain may possibly be yellow by formation of a compound of silver *arsenide* and silver nitrate, but as a rule moisture is present in sufficient amount to insure the decomposition of this compound.

Antimony will give a similar brown or black stain (not yellow), but presence of As may be conclusively demonstrated by making FLEITMANN'S TEST, which is conducted in the same way as the preceding, except that the hydrogen is evolved in alkaline solution, either by means of Zn and strong KOH solution ($Zn+2KOH=K_2ZnO_2+H_2$) or by sodium amalgam (made with arsenic-free mercury) and water ($NaHg_x+H_2O=NaOH+Hg+H$). In this case the SbH_3 is *not formed;* so a stain thus obtained constitutes a positive test for *arsenic.*

The MARSH-BERZELIUS TEST for arsenic is the most delicate of all and the one to which we resort in detecting As in the saliva or the urine. By this method one two-hundredth of a milligram or about 1/12800 of a grain can be easily shown as a brown deposit in the constricted tube at about the point *K*, Fig. 2. The apparatus used in this test is shown in Fig. 2, and consists of a small Erlenmeyer flask, or wide-mouth bottle, fitted as a hydrogen generator *A*, and connected with a drying-tube *B* filled with fused calcium chlorid, then with a tube of hard glass *C* drawn out to a very small diameter for half its length.

The generator *A* is charged with arsenic-free zinc, and dilute sulphuric acid (1/5) introduced through the thistle-tube *E*. After *all air has been driven from the apparatus,* light the escaping H at *T*, then the Bunsen burner *D*, and allow the generator to run for about twenty minutes, thus making a blank test of apparatus and reagents; if at the end of this time the hard glass is perfectly free from any deposit, the suspected liquid, which must have been freed from organic matter (process described in detail in chapter on Urine Analysis) may be introduced in portions of about 10 c.c. each.

The flame should be spread somewhat so as to heat at least

1 inch of the glass tube. This may be accomplished, in the absence of a burner-tip, by placing an inverted V-shaped piece of asbestos board 1 inch wide over the heated part of the tube.

The presence of arsenic increases the evolution of hydrogen, and unless the solution is added gradually the AsH_3 may be driven so rapidly past the flame as to escape decomposition, or the tube may become heated to such an extent that arsenic will not be deposited.

The escape of As at *T* may be noticed by the bluish color of the flame and by the characteristic garlic odor.

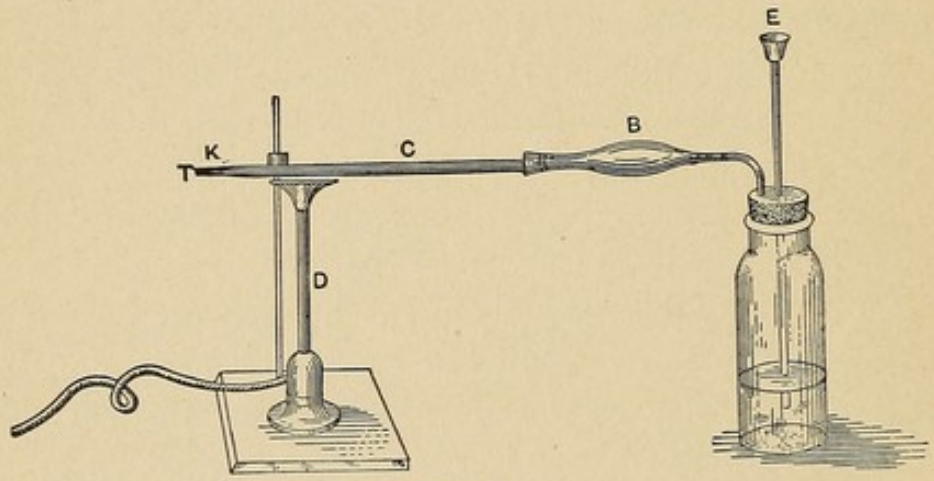


FIG. 2.

Antimony is similarly deposited as a dead-black stain instead of brown-black, and as Sb is less easily volatile than As the deposit will be nearer the flame, possibly on both sides of the flame. (For further differences between As and Sb see tests given on page 14.)

Arsenic compounds (As^v), as Na_2HAsO_4, are of but little interest from the dentist's standpoint.

All arsenic compounds are reduced by nascent H to arsenious combinations, then to elementary As, then to AsH_3 (arsine); hence the special tests given for arsenious compounds are applicable.

Free chlorin, nitric acid in alkaline solution, and potassium ferricyanid oxidize arsenious compounds to arsenic, and in this

condition the As is not easily volatilized and organic matter may be destroyed by deflagration (in presence of excess of nitrates) with but slight loss of arsenic.

Antimony, Sb (Stibium).

Atomic weight 120.2. Occurs native in Australia, and as the sulphid, Sb_2S_3, known as Stibnite.

The most common compound of antimony is the double tartrate of antimony and potassium ($KSbOC_4H_4O_6$), known as tartar emetic. A 2% aqueous solution may be used in the following tests:

To an antimony solution represented by $SbCl_3$ add H_2S water: Sb_2S_3 is precipitated orange-red. Test solubility of the precipitate in $(NH_4)_2S$ and in $(NH_4)_2CO_3$.

How does it differ from arsenic?

Upon the addition of HCl in excess to the ammonium sulphid solution the Sb is reprecipitated, but not necessarily as Sb_2S_3, but more usually as Sb_2S_5 or a mixture of the two sulphids.

Salts of antimony tend to form oxycompounds and are held in solution by excess of acid. The antimonious chlorid, $SbCl_3$, in solution with HCl is precipitated by excess of water as a white oxychlorid, $Sb_4Cl_2O_5$, also known as "powder of Algaroth." The antimonic chlorid in like manner precipitates the antimonic oxychlorid, $SbOCl_3$. Demonstrate by turning 1 or 2 c.c. of $SbCl_3$ solution into a large excess of water.

Fig. 3.

Marsh's test for As (or Sb) consists of a simple hydrogen generator with glass tip for burning the gas as shown in Fig. 3. In this apparatus Sb and As are converted into the gaseous hydrides, AsH_3 and SbH_3, and if a piece of cold porcelain is pressed down upon the flame, As or Sb will be deposited as metallic stains (mirrors)

upon the porcelain. To distinguish between As and Sb spots, the following tests will suffice:

Arsenic.	*Antimony.*
Brown-black, lustrous spots.	Dead brown or black surfaces.
Soluble in solution of hypochlorite of lime or soda.	Insoluble in solution of hypochlorite of lime or soda.
Easily volatilized.	Volatilized at red heat.

Antimony may be retained in the generator by the introduction of a piece of platinum-foil; the Sb being precipitated upon the platinum, to which it adheres quite strongly.

Tin, Sn (Stannum).

Atomic weight 119.0. Cassiterite, or tin-stone, nearly pure SnO_2, is by far the most important source. The free metal has been found associated with gold.

Tin, like arsenic and antimony, forms two series of salts, the stannous (Sn'') and the stannic (Sn^{IV}). A little HCl treated with excess of granulated tin till hydrogen is no longer given off furnishes a solution of stannous chlorid suitable for the following experiments:

$SnCl_2$ with H_2S gives brown precipitate of SnS, soluble in $(NH_4)_2S$, insoluble in $(NH_4)_2CO_3$.

$SnCl_2$ with $HgCl_2$ gives a white or gray precipitate as explained on page 7 under "Mercury" and is used as a test for presence of mercury. It may also be used as an alkaloidal precipitant.

Strong solutions of $SnCl_2$ in presence of metallic Sn keep fairly well, but dilute solutions without an excess of tin oxidize very rapidly to stannic combinations and cease to be of value as reagents.

Metallic tin is not dissolved by HNO_3, but is converted into a white, insoluble metastannic acid. This acid, upon standing, changes to normal stannic acid which is easily soluble in acids; hence, in making use of this reaction in the analysis of amalgam

alloys, it is not well to allow the nitric acid solution of the alloy to stand too long before filtering.

Metallic zinc thrown into a tin solution will precipitate the tin as follows: $SnCl_2 + Zn = ZnCl_2 + Sn$.

This reaction is used in the separation of tin from antimony in the second group; and in order to obtain the tin in soluble form, suitable for a final test, it is necessary to add HCl sufficient to first dissolve *all* the Zn present; otherwise it may remain adhering to the zinc.

Gold, Au (Aurum).

Atomic weight 197.2. Usually found uncombined, but mixed with various impurities.

Gold is insoluble in simple acids, but may be dissolved in nitrohydrochloric acid (aqua regia) with formation of auric chlorid. Gold also unites easily with Br or I, forming $AuBr_3$ or AuI_3. A half per cent solution of $AuCl_3$ may be used in the following tests:

H_2S with $AuCl_3$ gives dark brown Au_2S_3 (auric sulphid) soluble in yellow ammonium sulphid.

Gold is reduced to the metallic state by many of the other metals, as Pb, Cu, Ag, Sn, Al, Sb, Fe, Mg, Zn, and Hg; also by ferrous sulphate, stannous chlorid, and oxalic acid.

Add a freshly prepared solution of ferrous sulphate to a little acid solution of $AuCl_3$. Gold is precipitated as follows: $AuCl_3 + 3FeSO_4 = Au + Fe_2(SO_4)_3 + FeCl_3$.

Stannous chlorid precipitates from gold solution, the "purple of Cassius," consisting of a mixture of gold and oxid of tin.

Gold is only slowly precipitated by oxalic acid, but, as Pt is not precipitated at all by this reagent, it is possible to separate Au and Pt in solution as chlorids by this means.

KI will give a dark-green precipitate of AuI_2 provided the KI is in excess; if the gold is in excess, the precipitate is apt to be the yellow AuI (aurous iodid). In the presence of a considerable excess of KI the AuI_3 is kept in solution as the

potassio-auric iodid, $KIAuI_3$. The reduction of this double salt by sodium thiosulphate is made the basis of the method to determine the quantity of Au in a given alloy, as described in the chapter on Volumetric Analysis.

Platinum, Pt.

Atomic weight 194.8. Platinum solubilities are similar to gold; aqua regia forms the chlorid $PtCl_4$.

$PtCl_4 + H_2S$ gives a precipitate of sulphide of platinum almost black, soluble in yellow ammonium sulphid.

Platinum solution with NH_4Cl precipitates yellow ammonium platinic chlorid, $(NH_4)_2PtCl_6$, crystalline. Potassium chlorid also gives a yellow crystalline precipitate of K_2PtCl_6, isomorphous with the ammonium compound. (Plate I, Figs. 1 and 5). These reactions may be made quantitative by using neutral, fairly concentrated solutions and adding an equal volume of alcohol.

Both of these double salts are soluble in excess of alkali and reprecipitated by HCl.

Stannous chlorid reduces $PtCl_4$ to $PtCl_2$ but forms no precipitate. Metallic Zn will precipitate platinum as a fine black powder or spongy mass.

Separation of parts (a) and (b) of Group II.

A portion of the clear filtrate from group I containing a slight excess of HCl is tested for metals of group II by the addition of H_2S water.*

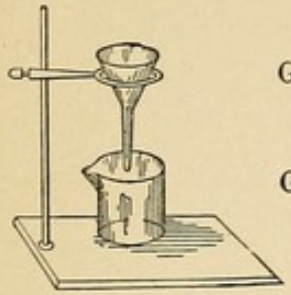
If a precipitate is obtained, warm the *whole* of the solution and pass in H_2S gas for from three to five minutes, which precipitates all metals of the group as sulphids. Filter.

Break point of filter-paper with glass rod and wash group II into beaker with warm $(NH_4)_2S$; digest hot for a few minutes.

---

* A preliminary test is made on a part of the solution because in the absence of Group II the analysis of Group III can be made more easily without the presence of H_2S.

Filter and wash the precipitate till wash-water shows only traces of Cl. Throw away all wash-water except the first.

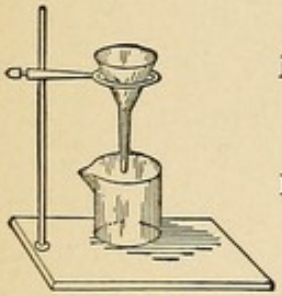


Group II (*a*). Cu, Cd, Bi, Hg and Pb.

Group II (*b*). As, Sb, Sn, Au, and Pt.

Analysis of Group II (*a*).

Dissolve the precipitate off the paper with hot dilute HNO_3.



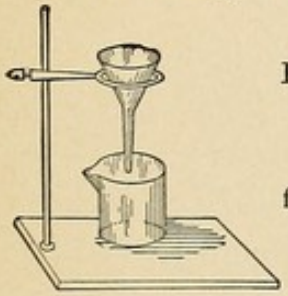
Hg, if present, will remain on paper, black.

Filtrate contains nitrates of Pb, Cu, Cd, and Bi

Test black residue on paper for Hg″ by dissolving in aqua regia and precipitating with $SnCl_2$. For reaction between $SnCl_2$ and $HgCl_2$ see page 7. Aqua regia may be made by mixing two or three parts of HCl with one of HNO_3. Free Cl is liberated which dissolves the HgS as $HgCl_2$.

$$3HCl + HNO_3 = NOCl + 2H_2O + Cl_2.$$

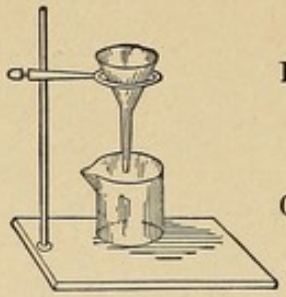
If lead was present in Group I, the filtrate above will contain traces which must be separated by adding a few drops of H_2SO_4 and allowing to stand at least fifteen minutes. Filter.



$PbSO_4$ remains on paper.

filtrate contains Cu, Cd, Bi.

To the filtrate add NH_4OH till alkaline; Bi separates as $Bi(OH)_3$, white. Filter.



$Bi(OH)_3$.

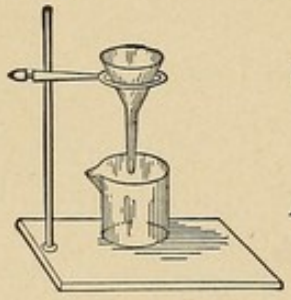
Cu and Cd.

Divide the filtrate (Cu and Cd) into two parts. A blue color indicates presence of Cu. With one part test for Cu by making it acid with acetic acid and adding K_4FeCy_6, which will give a brown precipitate of Cu_2FeCy_6. With the other part test for Cd by adding solid KCN very carefully till all blue color has disappeared; then a little H_2S water will give a yellow precipitate of CdS if cadmium is present.

Analysis of Group II (*b*).

To the ammonium sulphid add HCl till acid. A very fine white precipitate may be sulphur only.

Filter and wash. Throw away wash-water. Pierce filter and wash sulphids into large test-tube or small beaker. Add 10 c.c. of $(NH_4)_2CO_3$ and heat for a few minutes. Filter.

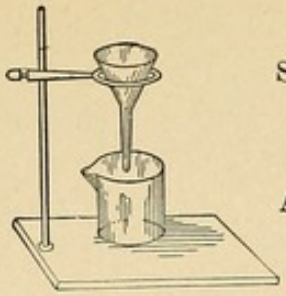


Sb, Sn, Au, Pt sulphids.

Arsenic sulphid.

Add HCl and Zn and make Gutzeit's test (page 10) and if necessary Fleitmann's (page 11) or Marsh's (page 13).

Dry this precipitate upon paper and place paper and precipitate in a porcelain evaporator, add concentrated HCl and heat. (This *must* be done under the hood.) Dilute and filter, when Au and Pt will remain undissolved.



Sb and Sn.

Au and Pt.

To the Sb and Sn solution add a *little* Zn and a piece of platinum-foil. The antimony and tin will both be reduced to the metallic state, the Sb being deposited on the Pt as a brown or black coating. Presence of Sb may be confirmed by removing the Pt, washing carefully, treating with $(NH_4)_2S$ and drying, when the coating will become Sb_2S_3, orange-red.

To the solution to be tested for Sn add HCl enough to dissolve *all* the Zn which has been added, filter and test filtrate with $HgCl_2$. (Page 17.)

Dissolve the insoluble residue of Au and Pt (the residue will be dark-colored if either of these metals are present) in aqua regia and divide solution into two parts.

Test one for gold with solution of $FeSO_4$, or a mixture of $SnCl_2$ and $SnCl_4$. (Page 15.)

Test the other for Pt by adding NH_4Cl, allow to stand overnight with addition of little alcohol, and precipitate of ammonium platinic chlorid will be obtained, yellow and crystalline. (See Plate I, Fig. 1.)

QUESTIONS ON GROUP II.

Why is it necessary to wash the precipitate of Group II practically free from Cl before dissolving in warm HNO_3?

How does the Hg found in Group II differ from the Hg in Group I?

How does the Pb found in Group II differ from the Pb in Group I?

Before making the final test for Sn, why is it necessary to dissolve *all* the Zn which has been added?

In precipitating Group II why should the solution be made acid with HCl before adding H_2S?

Why is it better to use H_2S gas rather than H_2S water in precipitating metals of Group II?

Before testing for Cd why add KCN to decolorize the copper solution?

REACTIONS OF GROUP III.

Iron, Fe (Ferrum).

Atomic weight 55.9. Occurs widely distributed in nature combined with oxygen as Fe_2O_3 or Fe_3O_4; also as sulphid, FeS_2, and as carbonate, $FeCO_3$.

Iron forms two classes of salts, ferrous, represented by ferrous sulphate, $FeSO_4$; and ferric, represented by ferric sulphate, $Fe_2(SO_4)_3$, or ferric chlorid, $FeCl_3$.

A solution for demonstrating the reactions of ferrous salts is best made by saturating cold dilute sulphuric acid with clean iron wire. A 3 to 5 per cent solution of fresh crystals of ferrous ammonium sulphate may be used. The ordinary ferrous sulphate or " copperas " is almost sure to contain some ferric salt. Use a 2 to 3 per cent solution of ferric chlorid and make the following tests, comparing the deportment of the ferrous and ferric solutions with each reagent. Write the reactions.

H_2S with pure ferrous salts gives no reaction; with ferric salts the iron is reduced to the ferrous combination, but gives no precipitate except sulphur.

$(NH_4)_2S$ gives with ferrous iron a black precipitate of FeS; with ferric it gives a precipitate containing FeS and S.

NH_4OH precipitates Fe″ as ferrous hydroxid, $Fe(OH)_2$; color white if perfectly pure, but usually a dirty green from admixture of ferric compounds. The presence of NH_4Cl prevents a *complete* precipitation as $Fe(OH)_2$.

With ferric salts, NH_4OH completely precipitates the iron as brick-red ferric hydroxid, $Fe(OH)_3$.

K_4FeCy_6 gives with ferrous salts a bluish-white precipitate of potassium ferrous ferrocyanide, $K_2FeFeCy_6$.

With solution of ferric salts the deep Prussian blue, ferric ferrocyanide, $Fe_4(FeCy_6)_3$, is thrown out.

With potassium ferricyanid, ferrous salts give dark-blue precipitate of ferrous ferricyanid, $Fe_3(FeCy_6)_2$. With ferric salts no precipitation occurs, but the color may change to green or brown.

KCyS or NH_4CyS gives no reaction with pure ferrous salts, but with ferric salts a deep red solution of ferric thiocyanate, $Fe(CyS)_3$, is produced. This red color is destroyed by addition of $HgCl_2$, not affected by HCl, and may be extracted from aqueous solution by shaking with ether in which the $Fe(CyS)_3$ is soluble.

Aluminum, Al.

Atomic weight 27.1. Aluminum constitutes a considerable part of the earth's crust as a constituent of clay, feldspar, mica, etc.

Its most important soluble salts are ammonia alum, $NH_4Al(SO_4)_212H_2O$, potash alum, $KAl(SO_4)_212H_2O$, and aluminum sulphate, $Al_2(SO_4)_3$. Use a 5% solution of either of these for the following tests:

$Al_2(SO_4)_3$ with $(NH_4)_2S$ and H_2O gives a white precipitate of $Al(OH)_3$. Write the reaction.

$Al(OH)_3$ is likewise produced by NH_4OH, Na_2CO_3, or NaOH; the precipitate is soluble in excess of fixed alkali hydroxids with formation of aluminates:

$$Al(OH)_3 + KOH = KAlO_2 + 2H_2O.$$

The alkaline aluminates may also be formed by fusion with Na_2CO_3 and KNO_3 and then may be dissolved in hot water.

From the solution of $KAlO_2$ the Al may be precipitated as $Al(OH)_3$ by excess of NH_4Cl (difference from Zn, page 27).

The presence of organic acids, tartaric, oxalic, etc., interferes with the precipitation of aluminium hydroxid and may entirely prevent it. The presence of ammonium chlorid favors its precipitation.

Chromium, Cr.

Atomic weight 52.1. Occurs as chrome iron ore or chromite, $FeOCr_2O_3$. Chromium forms two oxids, one basic, Cr_2O_3, the basis of chromic salts, as $Cr_2(SO_4)_3$, $Cr_2Cl_6(CrCl_3)$,* etc.; the other, CrO_3, is an acid anhydride, crystallizes as dark-red needles, and gives rise to two series of salts: neutral chromates, such as K_2CrO_4, and acid chromates or dichromates, $K_2Cr_2O_7$.

The soluble chromic salts most easily obtained are chrome alum, $KCr(SO_4)_2$, chromic sulphate, $Cr_2(SO_4)_3$, and chromic chlorid, $CrCl_3$. With a 5% solution of either of these the following may be demonstrated:

$Cr_2(SO)_3$ with $(NH_4)_2S$ gives greenish precipitate of $Cr(OH)_3$.

Similarly to Al the chromium hydroxid is precipitated by NH_4OH, alkaline carbonates or hydroxids; and then by boiling the $Cr(OH)_3$ with NaOH or KOH, or by fusing with Na_2CO_3 and KNO_3, chromates of the alkalis are produced.

The solid dichromate $K_2Cr_2O_7$ with strong H_2SO_4 gives, in the presence of chlorids, the reddish-brown gas CrO_2Cl_2 (chlorochromic anhydride or chromium dioxychlorid) used as a test for chlorids. (Page 46.)

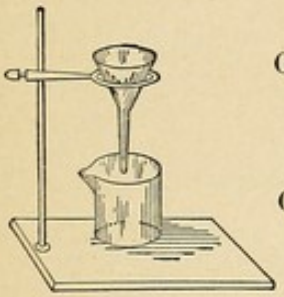
Analysis of Group III.

(Fe, Al, Cr. Phosphates and oxalates being absent.)

The filtrate from Group II must be freed from H_2S by boiling with a few drops of HNO_3 in a porcelain dish till a

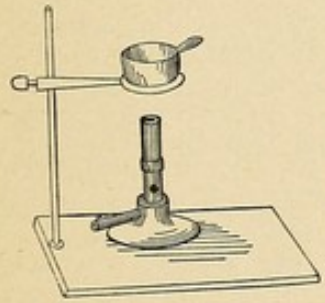
* There is a series of chromous salts, $CrCl_2$, $Cr(OH)_2$, etc., corresponding to a chromous oxid, CrO, but the oxid itself is not known.

drop removed by a glass rod does not blacken filter-paper wet with solution of lead acetate. This treatment also serves to oxidize the iron (reduced by H_2S) to ferric salt and at the same time concentrates the solution. To the clear solution thus obtained add 10 c.c. of NH_4Cl solution, then NH_4OH till alkaline, when the metals of this group will separate out as hydroxids: $Fe(OH)_3$ brick-red, $Al(OH)_3$ white, $Cr(OH)_3$ bluish green. Filter, wash carefully and dry precipitates, removing paper from funnel.

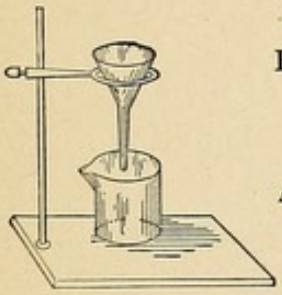


Group III.

Groups IV, V, and VI.



Scrape dried precipitate from paper in a crucible and cover well with a mixture of dry Na_2CO_3 and KNO_3 and fuse, keeping fusion liquid for at least three minutes. Cool. Boil the fused mass with H_2O; filter.



Fe.

Al and Cr.

Iron will remain on the paper; Al and Cr will be in solution as alkaline aluminate and chromate.

Divide filtrate (Al and Cr) into two parts. Test one portion for Al, by acidifying with HCl, adding $(NH_4)_2CO_3$ till alkaline and boiling, when Al will separate as a white flocculent precipitate of $Al_2(OH)_6$.

Test second portion of filtrate for Cr by acidifying strongly with *acetic* acid, boiling to expel CO_2 and adding a few drops of a solution of lead acetate. A yellow precipitate ($PbCrO_4$) indicates Cr.

Wash the precipitate remaining on the paper (Fe) and dissolve in dilute HCl. Divide resulting solution ($FeCl_3$) into two parts and confirm presence of Fe by testing one with K_4FeCy_6 (blue precipitate) and the other with KCyS (red solution).

If iron is found, determine in original substance whether ferrous or ferric, by use of tests described on pages 20 and 21.

QUESTIONS ON GROUP III.

Why boil off H_2S before precipitating the group with NH_4OH?

Why add HNO_3?

Of what use is the nitrate of potash (KNO_3) in the fusion of the hydroxids of Al and Cr?

In making the final test for Cr why is it necessary to add *acetic* acid, and why boil off the CO_2?

Why must HCl be added before making the final test for Al with $(NH_4)_2CO_3$?

REACTIONS OF GROUP IV.

Cobalt, Co.

Atomic weight 59.0. Use a 2% solution of nitrate. Crystalline salts of Co are usually of pink color; anhydrous salts are blue.

$Co(NO_3)_2$ with $(NH_4)_2S$ gives precipitate of CoS, black. Test solubility of this precipitate in HCl.

Make a borax bead by fusing a little borax in the looped end of a *clean* platinum wire. When a bead of clear "borax glass" has been obtained dip it in a little of the CoS just formed, and fuse again. The color of the bead when cold is a deep blue.

Note.—Be sure and make the fusion complete; the use of an insufficient

amount of heat will acount for much of the trouble experienced by students in obtaining satisfactory bead tests.

$Co(NO_3)_2$ with KNO_2 forms a double nitrite, $Co(NO_2)_2 2KNO_2$, soluble in water; but if acetic acid is added to strong acid reaction, the solution heated and then allowed to stand overnight, the Co is completely precipitated as another double salt, $Co(NO_2)_3,3KNO_2$, yellow and crystalline.

Nickel, Ni.

Atomic weight 58.7. Occurs associated with Co, sometimes with Fe as sulphid.

Use a 2% solution of the sulphate or nitrate. $NiSO_4$ with $(NH_4)_2S$ gives NiS, black. Test solubility in HCl.

The borax-bead test applied to NiS or other nickel salt gives a bead yellowish brown when cold, but the color is easily masked by other metals.

Ni salts with KNO_2 give the soluble double nitrite of similar composition to the Co salt, $Ni(NO_2)_2,2KNO_2$. The nickel salt, unlike the cobalt, is not easily decomposed, and is not precipitated by heating with acetic acid. Advantage is taken of this fact in effecting the separation of cobalt from nickel. (Page 27.)

Manganese, Mn.

Atomic weight 55.0. Occurs chiefly as the dioxid, MnO_2, pyrolusite.

Manganese salts are usually flesh-colored. The sulphate is easily obtained. A 3% solution may be used.

$MnSO_4$ with $(NH_4)_2S$ gives flesh-colored precipitate of MnS.

Test solubility in HCl. With a little of the precipitated MnS make a RED-LEAD TEST for Mn as follows:

Place in a test-tube a little red lead (Pb_3O_4). Add three or four cubic centimeters of nitric acid (about one part concentrated HNO_3 and one of H_2O), and boil well. Add, by means of a glass rod, a little of the washed MnS to the mixture in the tube and boil again. Now dilute with water till

tube is about three-quarters full, and allow to stand till liquid is clear. If Mn was present, the supernatant fluid will be a pink to red color due to the formation of permanganic acid, $HMnO_4$.

Note.—HCl or chlorids, even in small quantities, interfere with the reaction; hence it is recommended to make the test on the sulphid. Reducing agents must likewise be absent. When these precautions are observed the test is a very simple and extremely delicate one.

$MnSO_4$ with NaOH gives flesh-colored $Mn(OH)_2$ insoluble in excess of reagent (separation from Zn).

Upon fusion with a mixture of KNO_3 and Na_2CO_3 manganese salts produce green manganates, as Na_2MnO_4.

Zinc, Zn.

Atomic weight 65.4. Occurs chiefly as the carbonate $ZnCO_3$, calamine. A native carbonate of zinc is also known as smithsonite. The sulphid, ZnS (zinc blend), and the silicate are also natural sources of the metal.

The sulphate, $ZnSO_4$, also known as white vitriol, is perhaps the most common salt. The chlorid is a constituent of many commercial liquid disinfectants and antiseptics. The nitrate also is easily obtained.

A 2 or 3 per cent solution of any of these soluble salts may be used in the following tests:

$ZnSO_4$ with $(NH_4)_2S$ gives a white precipitate of ZnS.

Sulphid of zinc is the only white *sulphid* formed in the course of analysis of ordinary solutions, but the following white precipitates are formed: Sulphid of manganese is flesh-colored or dirty white. Aluminum hydroxid resembles sulphid of zinc in appearance and is precipitated by $(NH_4)_2S$. Yellow $(NH_4)_2S$ added to an acid solution will precipitate sulphur, white, very fine and difficult to filter out.

$ZnSO_4$ with NaOH (or KOH) gives a white gelatinous precipitate of zinc hydrate, $Zn(OH)_2$, soluble in excess of the reagent as Na_2ZnO_2 (sodium zincate).

Note.—Colorless gelatinous precipitates in slight amounts may escape detection, as it sometimes takes careful observation to see them, especially if the laboratory light happens to be poor.

Na_2ZnO_2 with H_2S or $(NH_4)_2S$ gives precipitate of ZnS.

From solution of Na_2ZnO_2 the Zn may be precipitated as $Zn(OH)_2$ by addition of NH_4Cl, but further addition of the NH_4Cl redissolves the precipitate (distinction from Al, page 22).

$ZnSO_4$ with K_4FeCy_6 gives white precipitate of zinc ferrocyanid (Zn_2FeCy_6) insoluble in NH_4OH.

Note.—The ferrocyanid and the sulphid are the only two zinc salts not soluble in NH_4OH. (Prescott and Johnson, page 179.)

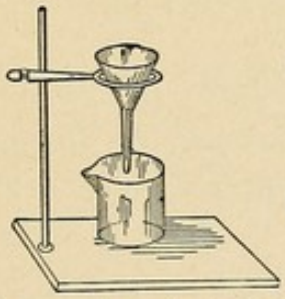
Soluble zinc salts and oxalic acid give crystals of ZnC_2O_4 which may be recognized under the microscope (Plate I, Fig. 2).

Analysis of Group IV.

(Co, Ni, Mn, Zn.)

(In the presence of phosphates, oxalates, borates, etc., examine this group by the scheme given on page 36.)

To the clear filtrate from Group III add $(NH_4)_2S$. A precipitate may be NiS, CoS, MnS, and ZnS. Wash the precipitate and treat with *cold dilute* HCl which will dissolve MnS and ZnS only.



CoS and NiS, black.

$MnCl_2$ and $ZnCl_2$ in solution.

Make a borax-bead test (page 24) of the precipitants. If a clear red-brown bead is obtained, Ni alone is present. If the bead is blue, Co is present, Ni may or may not be.

Separation of Cobalt and Nickel.

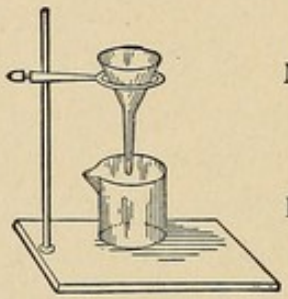
If Co is present, dissolve the black precipitate off the paper with aqua regia, evaporate in porcelain capsule practically

to dryness, dissolve in H_2O, add excess of acetic acid and potassium nitrite (KNO_2). Allow to stand over night, when Co will separate out as a yellow crystalline precipitate (page 25).

Filter and test filtrate for Ni with NaOH, which gives a pale-green precipitate of $Ni(OH)_2$ insoluble in excess of the precipitant.

Separation of Manganese and Zinc.

Boil the HCl solution of Zn and Mn to expel the H_2S, then add a decided excess of KOH or NaOH and allow to stand ten minutes *without* heating. Mn will separate out as $Mn(OH)_2$, while Zn will remain in solution as K_2ZnO_2.



Test precipitate with HNO_3 and Pb_3O_4. (Red-lead test for Mn, page 25.) Test filtrate for Zn by adding H_2S or a few drops of $(NH_4)_2S$, which will precipitate ZnS, white.

QUESTIONS ON GROUP IV.

Why dissolve the MnS and ZnS in *cold* and *dilute* HCl?

Why is it necessary to separate *all* the Mn before testing for Zn?

If traces of Co or Ni are dissolved by the HCl, how does it affect the final test for Zn?

In this analysis (in absence of phosphates, etc.) what important difference between the behavior of salts of Zn and Al?

Why is it necessary to allow time for *complete* precipitation of Co with KNO_2?

Why expel H_2S before separating Mn?

Where does this H_2S come from?

REACTIONS OF GROUP V.

(THE ALKALINE EARTHS Ba, Sr, Ca, Mg.)

Barium, Ba.

Atomic weight 137.4. Occurs chiefly as the sulphate, $BaSO_4$, heavy spar, and $BaCO_3$, witherite.

Use a 2% solution of the chlorid for tests.

$BaCl_2$ with $(NH_4)_2CO_3$ gives white precipitate of barium carbonate. Test solubility in acids. With soluble sulphates $BaCl_2$ produces $BaSO_4$ insoluble in HCl. (Test for sulphates.)

$BaCl_2$ with $K_2Cr_2O_7$ or K_2CrO_4 gives yellow precipitate of $BaCrO_4$. Barium salts moistened with HCl and held on a clean platinum wire give to the colorless flame of the Bunsen burner a green or yellowish-green color.

Strontium, Sr.

Atomic weight 87.6. Occurs as the carbonate, $SrCO_3$, strontianite, also as the sulphate.

Use a 3 to 4 per cent solution of the nitrate or chlorid for tests.

$Sr(NO_3)_2$ with $(NH_4)_2CO_3$ gives white precipitate of $SrCO_3$.

$Sr(NO_3)_2$ with H_2SO_4 or soluble sulphate gives white precipitate of $SrSO_4$, rather more soluble in water and more slowly formed than $BaSO_4$.

A saturated solution of $SrSO_4$ may be used to test for barium in presence of Sr salts.

$Sr(NO_3)_2$ with K_2CrO_4 gives precipitate of $SrCrO_4$, but with the acid chromate (dichromate) of potassium, $K_2Cr_2O_7$, no precipitate is formed except in concentrated solutions.

$Sr(NO_3)_2$ with oxalic acid gives a precipitate of strontium oxalate, SrC_2O_4, crystallizing in the so-called envelop form (Plate I, Fig. 3). Salts of Sr color the Bunsen flame crimson.

Calcium, Ca.

Atomic weight 40.1. Calcium is widely distributed and very abundant, limestone, chalk, marble, and calc-spar being natural carbonates; $CaCO_3$, gypsum, and alabaster are sulphates.

Calcium phosphate occurs in the mineral apatite and is also a principal constituent of animal bones.

Use a 3 or 4 per cent solution of $CaCl_2$ for tests.

$CaCl_2$ with $(NH_4)_2CO_3$ gives white precipitate of $CaCO_3$, easily soluble in acids.

$CaCl_2$ with oxalic acid or soluble oxalates gives a white precipitate of CaC_2O_4, similar in form to the SrC_2O_4 but much more difficult to obtain in the crystalline condition.

$CaSO_4$ is not precipitated except from moderately concentrated solution.

A saturated solution of $CaSO_4$ may be used to test for strontium salts in presence of Ca.

Magnesium, Mg.

Atomic weight 24.36. Principal sources are the carbonate, $MgCO_3$, magnesite, and a double carbonate, $CaMg(CO_3)_2$, dolomite. The sulphate $MgSO_4$ occurs in the mineral kieserite in the "Stassfurt deposit." "French chalk" (or talcum), soapstone, and meerschaum consist of magnesium silicate in varying states of purity.

Soluble salts easily procured are the sulphate, $MgSO_4$, crystallized with seven molecules of water as Epsom salts; the chlorid, $MgCl_2$; and the nitrate, $Mg(NO_3)_2$. A 5% solution of either of these may be used in the following tests:

Magnesium salts with $(NH_4)_2CO_3$ give a white precipitate of basic carbonate of variable composition. This precipitate forms *very* slowly in dilute solution, and in the presence of NH_4Cl the formation of soluble double salts prevents the precipitation altogether.

$MgCl_2$ with Na_2HPO_4 gives in fairly concentrated solution

a white precipitate of $MgHPO_4$. In presence of NH_4Cl and NH_4OH the alkaline phosphates precipitate magnesium-ammonium-phosphate, $MgNH_4PO_4,6H_2O$, even from *very* dilute solution (Plate I, Fig. 4).

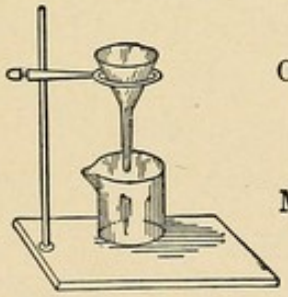
In case the precipitate has formed very slowly it may separate as small, almost transparent, crystals clinging to the sides of the beaker.

Ammonium oxalate does not precipitate magnesium solutions.

Analysis of Group V.

(Ba, Sr, Ca, Mg.)

To the filtrate from Group IV containing NH_4Cl and NH_4OH, add $(NH_4)_2CO_3$. (If NH_4Cl and NH_4OH are not present, add 10 c.c. of NH_4Cl solution and NH_4OH till strongly alkaline before proceeding with the analysis.) Ba, Sr, and Ca will be precipitated as carbonates; Mg will be held in solution by the ammonium chlorid. Filter.



Ca, Ba, Sr, carbonates.

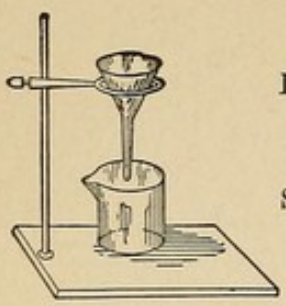
Mg.

Test the filtrate for Mg by adding Na_2HPO_4, when a white crystalline precipitate is $NH_4MgPO_4,6H_2O$.

To the carbonates on the paper add dilute acetic acid, which will dissolve the precipitate, forming acetates of the three metals.

Take a *portion* of the acetate solution in a test-tube and make a preliminary test for Ba by adding *acid* chromate of potassium ($K_2Cr_2O_7$). A yellowish precipitate will be $BaCrO_4$.

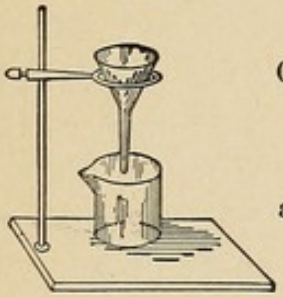
If *Ba is present*, add $K_2Cr_2O_7$ to the whole of the solution and filter out the $BaCrO_4$.



$BaCrO_4$.

Sr and Ca, acetates, $K_2Cr_2O_7$, etc.

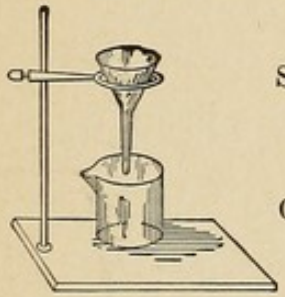
It is desirable to remove the excess of bichromate from the filtrate before testing for Ca and Sr.* To do this add NH_4OH till alkaline, then $(NH_4)_2CO_3$ will precipitate $SrCO_3$ and $CaCO_3$. Filter and dissolve off the paper with acetic acid as before.



$CaCO_3$ and $SrCO_3$, which treated with acetic acid will give

a solution of the acetates of Ca and Sr.

Reserve about one-fourth of this acetate solution. To the remainder add dilute K_2SO_4 solution, which will precipitate $SrSO_4$. (If only slight amounts of Sr are present, it may take some time to complete the precipitation. If a large amount of Ca is present, some $CaSO_4$ may also be thrown down.) Filter.



$SrSO_4$.

$Ca(C_2H_3O_2)_2$ or $CaSO_4$.

Test filtrate for Ca by adding ammonium oxalate, which will precipitate calcium oxalate, white.

* The object of removing the $K_2Cr_2O_7$ is to furnish a colorless solution wherein the Sr or Ca precipitates may be more clearly discerned. It is not absolutely necessary and, in case the amount of Sr and Ca is probably slight, might be omitted as the operation is always attended with some loss.

If there is any question about the precipitate thrown out by K_2SO_4 being Sr, make confirmatory test on reserved portion, either by flame test (page 29) or by adding $CaSO_4$ and allowing to stand twelve hours. $CaSO_4$ will precipitate Sr as $SrSO_4$, but of course cannot precipitate Ca.

QUESTIONS ON GROUP V.

Why add NH_4Cl before precipitating the group with $(NH_4)_2CO_3$?

Why dissolve the precipitated carbonates in acetic acid rather than HCl?

Why use the acid chromate of potassium ($K_2Cr_2O_7$) in testing for Ba rather than the neutral chromate (K_2CrO_4)?

Why precipitate Sr and Ca after separation of Ba with $K_2Cr_2O_7$?

REACTIONS OF GROUP VI.

(THE ALKALI METALS, K, Na, NH_4, Li.)

These bases are not precipitated by any group reagent and must be detected by individual tests. The following will be sufficient:

Potassium, K (Kalium).

Atomic weight 39.15. Occurs as carbonate in wood ashes, as nitrate in the "nitre beds" of India, etc., as chlorid from the Stassfurt deposit in Germany, as the mineral sylvite, also in the double chlorid of Mg and K (carnallite).

Nearly all potassium salts are soluble in water. The potassium platinic chlorid, K_2PtCl_6, the potassium acid tartrate, $KHC_4H_4O_6$, and a few others are only sparingly soluble and may be precipitated by addition to the solution of an equal volume of alcohol, in which they are quite insoluble.

The presence of potassium salts may be detected spectroscopically or by the violet color given to the flame observed through blue glass. Make comparative tests with known

solutions of sodium and potassium salts, using blue glass of sufficient thickness to obscure the yellow (Na) ray.

Note.—In making the flame test the best results are obtained by evaporating a little of the *original* solution to dryness, moistening with HCl and then taking up on a loop of clean platinum wire.

The platinic chlorid test may be made as follows:

Add a few drops of HCl to a little of the solution, then evaporate to dryness. Keep at a low red heat till all ammonium salts have been driven off, cool, and take up in a little (not more than 5 c.c.) distilled water. Add a few drops of H_2PtCl_6 and about 5 c.c. of alcohol. Set aside for some time.

K_2PtCl_6, yellow, will crystallize out recognizable under the microscope. (Plate I, Fig. 5.)

Sodium, Na (Natrium).

Atomic weight 23.05. Occurs principally as chlorid in sea-water and in mineral deposits, and to lesser extent as nitrate, Chili saltpeter, and as cryolite, the double fluorid of Al and Na, (Na_2AlF_6), found in Greenland.

Na may be detected by use of the spectroscope or by the *persistence* of the yellow flame obtained with a *clean* platinum wire and a colorless Bunsen flame. Make comparative test with small amount of known sodium salt.

Sodium salts are soluble with only a very few exceptions. The pyroantimonate $Na_2H_2Sb_2O_7$ may be precipitated in the cold by a freshly prepared solution of *potassium* pyroantimonate. (Prescott and Johnson, 228.)

From solution stronger than 3% and nearly neutral, the double acetate of uranyl and sodium ($NaC_2H_3O_2$, $UO_2(C_2H_3O_2)_2$) may be precipitated. (Plate I, Fig. 6.) As triple crystalline acetates may also be formed with Mg, Cu, Fe, Ni, and Co, it is recommended to first precipitate the bases of the first five groups, drive off ammonium salts, etc., as in the test for K with H_2PtCl_6.*

* Behrens's Manual of Microchemical Analysis, page 32.

Lithium, Li.

Atomic weight 7.03. The carbonate and chlorid are used in pharmacy as uric acid solvents.

The presence of lithium salts is easily shown after the precipitation of strontium by the intense carmine color given to the Bunsen flame.

The spectroscope furnishes a very delicate and positive test for this element.

Ammonium, NH_4.

Ammonium salts are generally soluble. H_2PtCl_6 precipitates the double chloride $(NH_4)_2PtCl_6$, similar in appearance and crystalline form to the corresponding potassium salt. (Plate I, Fig. 1.)

Ammonium salts are most easily detected by the evolution of ammonia gas (NH_3) whenever they are heated with fixed alkali, NaOH or KOH.

The test may be made upon the original solution by boiling in a test-tube with a little 10% NaOH, and the escaping NH_3 may be detected by the odor or, better, by suspending in the upper part of the tube a piece of *moistened red* litmus paper,* which is promptly turned blue by the " volatile alkali." The litmus-paper test is more delicate than the odor test. Care should be taken that the paper does not touch the sides of the tube, as it may come in contact with traces of NaOH.

Many ammonium solutions give off NH_3 gas without the aid of any fixed alkali. Common examples are the carbonate, acid carbonate, hydrate, sulphid, and sulphydrate.

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* Blue paper may be reddened by leaving it a few hours in a wide-mouth bottle after wetting the under side of the stopper with a drop or two of acetic acid.

Analysis of Groups III, IV, and V.

When phosphates, borates, or oxalates are present.

To the filtrate from Group II add NH_4Cl and NH_4OH in slight excess. Heat to boiling and add $(NH_4)_2S$ slowly (always keeping the solution at the boiling-point) until precipitation is complete. Filter as rapidly as possible and wash with hot water, adding occasionally a little $(NH_4)_2S$.

The filtrate, which may contain the barium and potassium groups, must be concentrated by evaporation, filtered if necessary and set aside.* The precipitate may contain MnS, ZnS, CoS, NiS, FeS, $Al(OH)_3$, and $Cr(OH)_3$ with phosphates or oxalates soluble in acids only. The color of the precipitate will give some indication of what is present. Test the precipitate for Mn by fusing a part with KNO_3 and Na_2CO_3.

Treat the precipitate with *cold dilute* HCl in which CoS and NiS alone are insoluble. Filter. Treat insoluble residue for Co and Ni according to direction on page 27.

The HCl solution, which may contain Mn, Zn, Fe, Cr, and Al as chlorids and phosphates and oxalates soluble in acids, and which is green or violet if much Cr is present, is boiled with a few drops of HNO_3 until all the H_2S is expelled.

Test a *small* portion of the solution for Fe exactly as in analysis of Group III given on page 24. Of the remainder of the solution take about one-third, and add dilute H_2SO_4.

A white precipitate may contain $BaSO_4$, $SrSO_4$, and possibly $CaSO_4$. Filter, wash precipitate, and fuse with a mixture of Na_2CO_3 and K_2CO_3.

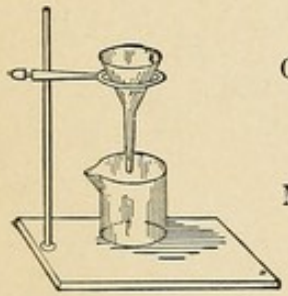
Note.—The mixture of the two carbonates in molecular proportions fuses at a lower temperature than either salt alone.

Filter and wash the carbonates thus formed, dissolve them in acetic acid and examine this solution for Ba, Sr, and Ca

* If Ni is present, the filtrate is frequently brown or black, since NiS is somewhat soluble in an excess of $(NH_4)_2S$, especially if much NH_4OH is present. The NiS may be precipitated, after evaporation, by acidifying with HCl.

as directed under the Ba group. To the filtrate from the precipitate produced by H_2SO_4, or to the solution in which H_2SO_4 has failed to give a precipitate, add three times its volume of alcohol; Ca if present is precipitated as white $CaSO_4$, and its presence may be confirmed by dissolving the precipitate in water and adding $(NH_4)_2C_2O_4$, which precipitates CaC_2O_4, white.

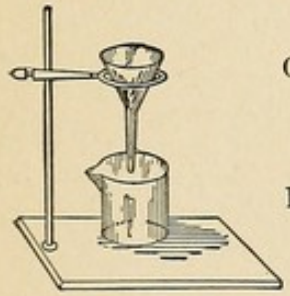
To the rest of the HCl solution add ferric chlorid carefully till a drop of the solution gives, when mixed with a drop of ammonic hydrate, a yellowish precipitate. To the solution add Na_2CO_3 or K_2CO_3 till the acid is nearly neutralized, then add excess of freshly precipitated $BaCO_3$, and allow to stand overnight. Filter.



Cr and Al as hydrates. (Fe as phosphate or hydrate and $BaCO_3$.)

$MnCl_2$, $ZnCl_2$, and possibly members of Group V.

Transfer the precipitate to a small beaker and boil for some time with NaOH or KOH. The Al will be converted into the aluminate $KAlO_2$. The phosphate will be more or less completely changed to potassium or sodium phosphate. Filter.



$Cr(OH)_3$, $BaCO_3$, etc.

$KAlO_2$ and Na_2HPO_4.

Test precipitate for Cr as on page 24. Add HNO_3 to filtrate till acid, then divide into two parts; test one for P_2O_5 with $(NH_4)_2MoO_4$.

Test the other for Al by adding NH_4OH till alkaline, when precipitate will be $AlPO_4$, insoluble in acetic acid.

To the solution of Mn and Zn chlorids add a little HCl and boil. Then make alkaline with NH_4OH, add $(NH_4)_2S$, warm slightly and filter. The precipitate (MnS and ZnS) may be dissolved in cold dilute HCl and tested for Mn and Zn as in analysis of Group IV, page 28.

OUTLINE SCHEME FOR ANALYSIS OF GROUP I.

To about one-third of a test-tubeful of the unknown solution add a few drops of HCl.

Ppt. = AgCl, HgCl, $PbCl_2$. Add hot H_2O.

Residue = AgCl, HgCl. Add NH_4OH.		Solution = $PbCl_2$. Test as on page 5.
Residue = HgCl. Test, page 6.	Solution = AgCl. Test with HNO_3.	

OUTLINE SCHEME FOR ANALYSIS OF GROUP II.

To the warmed filtrate from Group I add H_2S. A ppt. may be sulphids of As, Sb, Sn, Au, Pt, Cu, Cd, Bi, Hg, and Pb.

Filter and treat with warm $(NH_4)_2S$.

Residue is Group II (*a*), page 17, and consists of sulphid of Cu, Cd, Bi, Hg, and Pb. Treat on paper c warm dil. HNO_3.				*Solution* = As, Sb, Sn, Au, and Pt. Reprecipitate c HCl, filter and treat ppt. c strong $(NH_4)_2CO_3$ sol.			
Residue is Hg. Dissolve in aqua regia and test c $SnCl_2$ (page 17)	*Solution* Cu, Cd, Bi, and Pb. Add H_2SO_4 and filter.			*Residue* = Sb, Sn, Au, and Pt, sulphids. Treat c conc. HCl, dilute and filter.			*Solution* As. Make Gutzeit's or Fleitmann's test for As (page 10)
	Ppt. is $PbSO_4$	*Solution* is Cu, Cd, and Bi. Add NH_4OH and filter.		*Residue* Au and Pt. Dissolve in aqua regia and divide.		*Solution.* Sb and Sn Test for Sb c Pt foil and Zn.	
		Ppt. is $Bi(OH)_3$	*Solution* is Cu and Cd.	Pt. I. Test for Au c $FeSO_4$ (p. 19).	Pt. II. Test for Pt c NH_4Cl and alcohol.	Test for Sn in filtrate c $HgCl_2$ (page 19).	
			Test for Cu c HA and K_4FeCy_6 (page 18).	Test for Cd c KCN and H_2S			

OUTLINE SCHEME FOR ANALYSIS OF GROUPS III AND IV.

Take the clear solution in which H_2S fails to produce a precipitate and boi with a few drops of HNO_3 till H_2S is expelled. Add NH_4Cl and NH_4OH. Filter

Precipitate = Group III. Fe, Al, and Cr. Fuse c̄ Na_2CO_3 and KNO_3. Boil c̄ H_2O and filter.			*Solutions* = Groups IV, V, and VI. Add $(NH_4)_2S$ and precipitate. Group IV—Co, Ni, Mn, and Zn. Treat with cold dilute HCl.		
Residue = Fe. Test for Fe c̄ KCyS and K_4FeCy_6 (page 24).	*Solution* = Al and Cr. Divide solution and test for Al with HCl and $(NH_4)_2CO_3$ (page 23).	test for Cr with acetic and lead acetate (page 24).	*Residue* = Co and Ni. Make borax-bead test. Separate Co by means of KNO_2 (page 27).	*Solution* = Mn and Zn. Boil and treat c̄ KOH or NaOH.	
				Precipitate = $Mn(OH)_2$. Make red-lead test for Mn (page 25).	*Solution* = K_2ZnO_2. Test for Zn c̄ H_2S or $(NH_4)_2S$ (page 28).

OUTLINE SCHEME FOR ANALYSIS OF GROUPS III, IV, AND V.

(Phosphates, oxalates, borates, etc., being present.)

To filtrate from Group II add NH_4Cl and NH_4OH. Heat and add $(NH_4)_2S$. Filter rapidly.

Precipitate = MnS, ZnS, CoS, NiS, FeS, $Al(OH)_3$, $Cr(OH)_3$, also phosphates, etc., soluble in acids only. Fuse part of precipitate and test for Mn (page 28). Treat remainder c̄ cold dilute HCl.					Filtrate, members of Ba and K groups.
Residue = CoS and NiS. Make borax-bead test and separate Co if necessary, c̄ KNO_2 (page 27).	*Solution* = Mn, Zn, Cr, and Al. Divide solution into three parts of about 1/8, 2/8, and 5/8, respectively, and treat as follows:				
	I Test small portion for Fe (page 24).	II To second portion add dilute H_2SO_4.		III To third portion add $FeCl_3$ to combine c̄ H_3PO_4, etc., then add Na_2CO_3 or K_2CO_3, and $BaCO_3$ (page 37).	
		Precipitate may be $BaSO_4$, $SrSO_4$ or $CaSO_4$. Filter, wash, fuse c̄ Na_2CO_3 and K_2CO_3. Dissolve fusion in $\overline{HA}$ and analyze for Group V.	*Solution* = $CaSO_4$. Add alcohol; if precipitate occurs, filter, dissolve in H_2O, and test with ammonium oxalate.	*Precipitate* = Cr, Al, Fe, and $BaCO_3$. Boil precipitate c̄ NaOH and filter.	*Solution* = Mn and Zn. Reprecipitate Mn and Zn as sulphids, and test according to page 26.
				Residue = Cr, $BaCO_3$, etc. Test for Cr as on page 24.	*Solution* = $KAlO_2$. Test for Al as on page 23.

OUTLINE SCHEME FOR ANALYSIS OF GROUPS V AND VI.

To the clear filtrate from Group IV add $(NH_4)_2CO_3$.

<table>
<tr>
<td colspan="2">*Precipitate* = Ba, Sr, and Ca. Add $K_2Cr_2O_7$ if necessary to precipitate Ba.</td>
<td rowspan="2">*Solution* = Mg and Group VI. Test for Mg with Na_2HPO_4 (page 31). Make separate tests for metals of Group VI according to pages 33, 34, and 35 of the text.</td>
</tr>
<tr>
<td>*Precipitate* = $BaCrO_4$.</td>
<td>*Solution* = Sr and Ca. Reprecipitate Sr or Ca with $(NH_4)_2CO_3$ and test, or $CaSO_4$. Remove Sr with K_2SO_4 and alcohol, and test filtrate for Ca with $(NH_4)_2C_2O_4$ (page 32).</td>
</tr>
</table>

SECTION II.—ANALYTICAL REACTIONS OF THE ACIDS.

In the analytical processes thus far described we have considered only the separation and detection of the basic or metallic part of the salt, that is, we have analyzed a solution of ferric chlorid and found iron simply. It is necessary to find the chlorin. Before making any examination for acid, it will be possible to save a considerable amount of both time and labor by first carefully considering what acids are capable of forming soluble salts with the bases which have already been detected. To facilitate this consideration a table of solubilities will be found on the following page, by a careful study of which it will be possible to select such acids as are most likely to be present in the unknown solution under investigation, and also to neglect a number of acids which, from the solubility of their salts, together with the character of the solution (acid, alkaline, neutral and aqueous, or otherwise), will necessarily be absent.

In this connection it is well to remember that practically all nitrates and chlorates are soluble in water; sulphates are mostly soluble, except those of barium, strontium, and calcium. Phosphates (di- or trimetallic), silicates, oxalates, and borates are practically insoluble, except those of the alkaline

TABLE SHOWING THE SOLUBILITY OF SALTS.

	K	Na	NH_4	Mg	Ba	Sr	Ca	Mn	Zn	Co	Ni	Fe	Fe_2
Acetate	w	w	w	w	w	w	w	w	w	w	w	w	w
Arseniate	w	w	w	a	a	a	a	a	a	a	a	a	a
Arsenite	w	w	w	a	wa	wa	a	a		a	a	a	a
Borate	w	w	w	wa	a	a	a	a	a	a	a	a	a
Bromid	w	w	w	w	w	w	w	w	w	w	w	w	w
Carbonate	w	w	w	a	a	a	a	a	a	a	a	a	a
Chlorate	w	w	w	w	w	w	w	w	w	w	w	w	w
Chlorid	w	w	w	w	w	w	w	w	w	w	w	w	w
Chromate	w	w	w	w	a	wa	wa	w	w	a	a		w
Cyanid	w	w	w	w	wa	w	w	a	a	ai	ai	ai	
Iodid	w	w	w	w	w	w	w	w	w	w	w	w	w
Nitrate	w	w	w	w	w	w	w	w	w	w	w	w	w
Oxalate	w	w	w	a	a	a	a	a	a	a	a	a	a
Oxid	w	w		a	w	w	w	a	a	a	a	a	a
Phosphate	w	w	w	a	a	a	a	a	a	a	a	a	a
Silicate	w	w		a	a	a	a	a	a	a	a	a	a
Sulphate	w	w	w	w	i	i	wi	w	w	w	w	w	w
Sulphid	w	w	w	wa	w	w	w	a	a	a	a	a	a
Sulphocyanate	w	w	w	w	w	w	w	w	w	w	w	w	w
Tartrate	w	w	w	wa	a	a	a	wa	a	w	a	wa	w

	Cr_2	Al_2	Sb	Sn''	Sn^{IV}	Au	Ag	Hg_2	Hg	Pb	Bi	Cu	Cd
Acetate	w	w	w	w	w		wa	wa	w	w	w	w	w
Arseniate	a	a	a	a	a		a	a	a	a	a	a	a
Arsenite			a	a	a		a	a	a	a		a	
Borate	a	a		a			a			a	a	a	wa
Bromid	w	w	wa	w	w	w	i	ai	wa	wi	wa	w	w
Carbonate							a	a	a	a	a	a	a
Chlorate	w	w		w			w	w	w	w	w	w	w
Chlorid	w & i	w	wa	w	w	w	i	ai	w	wi	wa	w	w
Chromate	a		a	a			a	a	wa	ai	a	w	a
Cyanid	a					w	i		w	a	wa	a	a
Iodid	w	w	wa	w	w	a	i	a	a	wa	a	a	w
Nitrate	w	w		a	a		w	w	w	w	a	w	w
Oxalate	w	a	a	a	w		a	a	a	a	a	a	a
Oxid	a & i	a & i	a	a	a & i		a	a	a	a	a	a	a
Phosphate	a	a	a	a	a		a	a	a	a	a	a	a
Silicate	a	ai								a		a	a
Sulphate	w & a	w	a	w	w		wa	wa	wa	i	a	w	w
Sulphid			a	a	a	a	a	a	a	a	a	a	a
Sulphocyanate	w				w		i	a	w	a		a	wa
Tartrate	w	w	w	wa			a	a	a	a	a	wa	wa

w, soluble in water; a, insoluble in water, soluble in acids; i, insoluble in water or acids; wa, sparingly soluble in water, readily soluble in acids; wi, sparingly soluble in water and acids; ai, sparingly soluble in acids only.

metals. This latter statement is also true of carbonates, except that some of the carbonates will dissolve to appreciable extent in water containing CO_2. Chlorids, bromids, and iodids are nearly all soluble except those of the first-group metals. Sulphids are insoluble except those of Groups V and VI. Acid salts are usually more soluble than neutral salts.

In making qualitative tests for the acids it is not necessary to separate them one from the other, as it is in case of the metals; hence the tests are individual ones, usually made upon the original substance or solution, and often require confirmation before conclusive evidence is obtained. The grouping is therefore simply for convenience, as it thus becomes possible to exclude a considerable number of acids by a single general test.

Group I may include such acids as give effervescence when their dry salts are treated with *dilute* H_2SO_4, as H_2CO_3, H_2S, $H_2S_2O_3$, H_2SO_3, HCN.

Group II may include acids giving a precipitate with $AgNO_3$ in dilute HNO_3 solution, as HCl, HBr, HI, HCN, HCNS, HNO_2, HClO, H_4FeCy_6, H_3FeCy_6, $H_2S_2O_3$, H_2S, HPH_2O_2.

This second group may be further subdivided into three parts according to the color of the precipitate obtained (page 45).

Group III may include acids forming insoluble salts with BaCl or $CaCl_2$ and not found in Groups I or II, or H_2SO_4, $H_2C_2O_4$, H_3PO_4, H_3BO_3, H_2CrO_4, H_2SiO_3.

Besides the acids found in these groups there are three others of common occurrence: nitric (nitrates), chloric (chlorates), and acetic (acetates).

Detection of Acids of Group I.

(Acids effervescing with dilute sulphuric acid. H_2CO_3, H_2S, H_2SO_3, $H_2S_2O_3$, HCN.)

To a quarter test-tubeful of the unknown solution, or a little dry substance on a watch-glass, add dilute H_2SO_4. If

solution is very dilute, concentrate it before making test, as a slight amount of gas might be absorbed by the water. Watch carefully for any escape of gas and note any odor which may be given off.

Carbonates evolve CO_2, odorless, but if passed into lime-water or baryta-water will give white precipitate of $CaCO_3$ or $BaCO_3$.

Sulphids evolve H_2S, odor of rotten eggs. Confirm by adding a little dilute H_2SO_4 to the suspected powder (or solution) in a test-tube and holding over the mouth of the tube a piece of filter-paper wet with a solution of lead acetate. The test-tube may be warmed slightly to expel the gas, when a dark-colored stain will appear on the filter-paper, due to the formation of PbS.

Sulphites evolve SO_2, odor of burning sulphur. Sulphites in *neutral* solution may be further identified by the deep-red color produced with ferric chlorid. The color is discharged upon addition of dilute acids, HCl, H_2SO_4 (difference from HCyS.)

Thiosulphates *also* evolve SO_2, but at the same time the mixture becomes cloudy from precipitation of sulphur.*

Thiosulphates in neutral solution treated with ferric chlorid give a violet to purple color, fading (rapidly upon warming) to a colorless solution. In mixtures of sulphites and thiosulphates both acids may often be detected by the use of $FeCl_3$, the deep-red coloration of the mixed acids rapidly fading to the lighter red of $Fe_2(SO_3)_3$ (not to colorless solution).

Cyanids evolve HCN, odor of peach-stones. (Mercuric cyanid does not respond to this reaction.) Confirm by reactions given under Group II.

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* Sulphids may also precipitate sulphur in presence of compounds capable of oxidizing the H_2S, such as $FeCl_3$. In the absence of sulphates either H_2SO_3 or $H_2S_2O_3$ can be oxidized to H_2SO_4 by heating with HNO_3 and a precipitate of $BaSO_4$ obtained with $BaCl_2$.

Preliminary Tests for Common Acids of Groups II and III.

(In preparatory courses the acids given in this list may be sufficient.)

From the acids of Group II and III it may be desirable to select for laboratory practice, at least at the beginning of the acid work, the more common members of the groups. These will be HCl, HBr, HI, HCN, and H_2S of Group II and H_2SO_4, $H_2C_2O_4$, and H_3PO_4 of Group III; and tests for them may be made as follows:

Chlorids give with $AgNO_3$ in presence of HNO_3 a white curdy precipitate of AgCl, much more freely soluble in ammonia than any other acid of the group here given except the cyanid AgCN, but HCN is a member of the *first* acid group and would have been previously detected.

Bromids with $AgNO_3$ and HNO_3 give a precipitate of AgBr similar in appearance to AgCl, but with a slightly yellowish color and only sparingly soluble in NH_4OH.

The tests described on page 46 should also be made if bromids or iodids are suspected in the solution.

Cyanids, see Group I.

Sulphids will give a black precipitate with $AgNO_3$, and have been previously considered in Group I.

Sulphates may be detected by first acidifying the solution strongly with HCl (filtering out a precipitate if any occurs) and adding solution of $BaCl_2$; a white precipitate will then be $BaSO_4$, showing presence of sulphates in solution tested.

Phosphates in a solution containing HNO_3 and free or nearly free from HCl will give, with ammonium molybdate, a yellow crystalline precipitate of ammonium phosphomolybdate.

Oxalates may be detected, in a solution free from sulphates and which is slightly acid with acetic acid, by simple addition of calcium chlorid, which will precipitate CaC_2O_4, white and crystalline.

Detection of Acids of Group II.

(Giving precipitate with $AgNO_3$ in presence of dilute HNO_3.)

To the solution to be tested add a *very* slight amount of HNO_3 and a few cubic centimeters of $AgNO_3$ solution. A precipitate indicates acids of this group.

(*a*) The precipitate is white or nearly white, HCl, HBr (yellowish white), HI (yellow), HCN, HCNS, H_4FeCy_6 (slowly turns blue), HClO,* and HNO_2. ($AgNO_2$ is soluble in HNO_3 unless quite dilute.) Test the solubility of the silver precipitate in ammonia-water after decanting the supernatant fluid and it will be found that AgCl, AgCN, $AgNO_2$, Ag_3FeCy_6 are freely soluble; AgCyS, AgBr, Ag_4FeCy_6 are slightly or slowly soluble; AgI is practically insoluble.

(*b*) The precipitate is red-brown or orange, soluble in $NH_4OH = H_3FeCy_6$.

(*c*) The precipitate is black or turns black upon warming: H_2S (black immediately). HH_2PO_2 (starts to precipitate white, but rapidly turns black), $H_2S_2O_3$ (precipitates white, turns black slowly or upon heating).

Sulphids (H_2S) and **thiosulphates** ($H_2S_2O_3$) may also be detected as described under Group I, Acids.

To distinguish between the *white* precipitates obtained with $AgNO_3$ shake a little of the solution with a few centimeters of dilute indigo solution. The indigo is decolorized by hypochlorites (HClO) and by nitrites (HNO_2).

Hypochlorites liberate I from KI without the addition of acid.

Note.—Hypochlorite solutions are usually quite strongly alkaline, and in such cases a considerable amount of iodid is necessary to obtain the characteristic color in chloroform or with starch.

Nitrites liberate I from KI after the addition of acetic acid. They also give a brown coloration with acetic acid

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* Precipitate is AgCl. Reaction is $3NaClO + 3AgNO_3 = 2AgCl + AgClO_3 + 3NaNO_3$.

and a crystal of ferrous sulphate. (Nitrates require a stronger acid.)

Note.—This test is much more delicate than either of the others given, and if the solution is very dilute it is well to make it, even if the indigo color is not discharged.

With a few drops of $FeCl_3$, **sulphocyanates** or **thiocyanates** (HCNS) give a deep blood-red solution. The color is soluble in ether and may be discharged by $HgCl_2$. **Ferrocyanids** (H_4FeCy_6) give deep-blue precipitate.

If the test given under Group I is not conclusive, **cyanids** may be converted into sulphocyanids by addition of a few drops of $(NH_4)_2S$ and evaporation on the water-bath to dryness. It may be then dissolved in a little distilled H_2O, filtered and tested with $FeCl_3$.

Iodids and **bromids** (HI and HBr) may be detected in the same solution by adding Cl water, very cautiously at first, and shaking with chloroform. The Cl liberates, first, the iodin, which is dissolved by the chloroform with violet color. Excess of Cl decolorizes the iodin and liberates the bromin, which in turn is dissolved by the chloroform with yellow to red color.

Chlorids (HCl) may be distinguished from HBr and HI by the ready solubility of the silver precipitate in NH_4OH. If bromids and iodids are present, liberate the halogens by means of MnO_2 and H_2SO_4, pass the mixed gases into a solution of aniline in acetic acid (4 c.c. of saturated aqueous solution of aniline and 1 c.c. glacial acetic acid). Iodin gives no precipitate, bromin gives a white one, chlorin a black one. (Prescott and Johnson, page 336.)

This is a delicate and very satisfactory test for bromin, not so delicate for chlorin in the presence of bromids. For such cases the following *chloro-chromic anhydride test* is recommended. Neutralize the solution if necessary, evaporate to dryness, transfer residue to a test-tube of rather small diameter, add a little solid $K_2Cr_2O_7$, then concentrated H_2SO_4.

Decant the *fumes* into a wider test-tube containing a few centimeters of NH_4OH.

If the chlorochromic anhydride is evolved, ammonium chromote will be formed. Test by making acid with acetic acid, then adding acetate of lead. A yellow precipitate of lead chromate indicates chlorin in the original solution.

Acid Group III.

(Acids forming insoluble barium or calcium salts, not included in the Acid Group I or II.)

The members of this group may be separated from each other, although this is not necessary unless several members are present. H_2SO_4, $H_2C_2O_4$, H_2CrO_4, H_2SiO_3, H_3BO_3, H_3PO_4, separated as follows: To a little of the unknown solution add 2 or 3 c.c. of HCl; a white or gelatinous precipitate which is not dissolved by dilution with water and warming is probably silicic acid. Make a bead test with microcosmic salt; the particles of SiO_2 remain undisturbed by the hot bead, forming the so-called silicon "skeleton." Filter out the silicic acid and add $CaCl_2$ or a mixture of $BaCl_2$ and $CaCl_2$; a white precipitate will be $BaSO_4$ * (test for *sulphates*). The Ba and Ca salts of all remaining acids of the group being soluble in HCl.

Filter out the $BaSO_4$, and to the filtrate add NH_4OH, which will cause a precipitate of barium *oxalate, chromate, borate,* and *phosphate.* Filter, wash precipitate two or three times, reject wash-water, then transfer to test-tube by making a small hole in point of paper and forcibly washing through with the least possible amount of water; acidulate strongly with acetic acid, which will dissolve the phosphates and borates, leaving undissolved the oxalates (BaC_2O_4, white) and chromates ($BaCrO_4$, yellow.)

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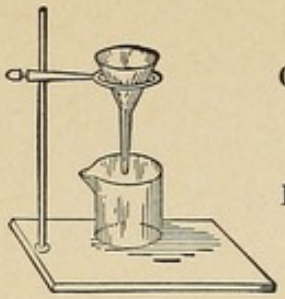
* If the HCl is too strong, $BaCl_2$ may be precipitated as such, but the precipitate in this case will form more slowly than the $BaSO_4$; it will have a crystalline appearance and will dissolve upon addition of water.



Oxalic and chromic acids as barium salts.

Phosphoric and boric acids.

Divide the filtrate into two parts, *a* and *b*. Test one part, *a*, for H_3PO_4 by adding to it an excess of ammonium molybdate in HNO_3, when a yellow precipitate (forming sometimes after several hours' standing) is ammonium phosphomolybdate (test for *phosphates*); the mixture may be warmed to hasten precipitation; the degree of heat should not exceed 40° C., as the ammonium molybdate might be decomposed, giving a yellow precipitate similar to the phosphomolybdate.

Note.—If As is present, it must be removed by H_2S before testing for H_3PO_4.

Test the other part, *b*, for H_3BO_3 by evaporating to dryness in a porcelain dish; then moisten with strong H_2SO_4, cover with a little alcohol and ignite. Boric acid will give to the flame (particularly the edge) of the burning alcohol a green color due to formation of ethyl borate. This color is more easily apparent if the dish is placed in a darkened corner.

A test for H_3BO_3 may also be made with turmeric paper, which if dipped into a solution of boric acid, or of a borate mixed with HCl or H_2SO_4 to slight but distinct acid reaction, and dried at 100°, becomes red; the red color becomes bluish black or greenish black when moistened with a solution of an alkali or an alkaline carbonate. If there is a suspicion that H_2CrO_4 and $H_2C_2O_4$ are both present, dissolve the precipitate of barium oxalate and chromate off the paper with dilute HCl; divide the filtrate into two parts and test one for H_2CrO_4 by addition of H_2O_2, which with chromates in presence of HCl produces a deep-blue solution and ultimately $CrCl_3$.

In the *absence* of chromates, the precipitate being white,

oxalates may be confirmed by coloring the second part of solution faint pink with dilute solution of $KMnO_4$ and warming, when the color will be discharged.

In the *presence* of chromates (the precipitate being yellow) it will be necessary to test the original solution for oxalates as follows: To a few centimeters of the unknown add alcohol; warm. The chromate will be reduced to $CrCl_3$. Add NH_4OH till alkaline and filter out the precipitate, $Cr(OH)_3$. The filtrate may be tested for oxalic acid as above, or with $CaCl_2$; a white precipitate being CaC_2O_4.

The remaining acids of importance not included in either of the three preceding groups are nitric, HNO_3, chloric, $HClO_3$, and acetic, $HC_2H_3O_2$.

Nitrates.—Saturate 5 c.c. of a very dilute nitrate solution with $FeSO_4$. Filter and carefully underlay the clear filtrate with concentrated sulphuric acid; a dark ring (pale red-brown to nearly black) at point of contact of the two liquids shows presence of a nitrate.

Chlorates.—A solution free from chlorids or hypochlorites treated with Zn and dilute H_2SO_4 will give a test for HCl if chlorates were originally present, the chlorate having been reduced by the nascent H:

$$2KClO_3 + 6Zn + 7H_2SO_4 = 6ZnSO_4 + K_2SO_4 + 2HCl + 6H_2O.$$

Boiling with sulphurous acid also reduces $HClO_3$ (and HClO) to HCl.

If the substance is in solid form, a *very small particle* may be warmed with concentrated H_2SO_4. Chlorates detonate and give off yellow fumes of ClO_2:

$$3KClO_3 + 2H_2SO_4 = 2KHSO_4 + KClO_4 + 2ClO_2 + H_2O.$$

Acetates give with ferric chlorid a red color which is not discharged by $HgCl_2$ (difference from sulphocyanate), but may be discharged by HCl (difference from sulphocyanate and meconate).

A more positive test is the formation of the ethyl ester

or acetic ether. A blank test for comparison should always be made, the method of procedure being as follows:

Take two test-tubes of practically equal diameter, mix in each equal volumes of alcohol and strong H_2SO_4; warm the tubes together; then into one introduce a few centimeters of the unknown solution, and into the other an equal volume of H_2O. Heat again to a boiling-point and compare the odors from the two tubes. The acetate is easily detected if present.

SECTION III.—ANALYSIS IN THE DRY WAY.

In the examination of solid substances much may be learned by a few simple tests directly applied to the substance, which has been reduced (if necessary) to the form of a powder.

Some of these are usually used as preliminary to the solution of the substance and regular analysis in the wet way. These tests may be made quickly and, with a little elaboration, will often give all the information required regarding an unknown substance.

The practical questions of actua experience are usually simple ones. It is not an analysis of an unknown solution possibly containing all the metals of one or more groups that interests an active practitioner, but a specific inquiry as to whether or no this or that preparation contains or does not contain the necessary or the undesirable ingredient, whether the thing is of the composition or of the strength represented, and a few minutes' work in the laboratory, especially if aided by the microscopical tests given in a subsequent chapter, will frequently be found sufficient to answer questions of this character.

The tests made in the dry way are not as delicate, nor are the results obtained (especially negative ones) as conclusive, as those of a systematic analysis of the substance in

solution, and in occasional cases it may be necessary to resort to the more tedious process.

Before undertaking the analysis of a substance, note carefully its physical properties of odor, color, and solubility; also whether it is magnetic, metallic, or crystalline.

The volatile acids, certain ammonium compounds, bromin and iodin may be detected frequently by their odor.

Colors of Salts and Solutions.

The following colored salts are *soluble* in water:

Black	Silver albuminate (argyrol, etc.)
Violet or purple	Chromic salts and permanganates
Red	CrO_3 and acid chromates, K_3FeCy_6, sodium-nitro-prusside, H_2PtCl_6
Reddish brown or purple-red	Manganic salts
Reddish yellow	Ferric salts and $AuCl_3$
Yellow	Neutral chromates of the alkalies, salts of uranium
Pale yellow	K_4FeCy_6 (Potassium ferrocyanide).
Pink	Salts of cobalt
Pale pink	Manganous salts
Green	Ferrous salts, nickel salts, certain copper salts
Dark green	Some chromic salts
Blue-green	Chromates
Blue	Cupric salts

The following colored substances are *insoluble* in water:

Black	Carbon and carbids, metals, many metallic sulphids, oxids of Cu, Fe, Mn, and Pb. Iodin is bluish black
Red	HgO, HgS, HgI_2, Pb_3O_4, As_2S_2
Brick-red	Amorphous phosphorus, Fe_2O_3
Light brown	PbO, litharge
Yellow	S, HgO, CdS, As_2S_3, PbI_2, Ag_3PO_4, ammonium phospho-molybdate, and chromates of the heavy metals, $PbCrO_4$, $BaCrO_4$
Green	Some copper compounds, Cu_2I_2, Paris green, etc., Cr_2O_3
Blue	Some copper compounds, Prussian blue, ultramarine; anhydrous salts of cobalt

METHODS OF EXAMINATION.

Powder the substance and apply tests described in this chapter, which will be considered in the following order:

A. Ignition on the platinum foil.
B. Closed-tube test.
C. Flame test on platinum wire.
D. Examination with the blowpipe on plaster slab.
E. Bead tests on platinum wire.
F. Special tests, distinguishing or confirmatory.

A. Ignition on Platinum Foil.

A piece of platinum foil about 1 inch square is pressed into the palm of one hand with the thumb of the other in such a way as to leave the foil slightly concave.

The Bunsen flame is turned down to about ½ inch in height, the air-supply being so regulated that combustion is perfect. The platinum foil, holding a few grains of the unknown substance and held by crucible-tongs, is heated carefully at first, and then the heat is gradually increased until no further apparent change takes place in the substance.

The majority of phenomena occurring under A are more easily observed in the test made with the closed tube, B, and will be given under that head. There is, however, some information more easily obtained by use of the platinum.

Observed Phenomena.	Indication.
The substance *melts* and steam is given off.	Water of crystallization, NH_4NO_3 or $H_2C_2O_4$, which entirely disappears.
The substance *burns* (*a*) at comparatively low temperature with blue flame and odor of SO_2 or burning matches.	Sulphur.
(*b*) With yellow flame and much smoke.	Fat, waxes, resins, etc.
(*c*) Blackens and then burns at fairly high temperature, leaving white or gray ash.	Carbonaceous matter other than fats, etc.
(*d*) Blackens without burning.	Formation of oxids of Fe, Co, Ni, or Cu.

Observed Phenomena.	Indication.
Vapors are given off:	
(*a*) Of a violet color.	Iodin.
(*b*) Of a red-brown color.	Br or nitrogen oxids.
(*c*) Of a greenish-yellow color.	Chlorin or ClO_2.
(*d*) White, practically odorless.	Some ammonium salts, NH_4Cl, $(NH_4)_2SO_4$, etc.
(*e*) White with odor of NH_3.	Ammonium carbonate.
(*f*) White with odor of garlic.	Arsenic.
(*g*) White and yellow with ammoniacal or empyreumatic odor.	Organic matter.
The substance decrepitates.	Water held mechanically by crystals, as NaCl, etc.
Examine residue on foil; add a drop or two of water and test with litmus-paper.	
If found to be acid.	Acid salts.
If alkaline without blackening.	Fixed alkali hydrates or carbonates.
If alkaline with blackening.	Carbonate formed by combustion of organic compounds.
Add a drop of dilute HCl, effervescence.	Carbonates.

B. Closed-tube Test.

Select a tube of soft glass about 5 or 6 inches in length. Seal one end and enlarge slightly. Into the bulb thus formed introduce a few grains of the unknown powdered substance. Heat carefully, making the following tests at various stages of the process. Note the odor of escaping gases.

Test for oxygen by inserting a glowing splinter into the tube.

Test for combustible gases by occasionally applying flame to the open end of the tube.

Bring to the mouth of the tube a clear drop of $Ba(OH)_2$ solution. If the drop becomes turbid, CO_2 is indicated.

Observed Phenomena.	Indication.
Steam condenses in cold part of tube.	See under A.
Oxygen is evolved.	A peroxid, chlorate, some oxids (as HgO), alkali nitrates.
Carbon Dioxid is evolved.	Carbonates, oxalates (at high temperature), organic matter.

Observed Phenomena.	Indication.
A Combustible Gas is formed:	
(*a*) Burning with a luminous flame, black residue remains in tube.	Hydrocarbons from organic matter.
(*b*) Burning with a blue flame.	CO from oxalates.
(*c*) Burning as in (*b*) and with odor of SO_2.	H_2S from moist sulphids.
A Sublimate forms in the cooler part of the tube. Examine under microscope.	
Colorless with partial decomposition.	Oxalic acid. Plate II, Fig. 1.
Color is *white* with production of garlic odor, crystalline.	As_2O_3. Plate II, Fig. 2.
Color is *white* when cold. Yellow when hot, crystalline.	$HgCl_2$. Plate II, Fig. 3.
Color is white—it sublimes directly without melting and blackens with NH_4OH.	HgCl.
A white sublimate which by treatment with slaked lime yields NH_3.	Ammonium salts. Plate II, Fig. 4.
A white sublimate of As_2O_3 with black residue in tube and odor of acetic acid.	Paris green.
Sublimate is gray, consisting of small globules which can be made to unite by rubbing.	Hg from HgO, amalgam, etc. Plate II, Fig. 5.
Sublimate consists of reddish yellow to red globules, yellow when cold.	Sulphur.
Sublimate darker than above and reddish yellow when cold.	Native Sulphid of arsenic.
Sublimate is brown to black "metallic mirror," soluble in NaClO.	Metallic arsenic.
Ditto; dead black, insoluble in NaClO.	Metallic antimony.
Sublimate is black accompanied by violet vapor.	Iodin. Plate II, Fig. 6.
Sublimate black, turning red when rubbed.	HgS, cinnabar.
No sublimate is formed, but the color changes to	
Yellow when hot, white when cold.	ZnO.
Reddish brown when hot, yellow when cold.	PbO or Bi_2O_3. (See D.)
Black when hot, red when cold.	HgO (Hg sublimes).
Black when hot, brick-red when cold.	Fe_2O_3.
Dark orange when hot, yellow when cold.	Chromates of Pb, etc.
Black residue without other visible manifestation.	Oxids of Cu, Co, etc. (See A.)
Substance melts without a sublimate being formed.	Salts of the alkaline metals.

C. Flame Test with Platinum Wire.

Introduce the substance on platinum wire into the edge of the flame. More satisfactory results are sometimes obtained if the solid is first moistened with HCl (page 34). The flame is colored as follows: by Na, yellow; K, violet; Li, carmine;

Sr, crimson; Ca, orange-red; Ba, yellowish green; Cu, usually bright green; $CuCl_2$, an intense blue; H_3BO_3, pale green; Sb, greenish blue; Pb, As, Bi, livid blue.

D. Blowpipe Test on Plaster.*

Smooth plaster slabs about 1 inch wide and 4 inches long are well suited for these tests. These may be prepared by making a magma of calcined plaster and pouring upon a glass plate. Before it hardens mark deeply with a spatula into slabs of desired shape and, after it is throughly dried, break as marked.

Make a little depression near one end of the slab and in it place a small amount of the substance to be tested; then if a fine oxidizing flame is made to play over the *surface* of the assay, characteristic coatings of oxid or sublimate may be obtained.

In many cases the character of the substance may be determined more easily by first moistening the assay with various reagents. Tetrachlorid of tin, cobalt nitrate, and "sulphur iodid" are the most valuable of the reagents so used. The "sulphur iodid" is not of definite composition, but a mixture of about equal weights of sulphur and potassium iodid.

D. I. *Examination without Reagents.*

Observed Phenomena.	Indication.
Substance melts to bright metallic globules with brownish-yellow deposit near assay. Requires high heat. Assay revolves.	Silver.
Substance melts to bright globule with coating on plaster, deep orange when hot, light yellow when cold.	Lead or bismuth (See D. II.)

* Substances sufficiently identified by previous tests have been omitted. This method will be found useful mainly in the identification of metals.

The Author was greatly aided in the preparation of this list by Mr. Geo. F. S. Pearce of the Harvard Dental School, who carefully verified each test.

Observed Phenomena.	Indication.
Substance remains or becomes black without melting. No coating on plaster.	Copper or iron. (See A; also F.)
Substance volatilizes with white fumes, but leaves dark stain; gray to black.	Antimony or arsenic. (See F.)
Substance melts with white or gray oxid on assay.	Tin. (See D. III.)
Forms a white or gray oxid *without* fusion. Coating on plaster is yellow over brownish black.	Cadmium.
Forms bulky white oxid with active combustion of assay.	Magnesium.
Forms gray coating easily volatilized.	Mercury for amalgams. (See D. II.)
Cherry-red—crimson to black according to amount of substance deposited. Odor of rotten horse-radish; coating not permanent.	Selenium.
White coating or white fumes at very high heat. Assay burns with bluish-white light.	Zinc. (See D. III.)
Silver-white. Assay remains unchanged.	Platinum, metallic.

D. II. *Cover substance with KI and S. Use oxidizing flame.*

Observed Phenomena.	Indication.
Dirty-white and light-gray coating. Treated with fumes of strong NH_3 and again placed in oxidizing flame gives bright-red color. Metallic globule is dull and brittle.	Bismuth.
Dirty white half an inch from assay. Brown directly under assay. No change when treated as above with strong ammonia fumes. Metallic globule is bright and malleable.	Lead.
No coating near assay. Lead-colored, one to one and a half inches, shading to yellow.	Mercury.
Coating bright red when hot, fading to yellow when cold.	Cadmium.
Fine brown coating, very volatile.	Antimony.

D. III. *Examination with Solution of Cobalt Nitrate.*

Heat substance on plaster in the oxidizing flame, moisten well with cobalt nitrate, and again apply oxidizing flame.

Observed Phenomena.	Indication.
Color is deep blue.	Aluminium.
Substance is infusible.	Infusible silicates. (See F.)
Color is fine blue. Substance fusible.	Alkaline silicate, borate, or phosphate.
Color is yellowish green.	Zinc.
Drab to bluish green.	Tin.

D. IV. *Examination with Tetrachlorid of Tin.*

Observed Phenomena.	Indication.
Coating pale blue to lavendar.	Bismuth.
Coating fine blue, in places almost black.	Antimony.
Delicate pink to red produced only by oxidizing flame.	Neutral and acid chromates.

E. Bead Tests.

The bead tests are made with borax, as described on page 24, or in a similar manner with microcosmic salt, $NaNH_4HPO_4$, which by action of the heat gives up NH_3 and H_2O, becoming sodium metaphosphate, $NaPO_3$. These substances fused on a loop of platinum wire unite with many of the metallic oxids, forming "beads" of various characteristic colors, some of the more important given below.

With Borax.

Co in the oxidizing flame gives an intense blue bead.
Ni gives a red-brown, yellow when cold.
Cu " a green, blue or bluish green when cold.
Cr " green.
Fe " a red, yellowish when cold.
Mn " an amethyst.

With Microcosmic Salt.

Cobalt, copper, nickel, and iron give colors similar to those obtained with borax. Manganese gives a violet bead when heated in the oxidizing flame, but a colorless one in the reducing flame.

F. Special Tests Distinctive or Confirmatory.

The oxids of copper and iron may be distinguished by adding a drop of HNO_3, warming gently to drive off excess of acid (high heat will decompose the nitrate, giving the oxid

again), and then adding a drop of solution of K_4FeCy_6. Fe will give dark-blue coloration; Cu will give a brown.

To distinguish between As and Sb stains, add a drop of hypochlorite solution (NaClO). The arsenic stain will dissolve; the antimony stain will remain unaffected (see page 14).

Antimony gives a very characteristic coating on plaster if treated with tetrachlorid of tin. The coating is bluish black near assay, fading away to a very delicate color at greater distance. It appears almost immediately and is permanent.

In case of suspected silicates make the "silica skeleton" with a bead of microcosmic salt (page 47).

PART II.

DENTAL METALLURGY.

INCLUDING THE CHEMISTRY OF ALLOYS, AMALGAMS, SOLDERS, AND CEMENTS.

SECTION I.—THE METALS.

THE metals occur in nature to quite an extent free, but more often combined with other elements. These combinations are chiefly as oxids, sulphids, carbonates, and silicates, and in one or more of these four forms may the great mass of metals contained in the earth's crust be found.

Metallic sulphates are found to a considerable extent. Calcium sulphate is of particular interest, occurring as gypsum, $CaSO_4.2H_2O$. Upon heating, the two molecules of water of crystallization may be driven off, leaving the anhydrous $CaSO_4$, or plaster of Paris, so largely used in dental laboratories. When water is added to the anhydrous powder it reunites in the proportions of the original crystallized salt and thereby occasions the "setting" of the plaster. Essig states that if in the preparation of plaster the heat is allowed to exceed 127° C., its affinity for water is impaired or destroyed and this effect will not be produced.*

As plaster sets, more or less expansion will take place, and if spread upon glass, the mass usually rises slightly in the center, producing a plate which is somewhat concave on the under

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* American Text-book of Prosthetic Dentistry.

surface. This tendency to expansion varies with different grades of plaster, as may easily be shown by a method suggested by Dr. George H. Wilson in the Dental Cosmos for August, 1905, page 940, which consists simply of filling small glass beakers with mixtures similarly prepared. Some samples were found to expand so slightly as not to injure the glass, others cracked, and some broke the beaker into fragments.

The method of mixing also affects the amount of expansion. In a valuable article on "Experiments in Plaster of Paris to Test Expansions," by Dr. Stewart J. Spence, in Items of Interest, 1902, page 721, it is shown that "not only do different plasters expand in differing degrees, but the same plaster expands very differently according to the stirring given it before pouring," and that long stirring increases the heat developed, the rapidity of setting, and the amount of expansion, but decreases the strength.

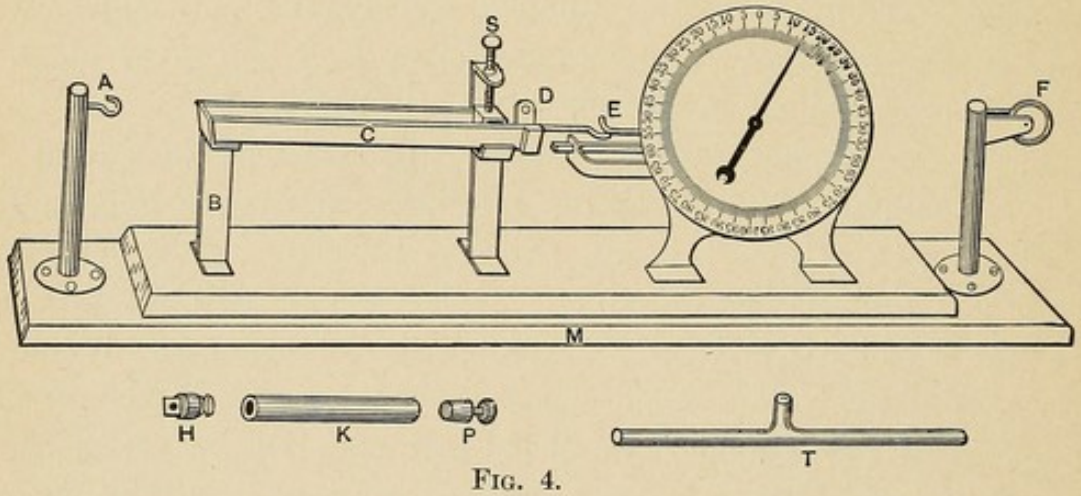

FIG. 4.

An apparatus which may be used for testing the expansion of plaster is represented by Fig. 4. The plug *H* is screwed into the cylinder *K*, which is then filled with the fresh mixture of plaster and water. The piston *P* is inserted and the whole placed in the trough *C*. A pin above the post *B* passes through the hole *H*, holding it perfectly firm at this end. At the other end the sliding section *D* is brought up firmly against the end of *P* and the needle set at *O*. Any expansion of the plaster

must manifest itself in the only direction possible by pushing out the piston *P* and indicating on the dial the degree of expansion. The needle is turned by a rack and pinion connected with *E*. The action is very easy, and the mechanism so arranged that the slightest expansion is readily indicated by the needle. By placing this apparatus on the second base *M* this same apparatus may be used to test the strength or contraction of dental floss, ligatures, etc. The silk is fastened firmly at *A*, then passed through the hollow glass tube *T*, which is of the proper length to allow the silk to be again fastened at *D*. It is then passed back of the dial and over *F*, and if desired a weight attached. The tube *T* is then filled with water, which by capillary attraction retains sufficient moisture to keep the thread wet. As the thread thickens it contracts longitudinally, pulling the needle to the left, and comparative tests may be made and continued for as long a time as necessary.

Various methods have been prepared to overcome the difficulties in manipulation of plaster, such as mixing the plaster with alum, marble-dust, or potassium sulphate. A compound on the market consists of a mixture of plaster and Portland cement. A mixture which has been very strongly recommended as an investment preparation consists of two-thirds plaster and one-third powdered pumice-stone.

Other natural sources of the metals are phosphates and chlorids, also smaller amounts of nitrates and comparatively slight amounts of bromids, iodids, and fluorids. The metals are extracted from their ores chiefly by reduction with some form of carbon. In case of the oxids this reduction takes place directly, according to this reaction: $2CuO + C = 2Cu + CO_2$.

In case the metallic combination is a sulphid, the ore is first "roasted" in the air, whereby the sulphur is burned off and an oxid is formed, which may then be reduced as above: $2CuS + 3O_2 = 2CuO + 2SO_2$.

The native carbonates are reduced to oxids by calcination, as $CaCO_3 + \text{heat} = CaO + CO_2$.

The silicates must first be changed to carbonates by fusion with alkali carbonates, then the reduction may be carried on as before:

$$MgSiO_3 + Na_2CO_3 = MgCO_3 + Na_2SiO_3;$$

then

$$MgCO_3 + heat = MgO + CO_2.$$

Properties of the Metals.

Metals are *malleable* in order as follows from gold, the most malleable, to nickel, the least: Au, Ag, Al, Sn, Cu, Pt, Pb, Cd, Zn, Fe, Ni.

Metals are *ductile* from most to least as follows: Au, Ag, Pt, Fe, Ni, Cu, Cd, Al, Zn, Sn, Pb.

Metals conduct heat and electricity in the same order until Sn is reached. From Sn the order given is correct for heat but not for electricity: Ag, Cu, Au, Al, Zn, Cd, Sn, Fe, Pb, Pt, Bi.

The melting-point of the various metals is of considerable importance in the preparation of alloys. The following table has been compiled from the latest available results. The degrees given are according to the centigrade scale:

Pt	2000°	Cu	1054°	Zn	420° (burns)
Ni	1450°	Ag	954°	Pb	326°
Cast steel	1375°	Al	700°	Cd	320°
Cast iron	1275°	Mg	500° (burns)	Bi	268°
Au	1075°	Sb	432°	Sn	238°

The expansion of the various metals under the influence of heat is fairly constant and there have been determined *coefficients of expansion*. These represent the amount of linear expansion of the metals due to a rise in temperature of 1° C., usually from 0° to 1°. The coefficients are not *absolutely* constant, and the amount of expansion observed between 0° and 1° may differ somewhat from that between 50° and 51°. The coefficients vary widely for the different metals; for instance, in passing from 0° to 100° mercury expands 1/16 of its linear measure, copper 1/598, and platinum 1/1123.

Hall's Dental Chemistry gives the following table of expansion from cadmium to platinum:

Cd	1/326	Ag	1/518	Ni	1/787
Pb	1/342	Cu	1/598	Fe (cast)	1/934
Zn	1/343	Bi	1/617	Sb	1/952
Al	1/432	Au	1/689	Pt	1/1123
Sn	1/448				

The only other general property of the metals directly affecting their use in dental practice is the electric or galvanic, that is, the electropositive or negative relations they sustain to one another.

The metals are electropositive to each other in the following order from zinc, the most positive, to platinum, the least: Zn, Cd, Sn, Pb, Fe, Ni, Bi, Sb, Cu, Ag, Au, Pt, and C.

Thus if a battery is constructed with Zn as represented in the cut (Fig. 5), and iron in place of the carbon, then the iron will be electronegative to the zinc, and hydrogen will be evolved from its surface; if, on the other hand, Fe is used in place of the zinc, and the carbon remains as in the cut, the Fe will be electropositive to the carbon, and oxygen will be evolved from its surface. This property of metals has a direct bearing upon dental science, because human saliva may be an exciting fluid for the generation of galvanic currents, its activity being increased by an abnormal reaction either acid or strongly alkaline, and it is only necessary to place in the mouth properly related metals, as amalgam fillings or otherwise, to produce the elements of a galvanic battery.

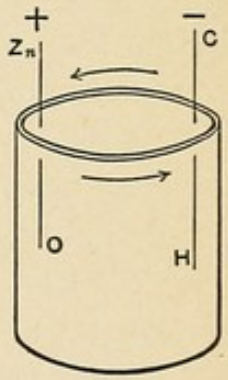

FIG. 5.

The currents thus generated are of course infinitesimal, but they are constant and may aid in the disintegration of fillings and in the solution of the constituent metals. Regarding the extent to which electric currents may exist in the mouth, see Miller's Microorganisms of the Human Mouth.

SECTION II.—ALLOYS.

An intimate union of two or more metals, usually produced by fusion, forms an *alloy*. Such a union of one or more metals with mercury is an *amalgam*.

An alloy designed to be used in the preparation of dental amalgams is known as an *amalgam alloy*.

Some metals can be fused together in all proportions, as Pb and Ag. Others can be made to unite only in limited proportions, as Pb and Zn. Lead will carry only 1.6% of zinc, while zinc will unite with only 1.2% of Pb. Excess in either case separates out.

The *properties* of an alloy are, as a rule, the modified properties of its constituent metals. An exception to this rule might be made of the sonorous quality of bell-metal and like alloys, this being hardly a property of the constituent metals at all.

Following are some of the more common alloys. The proportions given are general formulæ and may, as a rule, be varied considerably:

Aluminium bronze, yellow, resembles gold, Cu 92, Al 8.

Bell-metal, Cu 80, Sn 20.

Britannia metal, Cu 2, Sn 82, Sb 16.

Coin silver, Ag 90, Cu 10.

Dental alloys, see pages 71 and 73.

Dental gold, Cu 85, Zn 15.

German silver, Cu 50, Ni 30, Zn 20.

Composition of different samples of German silver may differ widely; some contain about 2.5% of iron and the amount of Cu may vary from 40 to 60%.

Solder, see page 79.

Sterling silver, must contain 92.5% Ag.

Type metal, Pb 78, Sb 15, Bi 7.

All alloys (excluding amalgams) are solid at ordinary temperatures with one exception; this one is an alloy of one part potassium with three parts sodium.

The *melting-point* of an alloy is often lower than that of the metals entering into its composition and usually lower than the mean melting-point of its constituents.

In *making alloys* the tendency to separation of the several metals is greater if the alloy is allowed to cool slowly; hence three essentials in the process are: *Complete fusion*, which makes possible *thorough mixing*, and after this has been attained *rapid cooling*. As the fused mass is to be cooled as quickly as possible after fusion is complete, it is desirable to use the least amount of heat practicable in effecting the desired result. To this end fuse first the metal with the lowest melting-point, then add other metals in the order of their melting-points. The more difficultly fusible metal will in a sense dissolve in the more easily fusible metal, an alloy is formed and its temperature has been kept far below the melting-point of the high fusing constituent. This general rule, however, may be modified by the proportion of metal used; thus, in making a silver-tin amalgam alloy containing 60% of silver it is better first to melt the silver under a flux of carbonate of sodium or borax to prevent superficial oxidation, then add the tin, and lastly any other metal to be used. The *mixing* is attained by stirring with a *wooden* stick and the *cooling* by turning quickly into a cold clean mold. For class work or in making small amounts (20 grams) of alloy, the Fletcher melting arrangement shown in Fig. 6 is very convenient. The metals are melted in the graphite crucible and then by tipping up the whole contrivance the melted metals flow back into the ingot mold. If the alloy is to be used in the preparation of dental amalgams it must be reduced to fine turnings or filings suitable for ready amalgamation. This is best accomplished in the laboratory by means of a coarse file, the ingot being held by a vise. The fine particles of iron must next be carefully removed with a magnet, and then the filings may be annealed if desired.

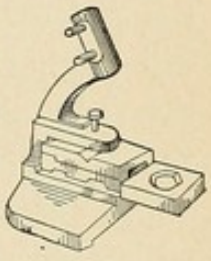

FIG. 6.

The *annealing* of the amalgam alloys may be accomplished by placing the freshly cut sample in a dry test-tube and keeping the test-tube in boiling water for ten or twelve minutes. It has been claimed that this process is one of superficial oxidation and the changes produced seem to be consistent with this theory. Again, it is claimed that the change is a molecular one of some sort due to change of temperature, and Prof. G. V. Black has shown that an alloy will anneal as rapidly in an atmosphere of nitrogen as of oxygen. The modification of properties produced by annealing varies somewhat with the composition of the alloy; for instance, the liability to discoloration is less in the annealed than in the unannealed sample if the alloy contains Ag and Sn, or Ag, Sn and Zn, but if Cu is a constituent the reverse condition has been found to exist.

According to Prof. Hall of Northwestern University "annealed alloys take up less mercury than unannealed and yield upon mixing a greater quantity of dirt, which consists of a lower oxid of tin." The amalgam made from an annealed alloy works more easily than from an unannealed.

The process of annealing up to a certain point seems to be, in general, beneficial; but beyond this point it may be detrimental, the amalgam being less strong and more liable to shrink.

Prof. Black has shown that while it may be possible to stop the process of annealing at such a point that a given alloy will neither shrink nor expand, it is easy to carry the process too far and the farther it has been allowed to go the greater the shrinkage. It is probably true that the exact effect of annealing will vary with the composition of the alloy, and with different proportions of metals in alloys of the same general composition.

Annealing of Gold.

When gold-foil is heated to redness it recovers the cohesive property which has been lost largely by hammering. It is recommended that the heating be done in an electric furnace or on plates of mica or platinum, thus insuring uniformity of effect throughout the mass which it is practically impossible to obtain by holding the metal in the flame. See Dental Cosmos, Vol. XLVII, page 233.

Non-cohesive gold or gold in which the cohesive property cannot be developed by heating may be prepared by alloying or treatment with carbon. **Corrugated gold** is of this variety and is prepared, according to Essig, by carbonization of unsized paper in intimate contact with the metal. See Essig, Dental Metallurgy, page 173.

In annealing *platinum* a high degree of heat is required, but the heat should be raised gradually, and in this case also the electric furnace furnishes an ideal method.

SECTION III.—AMALGAMS.

In general, amalgams may be made in three different ways: First, by direct union of the constituents as in the manufacture of sodium amalgam (page 69); second, by electrolysis of strong solutions of metallic salts in presence of mercury (as in copper amalgam, page 70), and third, by double decomposition as illustrated in the preparation of ammonium amalgam (page 69).

Amalgams possess the peculiar property of "setting" or hardening within a short time after mixing. This in some cases seems to be a process of crystallization, and in all cases is probably due to molecular rearrangement of some sort.

After an amalgam has "set" to a sufficient extent to make it hard to work it may be softened by application of gentle heat. Continued reheating is detrimental to the quality

of the amalgam, and should be avoided; this is particularly true of copper amalgam. It is also possible to sometimes restore the plastic quality of an amalgam by adding a further slight amount of mercury, but the union of the second lot of Hg after the first has partly hardened is very unsatisfactory and results in a weakened product.

Flow of Amalgams.—This property may be defined as the tendency to flatten or change shape under stress or pressure. It is common to most amalgams (copper amalgam being an exception according to Dr. Black), and is possessed by many alloys other than amalgams.

Tests for "flow" may be made with the "dynamometer" on cubes of alloy or amalgam measuring one tenth of an inch each way and the results expressed in percentage of increase or decrease of one dimension. The dynamometer used for this purpose is pictured in Fig. 7 and is a modification of the

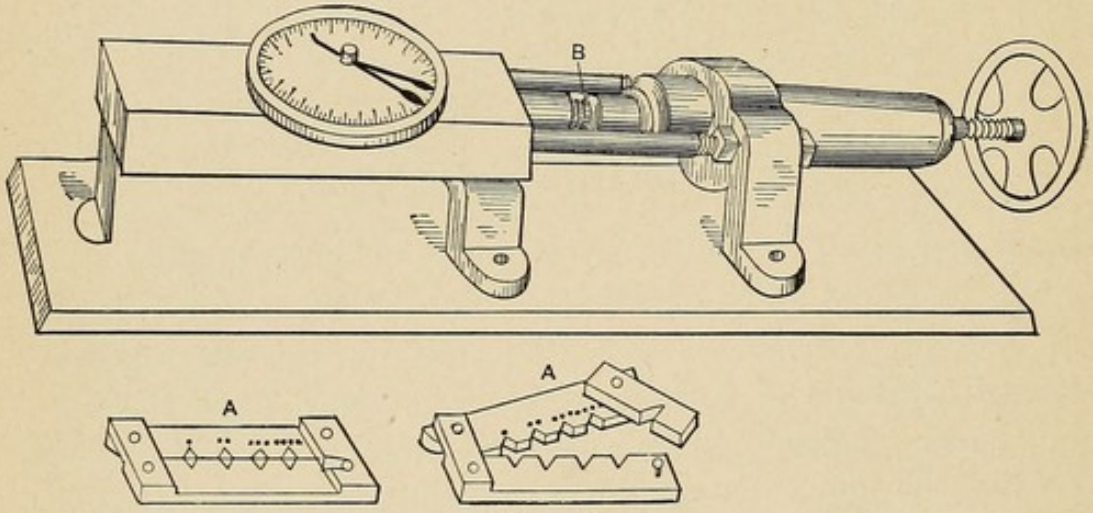

FIG. 7.

apparatus devised by Dr. Black and described on pages 408, 409 of the Dental Cosmos, Vol. 37, *A-A* being the molds in which the cubes of amalgams are set and *B* the point in the apparatus where the cube after setting is introduced with a pair of fine forceps. The dial is supplied with two hands, one which flies back the instant the cube breaks, the other remaining to indicate the number of pounds applied necessary

to crush the cube. The cubes of 1/10 inch are best suited for students' practice, with a dial constructed to record 250 pounds pressure. For accurate comparisons of thoroughly made amalgams the cubes must be made smaller.

Binary amalgams, as they are sometimes called, are those consisting of only one metal besides mercury. These are rarely used in dental practice, but from them the properties of the amalgamated metal are most easily observed.

Sodium amalgam may be made by direct union of the constituent elements. The mercury should be placed in an open dish under a hood, and the sodium added in *small* well-cleaned pieces.

The union is accompanied by a slight hissing noise, an elevation of temperature and evolution of vapor carrying more or less mercury, hence dangerous to breathe. An amalgam containing 1% sodium is a viscid liquid; if it contains 5% sodium it is a hard solid and intermediate percentages give varying degrees of firmness. Sodium amalgam if made with arsenic-free Hg is a very convenient reagent to use in making Fleitmann's test (page 11).

Ammonium amalgam has no use in dentistry, but it is of interest in that it is the nearest approach to which we may attain to the isolation of the purely hypothetical metal ammonium. It is easily made by adding sodium amalgam to a *cold* saturated solution of ammonium chlorid, thus illustrating the third general method of preparation of amalgams. It rapidly decomposes at ordinary temperature with the liberation of free hydrogen, ammonia-gas, and metallic mercury. The H thus liberated exhibits the properties of nascent H, indicating that in the amalgam it existed in true chemical combination, that is NH_4, rather than in any physical solution. At ordinary temperature ammonium amalgam is a soft, pasty, very porous mass, but at much reduced temperature it becomes solid and crystalline, although at $-39°$ (the freezing-point of Hg) H and NH_3 are still given off.

Copper amalgam is by far the most valuable of this class of amalgams. It may be made by amalgamating precipitated copper after moistening it with nitrate of mercury (Essig). The precipitated Cu may be prepared by metallic Zn in a slightly acid copper sulphate solution, but must be thoroughly washed with hot water to free it from zinc chlorid. The amalgamation may be effected by use of mortar and pestle. Rollins' method * by electrolysis of strong copper sulphate solution is rather unwieldy, but illustrates very well the second general process for the manufacture of amalgams.

Copper amalgam, according to Black, is absolutely rigid after it has once set and does not flow even to a slight extent. It is fine-grained and very hard. It is reduced in strength by reheating, does not expand or contract. In the mouth copper amalgam dissolves with comparative rapidity owing to the ready formation first of copper sulphid, then the oxidation of this compound to the sulphate. It blackens rapidly and in consequence of the tendency to dissolve just mentioned, it may penetrate the dentine and thus discolor the tooth itself.

Gold amalgam is readily made, but does not, by itself, harden well. An amalgam containing one part of gold to six of mercury will crystallize in four-sided prisms (Litch).

Platinum amalgam is very smooth, is formed with difficulty unless the Pt is *very* finely divided, and like gold does not harden well.

Silver amalgam, easily made but tends to expand.

Tin amalgam, alone shrinks badly.

Zinc amalgam, readily made, is white, but too *brittle* to be of service.

Cadmium amalgam may be easily made at ordinary temperature, "sets quickly and resists sufficiently, but fillings containing it, gradually soften and disintegrate and may

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* Details of this method may be found in the Boston Medical and Surgical Journal, February, 1886; also in Mitchell's Dental Chemistry.

stain the dentine bright yellow by formation of cadmium sulphid" (Mitchell).

Effect of Various Metals in Amalgam Alloys.

With the properties of these simpler combinations before us it becomes easy to understand the effect the addition of the various metals will have upon the properties of a silver-tin alloy; for practically *all* amalgam alloys are silver-tin alloys, either simple or combined with one or more other metals.

Silver is the most valuable constituent of amalgam alloys. It is essential to the proper setting and hardening of the amalgam. In an amalgam it tends to increase expansion and to hasten setting, while tin possesses the opposite characteristics. Combined with tin in the proportion of 65% silver to 35% tin, it forms an amalgam alloy perhaps more largely used than any other. It was this combination that Dr. Black succeeded in "annealing to zero," that is, so that upon testing, it showed neither expansion nor contraction.

Pure silver-tin alloys will flow from 2.5 to 10%.

Authorities seem to agree that if a Ag-Sn alloy contains 75% or more of silver it will expand only; while an alloy containing 50 to 61 or 62% of silver will shrink only; and one containing less than 50% of silver will first shrink and then expand.

The larger the proportion of tin the easier will the alloy cut, but the coarser will be the filings.

Zinc added to a silver-tin alloy tends to whiten the amalgam, hastens setting, increases the flow, and according to Essig, "causes a great but slow expansion."

Cadmium, see above.

Antimony gives a fine grain alloy and when the Ag is less than 50% is supposed to control shrinkage.

Bismuth will increase the flow of the amalgam; it is sometimes used in low-grade Ag-Sn alloys to control shrinkage.

Copper tends to diminish flow and gives a strength under pressure, sets quickly, gives better margins, and by some believed to have preservative influence on the tooth substance, but the more copper in an alloy the more rapidly does it discolor.

Gold —Three to seven per cent. of Au in a silver-tin alloy diminishes shrinkage, helps the color, adds to crushing strength. The filing from such an alloy will be very fine.

Dr. Black says 5% of gold gives a softer working property but retards setting of the amalgam, and makes it otherwise difficult to give a good finish to the filling (Dental Cosmos, Vol. 38, page 988).

Platinum, according to Black, is not a desirable addition to a silver-tin alloy. It gives an alloy, furnishing *very* fine filing, which produces a dirty working, slow-setting amalgam.

Excess o Mercury.—In the preparation of an amalgam from a dental alloy it is usual to add more mercury than the finished product requires and then squeeze out the excess between the fingers or otherwise. In filling a cavity, still more mercury is forced out, so that the composition of the deeper portions of a filling varies from the outer and probably accounts for the inequalities in expansion or contraction. The excess of Hg from the surface of a filling may be absorbed by a little hot gold or pure tin or by finely divided silver.

The excess of mercury which has to be squeezed out of an amalgam carries with it more or less of the constituent metals. Hall found that whatever the amount of mercury expressed, it carried just about 1% of tin. In the author's experience this amount has reached nearly $1\frac{1}{3}\%$ of tin. Silver is carried out to a much less extent than tin, so it is not impossible to carelessly make an amalgam and squeeze out enough mercury to change the proportion of Ag and Sn in the alloy. This change will, of course, be very slight, but we have seen that the contraction and expansion of amalgams may be affected by slight changes in composition.

Following is a short list of dental alloys, most of which may be easily prepared:

	Sn	Ag	Au	Cu	Zn	Sb
Arington's (S. S. White's)	57.5	42.5				
Chase copper-amalgam alloy	50	50		10		5
Chase's incisor	40	50				10
Flagg's submarine	35	60		5		
Fletcher's gold alloy (old)	56	40	4			
High-grade alloy (7½% gold)	41.5	49	7.5		2	
Harris' amalgam alloy	48.1	40		4.9	7	
King's Occidental	54.75	42.75			2.5	
Standard dental alloy (Eckfeldt)	40.6	52	4.4	3		
60% silver alloy	40	60				
Temporary alloy	88	10			2	

These formulæ have been selected from various sources with a view to giving the student opportunity to study effects obtained by varying percentages of Sn and Ag, and by introduction of other metals, Cu, Zn, etc.

Tests for Amalgams.

Color Test. — This is made upon a freshly amalgamated alloy, rolled into about the shape and size of a small pea, with a view to determine the amount of discoloration the amalgam is liable to undergo in the mouth.

A ball of amalgam carefully smoothed on at least one side is placed for forty-eight hours in a saturated solution of hydrogen sulphid, and after that time its color is compared with other amalgams similarly treated, or with amalgam of a similar composition which has not been treated at all.

Test for Expansion or Contraction.

Black has shown that tests of this nature to be of any value must be made in such a way that the *amount* of change in the volume can be measured, and that the simple method of packing glass tubes and using colored ink is wholly unreliable.

The author uses for this purpose an apparatus similar to one described by Prof. Vernon J. Hall. The amalgam is packed closely into a " well " in a steel block, then the block is placed in the apparatus so that a counterpoised steel plunger rests on the column of amalgam. This plunger is operated by a very long needle and attached at a point so near the pivotal support of the needle that a rise or fall of the plunger of 1/2500 of an inch moves the tip of the needle, at the scale, 1/16 of an inch, or one degree. If the needle rises half a degree, which may easily be read, it would indicate an expansion of the amalgam of 1/5000 of an inch.

There are two wells in each block and both of exactly the same depth. The figure given below will make this explanation easily understood, *A* being the steel block carrying the amalgam.

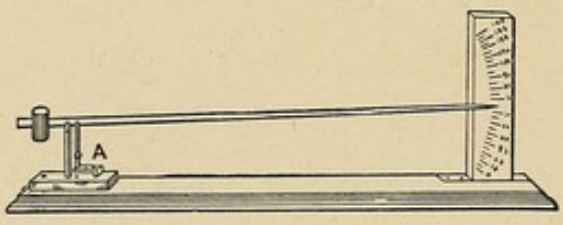


FIG. 8.

Test for Crushing Strength and Flow.—This test is made with Dr. Black's dynamometer (page 68) upon cubical blocks of amalgam which have been allowed to " set " for at least five days, and which measures 1/10 of an inch each way.

Specific gravity may be obtained by weighing the sample first in water, then in air, and dividing the weight in air by the difference between the two weights obtained.

It is instructive to make these tests on amalgam from alloys of varying composition, also on annealed and unannealed alloys of the same composition.

SECTION IV.—DENTAL CEMENTS.

Dental cements, largely used as temporary fillings and linings of cavities, contain oxid of zinc, oxid of copper (rarely sulphate of zinc) combined (at the time the cement is used) with phosphoric acid or with a solution of zinc chlorid.

There are six forms of dental cements which might be mentioned: the oxyphosphate of zinc, oxyphosphate of copper, artificial enamel, oxychlorid of zinc, oxysulphate of zinc, and tin cement. Of these, the last three are but little used.

Oxyphosphate of Zinc. — This is the most serviceable of the preparations of this class unless exception is made of the new artificial enamels, which have not been in use long enough to warrant positive assertions as to their comparative value.

The oxyphosphate cement is usually made by adding a powder, consisting of pure oxid of zinc, colored by a slight amount of other metallic oxids, to a liquid consisting of deliquesced phosphoric acid (or a solution of phosphoric acid in which zinc phosphate, and possibly slight amounts of other phosphates, have been dissolved), till a putty-like mass results, which rapidly hardens and becomes capable of receiving a considerable polish. When the phosphoric acid used is the glacial acid, the cement may be spoken of as a metaphosphate, as the glacial acid, before the addition of water, and to a certain extent afterwards, is actually metaphosphoric acid, HPO_3. The metaphosphoric acid by boiling in water or gradually without boiling becomes the orthophosphoric acid (H_3PO_4).

Arsenic is a frequent impurity in both zinc oxid and phosphoric acid, and if present is very liable to produce an irritating cement, sometimes causing considerable trouble, hence the material entering into the composition of any dental cement should be free from arsenic (see pages 10 to 13 for arsenic tests).

The purer the zinc oxid and the phosphoric acid, from

which the cement is made, the more durable it is found to be; so aside from any question of irritation, it is quite necessary for the sake of the cement itself that the ingredients be pure.

A pure ZnO may be made by calcining the precipitated carbonate of zinc, $Zn_5(OH)_6(CO_3)_2 + \text{heat} = 5ZnO + 2CO_2 + 3H_2O$. The heat should be below 500° F., because if two strongly heated, the color suffers, becoming yellowish.

Another method of making pure oxid of zinc is given as follows: Dissolve pure zinc in nitric acid, evaporate to dryness, and heat till fumes cease to be given off. The mechanical effect of the escaping oxids of nitrogen is said to leave the ZnO in the form of a *very* fine powder.

A pure phosphoric acid can be made from the ortho-acid by heating till the white fumes begin to come off, then heat to redness, cool, and dissolve in H_4O to a thick syrup. In mixing cements the powder should be worked into the liquid till the desired consistency is obtained.

Oxyphosphate cement and all cements having zinc oxid for a base tend to dissolve in the fluids of the mouth, lactic acid and ammonium salts being particularly good solvents for this class of compounds. The addition of ferric oxid to oxyphosphate cement increases resistance to disintegration. One part of ferric oxid to 6 to 10 of zinc oxid is recommended by Rollins in the International Dental Journal.

Oxychlorid of zinc is more easily soluble than oxyphosphate. It shrinks more, but is credited with a preservative action on dentine and hence used to some extent as a lining.

The powder of the oxychlorid cement is ZnO with sometimes a little borax, or silica, or both, added. A good oxychlorid cement will set in fifteen or twenty minutes, but keeps on growing harder for several hours. The following formula is recommended.

Oxychlorid Cement.

Oxid of zinc 10 grams, borax 0.1 gram, and powdered silica 0.2 gram.

Transfer to clay crucible and calcine for one half hour in furnace at bright-red heat. Pulverize, sift, and bottle. The liquid to be used with this powder consists of 10 c.c. of pure HCl *saturated* with pure zinc and filtered through glass wool.

Oxysulphate of Zinc.—This is used still less than the oxychlorid. It is non-irritating, dissolves easily, and is comparatively soft. The following formula is from Hall's Dental Chemistry.

Oxysulphate Cement.

Ten grams oxid of zinc, 4 grams sulphate of zinc. Dry, mix, calcine for one half hour and sift.

Liquid to be used with the powder may be made by dissolving 2 grams of zinc chlorid in 10 c.c. of water. This gives a turbid solution and should be shaken when used.

Oxyphosphate of Copper.—A preparation by this name which has been used to a considerable extent in the vicinity of Boston has been examined by the author and found to consist of the usual powder and liquid. The powder (coal-black) was composed of a mixture of the oxids of copper, iron, cobalt, and zinc, the liquid being phosphoric acid containing zinc phosphate in solution.

The cement resulting from this combination was found to be hard, showing practically no change of volume and resisting the solvent action of the saliva.

Tin Cement.

Dr. Arthur Scheuer, of Teplitz, Bohemia, recommends a preparation composed of a finely pulverized tin sponge and zinc oxid mixed with glacial phosphoric acid. "The powder

is of a light-gray color, becoming slightly darker when mixed with the acid, but regains its original color after setting. A tin-cement filling can be easily inserted and when polished it has a metallic appearance." (Dental Cosmos, May, 1904.)

Artificial Enamel.—Several preparations have recently been put on the market under this name with the claim that it makes a much harder cement and one which resists disintegration to a much greater extent than the ordinary zinc preparations.

The specifications of a German patent, under which one of these preparations is manufactured, claims that the powder consists of a mixture of the oxids of beryllium and silicon, together with alumina and lime. The liquid consists of a 50% solution of orthophosphoric acid in which aluminium phosphate and zinc phosphate have been dissolved.

When mixed in the usual manner these produce a cement which is much harder and less soluble than any of the preparations previously considered.

An advertisement of one of these preparations claims that its success is due to the use of a very valuable compound, without which it would be worthless, and so far as the author has had opportunity to investigate this subject, this statement seems to be true. A qualitative analysis confirms the claim of the patent specifications both in regard to the composition of the liquid and the presence of oxid of beryllium in the powder, and it is probable that the value of these preparations depends largely upon the proportion of beryllium entering into their composition.

Beryllium is one of the rare metals which occurs naturally with aluminium as a silicate. It forms basic compounds of such character as makes it suitable for use in dental cement.

The cement powders may be tested for beryllium as follows: Fuse a little of the powder with sodium carbonate (or the double sodium potassium carbonate); dissolve the fused mass in dilute hydrochloric acid; evaporate to dryness and

heat to 120° C. to dehydrate the silica; take up in water with a little HCl and filter; to the filtrate (probably containing Al, Be, Zn, and Ca) add a little ammonium chlorid, and an excess of ammonium carbonate, $Al(OH)_3$, $Be(OH)_2$, and $CaCO_3$, will be precipitated. The beryllium, however, is easily soluble in the excess of $(NH_4)_2CO_3$. Warm (not boil) and allow to stand for some time to insure complete separation of Al. (Note.—$Al(OH)_3$ is much less soluble in solution of $(NH_4)_2CO_3$ than in either NH_4OH or even NH_4OH and NH_4Cl.) Filter. Boil the filtrate for a long time, when the beryllium and some zinc will be precipitated. Filter and dissolve precipitate off paper in dilute HCl. To the filtrate containing $BeCl_2$ and $ZnCl_2$ add NH_4Cl in excess and NH_4OH, which will give a precipitate of $Be(OH)_2$. If Be and Zn only are present, the separation by boiling may be unnecessary.

The liquid may be tested for dissolved phosphates by diluting with water and adding ammonia till alkaline; if the mixture remains clear, phosphates of alumina, calcium, or zinc are absent. Care should be used, however, in the addition of the ammonia, as an excess of this reagent will redissolve phosphate of zinc.

If the ammonia is too strong, a precipitate of ammonium phosphate may be obtained, but this may be easily redissolved by the simple addition of water.

SECTION V.—SOLDERS.

Solders are alloys used in joining pieces of metal of the same or of different kinds. One of the constituent metals of the alloy forming the solder is usually the same as the surface upon which it is to be used, hence the various metals require solders of special composition; for instance, common solder is entirely unsuited for soldering aluminium or gold.

Common Solder is composed of tin and lead in different

proportions. The larger the proportion of tin the finer is the solder, and the following three grades may usually be obtained: "Fine" (tin two parts and lead one), "Common" (tin and lead equal parts), "Coarse" (tin one part and lead two parts).

In soldering metals it is absolutely essential that the surfaces are kept clean and free from superficial coating of oxids which may form easily with the elevated temperature employed in the process. Soldering acid and the various fluxes serve this purpose. Soldering acid is an acid solution of zinc chlorid usually made by taking a few ounces of strong hydrochloric acid and adding zinc as long as the metal dissolves. Among the substances which may be used as a flux to prevent oxidation, rosin and borax are the most common.

Soft Solders are those fusing below a red heat and include the common solders above mentioned, also the most fusible solders containing bismuth. These last are more properly fusible metals and will be discussed under that head.

Solders for Aluminium.—Aluminium solders with considerable difficulty owing in part to the low melting-point of the metal, also to the fact that aluminium is attacked by alkali, including borax, which makes it necessary to find some substitute for this convenient flux. Essig recommends a flux consisting of three parts of copaiba balsam, one part of Venetian turpentine, and a few drops of lemon-juice. The mixture is to be used in the same manner as soldering acid with a solder consisting of zinc 80 to 92 parts, aluminium 8 to 20 parts. Fused and finely powdered silver chlorid may also be used as a flux, the salt being reduced and the silver forming a superficial alloy. Richards recommends a solder for aluminium consisting of tin 29 parts, zinc 11 parts, aluminium 1 part, phosphor-tin 1 part.

Hall says that a solder which he has found very satisfactory may be prepared from aluminium 45 parts, tin 45, mercury 10; further, that the following formulæ suggested by Schlosser

are particularly adapted to soldering dental work since they resist the reaction of corrosive substances.

Platinum-Aluminium Solder.		Gold-Aluminium Solder.	
Gold	3 parts	Gold	5 parts
Platinum	0.1 part	Copper	1 part
Silver	2 parts	Silver	1 "
Aluminium	10 "	Aluminium	2 parts

For soldering articles of aluminium the following solder is given in the Pharmaceutical Era, January 10, 1895: Silver 2, nickel 5, aluminium 9, tin 34, and zinc 50 parts, to be used without flux.

Solder for brass requires a high heat for fusion and on this account is known as hard solder.

Edwinson gives the following formulæ: (1) copper 13 parts, silver 11; (2) copper 1 part, brass 1, silver 19; (3) brass 5 parts, zinc 5, silver 5. The flux for brass soldering is powdered borax, which may be mixed with water to a paste and applied with a feather or a small brush.

Solder for Gold.—Gold soldering is the most particular work of this class which the dentist has to do. There are a few requirements for a good gold solder which might be noted and which are also applicable to the other solders mentioned: (1) The color should be as nearly as possible that of the metals upon which it is to be used. (2) The solder should have a fusing-point but very slightly below that of the metal to be soldered. (3) The solder should flow freely.

Litch gives the following instruction for making a zinc-gold solder which will have the above-mentioned properties:

"To make the zinc-gold solder take 1 pennyweight of the same gold upon which it is to be used and add 1½ grains of zinc. If this is done in a crucible in the furnace, first fuse the gold (which should either be clean scraps or be cut from the plate; never use filings for this purpose), using but little borax; when thoroughly fused take the crucible in the tongs, drop the zinc into it, give the crucible a rather vigorous yet

skilful shake to assist in mixing its contents, but without causing any to be thrown out, and immediately pour into the previously prepared ingot mold. This must be done very quickly or the solder will require too high a heat for the fusion on account of a large proportion of the zinc being volatilized or oxidized and thus be lost as alloy."

Essig gives the following formulæ for alloys of gold employed in dentistry as solders:

No. 1. 14 Carats Fine.

American gold coin	$10.00
Pure silver	4 dwts.
Pure copper	2 "

No. 2. 14 Carats Fine.

American gold coin	16 dwts.
Pure copper	3 " 18 grs.
Pure silver	5 "

No. 3. 14 Carats Fine.

Pure silver	2½ dwts.
Pure copper	20 grs.
Pure zinc	35 "
18-carat gold plate (formula No. 11)	20 dwts.

No. 4. 15 Carats Fine.

Gold coin	6 dwts.
Pure silver	30 grs.
Pure copper	20 "
Brass	10 "

No. 5. 16 Carats Fine.

Pure gold	11 dwts.
Pure silver	3 " 6 grs.
Pure copper	2 " 6 "

No. 6. 16 Carats Fine.

Pure gold	11 dwts. 12 grs.
Pure copper	1 dwt. 12 "
Pure silver	3 dwts.
Pure zinc	12 grs.

No. 7. 18 Carats Fine.

Gold coin	30 parts
Pure silver	4 "
Pure copper	1 part
Brass	1 "

No. 8. 20 Carats Fine, for Crown and Bridge Work.

American gold coin (21.6 carats fine) $10 piece	258 grs.
Spelter solder	20.64 "

No. 9. 20 Carats Fine, Same Use as No. 8.

Pure gold	5 dwts.
Pure copper	6 grs.
Pure silver	12 "
Spelter solder	6 "

No. 10. 20 Carats Fine, for Crown and Bridge Work.

Zinc	1½ grs.
Pure gold	20 "
Silver solder	3 "

No. 11. Dr. C. M. Richmond's Solder for Bridge Work.

Gold coin	5 dwts.
Fine brass wire	1 dwt.

No. 12. Dr. Low's Formula for Solder for Crown and Bridge Work, 19 Carats Fine.

Coin gold	1 dwt.
Copper	2 grs.
Silver	4 "

Solder for Platinum.—Platinum utensils may be soldered with any good gold solder, and a flux may be used if desired. When, however, the solder, is used in connection with porcelain work, it must be pure gold or a gold and platinum alloy. A 25% platinum alloy has been found to give excellent results. The following in regard to gold and platinum alloy is from the Dental Review, August 1905:

" The colleges and text-books tell us the proper proportions of gold and platinum alloys, but they usually fail to tell us *how to do it.* In most cases the platinum appears in white spots on the plate without producing a proper alloy. Take a small piece of 22-carat gold and fuse it under the blowpipe. Then work in all the platinum you can in small pieces until it has taken up all that is required. It will produce a small button of a white alloy which is very brittle. Add this alloy in required proportions to the gold in the crucible and it will produce a real platinum alloy. By this method you can make clasp gold that is pretty nearly as stiff as a steel spring and yet will roll and work without fracture. (Mark G. McElhinney, Ottawa, Canada.)"

The gold " carat " signifies 1/24 part and is used as a measure of purity of an alloy, 22 carat gold being 22/24 pure gold. 20-carat gold is 20/24 pure, etc., etc. The amount of gold in

a given alloy may be determined with considerable accuracy by use of a device shown in Fig. 9 much used by jewelers, consisting of a series of standard alloys and a piece of stone upon which the test is made. The tips are standard alloys. Parallel markings are made on the stone with the alloy in question and with the tip supposed to correspond to it; then the addition of a drop of strong nitric acid to the marks and a careful comparison of their appearance will show if the two are of the same composition.

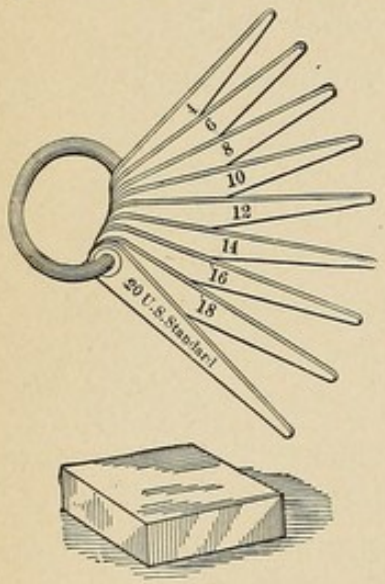


Fig. 9.

If the composition of an alloy is known the value in carats may be determined by the following

Rule to determine the carat of a given alloy: Multiply 24 by the weight of *gold* used and divide result by total weight of alloy. For instance, if an alloy is made containing 9 parts of gold and 3 of another metal, the total weight will be 12 and the calculations $24 \times 9 \div 12 = 18$. The alloy is an 18-carat gold.

Gold may be *raised* to a higher carat by the following rule: Multiply weight of alloy used by difference between *its* carat and that of the metal to be added. Then divide product by the difference between the carat of the metal added and that of required alloy. The figure thus obtained represents the total weight of *required* alloy. Subtract from this weight of material taken and difference in weight of pure or alloyed gold to be added. (From Hall's Dental Chemistry.)

To *reduce* gold to a required carat Essig takes the following rule from Richardson's Mechanical Dentistry: "Multiply the weight of gold used by 24 and divide the product by the required carat. The quotient is the weight of the mass when reduced, from which subtract the weight of the gold used, and the remainder is the weight of the alloy to be added."

Solder for Silver.—Solder for silver usually consists of alloys of silver and copper with sometimes zinc and sometimes tin. Litch recommends a silver solder made by alloying pure silver with one-third its weight of brass. "Brannt's Metallic Alloys" give alloys of silver and copper simply. Hall recommends silver 8 parts, copper 1, and zinc 2. In the preparation of solder containing copper, zinc, or tin, the use of a flux is necessary to prevent the formation of metallic oxid. For this purpose borax is usually employed. The silver constituting, as it does, the greater proportion of the alloy, should be melted first and be covered with considerable borax. When this has been thoroughly fused, the other metals may be added and mixed by agitation or by stirring with wood. Finally, the solder may be cast in the usual ingot mold.

Fusible Metals.

Under the head of fusible alloys properly come many of the alloys previously considered as solders. The fusible alloy usually contains lead or bismuth together with tin and occasionally cadmium. This may be mixed in proportions such that the melting-point may be anything desired down to 63° C. These alloys are largely used in the dental laboratory. Perhaps the most serviceable is known as Mellot's metal, which is composed, according to Essig, of bismuth 8 parts, tin 5, lead 3. This melts at about the temperature of boiling water. Wood's metal, melting at about 65° C., is composed of bismuth 4 parts, tin 1, lead 2, and cadmium 1. Rose's metal is bismuth 2 parts, tin 1, and lead 1. This melts at about 95° C.

Babbitt Metal, much used in the manufacture of dies, is composed of copper 1 part, antimony 2, and tin 8. The formula of common Babbitt metal on the market will be found to differ somewhat from the above and is not so well suited for dental purposes.

According to Essig's Dental Metallurgy, "Dr. C. M. Rich-

mond used a fusible alloy in crown and bridge work which he states is as hard as zinc and can be melted at 150° F. and poured into a plaster impression without generating steam. The formula of this alloy is as follows: Tin 20 parts, lead 19, cadmium 13, and bismuth 48. The following fusible-metal alloys are also suitable for the purpose."

Tin.	Lead.	Bismuth.	Melting-point of Alloy.
1	2	2	236° F. or 113° C.
5	3	3	202° F. or 94° C.
3	5	8	197° F. or 92° C.

The fusing-point of an alloy may be determined by melting under a liquid of sufficiently high boiling-point and then carefully noting the temperature at which the melted alloy solidifies. Care must be taken that the temperature of the alloy is exactly the same as recorded by the thermometer. To insure this, in the case of an alloy with low melting-point, it is usually sufficient to place the alloy in water or brine in a test-tube which is immersed in a beaker of similar fluid, then by raising the heat gradually with constant stirring and by taking the mean of two or three determinations, fairly accurate results are obtained.

SECTION VI. – RECOVERY OF RESIDUE.

Gold.—The gold scrap may be recovered in two ways: first, by fusion with suitable flux; second, by dissolving in aqua regia and precipitation of the metal. In the first method it is necessary to remove mechanically the impurities as far as possible, then mix the fairly clean gold waste with potassium nitrate and a little borax and fuse in a clay crucible. The gold will separate as a button at the bottom of the thoroughly fused slag.

In the second method the scrap gold is dissolved in aqua regia and the resulting solution of $AuCl_3$ is precipitated with ferrous sulphate or oxalic acid. The later precipitant, although

working more slowly than the iron, does not precipitate platinum, hence in case platinum is present it is the better reagent to use. The precipitated gold is next filtered, thoroughly washed, and fused in clay crucible under borax and potassium nitrate.

Silver.—The recovery of silver is best accomplished by dissolving the scrap or waste in nitric acid and precipitating as chlorid, then reducing the chlorid to metallic silver either by treatment with pure zinc or by fusion with sodium carbonate. If tin is present in the scrap the nitric acid will form metastannic acid, a white insoluble powder rather difficult to filter. Hence it is better to wash this by decantation several times with distilled water, which will remove practically all the silver. From the nitric acid solution the Ag may be precipitated by salt or hydrochloric acid. This precipitate must be washed till the wash-water is practically free from chlorin, then dried and fused with sodium carbonate, when a button of pure silver will be obtained.

If preferred, the precipitated chlorid of silver may be washed once by decantation, then agitated with pure zinc, when the following reaction takes place:

$$2AgCl + Zn = ZnCl_2 + 2Ag.$$

The finely divided Ag (in the form of nearly black powder) must be washed free from chlorin, carefully dried and fused under carbonate of sodium, or, after drying, it may be weighed and fused at once. If the silver residue contains mercury this may be driven off by heat before solution is attempted.

Mercury.—Mercury which has been used in making amalgams is best purified by distillation. Mercury which needs simply to be freed from dirt, dust, or slight traces of other metals may be purified as follows: If a piece of filter-paper is fitted closely in a glass funnel, a pin-hole made in the joint and the paper thoroughly wetted with water and the mercury to be purified placed on the paper, the heavy metal will

run through the pin-hole, leaving practically all the dirt clinging to the wet filter-paper. Such mercury may also be cleansed by filtering through chamois-skin.

In case the mercury contains slight amounts of other metals, if it is digested with a very dilute nitric acid, the acid will generally first dissolve the impurities and afterwards a little of the mercury itself. Then thorough washing of water will remove all excess of acid and all soluble salts which may have been formed. Pure mercury should have no coating of any sort on its surface, and if a few globules are allowed to run down a smooth inclined plane, they should leave no "tail" behind.

PART III.

VOLUMETRIC ANALYSIS.

VOLUMETRIC analysis is the determination of the quantity of a particular substance contained in a given sample by means of volumetric or standard solutions. By means of standard solutions it is possible to determine easily and quickly the strength of a peroxid of hydrogen solution, the percentage of silver in an amalgam alloy, or the amount of gold in a plate or solder, and it is volumetric analysis thus specialized and adapted to dental purposes that we shall consider.

The standard solution may be so prepared that it has an arbitrary or special value, such, for instance, as the silver nitrate solution usually used in determining the amount of chlorin in urine, 1 c.c. of this solution being equal to 10 milligrams of salt (NaCl); or its standardization may be made with reference to the molecular weights of the reagents employed, so that solutions of a similar nature will be of equivalent values. That is, a solution containing the hydrogen equivalent of the reagent, weighed in grams, per liter, is known as a *normal solution* and 10 c.c. of any normal acid will be of the same value in neutralizing an alkali as 10 c.c. of any other normal acid. On the other hand, 10 c.c. of a normal acid is equal to 10 c.c. of any normal alkali solution whatever the alkali may be.

The *normal factor* is the weight of reagent contained in *one cubic centimeter* of the normal solution.

The volumetric process and the use of the normal factor will be most clearly understood by the explanation of a specific example.

We will suppose that we have prepared a normal solution of NaOH and wish to ascertain the strength of a sample of dilute HCl. The normal solution will contain the molecular weight in grams of NaOH per liter or 40 grams absolute NaOH.

The molecular weight of HCl being 36.4 (36.37), a normal solution of HCl will contain 36.4 grams absolute HCl, and if a liter of normal NaOH were added to a liter of normal HCl *exact* neutralization would result.

$$\underset{40}{NaOH} + \underset{36.4}{HCl} = \underset{58.4}{NaCl} + \underset{18}{H_2O}.$$

The 1 liter of normal alkali (containing 40 grams NaOH) is exactly neutralized by 36.4 grams of HCl, or 1 c.c. of normal alkali by 0.0364 gram of HCl. 0.0364 is normal factor of HCl.

Now if by our process of analysis we find that it takes just 21 c.c. of the NaOH solution to exactly neutralize 10 c.c. of HCl solution, 1 c.c. of NaOH being equal to 0.0364 gram HCl, 21 c.c. of NaOH will be equal to 0.0364×21, or 0.7644 gram HCl, or 10 c.c. of the HCl solution contains 0.7644 gram of absolute HCl, equivalent, approximately, to 7.64%.

It has become apparent that in carrying out this process three things are absolutely necessary:

1. Methods for the preparation of standard solutions.

2. Apparatus for *accurate* measurements of both the standard solution and the unknown.

3. Means for determining just when the point of exact neutralization is reached. This point is known as the "*end point*" and is shown by "*indicators*" of various kinds.

Preparation of Standard Solutions.—Experience has shown that normal solutions are in many cases less convenient to work with than those much more dilute, both on account

of the keeping qualities of the standard solution and the accuracy of manipulation, and for the purposes of dental chemistry a *decinormal* or one-tenth normal solution represented by N/10 will generally be used.

In working with an N/10 solution the factor used in calculation of results will be one-tenth of the normal factor and is termed an *N/10 factor*. Other fractional proportions of the normal solution may be used as the centinormal, N/100, or seminormal, N/2. While the decinormal solution contains one-tenth of the hydrogen equivalent of reagent in grams per liter, and this amount is very easy to calculate, it is often very difficult to *weigh out* the exact amount required. For instance, we want an N/10 solution of HCl. HCl is a gas soluble in water and the strength of the solutions vary greatly, so we cannot weigh out 3.637 grams of absolute HCl to put in 1000 c.c. of water though we know this is just the amount necessary to produce our N/10 solution. Thus one of the first practical difficulties in making up standard solutions is to find some substance which can be weighed accurately and whose exact chemical composition may be relied upon.

Crystallized oxalic acid is such a compound, although with this care must be taken that the crystals are dry and yet contain all their water of crystallization; in other words, are actually represented by the formula $H_2C_2O_4,2H_2O$. Fused carbonate of sodium is another such compound. If the purest obtainable bicarbonate of soda is fused till no further change takes place, cooled, and powdered, the product is pure enough for the preparation of a standard solution for ordinary use.

For the preparation of volumetric solutions it is necessary to have first a balance which will weigh accurately to at least two decimal points; in other words, it must be sensitive to 1 centigram. Such a balance with silk bearings and which the author has found to answer the purpose very well was obtained of the L. E. Knott Apparatus Co., Boston (Fig. 10), at $12.00 list.

Secondly, a flask capable of holding 500 or 1000 c.c. carefully graduated on the neck; also one of 100 c.c. capacity.

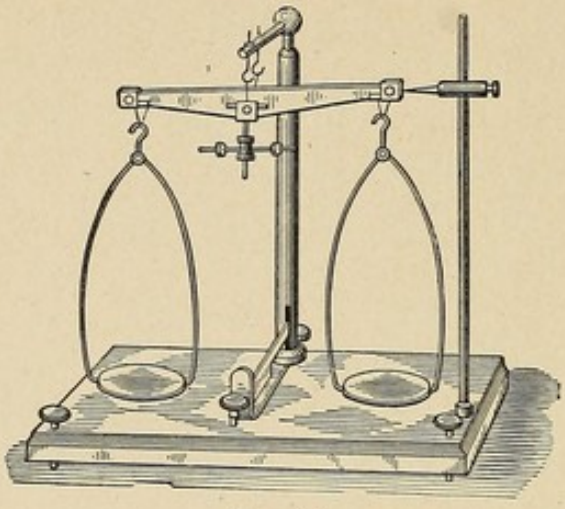


Fig. 10.

Graduated cylinders (Fig. 11) are not so well suited for the preparation of standard solutions, as the greater breadth of

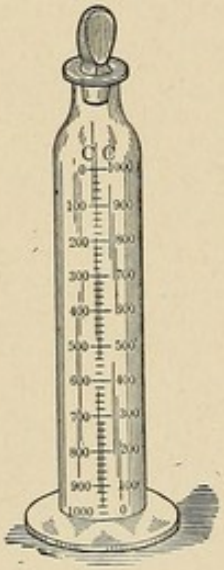


Fig. 11.

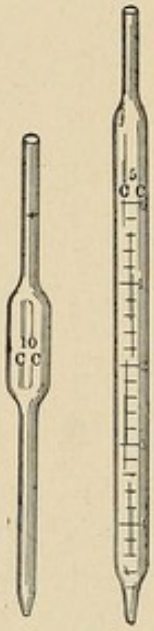


Fig. 12.

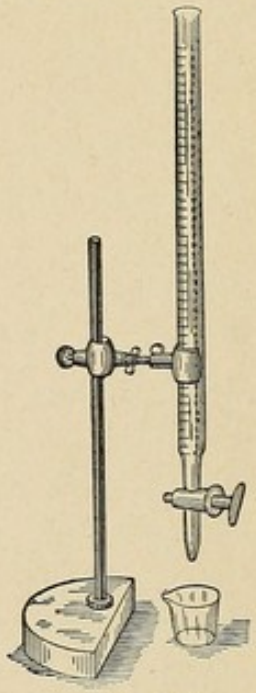


Fig. 13.

the column of liquid makes accurate reading much more difficult.

Small cylinders of 100 c.c. are useful in making up odd

amounts of solution, also 50 c.c., 100 c.c., and 250 c.c. graduated flasks are very convenient, although not absolutely necessary.

In the process of analysis it will be necessary to have pipettes (Fig. 12) measuring 5 and 10 c.c., also a burette (Fig. 13), from which the standard solution may be used. The burettes may be had in a variety of styles and sizes, a very serviceable one being of 25 c.c. capacity and graduated in tenths of c.c. It may have a glass stop-cock or it may be furnished with a glass tip with rubber connector and pinch-cock.

A set of measuring-instruments should be kept which have been carefully compared with one another; that is, the 1000-c.c. flask should be exactly filled by taking the 100-c.c. flask full to the mark just ten times, thus enabling one to accurately take aliquot parts of any given solution.

Indicators.

The third requisite for carrying out a volumetric process is a method for determining the end point of the reaction; that is, we must know when there has been a sufficient quantity of a standard solution added to an unknown solution. *Phenolphthalein* gives a red color with alkalis, which is discharged by the addition of acid till the solution becomes colorless as it becomes neutral or acid. *Litmus* gives a blue color with alkalis and a red with acids; *Methyl orange* can be used with carbonates and mineral acids; it does not work as well with organic acids. The color is pink in acid and yellow in alkaline solution. *Lacmoid* is useful in cases where the acid properties of such salts as alum or zinc chlorid might interfere with the use of litmus or phenolphthalein. The different indicators do not all change color at exactly the same point in the process of neutralization, and it is possible for a solution to be alkaline to litmus and acid to phenolphthalein at the same time. Hence uniformity in the use of indicators is desirable. In

physiological chemistry, Congo red, tropæolin 00, and dimethylamidoazobenzol are also used.

The end point may be indicated by excess of a standard solution if it happens to be highly colored, as potassium permanganate. Thin starch paste is used as an indicator in operations involving the use or liberation of free iodin. Other indicators will be considered as we have occasion to use them in the various analytical processes.

The processes of volumetric analysis may be divided into three classes: First, *acidimetry* and *alkalimetry*. Second, *oxidation* and *reduction*. Third, *precipitation*.

Acidimetry and Alkalimetry.

Acidimetry and alkalimetry includes all standardized solutions, either acid or alkaline, which may be used in neutralizing solutions of unknown strength of an opposite character. For instance, the strength of vinegar is determined by neutralizing a known volume with standard alkali.

For present purposes two standard acids and one standard alkaline solution will be sufficient. The first of these may be decinormal oxalic solution prepared from recently recrystallized and carefully dried acid. The composition of these crystals should be $H_2C_2O_4 2H_2O$, having molecular weight of 126. This being a dibasic acid it will be necessary to divide the molecular weight by 2 for a decinormal solution and then again by 10 to obtain the number of grams, which must be dissolved in 1 liter of water. For class use, each student may prepare 500 c.c. of this solution by dissolving 3.15 grams of pure crystallized oxalic acid in water and dilute to a half-liter. The graduated flasks are usually constructed to be used at a temperature of 60° F. or 15° C. and for accurate work solutions must be brought to this temperature. After the oxalic acid solution has been prepared the decinormal alkali may be made as follows:

Weigh out carefully 2½ grams of caustic soda or 3 grams of caustic potash and dissolve in something less than 500 c.c. of distilled water. After the solution has thoroughly cooled, fill a burette with it. Place 10 c.c. of standard acid previously prepared in a white porcelain dish of about 250 c.c. capacity, add 50 c.c. distilled water and 2 or 3 drops of phenolphthalein (2% phenolphthalein in alcohol and water, equal parts), then carefully run in from the burette with constant stirring the alkali solution until a permanent pink tint is produced.

The work will be more satisfactory if the titration is made for the appearance of color rather than the disappearance of color, as would have been the case had the standard acid run *into* the measured alkali solution. This process is known as "titration," and will hereafter be so designated.

The Calculation.—Supposing it has taken 8.2 c.c. of the alkali to exactly neutralize the 10 c.c. of N/10 acid, it follows that in the 8.2 c.c. is sufficient alkali to equal or to make 10 c.c. of an N/10 alkali solution; hence we may add 1.8 c.c. of distilled water to every 8.2 c.c. of alkali solution, thereby reducing it to decinormal strength. Practically we should take 410 c.c. of alkali solution and in a graduated fiask make it up to 500 c.c. with distilled water. It will be necessary to make several titrations and average the results before making the calculation.

From the standard alkali N/10 solution of HCl or H_2SO_4 may be prepared in a similar manner, it being impossible to accurately weigh either of these two acids. In titrating a carbonate, if an indicator, such as phenolphthalein, which is sensitive to carbonic acid, is used, it is necessary to keep the solution at a boiling temperature or at least bring it to a boil after every addition from the burette.

EXAMPLE OF ACIDIMETRY AND ALKALIMETRY.

Determine the strength of a sample of vinegar as follows:

Measure accurately into a white porcelain dish of 150–250 c.c. capacity 1 c.c. of the sample. This may be measured either with a carefully graduated 1-c.c. pipette or more accurately by diluting 10 c.c. of the sample to 100 c.c. in a graduated flask, then using 10 c.c. of the dilution for the titration, the titration to be performed with N/10 NaOH, using phenolphthalein as an indicator.

The molecular weight of acetic acid is, in round numbers, 60, hence the N/10 factor of acetic acid will be 0.006 (acetic acid being monobasic, $HC_2H_3O_2$), and to ascertain the strength of the sample of vinegar it is necessary to multiply the number of cubic centimeters used by this factor 0.006, which will give the amount of absolute acid calculated as acetic in 1 c.c. (practically 1 gram) of the sample. Thus if 8 c.c. of N/10 alkali were required to neutralize 1 c.c. of vinegar, multiplying the factor 0.006 by 8 would give 0.048 gram of absolute acetic acid in 1 c.c. of vinegar, which is equivalent to 4.8 %.

Analysis by Oxidation and Reduction.

If to a hot solution of oxalic acid containing sulphuric acid, permanganate of potash be added, the following reaction takes place:

$$2KMnO_4+5H_2C_2O_4+3H_2SO_4=K_2SO_4+2MnSO_4+10CO_2+8H_2O.$$

This reaction represents a very valuable method of volumetric analysis, but inasmuch as it is not a process of neutralization it cannot properly come under the head of acidimetry and alkalinity, but rather under a distinct classification, the determination involving *oxidation* and *reduction*.

Standard Permanganate Solution. — In the rection given above we may consider that, as the molecule of $K_2Mn_2O_8$ breaks

up, three of the eight atoms of oxygen are required to form the basic oxids K_2O and $2MnO$ (soluble in the acid as K_2SO_4 and $2MnSO_4$), while the remaining five atoms are liberated and constitute the active chemical agent whereby the oxalic acid is oxidized to CO_2 and H_2O. Hence to reduce this double molecular weight (316) to the hydrogen equivalent necessary for a normal solution, it is divided by 10 (five atoms of oxygen having a valence of 10).

The Decinormal Solution may be made by dissolving 3.16 grams of pure recrystallized and thoroughly dried crystals, if they can be obtained, in distilled water, and making the solution up to 1000 c.c., or it may be standardized by titration with the N/10 oxalic acid previously prepared; in this case one would proceed as follows:

Make a solution slightly stronger than the standard required, say about 3.5 grams of the ordinary pure crystals in a liter of water; with this fill a burette, place 10 c.c., measured from a pipette, of N/10 oxalic acid in an evaporating-dish or casserole, dilute with about 50 c.c. of water, add about 10 c.c. of dilute sulphuric acid, and heat the mixture nearly to the boiling-point. Then titrate with the permanganate from the burette. The permanganate will at first be rapidly decolorized, but as the operation progresses the color fades more slowly till at last a faint *permanent* pink color indicates that the "end point" has been reached.

The temperature must be kept above 60° C. throughout the titration or the oxidation will take place too slowly, and an apparent end point will be obtained before the reaction is completed.

If the solution turns muddy during the operation it is due to an insufficient amount of sulphuric acid and more should be added. The calculation is made as in the case of the N/10 NaOH described on page 95. The standard permanganate should be preserved in full, well-stoppered bottles and kept in a dark place.

It is better to have the $KMnO_4$ solution made up a day or two before it is standardized, thereby oxidizing traces of ammonia, etc., which the water may contain.

DETERMINATION OF PEROXID OF HYDROGEN.

In determining the strength of peroxid use 1 c.c. of the sample measured as in the case of vinegar (which see), dilute with 50 c.c. of distilled water, add 10 c.c. of dilute sulphuric acid, and titrate with the permanganate in exactly the same manner as detailed in the preceding paragraph, the reaction in this case being as follows:

$$2KMnO_4 + 5H_2O_2 + 3H_2SO_4 = K_2SO_4 + 2MnSO_4 + 5O_2 + 8H_2O.$$

The aqueous solutions of peroxid on the market used as antiseptics contain about 3% absolute H_2O_2 and yield approximately ten volumes of available oxygen; that is, 10 c.c. of solution will yield 100 c.c. of oxygen. The calculation may be made to express strength of the peroxid in terms of percentage of absolute H_2O_2 by multiplying the number of cubic centimeters of N/10 $KMnO_4$ decolorized by 1 c.c. of solution by 0.17, or to express the strength in volumes of available oxygen by multiplying the number of cubic centimeters of solution by 0.56 (more accurately 0.5594).

DECINORMAL IODIN.

A decinormal solution of iodin may be prepared by dissolving 12.68 grams of pure iodin crystals in one liter of water by the aid of about 18 grams of pure potassium iodid.

Iodin of sufficient purity may be obtained by carefully resubliming selected and carefully dried crystals of so-called "chemically-pure" iodin.

DECINORMAL SODIUM THIOSULPHATE.

$Na_2S_2O_3 \cdot 5H_2O$ = molecular weight, 248.24. This solution may be made by weighing directly 24.824 grams of the pure crystal-

lized salt, dissolving in water and diluting to 1000 c.c., or it may be standardized by titration with a decinormal iodin solution, the reaction being as follows:

$$2Na_2S_2O_3 + 2I = 2NaI + Na_2S_4O_6.$$

The indicator used is a very dilute starch paste which gives the characteristic blue color as soon as free iodin is in excess.

By means of these two standard solutions (iodin and sodium thiosulphate) a variety of determinations may be made with great accuracy. Any substance which will liberate iodin from potassium iodid may be quantitated by adding an excess of the potassium salt and titrating the free iodin with thiosulphate solution, using starch paste as usual for an indicator.

Peroxid of hydrogen may be thus determined as easily as by the permanganate method previously given. The process, being that of Kingzett, is given as follows by Sutton:

Mix 10 c.c. of peroxid solution to be examined with about 31 c.c. of dilute sulphuric acid (1–2) in a beaker, adding crystals of potassium iodid in sufficient quantity, and after standing five minutes titrating the liberated iodin with N/10 thiosulphate and starch. The peroxid solution should not exceed the strength of two volumes; if stronger, it must be diluted proportionately before the analysis.

In the case of a very weak solution it will be advisable to titrate with N/100 thiosulphate.

1 c.c. N/10 thiosulphate = 0.0017 gram H_2O_2 or 0.0016 gram.

VOLUMETRIC DETERMINATION OF ARSENIC.

Mohr's method of oxidation with iodin is a practical one. The titration is made with N/10 iodin and starch as usual, except that the solution should be at first neutral and then about 20 c.c. of saturated solution of sodium bicarbonate should be added to every 0.1 gram of As_2O_3 supposed to be in the unknown, thus giving a certain definite alkalinity. If

the solution is acid, neutralize with sodium bicarbonate, then make alkaline with more bicarbonate as above.

VOLUMETRIC DETERMINATION OF GOLD.

While gold is usually determined quantitatively by assay in a dry way (page 104) it may be determined very accurately by titration with thiosulphate solution. Fatka (Chem. Zeit.) recommends the following process based upon the facts that a neutral solution of gold salt with potassium iodid will give a greenish precipitate. When an excess of potassium iodid is used no precipitate is formed, but a solution of AuI_3 as $AuKI_4$ results. This is of a brown color and may be titrated with N/10 thiosulphate solution, when the following reaction takes place:

$$AuKI_4 + 2Na_2S_2O_3 = AuKI_2 + 2NaI + Na_2S_4O_6.$$

Process: 10 c.c. of gold solution containing approximately 2% of gold is treated with 4 grams of potassium iodid diluted to 100 c.c. with water and titrated with N/10 $Na_2S_2O_3$ solution, using starch as an indicator.

Analysis by Precipitation.

Because certain elements possess a selective affinity for other elements it is possible to determine many substances quantitatively by precipitation. That is, if silver nitrate is added to a mixture of a soluble chlorid and a chromate, the chlorin will combine first with the silver, forming AgCl, to the exclusion of the chromate. After the last trace of chlorin has been so combined, then the silver chromate will be formed, which is a salt with an intense red color; hence it is possible to determine the strength of solutions of soluble chlorids by titration with standard $AgNO_3$, using potassium chromate as an indicator. This process is used in analysis of drinking-water, of saliva and of urine, but for each of these it is desirable to have solutions of special strength.

A DECINORMAL SILVER SOLUTION

may be made by dissolving 17 grams of pure crystallized $AgNO_3$ in a liter of distilled water, and with this a

DECINORMAL SODIUM CHLORID SOLUTION

may be prepared as follows:

Weigh out 6 grams of the purest salt obtainable and dissolve in approximately 1 liter of distilled water. With a pipette measure 10 c.c. of this solution into a white porcelain dish, dilute to about 50 c.c. with H_2O, add two to five drops of neutral potassium chromate (K_2CrO_4) and add $AgNO_3$ from a burette till a faint pink color *persists*.

The calculation and dilution is made exactly as described on page 95 in the preparation of a standard NaOH solution. The silver nitrate solution used to determine chlorin in urine is usually prepared of such a strength that 1 c.c. precipitates just 10 grams of sodium chlorid. This is equivalent to 0.006065 gram of chlorin. A solution of this strength is produced when 29.075 grams of pure, fused silver nitrate is dissolved in sufficient distilled water to measure 1 liter of solution. If chlorin is to be determined in drinking-water, it is usually necessary to concentrate the water at least 1/5 its bulk and then use not more than one or two drops of neutral chromate as indicator. The standard silver nitrate for this titration should be very dilute. A convenient solution may be prepared by diluting the standard $AgNO_3$ for urine 1 to 10. In saliva the sample may be diluted with an equal volume of water and titrated the same as in the case of drinking-water. In all quantitative processes where silver chromate is used to determine the end point the solution must be practically neutral, as the formation of this salt is prevented by either acids or alkalis.

QUANTITATIVE ANALYSIS OF DENTAL ALLOYS CONTAINING Au, Sn, Ag, Cu, Zn.

Weigh accurately 0.5 of a gram of alloy which has been reduced to fine filings and from which all particles of iron have been carefully removed by a magnet, transfer to a beaker and dissolve in 15 c.c. of strong HNO_3 and 10 c.c. of H_2O by aid of gentle heat. Evaporate on a water-bath till *all* nitric acid has been expelled. If the sample contains tin or gold, complete solution will not be effected, but by watching the character of the sediment through the bottom of the beaker it is possible easily to determine when the alloy has been completely disintegrated.

After drying take up in H_2O and filter immediately through a small ashless filter and wash with warm water eight or ten times, using about 5 c.c. each time and allowing the liquid to run through the paper before the next portion is added. Reserve the residue on the paper for subsequent examination. Combine the filtrate and wash-water and make up to a definite volume, which may be 150 or 200 c.c., and titrate 10 c.c. for silver with N/10 NaCl solution, using potassium chromate as an indicator.

Estimation of Copper.

To 50 c.c. of the first filtrate (from which gold and tin alone have been separated) add HCl till silver is all precipitated. Filter, wash with warm water added in small portions eight to ten times. The filtrate with the wash-water, which should not exceed 100 c.c., is next warmed and H_2S gas passed in until all second-group metals are thrown out. Filter and wash precipitate thoroughly, reserving the wash-water and the filtrate for the determination of zinc. Dissolve the CuS in dilute HNO_3. Wash paper thoroughly in warm water, add Na_2CO_3 till the precipitate is nearly dissolved, then add 1 c.c.

of dilute NH_4OH. Titrate, to complete disappearance of blue color, with KCN solution previously standardized after this same method against pure copper wire. A little practice is required in determining the end point to give the process any degree of accuracy. An excess of ammonia should be avoided, as it interferes with the accuracy of the end point.

Copper also may be determined very easily by electrolysis of the faintly acid (H_2SO_4) solution, precipitating the Cu onto a platinum dish. The ordinary 110-volt current employed for electric lighting may be used by introducing a resistance of seven to ten 16-c.p. lamps. After the copper has been entirely deposited the residual solution is drained out of the platinum dish, a little alcohol added, which is also drained out, and by setting fire to the last traces of alcohol the precipitated copper is dried and in condition to weigh. Care must be taken to avoid oxidation of the finely divided Cu; if it turns black too much heat has been used and partial oxidation has taken place, which will of course increase the weight.

Estimation of Zinc.

If zinc alone is left, as is usually the case, evaporate to dryness the solution previously reserved, dissolve in H_2O, add a fairly strong solution of oxalic acid and an equal volume of strong alcohol. Allow to stand 15 to 30 minutes, filter, and wash with 70% alcohol till oxalic acid is removed, dry until the alcohol has disappeared, dissolve in dilute sulphuric acid, and titrate the solution with N/10 permanganate and calculate the zinc from the amount of oxalic acid found.

Estimation of the Tin and Gold.

The first residue containing the tin and gold is to be thoroughly dried. If gold is present the residue is not white but purple in color. After drying remove the precipitate from the filter-paper, burn the paper in a tared porcelain crucible, add the bulk of the precipitate, ignite, cool, and weigh. If the ig-

nited residue consists only of stannic oxid the weight of tin may be obtained by multiplying the weight of the ash by 0.788. If gold is present the weight of the gold should be deducted from the total weight of residue before making the calculation for tin.

The Assay of Gold and Silver in the Dry Way.

It is often more convenient to determine gold and silver by the fire assay than by the volumetric methods previously given. This is accomplished usually by fusion with an excess of lead and a borax flux. The mixture is kept at a high heat for upwards of thirty minutes, with a current of air passing over the surface of the molten metals. This serves to oxidize and carry away the baser metals, leaving the gold and silver with but a slight amount of lead, possibly a trace of copper and tin. The purification is completed by cupellation. When the traces of lead and other metals are absorbed by the cupel or are driven off as volatile oxids, the button of gold and silver is next cooled *very slowly* and carefully weighed. From this the silver may be dissolved by nitric acid unless the gold is in considerable excess, which would rarely be the case. If it should happen that the gold was present in sufficient quantity to prevent the solution of the silver in nitric acid a known weight of pure silver may be added in amount sufficient to increase the percentage of silver to 75 or over, fused, and then HNO_3 will dissolve out all the silver, leaving the gold.

The gold which has resisted solution may be found as small black particles or grains in the bottom of the crucible. This should be carefully washed with distilled water by decantation, very carefully dried and brought to a red heat, which will give a button of pure gold. This may be weighed and the weight subtracted from the weight of gold and silver button previously obtained.

PART IV.

MICROCHEMICAL ANALYSIS.

The advantages of microchemistry are many, as claimed by its enthusiastic advocates, and there are two particulars in which these 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 for 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.

Formation of crystals may be brought about in 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 which shall be comparable with those obtained at other times or by other individuals. The tendency of strong solution 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.

First the reagent should be of uniform strength, usually 1 or 2%; then place on a clean microscope-slide a small drop of the solution to be tested, and one of about equal size of the reagent to be used, and as close as possible to the drop of the "unknown" without touching it. 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 such 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 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 will be found upon the slide suitable for the test.

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, and the detail of the various tests will be given under classification furnished by the substances investigated.

Our study may include alloys and amalgams, teeth, tartar, dental anæsthetics, 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, and the student should make each test here indicated and carefully draw the crystals produced:

1. Calcium oxalate from 2% $H_2C_2O_4$ and $CaCl_2$ solutions (Plate III, Fig. 1).

2. Cadmium oxalate from 2% $H_2C_2O_4$ and $CdSO_4$ solutions (Plate III, Fig. 2).

3. Strontium oxalate from 2% $H_2C_2O_4$ and $Sr(NO_3)_2$ solutions (Plate I, Fig. 3).

4. Sodium oxalate by evaporation of aqueous solution, also by evaporation of urine containing $Na_2C_2O_4$ (polarized light) (Plate III, Fig. 3).

5. Urea oxalate from 2% $H_2C_2O_4$ and urea solution (Plate III, Fig. 4).

6. Ammonium-magnesium-phosphate from magnesium mixture * and sodium phosphate (Plate I, Fig. 4).

7. Ammonium platinic chlorid (Plate I, Fig. 1).

8. Ammonium phosphomolybdate from ammonium molybdate and phosphate of sodium (Plate III, Fig. 5).

9. Sodium urate by evaporation (polarized light) (Plate III, Fig. 6).

10. Crystals formed from cocain and potassium permanganate.

11. Crystals formed from carbolic acid and dilute bromine water (tribromphenol) (Plate IV, Fig. 1).

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

13. Crystals formed from morphine and Marme's reagent (Plate IV, Fig. 3).

14. Crystals formed from chloretone and sodium hypochlorite (Plate IV, Fig. 4).

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.

LOCAL ANÆSTHETICS.

In considering the chemistry of local anæsthetics we may divide them into two classes as follows:

1st. Those of definite or well-known composition, and

2d. Preparations of a proprietary nature, the composition of which is always problematical.

In the first class will be found cocain, eucain, tropacocain, acoin, ethyl chlorid, etc., which will be later alphabetically

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

considered. The second class contains a large number of preparations of all degrees 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 cocain as the anæsthetic, and frequently a little nitroglycerine as a cardiac stimulant to counteract the depressant effect of the alkaloid. Carbolic acid and oil of clove are also frequently used.

Many of the constituents of this class of anæsthetics 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 included in the following list of substances which have been extended to include a considerable number of preparations of common occurrence.

Acoin, asynthetic compound (chemically diparanisyl-monophenetyl-guanidine hydrochlorid, $C\begin{pmatrix}(NC_6H_4OCH_3)_2\\(NC_6H_4OC_2H_5)\end{pmatrix}HCl$) soluble in both alcohol and water. Strongly antiseptic and a valuable anæsthetic, especially in conjunction with cocain. Acoin should be used only in solution and this should be kept in a dark place.

Adrenalin, a valuable hæmostatic and frequently used in conjunction with dental anæsthetics, 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 chlorid, according to the following formula taken from a commercial sample:

Adrenalin chlorid, 1 part.

Normal sodium chlorid solution (with 0.5% chloretone), 1000 parts. This solution is usually diluted with the normal (0.6%) salt solution. According to the Druggists' Circular, preparations similar to the above are also marketed under the names of adrenol, adnephrin, hemostatin, supraredalin, etc.

Anesthol, or Anæsthol, is a mixture of ethyl chlorid and methyl chlorid, used as a local dental anæsthetic. The name is also applied to a *general* anæsthetic given by inhalation and consisting of a mixture of ethyl chlorid, chloroform, and ether.

Argyol, a compound of silver with albumin, soluble in water and recommended in place of ordinary silver nitrate solution. It contains 30% metallic silver.

Atropine, an alkaloid obtained from belladonna, usually used as the sulphate, $(C_{17}H_{23}NO_3)_2H_2SO_4$; the alkaloid is only sparingly soluble in water but the sulphate is easily soluble, dissolving in about half a part of water at ordinary temperature. A 1% 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 anæsthetic 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. Or a more conclusive test is to convert the alkaloid, which has been dissolved out with 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; when, if atropine is present, a dilation of the pupil occurs in fifteen minutes to an hour and a half's time, according to amount present.

Borax.—Sodium tetraborate, $Na_2B_4O_7$, is used in antiseptic solutions and may be detected by evaporating a little of the solution to dryness, adding a little HCl. Evaporate to dryness a second time, then add a very dilute HCl solution containing tincture turmeric. Upon drying this mixture 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.—Phenol, C_6H_5OH, obtained from the destructive distillation of coal-tar. A light oily liquid of specific gravity of 0.94–0.99. Carbolic acid is usually obtained as a white crystalline mass soluble in 15–20 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 from 5–8 per cent of water will cause liquefaction of the crystals and the preparation becomes permanently liquid. It is easily soluble in glycerine and strong solutions may thus be prepared. Carbolic acid is sometimes added to local anæsthetics 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 bromin-water, which gives white crystals of tribromphenol (see Plate IV, Fig. 1).

Chloral Hydrate, $CCl_3CHO.H_2O$, a crystalline solid composed of trichloraldehyde 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, when isobenzonitril is produced, which has a peculiarly disagreeable and characteristic odor. This test is 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 trichlorbutyl alcohol. Made from chloroform, acetone, and an alkali, and occurs as small white crystals, with taste and odor like camphor. It is dissolved by alchool and glycerine and to slight extent by water.

Tests.—A convenient microchemical test for chloretone devised by Mr. Niles, Harvard Dental School, '06, consists simply of treatment with a solution of hypochlorite of sodium. A precipitate is at once formed of a coarsely branching character which thus far seems to be characteristic of chloretone solutions (Plate IV, Fig. 4).

Chloroform, trichlormethan, $CHCl_3$, 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 a very volatile solvent for gutta-percha, caoutchouc, many vegetable balsams, camphor, iodin, bromin, and chlorin; it also dissolves sulphur and phosphorus to a limited extent.

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

Cocain 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 dental anæsthetics in 1 or 2% solution.

Tests.—Cocain solutions respond to the usual alkaloidal reagents. With 1% solution potassium permanganate gives pink plates in form resembling cholesterin (Plate IV, Fig. 5). Dilute cocain solution with picric acid gives a yellow precipitate which becomes crystalline on standing. Quite characteristic crystals also may be obtained from dilute cocain solutions and stannous chlorid in the presence of free HCl (Plate IV, Fig. 6).

Creosote.—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 ferric chlorid. Phenol is colored blue.

Cresol is the next higher homologue to phenol, having a formula $C_3H_4CH_3OH$, boiling at 198° C. It is largely used, usually together with allied compounds from coal-tar, as antiseptic and disinfectant solutions.

Ektogan.—Peroxid of zinc, ZnO_2, designed for external use (London, July 9, 1904).

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

β-Eucain is the hydrochlorate of benzoylvinyldiacetonealkamine, and occurs as a white, neutral powder, soluble in about 30 parts of cold water. It is used like cocain as a local anæsthetic, and is claimed to be less toxic, and sterilizable by boiling without fear of decomposition. It is applied mostly in 1 to 5% 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.)

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

Formaline, Formal, Formine, etc., are commercial names for a 40% 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 141, Exp. 14.)

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

Tests.—Upon heating strongly, it is decomposed, giving off odors of acrolein, which fact 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. Glycerine when present is apt to interfere with characteristic crystallization of many precipitates.

Heroin is a diacetic ester of morphine. It is usually obtained as the hydrochlorid 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 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.

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

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

$$BaO_2 + H_2SO_4 = BaSO_4 + H_2O_2$$
$$\text{or} \quad BaO_2 + H_2O + CO_2 = BaCO_3 + H_2O_2.$$

This latter reaction has the advantage of producing an insoluble barium compound and at the same time introducing no objectional acid. The peroxid of sodium, calcium, magnesium, and zinc may also be used; ZnO_2, however, is comparatively expensive and 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, as for this purpose an alkaline solution is re-

quired and the solution of Na_2O_2 in water produces both alkali and H_2O_2 according to the following reaction:

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

Sodium perborate (page 117), also sold as euzone, is a powder advertised to 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.

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 Chlorid, corrosive sublimate, $HgCl_2$, soluble in about 16 parts of water and 3 parts alcohol. A powerful antiseptic, in aqueous solution 1/1000 to 1/5000, and occasionally used in mouth-washes.

Tests.—A drop of the suspected solution with a trace of potassium iodid will give a red precipitate of mercuric iodid soluble in excess of either reagent. With lime-water or fixed alkaline hydroxids 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 chlorid mixed with a little methyl chlorid and chloroform is said to be the composition of a local anæsthetic sold under the name of methethyl (U. S. D.).

Methyl Chlorid, CH_3Cl, is a colorless gas which condenses to a liquid at 23° C. Methyl chlorid is easily soluble in alcohol,

somewhat in water, and is used in a similar manner to ethyl chlorid.

Morphine, $C_{17}H_{19}NO_3$, alkaloid from opium. It is usually used as a 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 amyl alcohol. Upon evaporation of the alcohol the residue may be tested with Fröhde's reagent (sodium molybdate 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 III, Fig. 5. Dental anæsthetics containing morphine will give precipitates with the usual alkaloidal reagents. Marme's reagent (CdI_2) gives crystals represented on Plate IV, Fig. 3.

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

Oil of Clove, oil of Gaultheria, and other essential oils may be detected by the same process of fractional evaporation as suggested under 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, $C_6H_3OH(NH_2)COOCH_3$, methylparaamidometaoxybenzoate, used as an anæsthetic and antiseptic, is without odor, color, or taste, is slightly soluble in water and easily soluble in alcohol or ether.

Phenol.—See Carbolic Acid.

Potassium Hydroxid, 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 from petroleum,

boiling at about 18° C., used as a spray for the production of low temperature, similarly to methyl or ethyl chlorid. It is readily inflammable and the vapor mixed with certain proportions of air is explosive. It should be kept in a cool place.

Silver Nitrate, $AgNO_3$, crystallizes in colorless plates without water of crystallization; used as an antiseptic, disinfectant, or escharotic. It is freely soluble in water and may be detected by the ordinary methods of qualitative analysis (page 6).

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

Sodium Perborate, a powder said to be the composition $NaBO_3 \cdot 4H_2O$, which will furnish 10% of available oxygen and produce H_2O_2 with water; very stable and recommended as a bleach-powder.

Sodium perborate may be made * by thoroughly mixing 78 grams Na_2O and 248 grams of crystallized H_3BO_3 and stirring the mixture gradually into 2 liters of cold H_2O. The sodium perborate, $Na_2B_4O_8 + 10H_2O$, is formed spontaneously and settles out from the solution as a white crystalline powder. Its solubility is increased by addition of weak organic acids, citric or tartaric.

Sodium Peroxid, Na_2O.—A white powder easily soluble in water, usually with evolution of more or less oxygen and formation of hydrogen dioxid.

Somnoform.—A general anæsthetic administered in manner similar to chloroform; introduced by Dr. Rolland, of Bordeaux; consists of 60% ethyl chlorid, 35% ethyl bromid, and 5% methyl bromid. (Dental Cosmos, Vol. XLVII, page 236.)

* Dental Cosmos, Nov. 1905, page 1381.

Tannic Acid, or tannin, sometimes called gallotannic acid, is an astringent organic acid obtained from nutgalls. It may be obtained as crystals carrying 2 moleucles of water, $HC_{14}H_9O_92H_2O$. Tannic acid is a white or slightly yellowish solid soluble in about one part of 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, $C_{10}H_{14}O$, a phenol, occurring in volatile oils of thymus *vulgaris* (Line). Melts at 44° C.; sparingly soluble in water, easily in alcohol and ether.

Tests.—It may usually be detected by its odor or by a small crystal dissolved in 1 c.c. of glacial acetic acid, then 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.)

Trichloracetic Acid occurs as deliquescent crystals, readily soluble in water. Distils at 195° C. and is a powerful caustic. Dilute solutions are recommended for treatment of pyorrhœa.

Tropa-cocain is an alkaloid originally isolated by Giesel from the leaves of small-leaved coca-plant of Java and introduced by Arthur P. Chadbourne, Harvard Medical School. Used hypodermically in normal salt solution. It is probably superior to cocain, 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.

TEETH AND TARTAR.

The chemical examination of teeth and tartar, while coming more properly under the head of physiological chemistry, will be considered in part in this place, as the tests made, especially on tartar, are practically all microchemical. 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 fluorid 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 chlorid 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, fluorids, and chlorids. An increase in the percentage of calcium phosphate or fluorid increases the hardness of the tooth, while an increase of calcium carbonate decreases the hardness.

In tartar, potassium sulphocyanate, ferric phosphate, sulphites, and uric acid have been found 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

Tartar from patients with pyorrhœa alveolaris has been found to contain oxalates and urates, not necessarily together, but often one or the other. The deficient oxidation and high

acidity usually occurring in such cases is conducive to the production of large amounts of oxalic or uric acids (most generally the latter) whether these substances have etiological relations to pyorrhœa or not.

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 (page 53) and may be determined by accurately weighing out 1 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 obtained 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 HNO_3; then to a few drops of the clear solution add an excess of ammonium molybdate in nitric acid. A yellow crystalline precipitate of ammonium phosphomolybdate will separate. Avoid heating above 60° C., as the ammonium molybdate may decompose and precipitate a yellow oxid of molybdenum.

Carbonic Acid may be detected by liberation of CO_2 and passing the gas into lime-water as described on page 43 or with closed tube and drop of baryta-water, page 53.

Chlorin 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 HCl solution (1) an excess of ammonia. Calcium phosphate 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 NH_4OH, dissolve in dilute HCl, and add Fe_2Cl_6 carefully till a drop of the solution gives, when mixed with a drop of NH_4OH, a yellowish precipitate. Nearly neutralize with Na_2CO_3 and add $BaCO_3$, which precipitates ferric phosphate. Filter, heat the filtrate, precipitate the barium with dilute sulphuric acid, and filter. From the filtrate calcium is precipitated as white calcium oxalate by making alkaline with NH_4OH and adding $(NH_4)_2C_2O_4$ as long as a precipitate is formed. Filter and add to the filtrate sodium phosphate, which precipitates magnesium as ammonio-magnesium phosphate, white.

PART V.

ORGANIC CHEMISTRY.

SEC. I.—THE HYDROCARBONS AND SUBSTITUTION PRODUCTS.

Our work up to this point has been confined to inorganic chemistry (excepting a few microchemical tests for organic substances). We are now to enter upon the study of organic or as some define it "carbon" chemistry, and the chemistry of the carbon compounds is a domain so vast that its magnitude almost overwhelms us.

We shall touch but lightly 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.

The carbon compounds contain the elements of C and H, and when these two only are present they are *hydrocarbons*. They more frequently contain C, H, and O, and when the H and O 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 C, H, O, and N and often in addition S or P. Many other elements, notably the halogens, and often the metals, may be found in organic compounds.

The question of its composition is then the first one presenting itself in the consideration of an organic substance.

Qualitative Tests.

Carbon.—The presence of this element may be shown by the "carbonization" obtained in the preliminary test on platinum foil (page 52).

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

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

(*a*) Conversion into a cyanogen compound;

(*b*) Conversion into free ammonia.

(*a*) A small piece of thoroughly dried albumen is placed in a matrass described on page 10, together with a little metallic potassium, and heated to redness for a few minutes. (Metallic sodium will work as well in most cases.) An alkali cyanide is formed which may be dissolved in water after breaking the tube, and by addition of a little yellow ammonium sulphid and evaporation to dryness on a water-bath will be changed to sulphocyanate, NH_4CNS. If the dry residue is taken up with dilute HCl, filtered, and tested with a drop of ferric chlorid solution, the presence of the sulphocyanate is at once shown by the red color produced.

(*b*) Most nitrogenous substances may be made to evolve ammonia-gas by simply heating in a test-tube with several times its bulk of soda-lime. Test for NH_3 by moistened red litmus paper or 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 when NH_3 is evolved, which may be detected by litmus paper or odor as above.

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 HNO_3 or mixture of $KClO_3$ and HCl. The resulting sulphate or phosphate is detected by the usual qualitative methods (page 44).

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

Halogens.—Cl, Br, and I cannot be detected in organic combinations by the ordinary qualitative test with $AgNO_3$ and dilute NHO_3, but must first be converted into corresponding inorganic haloid salts. This may be done by heating the organic substance strongly with pure lime, when calcium chlorid, bromid, etc., will be formed, which may be dissolved in water and tested in the usual way.

A test for chlorin or iodin may also be made by heating with copper oxid on a platinum wire in the Bunsen flame, chlorin giving first a blue then a green color to the flame. Iodin gives a green only (Beilstein).

Exp. 1. Test for presence of C, H, and S in dried albumen.

Exp. 2. Test for S by the caustic soda test.

Exp. 3. Test for P in casein precipitated from milk.

Exp. 4. Make tests for the halogens with a few drops of chloroform.

The Hydrocarbons.

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

of a single 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 H is replaced by a chlorin atom, for instance, we have a *substitution product*. Its formula will be CH_3Cl, its name monochlormethan or *methyl* chlorid. If two molecules of methyl chlorid are brought together and the Cl removed by metallic sodium the residual molecules (methyl radicals) will unite, forming a new hydrocarbon, as follows:

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

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

CH_4, methan or methyl hydrid (CH_3H).

C_2H_6, ethan or ethyl hydrid (C_2H_5H).

C_3H_8, propan or propyl hydrid (C_3H_7H).

C_4H_{10}, butan or butyl hydrid (C_4H_9H).

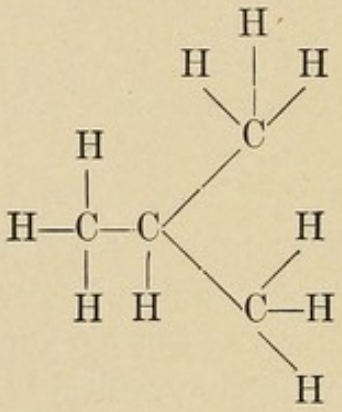
C_5H_{12}, pentan or amyl hydrid ($C_5H_{11}H$).

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.

Isomers.—When two or more compounds are of exactly the same molecular composition in regard to numbers and kind of atoms, they are isomeric substances or isomers.

Thus we may have a normal butan represented graphically by $H\text{–}\overset{H}{C}\text{–}\overset{H}{C}\text{–}\overset{H}{C}\text{–}\overset{H}{C}\text{–}H$ (C_4H_{10}), then we may have an isomeric or isobutan represented by



also C_4H_{10}, but having different physical and chemical properties from the normal compound. The greater the number of carbon atoms in the molecule the more numerous the possible isomers.

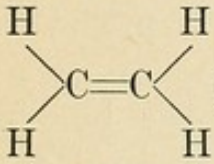
Polymers.—When one compound has a formula which may be regarded as a multiple of another it is said to be a polymer of it; thus paraform, a white crystalline solid, $(CH_2O)_3$, is a polymeric form of the gaseous formaldehyde CH_2O.

The hydrocarbons of the paraffin series are known as *straight chain* or *aliphatic hydrocarbons*, their graphic formulæ consisting of "chains" of carbon atoms, as butan, $-\overset{|}{\underset{|}{C}}\text{–}\overset{|}{\underset{|}{C}}\text{–}\overset{|}{\underset{|}{C}}\text{–}\overset{|}{\underset{|}{C}}\text{–}$, in distinction from the closed-chain or cyclic compounds as rep-

resented by the "benzole-ring" (page 161) carbon nucleus with the C 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 Cl, for instance, without first giving off an equivalent number of atoms of H. 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.

If two carbon atoms are united by a double bond, as in



(C_2H_4), chlorin may be added directly by the breaking of the double bond, forming ethylene chlorid, $C_2H_4Cl_2$.

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}.

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_2H_6, butylene, C_5H_{10}, etc. They are unimportant in dental or 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.

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≡C—H, (C_2H_2), made from calcium carbid

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* Notice that while addition products of saturated hydrocarbon cannot be formed, substitution products are easily possible. See page 125.

and water. It is poisonous, combining directly with the hæmoglobin 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.

These are substitution products. Among the compounds of this class used in dental medicine the following may be mentioned:

Methyl Chlorid, CH_3Cl, may be made from methyl alcohol, zinc chlorid, and hydrochloric acid. It is a colorless gas, condensing to a liquid at 23° C.; used as a spray in producing local anæsthesia (page 115).

Ethyl Chlorid, 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 anæsthetic in similar manner to methyl chlorid. Its higher boiling-point makes it the more convenient of the two preparations (see page 113).

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

Methylene Chlorid, CH_2Cl_2, has been used as a general anæsthetic, usually mixed with more or less chloroform and alcohol.

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

Iodoform, HCl_3, tri-iodomethan, is a much-used and very valuable antiseptic. It is a light-yellow crystalline powder with characteristic persistent odor (Plate V, Fig. 1).

Iodoform may be made by heating in a retort two parts of potassium carbonate, two of iodin, one of strong alcohol, and five of water, till the mixture is colorless.

Iodoform is also produced from action of above reagents

with acetone in place of alcohol. This reaction is a very delicate one and advantage is taken of it in testing for acetone in saliva, which see.

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

Chloroform, $CHCl_3$, trichlormethan, is a general anæsthetic prepared by distilling a mixture of chlorinated lime and acetone. Alcohol and water were formerly used in place of acetone (see Exp. 10, p. 130). While it is not regarded as inflammable, its heated vapor can be made to burn with a greenish flame.

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

Experiments with the Hydrocarbons and Haloid Derivations.

Exp. 5. 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. 6. 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. 7. 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.

$$CaC_2 + 2H_2O = Ca(OH)_2 + C_2H_2.$$

Exp. 8. Conduct a little of the acetylene gas into an ammoniacal cuprous chlorid solution. What is the red precipitate?

Exp. 9. If the evolution of gas 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.

Exp. 10. 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.

$$4C_2H_5OH + 8Ca(ClO)_2 = 2CHCl_3 + 3Ca(CHO_2)_2 + 5CaCl_2 + 8H_2O.$$
(Formate of Ca)

Exp. 11. Place in a test-tube about 1 gram of crystallized carbonate of sodium, about half as much iodin 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 iodin 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? Explain their relation to marsh-gas.

SECTION II.—ALCOHOL.

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. Alcohols may be considered as compounds containing an alkyl radical

and a distinctive alcohol group, and are primary, secondary, or tertiary according to relative position of the group. (See page 132.)

Alcohols are mono-, di-, tri-atomic, etc., according to the number of alcohol groups they contain.

$\left.\begin{array}{l} CH_2OH \\ | \\ CHOH \\ | \\ CH_2OH \end{array}\right\}$, glycerine, is thus a triatomic alcohol, while mannite, $CH_2OH–CHOH–CHOH–CHOH–CHOH–CH_2OH$, or $C_6H_8(OH)_6$, is a hexatomic alcohol.

Methyl Alcohol, CH_3OH, $(H–CH_2OH)$,* wood spirit, carbinol, is a product of the destructive distillation of wood or 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, and boils at 66°.

Ethyl Alcohol, C_2H_5OH, $(CH_3–CH_2OH)$, methyl carbinol, grain alcohol, or ordinary alcohol is made by fermentation of solutions of various carbohydrates and purified by distillation. Carbon dioxid is evolved as follows:

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

95% alcohol has a specific gravity 0.8164, boils at about 78° C., dissolves many inorganic salts, vegetables, waxes, resins (not gums), oils, etc. Miscible with water, ether, or chloroform.

Amyl Alcohol, $C_5H_{11}OH$, $(C_4H_9–CH_2OH)$, isobutyl carbinol, 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.

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

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

The alcohols just considered are all primary alcohols; that is, the –OH group has been introduced into the hydrocarbon in place of an H of a $-CH_3$ group, leaving a characteristic grouping for this class of compounds, $-CH_2OH$.

The hydroxyl derivatives (alcohols) of isopentan are well suited to illustrate the three (primary, secondary, and tertiary) characteristic alcohol groupings.

$$\begin{matrix} CH_3 \\ \\ CH_3 \end{matrix}\!\!\rangle CH\text{–}CH_2\text{–}CH_3 \text{ is isopentan}$$

and by introducing the OH group (hydroxyl) into the CH_3 group there is formed a *primary* amyl alcohol,

$$\begin{matrix} CH_3 \\ \\ CH_3 \end{matrix}\!\!\rangle CH\text{–}CH_2\text{–}CH_2OH, \text{ or isobutyl carbinol,}$$

and the *primary* alcohol grouping is $-CH_2OH$. By introducing hydroxyl (OH) into the CH_2 group we should have –CHOH– as a characteristic combination in *secondary* alcohols,

$$\begin{matrix} CH_3 \\ \\ CH_3 \end{matrix}\!\!\rangle CH\text{–}CHOH\text{–}CH_3, \text{ methylisopropyl carbinol;}$$

and lastly, by putting the OH in place of the H of the CH group of the hydrocarbon, we should have $(CH_3)_2{=}COH\text{–}CH_2\text{–}CH_3$, a tertiary alcohol with the group $\equiv COH$ as its characteristic.

Oxidation of the Alcohols.

Aldehyds.

The first step in the oxidation of an alcohol consists not in the addition of oxygen but in the withdrawal of hydrogen; thus the oxidation of methyl alcohol produces formaldehyd (CH_2O) and water.

$$CH_3OH + O = CH_2O + H_2O.$$

Aldehyds may be considered compounds containing an alkyl radical and a distinctive group, $-C\begin{matrix}H\\ \\ \|O\end{matrix}$; thus $\begin{matrix}H\\ |\\ CHO\end{matrix}$ is formaldehyd, $\begin{matrix}CH_3\\ |\\ CHO\end{matrix}$, in acetaldehyd, etc. (compare Alcohol, page 131).

Formaldehyd coagulates albumen and hardens gelatine; when used as a preservative it renders the proteids tougher and less digestible.

Formaldehyd polymerizes, producing the paraform or paraformaldehyd of trade, trioxymethylene, with a probable forula of $(CH_2O)_3$. It also forms one lower polymer $(CH_2O)_2$ and at least one higher, formose, a substance allied to glucose.

Acetaldehyd, aldehyd, CH_3–CHO or C_2H_4O, the aldehyd 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 etherial odor and boils at 22° C.

Paraldehyd, $(C_2H_4O)_3$, a polymer of acetic aldehyd, 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, trichloraldehyd, is an oily liquid formed by action of dry Cl gas on pure alcohol; soluble in ether and chloroform, boiling at 94° C. to 98° C., and forming, with a molecule of H_2O *chloral hydrate*, $CCl_3CHO.H_2O$, a crystalline solid, and this is the "chloral" of the pharmacopœia (see page 111).

Chloral hydrate is decomposed by caustic soda or potassa with liberation of chloroform (see Exp. 20, p. 142):

CCl_3–CHO + KOH = $CHCl_3$ + KCOOH (potassium formate).

Upon warming a drop or two of aniline oil an excess of alcoholic potash chloral hydrate forms, first, chloroform, then penylisocyanid, C_6H_5NC, the persistent disagreeable odor of which furnishes a delicate test for chloroform or chloral (see Exp. 21, p. 142). By using $CHCl_3$ 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 acetanilid. Other aldehyds 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 lecture.

Ketones.

The oxidation of *secondary* alcohols (page 132) will not yield aldehyds, but a class of substances known as *ketones:*

$$\underset{\substack{\text{A secondary alcohol.}\\ \text{Methyl isopropyl carbinol.}}}{(CH_3)_2–CH\text{-}CHOH–CH_3} + O = \underset{\text{Methyl isopropyl ketone.}}{(CH_3)_2–CH–C{:}O–CH_3} + H_2O,$$

or
$$\underset{\text{Isopropyl alcohol.}}{CH_3–CHOH–CH_3} + O = \underset{\text{Dimethyl ketone.}}{CH_3–CO–CH_3} + H_2O.$$

The converse of each of these reactions is possible, and by reduction of a ketone with nascent H (sodium amalgam) the secondary alcohol will be formed:

$$\underset{\text{Acetone.}}{CH_3–CO–CH_3} + H = \underset{\text{Isopropyl alcohol.}}{CH_3–CHOH–CH_3}.$$

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

$$\underset{\text{Acetaldehyd.}}{CH_3–CHO} + H_2 = \underset{\text{Ethyl alcohol.}}{CH_3–CH_2OH}.$$

Note that the grouping peculiar to ketone is $=CO$.

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

It occurs in the blood and urine of patients suffering from advanced diabetes. According to von Noorden the acetone found in the blood is formed by an intracellular process and indicates an acid auto-intoxication and an insufficient *utilization* of carbohydrates. Acetone may be found in the saliva, and that (in the experience of the author) sometimes when it cannot be found in the urine (for test, see Acetone under Saliva and Urine).

Another ketone of interest is lævulose, fruit-sugar, $CH_2OH–CHOH–CHOH–CHOH–CO–CH_2OH$, which with glucose will be studied later.

While the oxidation of a primary alcohol will produce an aldehyd and the oxidation of a secondary alcohol will produce a ketone, the tertiary alcohol, by action of an oxidizing agent, is split into two new carbon compounds, that is, the chain is broken and simpler ketones and acids are formed.

SECTION III.—ETHERS.

Ethers may be regarded as oxids of the hydrocarbon radicals, as $\begin{matrix} C_2H_5 \\ C_2H_5 \end{matrix}\!\!>\!O$, or as anhydrids of the monatomic alcohols, H_2O having been removed from two molecules of the alcohol:

$$2C_2H_5OH–H_2O=(C_2H_5)_2O.$$

Ethers may be simple, mixed, or compound. 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.

The compound ethers are compounds of alcohol radicals with acid radicals, that is, the salts of alcohol radicals. The acid may be either organic or inorganic; thus we have nitric

ether, ethyl nitrate, $C_2H_5NO_3$, and we have acetic ether, ethyl acetate, $C_2H_5C_2H_3O_2$. The compound ethers are often called esters and form a large and important class of organic compounds.

Methyl Ether.—Methyl oxid, $(CH_3)_2O$, 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 liquefies at $-20°$ C. (Bernthsen). It has been used as a general anæsthetic, and the anæsthesia 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 anæsthesia.

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

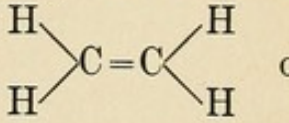
Ethyl Ether.—Ethyl oxid, $(C_2H_5)_2O$, consisting of 96% by weight of the "æther" of the pharmacopœia (the other 4% being alcohol and a little water). Ether is a general anæsthetic, widely used. It is made by the action of sulphuric acid on ethyl alcohol, and from this fact has been known as sulphuric ether, but this name is, of course, incorrectly used, sulphuric ether being properly an ethyl sulphate $(C_2H_5)_2SO_4$.

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 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 suffered itself 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 H_2SO_4 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, freed from water and traces of acid by redistillation over a mixture of lime and calcium chlorid. Ether, according to to the U. S. P. requirements, is "a transparent, colorless, mobile liquid with characteristic odor and a burning and sweetish taste"; specific gravity of 0.725 to 0.728 at 15° C. and boiling at about 37° C.

It is readily inflammable, and this fact, together with its easy volatility, makes it necessary to use considerable care when handling it. Absolute ether boils between 34° and 35° C.

The action of sulphuric acid upon alcohol needs careful regulation; and there are three other possible products in addition to the ethyl oxid 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_nH_{2n}, and containing "double-bonded" carbon atoms,  or $CH_2=CH-CH_3$. These are unsaturated hydrocarbons (see page 127). Ethylene is produced by the action of an excess of concentrated H_2SO_4, which abstracts H_2O from each molecule of alcohol ($C_2H_5OH-H_2O=C_2H_4$), whereas in the preparation of ether the more dilute acid abstracts H_2O from *two* C_2H_5OH.

Compound Ethers or Esters.

One of the most important of this class of compounds, from a dental standpoint, is the benzoyl-ecgonine methyl ester or cocain, $CH_3N\begin{cases} C_5H_7 \\ | \\ CH.C_7H_5O_2.CH_2–CO_2CH_3 \end{cases}$. While of considerable interest, the elucidation of the exact chemical relationship of this compound to tropacocain, etc., is beyond the scope of this work.

Another methyl ester of much simpler chemical composition is methyl salicylate, $CH_4–CH–COOCH_3$.

Salicylic acid is $CH_4–OH–COOH$ (oxybenzoic acid), and its methyl ester constitutes the methyl salicylate of the U. S. P. It is identical with the volatile oil of betula and with 90% of the oil of gaultheria (wintergreen). This latter oil is much used as a flavor in dental preparations, tooth-washes, powders, etc.

Ethyl Acetate, $CH_3–COO.C_2H_5$, 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 (page 49).

The acetic ether of the U.S.P. is "a liquid composed of about 98.5% of ethyl acetate and 1.5% 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% alcohol: odor of pineapple indicates butyric acid." (Hewes.)

Ethyl Nitrite, $C_2H_5NO_2$, may be made by heating sodium nitrite with concentrated sulphuric acid and alcohol, also by the reduction of nitric acid by copper in presence of alcohol and sulphuric acid. The ethyl nitrite is distilled, and must be collected in a receiver surrounded by a freezing mixture of ice and salt. Pure ethyl nitrite boils at 18° C. and has a gravity of 0.900. An alcoholic solution constitutes sweet spirits of nitre, the spiritus ætheris nitrosi of the U. S. P.

This preparation should, according to Dr. E. R. Squibb, contain 4.5% ethyl nitrite.

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

The **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 gylcerin or glycerol.

Glyceryl butyrate or butyrin, $CH_3–(CH_2)_2–COOC_3H_5$, constitutes (together with smaller quantities of the glyceryl esters of capric, caproic, and caprylic acids) about 7% 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.

Glyceryl Palmitate, $C_3H_5(C_{16}H_{31}O_2)_3$ tripalmitin, **glyceryl stearate,** $C_3H_5(C_{13}H_{33}O_2)_3$, tristearin, and **glyceryl oleate,** $C_3H_5(C_{48}H_{33}O_2)_3$, 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 monopalmatin, $C_3H_5(OH)_2$–$C_{16}H_{31}O_2$, etc., are possible and may be prepared by synthesis. Triolein is liquid at ordinary temperature, solidifies at $-6°$ C., is a "double-bonded" compound, hence forms addition-products with the halogens as stearin and palmitin cannot do, they being "saturated hydrocarbons."

The amount of chlorin or bromin which a fat or oil can thus absorb is an index of the proportions of fatty acids of this class contained in them, and hence becomes a valuable method of identification. Olive-oil and lard-oil contain large amounts of olein.

Tripalmitin melts at 66° C., is usually obtained from palm-oil. Tristearin melts at 72° C., occurs with palmitin and olein in beef-fat, mutton-tallow, etc., the consistence 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:

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

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

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

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

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.

Experiments with Alcohols, Aldehyds, and Ethers.

Exp. 12. To about 75 c.c. of a 10% glucose solution add a little yeast, and allow to stand for twenty-four hours at a temperature 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 130, Exp. 11. The production of CO_2 may also be demonstrated if the gases evolved during the fermentation are passed into clear lime-water:

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

Exp. 13. Formaldehyd may be made by the partial oxidation of methyl alcohol (wood-spirit) by passing the vapor of the alcohol mixed with air slowly over a red-hot spiral of platinum wire. With a little of the solution so prepared, or with the 40% solution known as " formaline," make the following test:

Exp. 14. Mix about 1 c.c. of solution to be tested with four or five times its volume of milk in a test-tube. 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 formaldehyd. It is necessary that the sulphuric acid should contain a trace of iron: this the *commercial* acid usually does. It is also 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. 15. 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 acetaldehyd produced by oxidation of the alcohol. Note also the reduction of the dichromate to $Cr_2(SO_4)_3$, as follows:

$$K_2Cr_2O_7 + 4H_2SO_4 + 3C_2H_5OH = K_2SO_4 + Cr_2(SO_4)_3 + 3C_2H_4O + 7H_2O.$$

Exp. 16. Apply to dilute solution of both formic and acetic aldehyd Tollen's test for aldehyd, which is made as follows: Into a clean test-tube which has been rinsed with caustic soda solution place 5 c.c. of a reagent made by dissolving 3 grams silver nitrate in 30 c.c. of ammonia-water and adding 3 c.c. of sodium hydroxid. Add the solution to be tested when the silver is reduced, forming a metallic mirror on the inner surface of the test-tube.

Exp. 17. 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.

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 H_2SO_4, as follows:

$$C_2H_5HSO_4 + C_2H_5OH = (C_2H_5)_2O + H_2SO_4.$$

Exp. 18. The production of compound ethers may be demonstrated by the test for acetic acid forming ethyl acetate, page 49, or by the following experiment used to detect butyric acid in gastric contents:

Exp. 19. Mix in a test-tube 5 c.c. of a dilute ($\frac{1}{2}\%$) 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 ethylbutyrate (pineapples).

Exp. 20. 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. 21. 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

* 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% aqueous solution of NaOH.

only of fresh aniline oil. Heat till the mixture just begins to boil and note the odor of the nitril.

SECTION IV.—ORGANIC ACIDS.

If the oxidation of an alcohol is carried beyond the formation of aldehyd or ketone, i.e., if the aldehyd 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); but the second atom of oxygen attaches itself to the molecule and does become a part of the new substance (the acid):

$$\underset{\text{Alcohol.}}{CH_3\text{-}CH_2OH} + O = \underset{\text{Aldehyd.}}{CH_3\text{-}CHO} + H_2O \qquad \underset{\text{Acetic aldehyd.}}{CH_3\text{-}CHO} + O = \underset{\text{Acetic acid.}}{CH_3\text{-}COOH}$$

The group –COOH is known as carboxyl and is the characteristic grouping of the acids. The H of the carboxyl differs from the other atoms of H in the molecule in that it is united to oxygen rather than to carbon, and constitutes the basic or replaceable H 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 will 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 proprionate;
C_3H_7COOH = butyric acid or hydrogen butyrate;

C_4H_9COOH = valerianic acid or hydrogen valerianate;
$C_{15}H_{31}COOH$ = palmitic acid or hydrogen palmitate;
$C_{17}H_{35}COOH$ = Stearic acid or hydrogen stearate.

Of the *Acrylic Acid Series*

$C_{17}H_{33}COOH$, or oleic acid, is the only one of interest.

Dibasic Acids.

$\begin{matrix}COOH\\|\\COOH\end{matrix}$
Oxalic acid.

$\begin{matrix}COOH\\|\\CH_2\\|\\COOH\end{matrix}$
Malonic acid.

$\begin{matrix}COOH\\|\\CH_2\\|\\CH_2\\|\\COOH\end{matrix}$
Succinic acid.

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 hydroxyformic acid, HO–COOH;

Glycolic acid as hydroxyacetic acid, $\begin{matrix}CH_2OH\\|\\COOH\end{matrix}$;

Lactic acid as hydroxypropionic acid, $\begin{matrix}C_2H_4OH\\|\\COOH\end{matrix}$;

Malic acid (from apples) as hydroxysuccinic acid, $\begin{matrix}CHOH–COOH\\|\\CH_2–COOH\end{matrix}$.

Tartaric acid is dihydroxysuccinic 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 $C_3H_4OH–(COOH)_3$.

Formic Acid (H.COOH), originally distilled from the bodies of ants (formica), is a colorless, very soluble liquid. It may also

be made by passing CO over hot KOH and by heating glycerine and oxalic acid.

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 $4\frac{1}{2}\%$. Glacial acetic acid is a commercial name of the acid containing 1% or less of water: it is a colorless solid at a temperature below 15° C. The U. S. P. acetic acid contains only 36% (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 chlorin, forming respectively the mono-, di-, or trichloracetic acids, the trichloracetic acid being used to a considerable extent in dentistry (page 118).

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, $2CO_2$ and $2H_2$. It is an occasional constituent of the gastric contents, and may be detected by formation of the ethyl ester (page 138). The pure acid is a heavy, colorless liquid with characteristic odor, soluble in H_2O in any proportion. See page 139 for the glyceryl ester of butyric acid (butyrin); also for stearic and palmitic acids.

Oleic Acid, closely allied to palmitic and stearic, does not belong to the saturated hydrocarbons but is a double-bonded acid, hence an unsaturated compound (page 127).

Dibasic acids contain two carboxyl groups. These are referable to, and in many cases may be formed from, the diatomic alcohols. Thus glycol, $\begin{matrix}CH_2OH\\|\\CH_2OH\end{matrix}$, upon oxidation yields glycollic acid, $\begin{matrix}CH_2OH\\|\\COOH\end{matrix}$, and oxalic acid, $\begin{matrix}COOH\\|\\COOH\end{matrix}$.

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, ($H_2C_2O_4.2H_2O$); 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 tomatoes, rhubarb, spinach, and asparagus; grapes, apples, and cabbage 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 KOH and NaOH, precipitating the acid as CaC_2O_4, and decomposing the salt by H_2SO_4. The acid is then purified by repeated crystallization.

Succinic Acid, $COOH(CH_2)_2$–COOH, which may be obtained by the saponification of ethylene cyanid, $C_2H_4(CN)_2$, 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, according to some authorities, in the urine (Hammarsten).

Oxyacids.

Several acids of this class are of importance to the student of dentistry.

Lactic Acid.—Oxypropionic acid, or i*-ethylidene lactic acid, CH_3–CHOH–COOH, is ordinary lactic acid produced by

* Optically inactive.

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 and brain tissue (Foster).

Sarcolactic or paralactic acid, d*-ethylidene lactic acid, occurs in meat extract. The presence of this acid 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 lactophosphates 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. This statement as yet lacks confirmatory demonstration.

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 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 H 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 dioxid 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 by 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

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* Dextrorotary.

von Noorden, in other cases of perverted oxidation (not insufficient oxidation).

Tartaric Acid is a dihydroxysuccinic acid, $COOH-(CHOH)_2-COOH$, obtained from grape-juice. The double tartrate of sodium and potassium (Rochelle salts), $KNaC_4H_4O_6$, is much used in medicine.

Tartaric acid combines with potassium and antimony to form tartar emetic, $(KSbOC_4H_4O_6)_2H_2O$.

The "*scale salts of iron*," "ferri et ammonii tartras" and "ferri et potassii tartras," are prepared by dissolving freshly precipitated ferric hydroxid 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, which are only sparingly soluble in water. Its commercial source is the wine-vat.

Amido Acids,

also called amino acids, are characterized by an NH_2 group in place of H– ; for example, acetic acid is $\begin{matrix}CH_3\\|\\COOH\end{matrix}$. Amido-acetic acid is $\begin{matrix}CH_2NH_2\\|\\COOH\end{matrix}$. These acids are of particular interest because of their close relationship to the proteids, many of them being among the cleavage products of proteid hydrolysis.

Amido-acetic Acid, also called glycocoll and glycin, is obtained with other amido acids by boiling glue with either acids or alkalis.* It is also obtained, by the hydrolysis of glycocholic acid, from the bile.

Hippuric acid (Plate V, Fig. 4) consists of benzoic acid united chemically to glycocoll, and may be produced synthetically by the union of these two substances.

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* Bernthsen, Organic Chemistry.

Amido-valerianic Acid, $CH_2(NH_2)$–$(CH_2)_3$–COOH, may be obtained with glycocoll from elastin, the proteid of the elastic fibres, of tendons, etc.* Isomeric with amido-caproic acid is *leucin,* an amido-isobutyl-acetic acid,† $\begin{matrix}CH_3\\CH_3\end{matrix}\!\!>\!CH$–$CH_2$–$CH(NH_2)$–COOH. Leucin is a cleavage product in the decomposition of proteids, including keratin and collagen. It results from the tryptic digestion of the hemipeptones and is regarded, as are other amido acids, as antecedent of urea (Plate V, Fig. 2).

Dibasic Amido Acids.

Of this class of compounds two may be mentioned: amido-succinic, aspartic or asparaginic acid, COOH–CH_2–$CH(NH_2)$–COOH, may be obtained from animal and vegetable proteids and in the pancreatic digestion of fibrin.

Glutaminic Acid is an amido-glutaric (pyrotartaric) acid, and occurs similarly to aspartic acid, except that it is not formed by pancreatic digestion.

Amins, or Substituted Ammonias.

If one or more of the H atoms of ammonia, NH_3, be replaced by a hydrocarbon group, the resulting compound is an amin; thus CH_3–NH_2 is methylamin, and $(CH_3)_2NH$ is dimethylamin. Trimethylamin, $(CH_3)_3N$, has been found among the decomposition products of fresh brain, human liver, and spleen.‡ It is poisonous and possesses a strong, fishy odor. At ordinary temperature it is a gas, but, like ammonia, is freely soluble in H_2O and forms a variety of salts.

Diamins are derived from two molecules of ammonia, as ethylene diamin, $C_2H_4\begin{matrix}NH_2\\NH_2\end{matrix}\!\!>$.

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* Foster, Chemical Basis of the Animal Body.

† Novy, Physiological Chemistry.

‡ Vaughn and Novy, Cellular Toxins.

To this class of compounds belong many of the "ptomains," produced by the putrefaction of organic matter, as putrescin, butylene diamin, CH_2NH_2–$(CH_2)_2$–CH_2NH_2, and cadaverin, penta-methylene diamin, CH_2NH_2–$(CH_2)_3$–CH_2NH_2. A large number of the ptomaines are aromatic compounds and as such will be referred to later.

Amids.

If the hydrogen of NH_3 be replaced by an oxygenated or acid radical, an amid results; thus $NH_2(C_2H_3O)$ is acetic-amid, or this compound may be regarded as acetic acid, CH_3–COOH, in which the OH has been replaced by NH_2. This group (NH_2) is known as the amido group, and characterizes a large number of very important compounds (see Amido Acids, page 148).

Hydrazines.

From diamid, 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, or phenyl-hydrazine, C_6H_5NH–NH_2.

This latter compound forms, with the monosaccharides and with many of the disaccharides, 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).

Experiments with Organic Acids.

Experiments 18 and 19 may be used as illustrating the laboratory test for acetic and butyric acids. In addition a test for lactic acid may be made with ferric chlorid test, which is also applicable to gastric contents.

Exp. 22. Dilute a few drops of neutral ferric chlorid solution until no color is discernible, then to 10 c.c. of this dilution

add 4 to 5 drops of 1/2% solution of lactic acid. A greenish-yellow color constitutes the 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. 23. Introduce into a small flask (250 c.c. capacity) about 30 c.c. of anhydrous glycerine 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 glycerine; 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 142. The silver solution will be reduced, but difficulty will be experienced in obtaining the mirror.

Exp. 24. From a mixture of formic acid, alcohol, and sulphuric acid, ethyl formate may be evolved in a manner similar to that in the production of ethyl acetate (page 49). Compare the odors of these two ethers.

Exp. 25. 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. 26. In separate test-tubes, insoluble oxalates may be produced by adding a solution of ammonium oxalate to a solution of (*a*) calcium chlorid, (*b*) silver nitrate, (*c*) zinc sulphate, (*d*) copper sulphate, (*e*) lead nitrate.

Exp. 27. To 1/3 test-tube of cider vinegar add a few cubic centimeters of basic acetate of lead solution; a bulky precipitate of lead malate separates out.

Exp. 28. Take about 5 c.c. each of alcoholic solution of stearic and oleic acids and treat separately with about 2 c.c.

of 1% iodin solution (alcoholic); allow to stand for some time, and explain *fully* the difference in deportment exhibited by the two fatty acids.

Exp. 29. To a dilute solution of ferric chlorid add a little acetic acid; divide the solution in two parts, and to one add mercuric chlorid and to the other HCl, and note results.

SECTION V.—CYANOGEN 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.

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. HCN 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, $K_4Fe(CN)_6 + 5H_2SO_4 = 6HCN + FeSO_4 + 4KHSO_4$. 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 H_2O, and a 2% aqueous solution constitutes the acidum hydrocyanicum dilutum of the pharmacopœia, also known as prussic acid.

The organic cyanides are known as *nitrils* or *isonitrils*, according as the hydrocarbon radical is attached directly to the C or to the N or the cyanogen group. That is, methyl cyanid would be represented by CH_3–CN, while the isocyanid 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 isocyanids are of

much greater interest to the student of dental medicine owing to their relation to the isocyanates and to urea.

Phenyl-isocyanid, 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 which is utilized as a test for chloroform or chloral hydrate (page 142); or, with chloroform and potassium hydrate, the production of isocyanid may become a test for aniline, acetanilid (antifebrin), etc.

Isocyanic Acid, O=C=N–H (carbimid), 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 Ag and Hg.

Thiocyanic Acid or Sulphocyanic Acid.—In this acid and its salts the atom of S replaces the oxygen of the cyanate in the empirical symbol (HCNS), but graphically the S is attached to the basic element (metal or H) rather than to C; thus, K–S–C≡N, that is, the sulphocyanate is not an isocompound. For occurrence and relations of HCNS in the human body, see chapter on Saliva.

Urea.

This substance forms about 50% of the total solids and about 85% of the nitrogenous matter contained in the urine. When we consider that only 5% of the nitrogenous waste passes off in the feces and 95% in the urine, the importance of urea as an index of the nitrogen excreted, and of proteid 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 decom-

position of potassium cyanate and ammonium sulphate and subsequent evaporation of the solution to dryness:

$$2CNOK + (NH_4)_2SO = OCN.NH_4 + K_2SO_4.$$

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

$$O=C\begin{matrix}NH_2\\NH_2\end{matrix} \text{ (urea).}$$

Urea is the amid of carbonic acid, $O=C\begin{matrix}OH\\OH\end{matrix}$, and from this type may be explained the rapid transformation of urea into ammonium carbonate in stale urine. $O=C\begin{matrix}NH_2\\NH_2\end{matrix}$ with one molecule of H_2O becomes $O=C\begin{matrix}ONH_4\\NH_2\end{matrix}$ or ammonium carbamate, and this, by addition of a second molecule of water, becomes $O=C\begin{matrix}ONH_4\\ONH_4\end{matrix}$ 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 ammelid, 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:

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

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

Biuret, $H\text{–}N\langle^{CO\text{–}NH_2}_{CO\text{–}NH_2}$, may be obtained by heating urea. When pure, it occurs as white, needle-shaped crystals. With NaOH and 1% CuSO it gives the characteristic violet and rose-red shades obtained in the biuret reaction (Piotrowski's proteid test). Exp. 74, page 185.

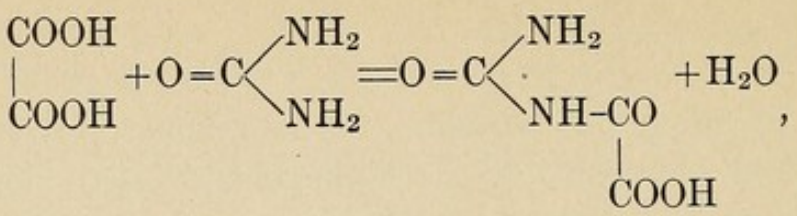
Urea Nitrate may be precipitated from fairly concentrated urine by addition of HNO_3. It separates in hexagonal crystals or plates, easily recognizable under the microscope (Plate V, Fig. 3).

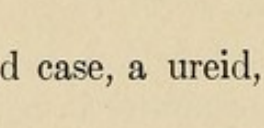
Urea Oxalate.—Upon addition of a solution of oxalic acid to concentrated urine, crystals of oxalate of urea are precipitated. They are rather more easily obtained in characteristic forms (Plate III, Fig. 4) than are the crystals of nitrate, and, in consequence, treatment with oxalic acid constitutes 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.—The hydrogen of the amido group may be replaced by alcohol radicals forming what are known as alkylated ureas; thus, $O{=}C\langle^{NH_2}_{NHCH_3}$ is methyl urea, $O{=}C\langle^{NH_2}_{NHC_2H_5}$, ethyl urea, and one, two, three or all four of the H atoms may be so replaced.

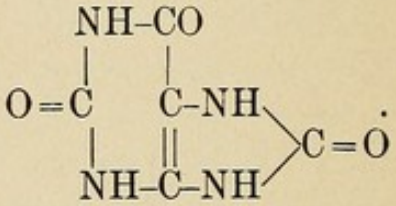
When, in place of an alcohol radical, the *acid* radical is introduced, a class of compounds known as "ureids" result; thus, $O{=}C\langle^{NH_2}_{NH(C_2H_3O)}$ (acetyl urea).

In case of a dibasic acid, such as oxalic, $\begin{matrix}COOH\\|\\COOH\end{matrix}$, entering into the reaction, one or both (OH) groups may be split off, forming in the first instance a ureid acid, as $O{=}C\langle^{NH_2}_{NH.CO,COOH}$, oxaluric acid,



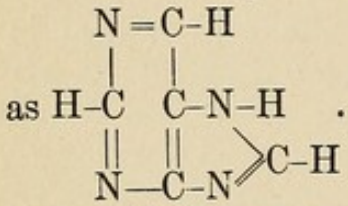
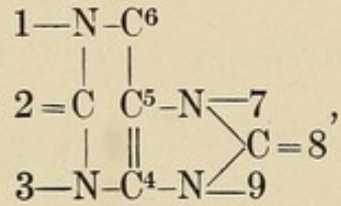
or, in the second case, a ureid, as  parabanic acid.

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

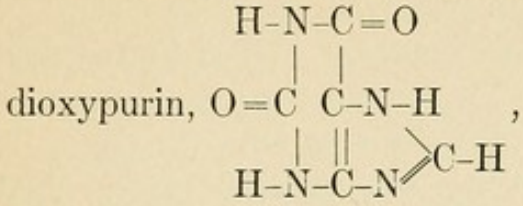
Uric Acid, trioxypurin, $C_5H_4N_4O_3$. Its relation to urea may be shown by the graphic formula  .

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

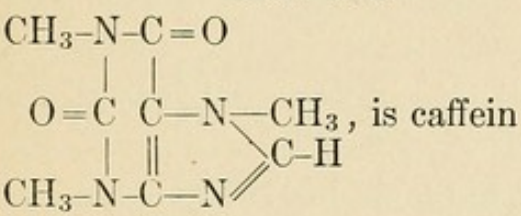
These bases are of great physiological interest, in that they form an unquestioned link between the decomposition products of the proteids, nuclein, etc., on the one hand, and uric acid and the urates on the other.

Purin is represented by the formula $C_5H_4N_4$, or graphically as  . If we now break all double bonds except those linking two carbon atoms (4 and 5), we obtain a graphic nucleus,  , 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 imido, 6 oxypurin), adenin (6 amido purin), and heteroxanthin (7 methyl xanthin) have been found in urine, and, in cases of leukæmia, many of them in increased amounts, notably xanthin, hypoxanthin, and adenin (Witthaus).

Uric acid occurs in the urine, traces in the blood and occasionally in saliva as urates. It is a dibasic crystalline acid, colorless when pure; but in urinary sediment it occurs generally as crystals yellow to red, "whetstone"-shaped, and in various other forms (Plate V, Figs. 5 and 6). The "brick-dust" deposit occasionally found in urine consists of uric acid. It is insoluble in alcohol and nearly insoluble in water; but its solubility in water is increased by the presence of urea.

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.

Cl hot yields cyanuric acid, $C_3H_3(OH)_3$. Cl or Br cold

forms oxalic acid, alloxan, $(CO\langle^{NHCO}_{NHCO}\rangle CO)$, parabanic acid, $(CO\langle^{NH-CO}_{NH-CO}\rangle)$ and ammonium cyanate. HNO_3 in the cold forms alloxan, alloxantin, and urea (Witthaus).

Uric acid may be detected by the murexid test. See Exp. 39, page 160.

Note.—Murexid is a definite chemical compound ($C_8H_5N_5O_6$) and may be produced from alloxantin, an oxidation product noted above.

While uric acid is practically insoluble in H_2O and the acid 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 insolu-

bility 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, p. 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.'"

Experiments with Cyanogen Compounds and Urea.

Exp. 30. In a test-tube dissolve ½ gram or *less* of potassium ferrocyanid in about 4 c.c. of H_2O. Add a little H_2SO_4 and boil, conducting the gas evolved into another test-tube 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.)

$$2K_4FeCy_6 + 6H_2SO_4 = K_2Fe(FeCy_6) + 6KHSO_4 + 6HCy.$$

Exp. 31. 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. 32. To the other half of the HCy from Exp. 30 add a little solution of ferrous sulphate; also a few drops of ferric chlorid solution; then a little KOH solution; mix thoroughly and acidify with HCl, a blue precipitate, in $Fe_4(FeCy_6)_3$. This constitutes a test for HCy or any soluble cyanid.

Exp. 33. To a few drops of KCN solution add a little yellow ammonium sulphid, $(NH_4)_2S$, and evaporate to dryness. Dissolve in water; acidify with HCl and add Fe_2Cl_6 solution.

Exp. 34. 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 154.)

Exp. 35. Heat a few crystals of urea in a test-tube till it fuses and no more gas is given off; cool, and dissolve the fused mass in water; add one or two c.c. of strong NaOH solution, then not more than one or two drops of a 1% $CuSO_4$ 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:

$$2\,O{=}C\begin{matrix}NH_2\\NH_2\end{matrix} = \begin{matrix}O{=}C\begin{matrix}NH_2\\NH\end{matrix}\\O{=}C\begin{matrix}\diagup\\NH_2\end{matrix}\end{matrix} + NH_3.$$

Exp. 36. Produce crystals of urea nitrate and oxalate (page 155) and examine under the microscope. Repeat with urea obtained from urine.

Exp. 37. 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 154.

Exp. 38. Heat a third of a test-tube of urine with barium hydroxid (baryta-water); test vapor with red litmus for NH_3.

Exp. 39. *Murexid test* for uric acid: Place a very small quantity of uric acid on a porcelain crucible cover, or in a small evaporating-dish. Add two or three 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 purple-violet upon further addition of a drop of KOH solution, the color disappearing

* For method of making potassium cyanate, see Preparation of Reagents and Organic Compounds, in the Appendix.

upon standing or upon the application of heat. (Difference from xanthin, which also gives a much redder color.)

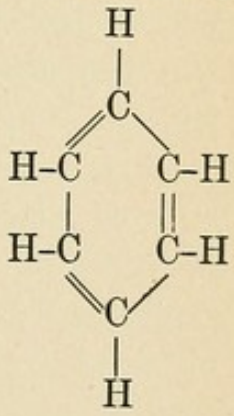
Exp. 40. Repeat No. 37, using caffein in place of uric acid.

Exp. 41. 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 158).

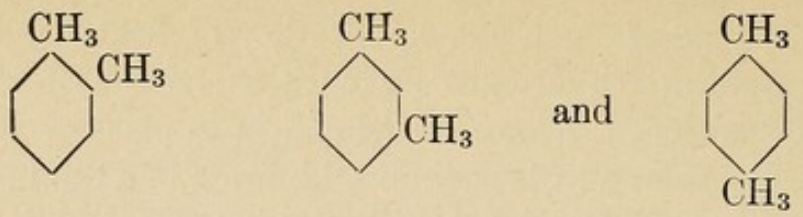
SECTION VI.—CLOSED-CHAIN HYDROCARBONS.

In illustrating the simpler relationship 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 which we are to consider.

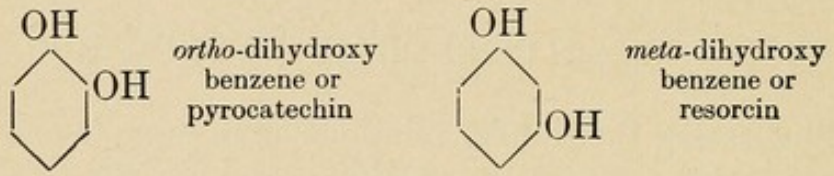
The hydrocarbons of the aromatic series have, for a general formula, C_NH_{2N-6}, the simplest being *benzene* or benzol, C_6H_6; and from this we may consider that the aromatic compounds are derived. The structure of the benzene molecule is represented by "Kekulé's" benzene ring. Note that there are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



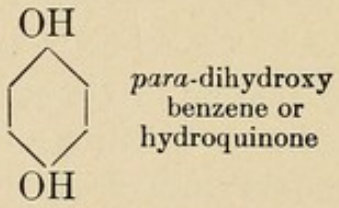
The next higher homologue of the series will be C_7H_8; this is methyl benzene, ($C_6H_5CH_3$), or toluene. The dimethyl benzenes are the xylenes, three in number, $C_6H_4(CH_3)_2$, or C_8H_{10}. Note the possibility here existing for isomeric compounds, as follows:



These three possible positions of the *second* substitution are known as ortho-, meta-, and para-; thus, in the more familiar dihydroxybenzene we have



and



Of these three dihydroxybenzenes, the ortho compound, pyrocatechin, is of particular interest. 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 a greater part (60 to 90 per cent U. S. D.). Guaiacol and various compounds produced from it have been widely recommended for tubercular diseases.

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

Benzene, C_6H_6, is a colorless liquid from the "light-oil" obtained by distillation of coal-tar. It boils at 80°, has a gravity of 0.899, soluble in ether, alcohol, and chloroform, but insoluble in H_2O. It may be made pure by distilling an intimate mixture of benzoic acid and quicklime, and at a tem-

perature of about 5° C. may be obtained as a crystalline solid, $C_6H_5COOH + CaO = CaCO_3 + C_6H_6$. (See Exp. 40.)

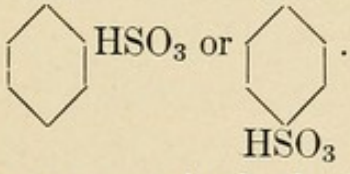
Nitro-benzene, $C_6H_5NO_2$, may be produced by treating benzene with a mixture of nitric and sulphuric acid at reduced temperature. (Exp. 42, page 167.) It is a yellow oily liquid with the odor of bitter almonds, commercially known as oil of mirbane, and used in the manufacture of aniline.

Amido-benzene, $C_6H_5NH_2$. By reaction of nitrobenzene with nascent hydrogen, the NO_2 group becomes an NH_2 group and amidobenzene or aniline is produced. Aniline is a colorless liquid, also called aniline oil, and is the basis of a large number of complex compounds, the aniline dyes.

Methyl-benzene or Toluene, $C_6H_5CH_3$, is a colorless liquid obtained from coal-tar and a valuable reagent in organic chemistry. It may be oxidized to benzoic acid, C_6H_5COOH.

Dimethyl-benzene or xylene, $C_6H_4(CH_3)_2$, is a valuable solvent much used in chemical and bacteriological laboratories. There are three isomeric forms, the ortho-, meta-, and para-.

Phenol, carbolic acid, is oxybenzene, C_6H_5OH, obtained from the distillation of coal-tar, and used as an antiseptic and disinfectant. For properties and test, see page 111. Phenol acts like an acid, in that it forms salts with the metallic bases, C_6H_5OK, potassium phenolate.

Phenol Sulphonic Acid.—When phenol is treated with several times its volume of cold, strong H_2SO_4, phenol sulphonic acid results, 

or

. If the mixture is heated for some time over a water-bath, the disulphonic acid results. This acid, warmed with a nitrate and the mixture treated with excess of ammonia, yields ammonium picrate, and constitutes a delicate test for nitrates present in drinking-water. (See Exp. 49, page 168.)

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.

Picric Acid is trinitrophenol, $C_6H_2.OH.(NO_2)_3$. It may be formed by action of strong HNO_3, or mixture of H_2SO_4 and HNO_3 on phenol. It occurs as yellow plates slightly soluble in H_2O, 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.

Phloroglucin, used in physiologial chemistry as a reagent with vanillin, is a trihydroxybenzene, $C_6H_3(OH)_{3(1.3.5)}$. It crystallizes in rhombic prisms, soluble in water, alcohol, and ether.

Benzoic Acid, C_6H_5COOH, originally produced from gum benzoin, but may be made from hippuric acid (q. v.), and this (from urine of horses) formerly constituted a commercial source. It is chiefly prepared, however, from toluene; it crystallizes in colorless plates or long prismatic crystals (from solution). Sparingly soluble in cold water, more soluble in hot water, and easily in alcohol. It sublimes and is inflammable, burning without residue.

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

Benzaldehyd, C_6H_5–CHO, is a colorless liquid, soluble in alcohol and ether, and sparingly in water. The U. S. P. oil of bitter almonds is practically benzaldehyd; it is a volatile oil, very poisonous, and upon standing deposits benzoic acid from partial oxidation.

Salicylic Acid, orthohydroxybenzoic acid, C_6H_4–OH.COOH, a white crystalline powder, odorless, irritating to mucus 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 CO_2 on sodium phenate and subsequent decompo-

sition of the sodium salicylate. By heating rapidly the acid may be changed into carbolic acid and CO_2.

Salicylates have been used to considerable extent in various uric-acid diseases. Methyl salicylate constitutes 90% of natural oil of wintergreen (page 138). The alcoholic solution is essence of checkerberry.

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

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

Anilin, amidobenzene, $C_6H_5NH_2$, may be made by reduction of nitrobenzene by nascent hydrogen. This substance is important from a commercial rather than from a medical standpoint, as it forms the basis of the anilin dyes. When pure it is a colorless liquid, but changes quite rapidly when exposed to the light. Used in testing for chloral and chloroform. Slightly soluble in water, and easily soluble in alcohol and ether. At 8° C. it becomes a crystalline solid.

Hippuric Acid, benzoyl glycocoll, $C_6H_5.CO.NH.CH_2–COOH$, occurs in traces in human urine, to a considerable extent in the urine of the herbivora, and not at all in that of the carnivora. It crystallizes in prismatic needles (Plate V, Fig. 4), 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 hydrolysed by the urease of fermenting urine, forming benzoic acid and glycocoll (amido-acetic acid):

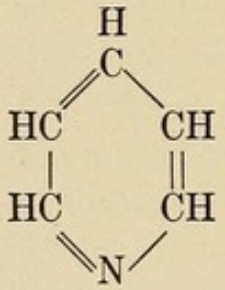
$$C_6H_5.CO–NH–CH_2–COOH + H_2C$$
$$= C_6H_5COOH + CH_2NH_2COOH.$$

Tryosin, $C_6H_4.OH.–CH_2CH(NH_2)–COOH$, may be crystallized as fine silky needles. It is formed from proteid sub-

stance, particularly casein and fibrin, both by the action of proteolytic enzymes and by putrefactive processes. It rarely occurs in urinary sediment in bundles or sheaves (Plate VI, Fig. 1), and when found it is usually indicative of acute liver disease, phosphorus poisoning, etc.

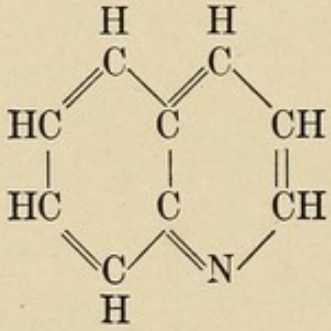
Heterocyclic Compounds.—The closed-chain or cyclic compounds are known as isocyclic or homocyclic when the atoms constituting the "ring" or nucleus of the molecule are all of the same sort (carbocyclic, if all of carbon), as has been the case in all the aromatic compounds which we have thus far taken up, i.e., the structure of compounds has been based upon the six-carbon or benzene ring. If the ring is made up of atoms of different sorts the compound is heterocyclic, and one or two of these are of importance.

First, pyridin, C_5H_5N, which may be regarded as benzene in which one CH group has been replaced by an atom of nitrogen:



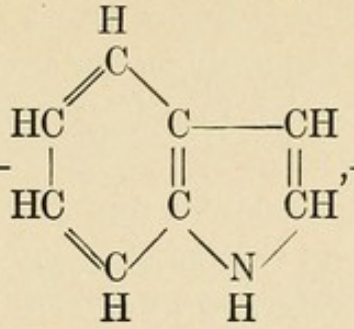
It is a liquid miscible with water, boiling-point 115° C.

Second, quinalin, C_9H_7N, a colorless liquid.



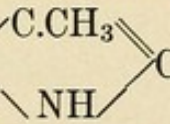
And upon one or the other of these two bases may be constructed the graphic formula of many of the vegetable alkaloids.

A certain number of alkaloids, such as caffein, thein (trimethylxanthin), are referable to the purin nucleus (page 156).

Indol, C_8H_7N,—  —is produced from pro-

teid 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 K_2SO_4, forms indoxyl-potassium sulphate, $C_8H_6NKSO_4$, and as such is eliminated (in part) by the kidneys. 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 $BaCl_2$ in alkaline solutions, but may be decomposed by prolonged boiling with HCl and then precipitated as usual.

The oxidation of indoxyl produces indigo blue, and this fact is utilized in the qualitative test for indoxyl in urine (q. v.).

Skatol, methylindol, C_6H_4  , occurs in similar

manner to indoxyl, and likewise passes into the urine as an ethereal sulphate (skatoxyl-potassium sulphate). Skatol is a constituent of the fæces and possesses a strong fæcal odor.

Experiments with Aromatic Hydrocarbons.

Exp. 42. Into a small and thoroughly dry flask (250 c.c.) introduce about 50 grams of a mixture consisting of one part of benzoic acid and two parts of quicklime; connect with a condenser and heat. Benzole (benzene) distils over:

$$CaO + C_6H_5COOH = CaCO_3 + C_6H_6.$$

Exp. 43. 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 little pure benzene by immersing a few cubic centimeters, contained in a narrow test-tube, in a freezing mixture of ice and salt.

Exp. 44. 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. 45. To a few cubic centimeters of a 3% carbolic acid solution add dilute bromin water. A yellowish-white crystalline precipitate of tribromphenol is produced. (See page 111.)

Exp. 46. To an aqueous solution of carbolic acid add a few drops of solution of ferric chlorid.

Exp. 47. Boil 15 c.c. oil of wintergreen with 20% 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. 48. To a dilute solution of sodium salicylate, or saturated aqueous solution of salicylic acid, add a few drops of Fe_2Cl_6. Slight amount of salicylates in the urine will produce this color when test is being made for diacetic acid (q. v.).

Exp. 49. Evaporate a few drops of a 1% 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 and 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.

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* For method of preparation of phenoldisulphonic acid, see Appendix.

PART VI.

PHYSIOLOGICAL CHEMISTRY.

SECTION I.—FERMENTS.

THE study of Physiological Chemistry includes the substances which 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, with a view simply to enable 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 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,* and they may be classified as organized and unorganized ferments.

The organized ferments are possessed of a definite organism and are represented by the yeast-plant, the lactic acid bacillus, and many other micro-organisms capable of bringing about these changes. The unorganized ferments are known as ENZYMES, and are of the nature of protein substances lacking a specific organization. They exist within the cell, oftentimes as a *zymogen* or parent-enzyme, from which the enzyme itself

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

is produced, as illustrated by the pepsinogen (zymogen) existing in the stomach-wall, which, by action of HCl of the gastric juice, becomes the pepsin (enzyme) in the stomach (q. v.).

"Hydrolysis" is a term used to describe the breaking-up of complex molecules and the utilization of a molecule of water in the production of the new compounds. 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, $C_6H_{12}O_6$. $C_{12}H_{22}O_{11} + H_2O = 2C_6H_{12}O_6$. Many enzymes produce molecular changes in this way, and in consequence are called hydrolytic enzymes. The name of the substance acted upon may also be used to designate an enzyme; thus a proteolytic enzyme produces a cleavage of proteid substances. A lipolytic enzyme (lipase) splits the fat molecule, etc.

The name of a specific enzyme usually ends in "ase," as lipase (as above); zymase, the enzyme contained in yeast; urease, the urine ferment, etc. Their action is effected in marked degree by the character of the media in which they work, and by the temperature employed. Hydrolysis may also be brought about by chemical action, as illustrated by the conversion of starch into glucose by boiling with very dilute mineral acid, HCl or H_2SO_4.

SECTION II.—CARBOHYDRATES.

Classification:

Sugars	Dextrose, Lævulose, Galactose	Monosaccharids.
	Saccharose, Maltose, Lactose	Disaccharids.
Starch	Starch, Glycogen	Polysaccharids
Gum	Dextrin	
Cellulose		

Characteristics.—The monosaccharids 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 lævulose is a "ketose." The monosaccharids above mentioned have the formula $C_6H_{12}O_6$. 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).

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

The polysaccharids of the above group have the general formula $(C_6H_{10}O_5)x$. They lack the sweet taste which the sugars possess in varying degrees. They may all be converted by action of acids into monosaccharids, although the change is effected in cellulose with some difficulty.

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 disaccharids; also from many of the polysaccharids. 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 when hydrolysis takes place, as follows: $C_6H_{10}C_5 + H_2O = C_6H_{12}O_6$. Glucose contains an aldehyde group, $-COH$, in consequence of which it is sometimes termed an aldose, in distinction from the ketones, which contain the $\overset{|}{C}O$ group, of which lævulose is an example.

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

Glucose responds to Molisch's test for carbohydrates, which is made with an alcoholic solution of α-naphthol and concen-

trated sulphuric acid. (Exp. 51.) It may be distinguished not only from other carbohydrates but from other sugars by heating with Barfoed's solution (copper acetate in dilute acetic acid), which is reduced with precipitation of Cu_2O.

Heated with phenylhydrazine solution nearly to the boiling-point of water, glucose forms phenylglucosazon, which crystallizes, as the mixture cools, in characteristic yellow needles, usually arranged in bundles or sheaves. (Plate VI, Fig. 2.)

Osazones are the various compounds formed by the different sugars and phenylhydrazine when treated as above. They crystallize in fairly distinctive forms and furnish valuable tests for the sugars, this method being considered at least ten times more delicate than Fehling's test. Glucose readily undergoes alcoholic fermentation, yielding C_2H_5OH and CO_2. (See Exp. 58.)

Lævulose, $C_6H_{12}O_6$, 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. Lævulose 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.

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.

Cane-sugar, $C_{12}H_{22}O_{11}$, sucrose or saccharose, obtained from the sugar-cane (various varieties of sorghum), also from the sugar-beet (*Beta vulgaris*) and the sugar-maple (*Acer saccharinum*). Cane-sugar is a white crystalline solid soluble in about $\frac{1}{2}$ 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 phenylhydrazin; but it is easily hydrolyzed with the formation of dextrose and lævulose, 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 lævulose as above, these latter sugars being easily fermentable.

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: $C_{12}H_{22}O_{11} + H_2O = 2C_6H_{12}O_6$. It is formed in the fermentation of barley by diastase (the ferment of malt), and with phenylhydrazine it produces an osazone distinguished from glucosazone and lactosazone by its microscopical appearance (Plate VI, Fig. 3) and its melting-point.

Lactose, $C_{12}H_{22}O_{11}$, obtained from milk, is a disaccharid with far less sweetening power than sucrose. It forms an osazone which crystallizes in small burr-shaped forms (Plate VI, Fig. 4). 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.

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 VII.)

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 chlorid of zinc or of magnesium, dilute HCl or H_2SO_4, 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 iodin (Exp. 169, *c*); next *erythrodextrin*, which gives a red color with iodin solution; then *achroo-* and *maltodextrin*, which give no color with iodin, but react slightly with Fehling's copper

solution; then *maltose,* also negative with iodin, 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; is formed from starch, as indicated above; constitutes to a considerable extent the "crust" of bread; is soluble in water, the solution giving a red color with iodin; and is further 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 oyster is 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% 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 proteids. 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 fibre, and which may be found in the laboratory in nearly a 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. It is converted into monosaccharids by acids

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* Foster's Text-book of Physiology.

only after first treating with concentrated H_2SO_4, which partially dissolves it. Cellulose aids digestion in a purely mechanical way; treated with a mixture of nitric and sulphuric acids, it is converted into nitro-substitution products which are known as guncotton. The soluble cotton from which collodion is prepared is a mixture of tetra- and pentanitrates, while the more explosive but insoluble guncotton is a hexanitrate, formerly known as trinitrocellulose.

Experiments with Carbohydrates.

Monosaccharids.—Exp. 50. 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 page 123.) Write reactions.

Exp. 51. *Molisch's Test for Carbohydrates.*—To a few cubic centimeters of a 3% glucose solution add a few drops of an alcoholic solution of α-naphthol, and carefully underlay the mixture with strong H_2SO_4.

Exp. 52. To a few cubic centimeters of $CuSO_4$ solution in a test-tube add a little NaOH. Boil and write reaction.

Exp. 53. Repeat Exp. 52 with the addition of Rochelle salt; if solution remains clear on boiling, add a few drops of a glucose solution.

Exp. 54. *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.

Exp. 55. Repeat Exp. 54, using diabetic urine.

Exp. 56. Repeat Exp. 54 without heat and allow to stand twenty-four hours.

Exp. 57. *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.

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* For preparation, see Appendix.

Exp. 58. *Fermentation Test.*—Fill the "fermentation-tube" (Fig. 14) 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.

FIG. 14.

Exp. 59. *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.

Disaccharids.—Exp. 60. Use dilute solutions of cane-sugar, milk-sugar, and maltose, and make on each Fehling's test (Exp. 54), Barfoed's test (Exp. 57), and the phenylhydrazine test (Exp. 59). Sketch the different osazone crystals obtained.

Exp. 61. 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. 54–57 and 59. Explain results.

Polysaccharids.—Exp. 62. Examine potato, corn, and wheat starch under the microscope. Sketch the granules of each in note-book, and, while still on the slide, treat with a dilute iodin solution. Note changes in appearance of granules.

Exp. 63. 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% solution of dextrin and a solution of glycogen * as follows:

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* For the isolation of glycogen, see Appendix.

(*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 iodin 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% alcohol.

(*e*) Tabulate results of the tests, and formulate method of distinguishing these three substances from one another.

SECTION III.—FATS AND OILS.

Natural fats and oils of animal or vegetable origin are mixtures of several compound glyceryl ethers or esters (see page 139), and by subjecting them to cold and pressure they may be separated into two portions, one solid with comparatively high melting-point, and the other liquid at ordinary temperature. 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 stearopten 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 carbondisulphid, less easily by alcohol; crystallizing on evaporation of the solvent. (Plate VI, Fig. 5.) 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. (For saponification of fats see page 140 and Exp. 67 below.)

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).

Experiments with Fats and Oils.

Exp. 64. Test solubility of olive-oil in water, ether, chloroform, and alcohol, carefully avoiding the vicinity of a flame.

Exp. 65. 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. 66. Dissolve a little solid fat in warm alcohol and examine with the microscope, and micropolariscope the crystals, which separate on cooling.

Exp. 67. *Saponification.*—To about 2 grams of solid fat (butter or lard), placed in a narrow beaker, add 10 or 15 c.c. of alcoholic solution of potassium hydroxid. 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 chlorid. What takes place?

(*b*) To another portion add a few cubic centimeters of a solution of calcium or magnesium chlorid. 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 filtrate.

Exp. 68. Fatty acids.

(*a*) Dissolve a portion of the above precipitate (67, *c*) by warming with strong alcohol. Test the reaction of the solution. Examine the crystals, which separate upon standing, with microscope and micropolariscope. (Plate VI, Fig. 6.)

(*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 this.

Exp. 69. Neutralize the filtrate of 67, (*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 glycerine on a platinum loop: green bead.

Exp. 70. *Emulsification.*—(*a*) Put 1 to 2 c.c. of a solution of sodium carbonate (0.25%) in a watch-glass, and place in the centre of 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% Na_2CO_3 solution + 2 drops neutral oil.
2. 10 c.c. of a 0.2% Na_2CO_3 solution + 2 drops rancid oil.
3. 10 c.c. of soap-jelly (see 68 *b*) warm + 2 drops neutral oil.
4. 10 c.c. of albumin solution + 2 drops neutral oil.
5. 10 c.c. of gum-arabic solution + 2 drops neutral oil.
6. 10 c.c. of water + 2 drops 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.

SECTION IV.—PROTEIDS.

Protein or proteid substances are general terms used to designate the nitrogenized bodies which constitute the greater proportion of animal tissue. There have been many classifications of the proteids, some based solely on their physical properties, others based on what we know of their chemical composition. This latter method has the advantage of being the more scientific, but is not entirely satisfactory owing to our very limited knowledge. The most useful system at present is a combination of both of the above methods. The following is after Hofmeister, "Ergebnisse der Physiologie," Jahrg. I.

The following chemical groups have been isolated from the true proteid:

I. Groups of the Aliphatic Series.

A. Groups containing C, N, H.

The only representative known is the guanidin radical $(CNH) \cdot NH_2$.

B. Groups containing C, N, H, O.

1. Amido-acids.

(*a*) Monamido-acids

1. Monobasic monamido-acids, $C_nH_{2n+1}NO_2$.

C_2 is glycocoll.

C_3 is alanin.

C_5 is amidovalerianic acid.

C_6 is leucin, which occurs universally.

2. Dibasic monamido-acids, $C_nH_{2n-1}NO_4$.

C_4 is asparaginic acid.

C_5 is glutaminic acid.

(*b*) Diamido-acids (all monobasic acids).

C_2 is diamidoacetic acid (rare).

Argynin (guanidin-α-amidovalerianic acid). Here the diamido-acid is combined with the guanidin radical,

$$NH_2 \cdot NH \cdot C \cdot NH \cdot CH_2 \cdot (CH_2)_2 \cdot CH \cdot NH_2COOH.$$

Lysin (α-ε-diamidocapronic acid),

$$NH_2 \cdot CH_2 \cdot (CH_2)_3 \cdot CH \cdot NH_2 \cdot COOH.$$

2. Amido-alcohols.

Glucosamin, $C_6H_{11}O_5(NH_2)$, a hexose into which NH_2 has entered the carbohydrate group of the proteid molecule.

C. Groups containing C, N, H, O, S.

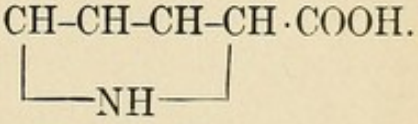
Cystein, amidothiolactic acid, $CH_2 \cdot SH \cdot CH(NH_2) \cdot COOH$.
Cystin, the sulphid of cystein, $C_6H_{12}S_2N_2O_4$.
α-thiolactic acid.

II. Groups of the Aromatic Series.

A. Phenylalanin, $C_6H_5 \cdot CH_2 \cdot CH(NH_2) \cdot COOH$.
B. Tyrosin, $C_6H_4 \cdot OH \cdot CH_2 \cdot CH(NH_2) \cdot COOH$.

III.

A. Pyrrol group.

1. α-pyrrolidin carbonic acid, 

B. Indol group.

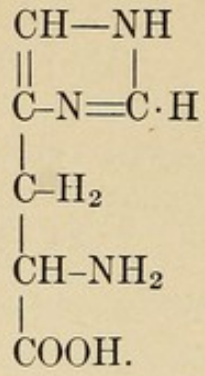
1. Indol, see page 167.
2. Skatol (methyl indol), see page 167.
3. Tryptophan (indolamidopropionic acid), $C_{11}H_{12}N_2O_2$.
4. Skatosin, $C_{10}H_{16}N_2O_2$.

C. Pyridin group.

Pyridin, see structural formula on page 166.

D. Pyrimidin group.

Histidin: structural formula probably



Excepting the carbohydrate group, and perhaps the pyridin and pyrimidin groups, absent in a few special instances, all typical proteids contain at least one representative from each group.

The further partial classification, based upon physical and chemical properties rather than chemical composition, has been adopted for the work, as follows:

A. Simple Proteids.

Albumin: Serum albumin, Egg-albumin, Lactalbumin (Myo-albumin).

Globulin: Serum globulin, Fibrinogen, Myosinogen, Myoglobulin.

Histon.

B. Derived Proteids

Through action of enzymes: Fibrin, Myosin.

Through action of acids, etc.: Acidalbumin, Syntonin, Alkali-albuminates.

Through action of digestive ferments: Proteoses, Albumose (Deutero-, Hetero-, etc.), Globulose, Myosinose, etc., Peptones.

C. Proteïde, or Compound Proteid.

Nucleoproteid: Nucleohiston.
Nuclein.
Glycoproteid: Mucin and others.
Nucleoglycoproteid.
Chromoproteid: Hæmoglobins.

D. Pseudo-Nucleoalbumin.

Casein: Ovovitillin.

E. Albuminoids.

Collagen.
Elastin.
Keratin.
Reticulin and others.

Albumins.

The albumins are conveniently represented by egg-albumin and serum-albumin. They are soluble in water, respond to the general proteid reactions (Exp. 71, 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 albumins known as acid or alkali albumin or albuminate. 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.

If the hydrolysis of albumin is brought about by HCl at the body temperature, it causes the molecule to split into two proteids, one known as antialbuminate and the other as hemi-

albumose, these in turn becoming respectively antialbumid and hemipeptone. Sulphuric acid at a boiling temperature produces a similar change, except that the hemipeptone is further changed to leucin and tyrosin. Digestive ferments, pepsin, and trypsin produce antialbumose, hemiantipeptone, and hemialbumose, but trypsin alone converts the hemipeptone into leucin and tyrosin.

Albumin normally occurs in all the body fluids except in 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 is always abnormal, although in the majority of cases it has no serious significance unless present in more than the slightest possible trace.

Globulin occurs associated with albumin in blood-plasma. It 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 proteid tests and reactions. The albumin of albuminous urine always consists of a mixture of these two proteids, 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 chlorid, will serve to effect the solution. Globulin is thrown out of solution by action of carbon dioxid as a white flocculent precipitate. By dialysis the inorganic salts necessary for its solution will be removed and the proteid will be precipitated. It is also thrown out by saturation of sodium chlorid or magnesium sulphate. Globulin is coagulated by heat at practically the same temperature as serum-albumin, i.e., 75° C.

General Proteid Reactions.

Exp. 71. Test dried egg-albumin for C, H, S, and N, according to the methods described on pages 123 and 124. Test casein for phosphorus, and dried blood for iron.

There are several reactions which are common to nearly all

proteids. For the following tests use a solution of egg-albumin (1/50) in water, as a general type of a proteid.

1. *Color Reactions.*

Exp. 72. *Xanthoproteic Test.*—To 10 c.c. of the 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 proteid 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. 73. *Millon's Test.*—Add a few drops of Millon's reagent* to a part of the albumin solution. A precipitate forms, which becomes brick-red upon heating. The liquid is only colored red in the presence of non-coagulable proteid or minute traces of albumin.

Exp. 74. *Piotrowski's Test.*—To a third portion add two drops of a *very* dilute solution of $CuSO_4$, and then 5 to 10 c.c. of a 40% solution of NaOH. The solution becomes blue or violet. Proteoses and peptones give a rose-red color (biuret 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 proteids. This test responds with all proteids.

2. *General Precipitants.*

Proteids are precipitated from solution by the following reagents (peptones are exceptions in some cases):

Exp. 75. *Acetic Acid and Potassic Ferrocyanid.*—Make a portion strongly acid with acetic acid, and add a few drops of potassic ferrocyanid solution. A white flocculent precipitate is formed (not with peptones).

Exp. 76. *Alcohol.*—Add one or two volumes of alcohol.

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* Mercuric nitrate in nitric acid. For the preparation of this and other reagents, see Appendix.

Exp. 77. *Tannic Acid.*—Make the solution acid with acetic acid, and add a few drops of tannic-acid solution

Exp. 78.—*Potassio-mercuric Iodid.*—Make acid another portion with HCl, and add a few drops of the reagent.

Exp. 79. *Neutral Salts.*—Certain neutral salts precipitate most proteids. $(NH_4)_2SO_4$ added to complete saturation to proteid solutions, faintly acid with acetic acid, precipitates all proteids, with the exception of peptones.

Experiments with Albumin and Globulin.

The albumins and globulins respond to all the foregoing general reactions.

Exp. 80. 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 (1/10) solution of egg-albumin.

Exp. 81. *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 proteids respond to this test?

Exp. 82. *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 proteids respond to this test?

Exp. 83. *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, 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. 84. *Action of* $MgSO_4$.—Perform an experiment similar to Exp. 83, using solid $MgSO_4$ instead of $(NH_4)_2SO_4$. With what results?

Exp. 85. *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?

Globulins.

The following tests serve to distinguish the globulins from other proteids. The tests are made upon blood-serum, which contains an albumin (serumalbumin) as well as a globulin (paraglobulin).

Exp. 86. *Action of CO_2.*—To 5 c.c. of the 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. 87. *Precipitation by Dialysis.*—Into a parchment dialyzing-tube, previously soaked in distilled water, pour 20 c.c. of the 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. 88. Pour serum, drop by drop, into a large volume of distilled water (in a beaker). What takes place? Explain.

Exp. 89. *Precipitation by Magnesium Sulphate.*—Saturate about 5 c.c. of the serum with 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.

Albuminates.

1. *Alkali Albuminate.*

Exp. 90. To a solution of egg-albumin add a few drops of a 0.5% solution of NaOH, and warm gently for a few minutes. With the solution of alkali albuminate thus obtained perform the following tests:

(*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.

2. *Acid Albuminate.*

Exp. 91. Add a small quantity of a 0.2% 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% 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 albuminates, and compare results. How distinguish between them?

The Proteoses (albumoses) may be considered as the next well-defined proteid product of proteid digestion following the albuminate. That is, leaving out the many intermediate products between which sharp lines of demarkation 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 proteids at a corresponding point of decomposition or peptic digestion. Albumose may be coagulated by heat at a temperature ranging upwards from 56° C., but, unlike albumin, as the temperature approaches the boiling-point the albumose goes again into solution, and at a boiling temperature may be separated from albumin by filtration. As the filtrate cools,

albumose will again precipitate. The albumose is also precipitated by nitric acid, by ferrocyanid of potassium and acetic acid (the precipitate in both cases being dissolved by heat), and the other general proteid precipitates. 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 proteids.

Peptones are the final products of *peptic* digestion of the proteids. They are soluble substances which give the biuret test similarly to the proteoses, but are not precipitated by heat, by nitric acid, by potassium ferrocyanid and acetic acid, nor by saturation with ammonium sulphate.

Experiments with Albumose and Peptone.

Albumoses (Hemialbumose).—This name includes four colsely 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. 92. Make a solution 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. 72, 73, 74, 81, 82. If no precipitate forms with HNO_3 in Exp. 81, add a drop or two of a saturated solution of common salt. (Deuteroalbumose gives this reaction only in the presence of HCl.)

Exp. 93. Saturate some of the solution with $(NH_4)_2SO_4$. Report the result.

Exp. 94. To some of the solution add 2–3 drops of acetic acid, and then a saturated solution of NaCl. A precipitate forms, which dissolves on heating, to reappear on cooling.

Exp. 95. Using the peptone solution prepared in manner above described from commercial peptone, repeat the experiments indicated in Exp. 92.

Exp. 96. *Effect of heating.*—Boil some of the peptone solution. Report the result.

Exp. 97. *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. 98. *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 proteids. A report of the results, together with the methods employed, is to be made.

Casein is the principal proteid found in milk. It exists in combination with calcium salts as caseinogen. This combination is broken up and the casein precipitated by the action of rennin and other enzymes, by acids, and by certain inorganic salts.

Casein is classified as a pseudo-nucleo-albumin. The nucleoproteids, so named because true nuclein may be obtained from them, are constituents of the cell nuclei, and differ in composition from ordinary proteids by containing from 0.5 to 1.6% of phosphorus. Casein from cow's milk contains, according to Hammarsten, 0.85% of phosphorus. It is a *pseudo*-nucleoalbumin 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 *proteid* substance worthy of note in milk. This may be found in the filtrate after separating the casein. The total proteids contained in human milk average from 1.5 to 2.5 per cent., while in cow's milk the proteids 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 proteids), and of cream and milk-sugar (to increase fat and lactose).

The following table shows comparative composition:

	Reaction.	Total Solids.	Proteids.	Sugar.	Fat.	Ash.
Human milk.....	Alkaline	13.00%	2.70%	6.10%	4.00%	.20%
Cow's milk.....	Acid	14.00%	4.15%	4.90%	4.25%	.70%

Mucins have been classed as glycoproteids, indicating that by hydrolytic cleavage (from heating with dilute mineral acids) they will yield a simple proteid and a carbohydrate resembling glucose in its chemical reactions, particularly toward alkaline copper solutions. Other glycoproteids, many of which have been classified as mucins, are the mucoids, resembling mucin, and the chondroproteids, which yield chondroitin.

The true mucins form a mucilaginous solution, and are precipitated by acetic acid; contain C, H, N, S, and O. Mucoids are not precipitated by acetic acid, while the chondroproteids may be.

The "mucin" obtained from tendons, and much used to study the physical properties of the mucins, is a chondroproteid. Mucin from the navel-cord is probably a true mucin, and furnishes the most convenient supply for laboratory investigations.

The mucin from the submaxillary gland is (according to Hammarsten) also a true mucin, although the statement has been disputed.

Mucin is not coagulated by heat, but responds to the general proteid reactions. It is of a faintly acid character and is combined with the alkali or alkaline earth bases. Upon decomposition by dilute acids (hydrolysis) it yields a reducing substance and an acid albuminate; by further decomposition leucin, tyrosin, and lævulinic acid are formed.*

Experiments with Milk and Mucin.

Exp. 99. Examine microscopically whole milk, skim-milk, and cream. Note the relative amounts of fat in the three varieties.

Exp. 100. 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. 101. With a lactometer take the specific gravity of whole milk and skim-milk and explain the difference in results.

Exp. 102. Test the reaction of milk with litmus.

Exp. 103. Dilute some milk with six or seven times its volume of water, and add acetic acid drop by drop till the casein is precipitated. Filter and reserve the precipitate. Test the filtrate for proteids, if any remain; determine if possible their character.

Exp. 104. Test another portion of the filtrate for carbohydrates, determining the variety present.

Exp. 105. 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. 106. Take a portion of the precipitated casein from

* Simon, Physiological Chemistry.

Exp. 103, digest at 40° C. with pepsin HCl for twenty minutes or half an hour. While digesting, test other portions of casein, for solubility in water, in dilute acid and dilute alkali. Test also 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. 107. To a little skim-milk contained in a test-tube add a saturated solution of ammonium sulphate.

Exp. 108. 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% HCl.

Exp. 109. Make color-tests for proteids.

Exp. 110. Boil a little mucin solution with dilute HCl for several minutes. Cool, neutralize, and test for sugar.

SECTION V.—BLOOD, BONE, MUSCLE, ETC.

Blood.

The blood, carrying oxygen and other forms of nutrition to all parts of the body, and returning carbon monoxid and the waste products of cellular activity, is an exceedingly complex substance. The composition of the blood itself, however, may be grossly 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%. This is largely proteid, consisting of serum globulin, serum albumin, a slight amount of nucleoproteid, and fibrinogen; also a fibrin ferment, thrombase or thrombin, by the action of which the fibrin is separated as a "clot" which mechanically carries down the corpuscles. As the clot contracts, the "serum" separates as a clear, amber-colored liquid, consisting of serum globulin (paraglobulin), serum albumin, and the fibrin ferment.

* For preparation of mucin solution see Appendix.

Fibrin.—The fibrin may be obtained free from corpuscles by whipping the fresh blood. Under this treatment the fibrin separates as shreds, while the remaining constituents constitute the "defibrinated blood." The presence of lime-salts is essential to the coagulation of the blood, i.e., the decomposition of the fibrinogen and separation of fibrin, in much the same way as in the decomposition of caseinogen and precipitation of casein from milk.

Fibrin, as usually obtained, is in the form of brown, stringy, and "fibrinous" masses, and is kept for laboratory use under glycerine. 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.

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, nucleoalbumin, and a globulin. (Hammarsten.) Upon and all through the stroma is the hæmoglobin, which, together with its oxygen compound oxyhæmoglobin, is responsible for the *color* of the blood. Oxyhæmoglobin may be obtained as silky, transparent crystals of blood-red color.

From hæmoglobin may be derived the blood pigment *hæmochromogen*, containing iron, and this by oxidation is converted into hæmatin. 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. Kirk of Philadelphia, 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.

CO Hæmoglobin.—Hæmoglobin forms with carbon monoxid (from water-gas or other sources) a definite and very stable

compound, being even stronger than the oxyhæmoglobin, to which reference has previously been made. Blood containing carbon monoxid hæmoglobin is of a bright-red color, which darkens in the air much more slowly than ordinary blood.

Hæmin, or Teichmann's hæmin crystals, is the hydrochloric acid compound of hæmatin. (See Exp. 115.)

The form of the red corpuscle is that of a biconcave disk without nucleus; by action of water it becomes swollen, and the hæmoglobin may be washed away, leaving the "stroma." The diameter of the red corpuscles of human blood is about $\frac{1}{3200}$ of an inch. Of the domestic animals, the corpuscles of the dog approach most nearly to this 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 VII, 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 7,500 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, aggregations of which 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 are not to be distinguished chemically.

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 chlorin, fluorin, and of sulphates, the proportion being practically the same as given for dentine, under Teeth, on page 119. Because in some diseases, in which the bones are softened or decalcified (as osteomalacia), the relation of the CaO and P_2O_5

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: $3Ca_3(PO_4)_2.CaCO_3$.

If, by treatment with dilute hydrochloric acid, the mineral constituents are entirely dissolved out of bone, there remains a substance from which glue (gelatine) is derived, of similar composition to collagen, from connective tissue, and known as *ossein*. Neither of these (ossein or collagen) are soluble in water or in dilute acids.

Gelatine is made by hydrolysis of ossein or collagen brought about by *prolonged* boiling with dilute mineral acids. Gelatine, if first treated with cold water till soft, may be dissolved in hot water. The solution is precipitated by mercuric chlorid, alcohol, tannic and picric acids. It responds but feebly to the general proteid reactions, but, by digestion with either pepsin or trypsin, compounds are obtained analogous to those resulting from similar proteid digestion.

Experiments on Blood and Bone.

Exp. 111. 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. 112. *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. 113. *Hæmoglobin 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 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. 114. A spectroscope will be found ready for use in the laboratory, and the absorption-bands given by oxyhæmoglobin and hæmoglobin will be demonstrated. The student may prepare solutions for examination as follows:

(*a*) *Oxyhæmoglobin.*—Use dilute blood (one part of defibrinated blood in fifty parts distilled water).

(*b*) *Hæmoglobin* (reduced hæmoglobin).—Add to blood a few drops of strong ammonium sulphid, or one or two drops of freshly-prepared Stokes's reagent.* Note the change in color produced by the addition of the reducing agent. Shake with air and note the rapid change to oxyhæmoglobin.

(*c*) *Hæmochromogen.*—To a little of the hæmochromogen, reduced with ammonium sulphid, add a few drops of concentrated NaCl, and note the spectrum of reduced hæmatin or hæmochromogen.

(*d*) *Carbonmonoxid Hæmoglobin.*—Pass a current of illuminating-gas through a dilute oxyhæmoglobin solution for a few minutes and filter. Note the change of color. Try the effect on the solution of (1) ammonium sulphid; (2) Stokes's reagent; (3) shaking with air. Note the stability of the compound.

Exp. 115. *Hæmin 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 (chlorid of hæmatin). Similar crystals can be obtained by using an alkaline iodid or bromid in place of NaCl.

Exp. 116. *Coagulation of Blood.*—Observe the phenomena of coagulation as it takes plase (*a*) in a test-tube, (*b*) in a drop of blood examined under the microscope. Explain fully.

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* Stokes's reagent consists of two parts of ferrous sulphate and three parts of tartaric acid dissolved in water and ammonia added to distinct alkaline reaction. There should be no permanent precipitate.

Exp. 117. *Proteids of Blood-plasma.*—(*a*) Serum-albumin. (*b*) Serum-globulin. Using blood-serum, separate and identify these two proteids.

(*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 distinguished from paraglobulin easily 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. 118. *Fibrin.*—(*a*) Note its physical properties.

(*b*) Note action of 0.2% hydrochloric acid.

(*c*) Apply the proteid color tests.

Exp. 119. Examine microscopically and sketch structure of bone and teeth.

Exp. 120. *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 hydration 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. With the solution perform the following experiments. (If too little gelatin is obtained for all the tests, a solution will be provided.)

Gelatin may also be prepared from tendons which consist

almost wholly of white fibres. Collagen is the substance of which white fibres are made up.

Exp. 121. With a solution of gelatin make the usual tests for proteid.

Exp. 122. Precipitate gelatin from dilute solution with the following reagents:

(*a*) Tannic acid.
(*b*) Alcohol.
(*c*) Acetic acid and potassium ferrocyanid.
(*d*) Mercuric chlorid.
(*e*) Picric acid.

Muscle.

The chemistry of muscle is complex. It 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, in distinction from the fluid obtained in the same manner from dead muscle, which is called muscle serum. The chemical reactions of these solutions differ, due to the formation of sarcolactic acid in the dead muscle. The proteids differ in certain respects. Myosin is the most essential constituent of muscle plasma, and corresponds to the fibrin of the blood-clot. It exists as a parent proteid myosinogen, or myogen, from which it may be precipitated by saturation with salt or magnesium sulphate. Myosin has many of the properties of the globulins, but differs in the very important particular of not being precipitated by dyalization. Among the more important extractive bodies obtained from muscle are creatin, carnin, inosite, glycogen, and lactic acid. Creatin is a xanthin body, being chemically a methyl-guanidin-acetic acid, which may appear in the urine as creatinin. (Creatinin is creatin minus H_2O.)

Carnin is a white crystalline substance obtained from meat extract and converted by oxidation by nitric acid, chlorin or

bromin into hyopxanthin or sarkin. Its chemical constitution is not postively known.

Inosite, $C_6H_{12}O_6 + H_2O$, is a hexahydroxybenzene, $C_6H_6(OH)_6 + H_2O$. It has a sweet taste, and was formerly erroneously classed with the carbohydrates. It is capable of yielding lactic and butyric acids(?).

Glycogen occurs in slight amounts in muscle, but decomposes after death, with formation of a reducing sugar. (Compare page 174.)

Lactic Acid is a constituent not only of muscle but also of various glands, of the bile, and of blood. For the chemistry of this substance, see page 146.

Keratin.

Keratin is the characteristic constituent of the hair, nails, feathers, and horn. It contains a considerable proportion of loosely combined sulphur. It is insoluble in dilute acids and unaffected by any of the digestive ferments; it does, however, dissolve in the caustic alkali solutions, and may be used as the source of leucin, tyrosin, cystin, and other well-known products of proteid digestion.

Experiments with Muscle and Keratin.

Exp. 123. Place 25 grams of fresh finely chopped muscle in a beaker with 75 c.c. of 5% solution of common salt, and allow to stand for about one hour, with frequent stirrin . In the meanwhile perform Exp. 124. Then filter off the liquid and make the following tests with the filtrate:

(*a*) Test for proteids.

(*b*) Having found proteids, 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

44°–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 30°–40° C. This is due to soluble myogenfibrin. Now filter off the coagulated myosin.

Heat filtrate to 55°–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 70°–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 proteids. 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. 124. 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 chlorid solution. The presence of lactic acid is shown by a bright-yellow color.

Exp. 125. Creatin may be most conveniently prepared from a strong solution of Liebig's extract. Dissolve the extract in twenty parts of water and remove excess of lead; concentrate to a fine syrup over a water-bath and allow to stand in a cool place, when creatin crystals will separate out. Two or three days' time may be required before the crystals are obtained. They may be washed with 88% alcohol and purified by recrystallization from water. Hypoxanthin and sarcolactic acid may be obtained from the mother liquor.*

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* Lea's Chemical Basis of the Animal Body.

Exp. 126. Creatinin may be prepared from creatin by boiling for ten or fifteen minutes with very dilute sulphuric acid. Neutralize the acid with $BaCO_3$, evaporate and filter to dryness on a water-bath, and extract the creatinin with alcohol. Upon evaporation the creatinin is obtained in the form of crystals.

Keratins are characterized by their insolubility, and by their high content of loosely combined sulphur.

Exp. 127. Test solubility of keratin (nail or horn) in water, acids, alkalies, gastric and pancreatic juice.

Exp. 128. 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?

PART VII.

DIGESTION.

SECTION I.—SALIVA: PROPERTIES AND CONSTITUENTS.

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 chemical standpoint we are much more interested in the mixed saliva and its constituents than the differences in the product 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 dializable through the salivary glands, and hence furnishes a reliable index of metabolic processes which are being carried on within the system. In order for this fact to be of practical value, two things are obviously of prime importance: First, methods of analysis

which are not too complicated and 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 Professor Michaels and Dr. Kirk that this subject of salivary analysis promises much, and is certainly worthy of careful investigation.

The *quantity* of saliva secreted in twenty-four hours is variously estimated from a few hundred to 1500 c.c.; 1200 to 1500 is the more probable amount. The quantity is diminished in fevers, severe diarrhœa, 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 iodid, or pilocarpin. The flow of saliva is also increased by action of the sympathetic nervous system, during pregnancy, and by local inflammatory process.

Physical Properties.—The physical properties of saliva include its appearance, specific gravity, reaction, color, and odor.

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 dioxid.

Specific Gravity.—Specific gravity ranges from 1002 to 1009, the total solids being only from 0.6 to 2.5 per cent.

Reaction.—The reaction is normally alkaline to litmus-paper or to lacmoid. Normal saliva, however, fails to give an alkaline reaction with phenolphthalein due to the presence of free CO_2, which may be present to the extent of 19 parts in 100, by volume. If the sample be subjected to even a slight

degree of heat the acid gas is expelled; then the usual pink color may be obtained with this indicator. Saliva mey be acid upon fasting, particularly before breakfast and also after much talking. Acid conditions may exist which are local in their character and due to lactic acid fermentation. Acid salivas may also be met with in cases of rheumatism, mercury salivation, and diabetes. By exercise of the glands, as during the chewing of food, the alkalinity is increased; oftentimes the reaction changes from faintly acid to alkaline during this process, the proportion of alkaline salts becoming greater, although the total solids as a whole are slightly diminished. This fact of the change in the reaction from acid to alkaline has been explained by ascribing the acidity due to fermenting particles in the mouth; and the continued process of chewing and swallowing washes this away, or, in other words, that the change in reaction is a mechanical one rather than a change of the chemical composition of the secretion. This explanation seems to be a superficial one and without sufficient experimental foundation.

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 pyorrhœa there is usually a peculiar fetid odor easily recognized. In other pathogenic conditions the odor may be slightly ammoniacal, or occasionally resemble the odor of acetone or garlic.

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, ammonium salts, potassium sulphocyanate, alkaline phosphates, and chlorids, with traces of carbonates; and, in the sediment, epithelium cells, occasional leucocytes, and fat globules. The *abnormal* constituents will include glycogen, urea, 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.

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 proteid; 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 much in the same individual in health and disease. The changes in the characteristics of salivary mucin is an almost unexplored field of dental research, and the importance 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 may also give rise to mucic acid, which, according to Dr. G. W. Cook of Chicago, is a probable factor in tooth erosion. (See *Dental Review*, May 1906, p. 461.)

Albumin.—Albumin is present in very small quantities increased during mercurial ptyalism, usually in cases of pyorrhœa, and, according to some authorities, in various albuminurias. It may be detected by 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." *

Ptyalin.—Ptyalin is the principal ferment of the saliva; it converts starch, by hydrolysis through the various dextrins, (page 173) to maltose. The maltose in turn being 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 media. 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. 130.)

Ammonium Salts.—Ammonium salts occur chiefly as chlorid, probably to some extent as sulphocyanate, and occasionally as oxalate. Professor Michaels says that ammonia must be considered as a more completely oxidized form of nitrogen than urea, hence its relative increase is observed in all diseases which occasion an excess of nitrogen and urea, as in tuberculosis and all hypoacid diatheses. There is a decrease of ammonia whenever the nitrogen fails to reach the stage of oxidation repre-

* Dr. Joseph P. Michaels. S. S. White's reprint of paper read before International Dental Congress, Paris, 1900.

sented by urea. This condition is accompanied by uric acid and other products of deficient oxidation, and characterizes the hyperacid state. The ammonia may be detected by a microscopical examination of the dried saliva, although the ammonium salts do not polarize light (Plate VIII, Fig. 1), also by the reaction with Nessler's reagent, which produces a yellow color.

Potassium Sulphocyanate is peculiarly a constituent of the saliva, although it occurs in traces in the blood, urine, etc. In a state of health, according to Dr. Michaels, the ammonium salts and the sulphocyanates are present in very slight amounts, and the color-tests, with Nessler's solution and with ferric chlorid, 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 chlorid, and distinguished from meconates and acetates, as indicated by Exp. 131. 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 proteids, and exist in the urine in quantities variously estimated from 20 to 80 milligrams per liter, while in saliva it has been estimated as 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 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 pyorrhœa it is also claimed to be present in considerable quantity.

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 present in only very slight quantities, if at all. Chittenden says that human mixed saliva contains normally no sodium carbonate whatever.

As explained by Dr. Kirk, the normal reaction by which over-acidity 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. 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% of sugar in the urine, and there was obtained a very few osazone crystals by subjecting a considerable quantity of saliva, after concentration, to the phenylhydrazine test.

Urea has been repeatedly found in the saliva of patients suffering from chronic nephritis.

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. In the experience of the author 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.)

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* International Dental Journal, February, 1904.

Chlosterin and lecithin have been found by Professor Michaels in pathological saliva, and leucin has been found by Michaels in a case of lupus and, according to Novey, in a case of hysteria.

Of the crystalline salts which may be separated by evaporation of dialyzed saliva, the sodium oxalate and the lactates or lacto-phosphates 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.

SECTION II.—ANALYSIS OF SALIVA.

In the systematic examination of saliva the first thing to be done is to start a few cubic centimeters dialyzing (page 212) for subsequent examination of crystals by polarized light. "The five-drop test" of Professor Michaels may next be made as follows: On a porcelain tile place 5 large-sized drops of the saliva under examination.

Sulphocyanate Test.—To the first drop add one drop of 5% ferric chlorid, slightly acid with HCl; a reddish coloration constitutes the test for sulphocyanates. (For properties of ferric sulphocyanate, see Exp. 131, page 215).

Ammonium Salts.—To the second drop add one drop of Nessler's reagent: a yellow to brown shows the presence of ammonium salts. If a precipitate forms by the addition of Nessler's reagent, it indicates either a large amount of ammonia or the presence of urobilin. If due to urobilin the precipitate is of a rose color after desiccation. Ammonium salts are usually seen in the evaporated drop examined by polarized light. Plate VIII, Fig. 1.

Chlorids.—To the third drop add a small drop of a 5% solution of neutral chromate of potassium, K_2CrO_4. Mix with a glass rod, and add one drop of a $^1/_{10}\%$ solution of silver nitrate. This constitutes the chlorin test which, if present in normal quantities, will give a reddish precipitate, gradually

becoming white. Should the precipitate remain red it shows the chlorin deficient or less than normal in amount. If the precipitate rapidly turns white, or if a white precipitate is formed to the exclusion of the red, chlorin is increased in amount. High chlorin is indicative of hypoacid diathesis.

Glycogen, etc.—The fourth drop may be tested for glycogen by the addition of one drop of an aqueous solution of iodin and potassium iodid. This must be left for some time, as the test is not obtained until the drop is dried, then, if the color is a feeble violet around the edge, glycogen is indicated. If the color is a strong brown-red, erythrodextrin, if gray or black, a reducing sugar is indicated.

Acetone.—In the fifth drop dissolve a small crystal of potassium carbonate, then add a drop of Gram's reagent, when a marked odor of iodoform will indicate the presence of acetone. Should this odor be obtained, it is better to repeat this test upon a microscope slide, and examine carefully for the characteristic hexagonal crystals of iodoform (Plate V, Fig. 1).

Specific gravity may be taken with a urinometer, reading from underneath the surface of the liquid. If the quantity of saliva should be small, it may be diluted with an equal volume of water, and then the last two figures multiplied by two will give the gravity of the undiluted sample.

The *reaction* may be taken by placing a drop on litmus-paper. *Appearance* and *color* are noted in the sample when perfectly fresh; then, if a small vial is nearly filled, tightly corked, and set aside for twenty-four to forty-eight hours, the tints previously referred to may appear. The color may be a 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, which was when fresh hypoacid in character, is, after 48 hours, usually markedly opalescent and of offensive odor, while a hyperacid saliva may have become clear or cloudy but without odor.

Total Solids may be obtained by evaporating over a water-bath 10 c.c. of thoroughly mixed sample and weighing the residue. If this is done in a platinum dish the residue may be ignited till a white ash is obtained and again weighed, and the result will represent the entire mineral constituents in the saliva, except traces of CO_2 and of chlorids which may have been lost during ignition. If a portion of the saliva is carefully filtered, the epithelium cells, leucocytes, etc., will be separated. Then, if the total solids in the filtrate are determined, the difference will be the weight of the epithelium, etc.

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 has been tightly tied a piece of parchment (Fig. 15), 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 which existed

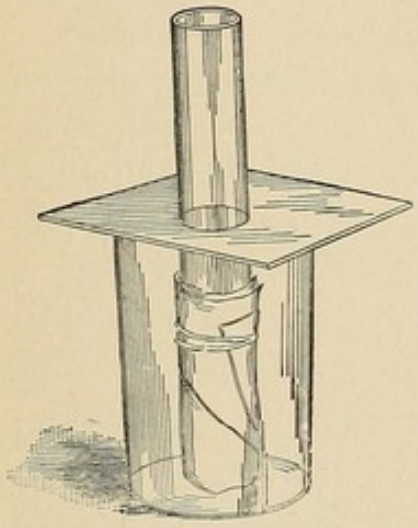

Fig. 15.

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), fill each cell full of the dialyzed saliva. Put number 1 in a warm place that it may evaporate rapidly, leave number 2 exposed to the air at the room temperature, it will dry in half to three-quarters of an hour. Place number 3 under a large beaker, or small bell-jar, and cover number 4 with a cover-glass, and from time to time examine the crystals that may be formed. Numbers 3 and 4 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 sodium oxalate, lactates, and lacto-phosphate of lime and magnesia, and rarely urates of the alkalis. (For forms of these crystals see Plate VIII, Figs. 3 and 4, and Plate III, Fig. 3.)

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.

Albumin may be demonstrated in the filtrate, from which mucin has been separated by underlying with strong nitric 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.

Ptyalin.—The presence of ptyalin may be demonstrated, and the conditions influencing its amylolytic action studied, by the two following experiments.

Exp. 129. *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 iodin, and finally to achroo-dextrin, which gives no reaction with iodin, 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 iodin 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, due to erythrodextrin alone (starch being absent), and finally no reaction at all with iodin, proving the absence of starch and erythrodextrin. The fluid now contains achroo-dextrin and maltose. Test for the latter with Fehling's solution and with Barfoed's reagent.

Exp. 130. *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% 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% 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% 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. 131. In three separate test-tubes place a few cubic centimeters of dilute solutions of KCyS, of meconic acid, and of acetic acid; add to each a few drops of ferric chlorid, 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-chlorid solution. Formulate a method of distinguishing from the sulphocyanates, meconates, and acetates.

Tests for Abnormal Constituents.

Acetone, glycogen, and dextrin have already been considered. Urea may be demonstrated as follows: To a given volume of saliva add twice as much alcohol, this serves to precipitate proteids. Filter and evaporate on a water-bath till original volume is reached, or evaporate to less than original volume, and make up with distilled water. Then determine urea with Squibb's apparatus, as used for urine, except that in this case it will be necessary to replace the 2-c.c. pipette with a small burette, and introduce 10 c.c. of the prepared saliva. Then it will be necessary to allow for these 10 c.c. by subtracting this amount from the volume of water received in the graduated cylinder, and the remaining number of cubic centimeters, multiplied by two, will correspond to the urea in 20 c.c. of the sample. The percentage shown on the card, divided by ten, will give the per cent. of urea required.

Lactic, butyric, and *acetic acids* may each be tested for qualitatively by the methods given under gastric digestion (q. v.).

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 HCl, throw in a few very small pieces of copper-turnings, which have been recently cleaned in dilute HNO_3, 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 chlorid, 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 chlorid of gold shall be suspended directly above the turnings and perhaps a half 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 chlorid 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 fibres, etc. An excessive amount of blood, of 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.

SECTION III.—GASTRIC 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 œsophagus. 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 1%.

The first step in the gastric digestion is probably the union of the stomach HCl with the proteids, forming acid albumins or allied bodies which are changed by pepsin, which is the active digestive ferment of the stomach, into the albumoses, and slight amounts of the various peptones, following practically the changes produced experimentally on page 219.

Pepsin is an active proteolytic enzyme occurring in the cells of the stomach-wall as pepsinogen, which is decomposed by the HCl with the formation of free pepsin. Pepsin works only in faintly acid solutions, and in the stomach carries the digestion of proteids but little beyond the stage of the proteoses.

Hydrochloric acid, obtained from the fundus glands by an interchange of radicles between alkaline chlorids and the carbonates of the blood.* The quantity present varies from 0 to $^3/_{10}$ per cent, 0.18% being about the most favorable for peptic activity. Aside from HCl, various organic acids may be present in the stomach contents; lactic acid, butyric acid, and acetic

* Long's Physiological Chemistry.

acid are the most important of this class, tests for which are referred to under analysis of gastric contents.

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 peculiarly the curdling of milk, i.e., the decomposition of caseinogen (Exp. 137), and consequent coagulation of the casein. A third enzyme, existing in the stomach in very small quantities, is a gastric lipase, or stomach steapsin, a fat-splitting enzyme, the action of which is comparatively weak, and of but slight importance.

Analysis of Gastric Contents and Experiments with Pepsin.

The following solutions will be found in the laboratory:

A. *A .2% 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 two grams of pepsin in 1000 c.c. of water.

C. *A Pepsin-hydrochloric-acid Solution.*—Prepared by dissolving two 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 glycerine extract of the mucous membrane of the stomach.

Exp. 132. 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% HCl + a few shreds of fibrin.

(*c*) 3 c.c. pepsin solution + 10 c.c. 0.2% HCl, and a few shreds of fibrin.

(*d*) 3 c.c. pepsin solution + 10 c.c. 0.2% HCl, boil, and then add a few shreds of fibrin.

(*e*) 3 c.c. pepsin solution + 10 c.c. 0.2% 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. 133. Filter *c*. Neutralize with dilute Na_2CO_3. Filter again. Why? Test the filtrate for the biuret reaction.

Exp. 134. To 5 grams fibrin add 30 c.c. of the pepsin solution and 100 c.c. 0.2% 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. 135. Dissolve the last precipitate of Exp. 134 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.

Exp. 136. To the last filtrate of Exp. 134 add an equal volume of 95% 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 so dissolved?

(*c*) Try the biuret reaction.

Exp. 137. *Demonstration of the Rennet Enzyme.*—Place 10 c.c. milk in each of three test-tubes. Label the test-tubes 1, 2, 3.

To 1 add a drop of neutralized glycerine extract of the mucous membrane of the stomach (made from the stomach of the calf).

To 2 add a drop of a neutralized glycerine 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 glycerine 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. 138. *Digestion of Casein.*—Determine the products of the digestion of the curd from the last experiment.

Exp. 139. *Tests for Free Hydrochloric Acid.*—Try each of the following tests with (*a*) HCl (0.2%, 0.05%, and 0.01% successively); (*b*) lactic acid (1%); (*c*) mixtures containing equal volumes of (*a*) and (*b*). Tabulate the results.

(*a*) *Dimethylamidoazobenzol.*—Use one or two drops of a 0.5% 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 two or three drops of the solution to be tested in a porcelain dish, add one or two 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 resorcin and a gram of cane-sugar in 100 grams of 94% 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 vermillion red color is obtained. This gradually fades on cooling.

(*d*) *Tropæolin OO.*—Use one or two 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. 140. To 5 c.c. egg-albumin in solution add 1 c.c. of 0.2% 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 pepton in place of the egg-albumin.

Exp. 141. *Tests for Lactic Acid.*—Uffelmann's reagent. Mix

10 c.c. of a 4% solution of carbolic acid with 20 c.c. of water, and add a drop or two of ferric chlorid.

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 three or four drops of a 10% ferric-chlorid solution to 50 c.c. of water. Such a solution has a *very faint* yellow color, which is distinctly intensified by lactic acid.

Using 5 c.c. of this near y colorless solution for each experiment, note the effect of (*a*) 0 2% HCl; (*b*) acid phosphate of sodium; (*c*) alcohol; (*d*) glucose; (*e*) cane-sugar. What conclusions do you reach concerning the value of this list, 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 two 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. 142. Test for butyric acid; see ethyl butyrate, page 138.

Exp. 143. Test for acetic acid; see acetates (page 49).

Exp. 144. 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 Erlenmyer flask, add 2 or 3 drops of a solution of dimethylamidoazobenzene. 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 entirely from fermentations which are inhibited by the hydrochloric acid.

SECTION IV.—PANCREATIC DIGESTION AND BILE.

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 principal ferments, pepsin and rennin, in the pancreatic juice, three active ferments. First, a proteolytic enzyme, known as *trypsin*, which continues the work of the gastric juice, and converts the proteoses into peptones, tyrosin, leucin, etc. The pancreatic juice is a much more energetic digestive agent than the gastric juice, but differs in that it is an alkali secretion, and neutralizes any acid which may have been obtained from the stomach. Next, the amylolytic enzyme known as *amylopsin*. 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.

Steapsin is the fat-splitting enzyme of the pancreatic juice. It splits the fat, as indicated on page 140, into glycerine 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.

Bile.—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.—Its composition is very complex, but there are two acids and two coloring matters which are of particular importance, and derivatives of which indicate the presence of bile in saliva, urine, blood, etc. The acids are taurocholic and glycocholic, existing principally as sodium or potassium salts. The coloring matters are bilirubin and biliverdin; the former predominates in human bile and the latter

in ox bile. Glycocholic acid upon hydrolysis splits into a simpler acid (cholalic) and glycocoll, glycocoll being an amido-acetic acid (page 148), which is undoubtedly an antecedent of urea. Both of 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 pathologic conditions either of the liver- or bile-ducts, causing obstruction 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.

Experiments with Pancreatic Juice.

Exp. 145. *Proteolytic Action.*—To 25 c.c. of a 1% 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 to 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% 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. 146. *Formation of Leucin and Tyrosin.*—Perform a similar experiment, using boiled fibrin and adding a few drops of a 20% solution of thymol, or a few drops of chloroform water. Why use boiled fibrin, and why add thymol or chloroform? Digest for forty-eight hours, and then examine as follows: Filter, neutralize, and concentrate by evaporation on the water-bath. Crystals of tyrosin (and possibly leucin) usually separate. Examine microscopically.

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* Ogden.

Exp. 147. *Amylolytic Action.*—To some starch paste in a test-tube add a drop or two or the pancreatic extract and place in the water-bath at 40° C. After a few minutes test for sugar and report the result.

Exp. 148. *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. 149. *Emulsifying Action.*—To 10 c.c. of a 0.2% solution of Na_2CO_3 add a few drops of the mixture used in Exp. 148. 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. 150. *Color.*—Note the difference in color between human bile and ox bile. Explain.

Exp. 151. *Reaction.*—Dilute some bile with four parts of water. Immerse a strip of red litmus-paper, then remove and wash with water. Note the recation.

Exp. 152. *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. 153. Filter (152) and test the filtrate for proteids. Report the result.

Exp. 154. *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. 6.)

Exp. 155. *Bile-pigments.*—(*a*) *Gmelin's Test.*—Take some bile in a wine-glass and underlie 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*) *Iodin Test.*—Place 10 c.c. of dilute bile in a test-tube, and add slowly two or three cubic centimeters of dilute tincture of iodin, so that it forms an upper layer. A bright green ring forms at the line of contact.

Exp. 156. *Cholesterin.*—Examine under the microscope the cyrstals obtained by the cautious evaporation of the alcohol-ether filtrate of Exp. 154. For color reactions refer to demonstrations.

Exp. 157. *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.

PART VIII.

URINE.

SECTION I.—PHYSICAL PROPERTIES OF 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 very slight amounts, including, perhaps, albumin and sugar, chlorid of sodium, sulphates and phosphates of the alkalis 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; occasionally albumose, peptones, lactates, lacto-phosphates, oxalates, carbonates, hippuric acid, also organic compounds, resulting from insufficient or imperfect oxidations, as amido-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. As has been previously stated by the author,* if there are diseases of the oral cavity which may have their

* International Dental Journal, January, 1905.

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 his case, and at present a quantitative analysis of the urine is of greater value than any other laboratory aid. 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 the bottle or other receptacle, in which the twenty-four-hour sample is collected. 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 normally is about 1200 to 1400 c.c. for an adult female and 100 or 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 any 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, due to 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.—Aside from these variously colored samples 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 parasitic disease of the blood. 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 ob-

tained which, after acidifying with dilute acetic acid, will be suitable for an albumin test.

Specific Gravity.—The gravity is most conveniently taken with a urinometer (Fig. 16). Care should be taken in the selection of this instrument that the scale graduation is accurate. The fact that the instrument will sink in distilled water at the proper temperature (usually 60° F., 15½° C.) to the 0 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.

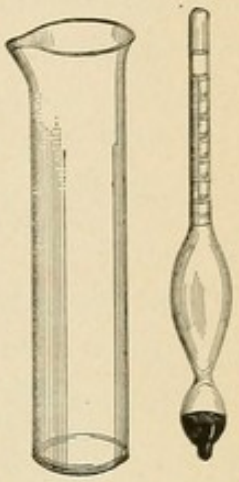

FIG. 16.

Reaction.—The reaction of urine is normally acid to litmus-paper, due to the presence of acid sodium phosphate. 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, 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% 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 HCl 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."

SECTION II.—NORMAL CONSTITUENTS.

THE more important normal constituents of the urine are urea, uric acid (combined as urates), chlorids, phosphates, sulphates, coloring matters (urophain and indoxyl); traces of mucin, organic acids, carbonates, hippuric acid, creatin, and creatinin may also be present. The total normal solids are composed approximately of 50% urea, 25% chlorid 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 by $2\frac{1}{3}$,* which will give 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 been already considered (page 153).

Detection.—A qualitative test for this substance is obvi-

* Coefficient of Haeser.

ously superfluous, although such may be made by obtaining the crystals of urea nitrate or oxalate (page 155). 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 fæces, and traces only by other avenues. The urea may be quantitatively determined by various methods, the hypobromite method is the most practical.

Quantitative Determination.—There are various forms of apparatus used in connection with this process, but the one

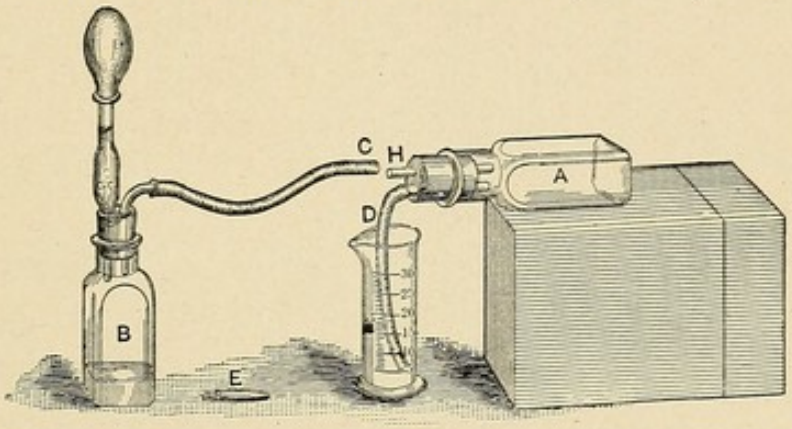

FIG. 17.

devised by Dr. Squibb will probably give the most accurate results with the least practice. The use of this apparatus may be best explained by reference to the above cut.

The first step in the use of this apparatus is to completely fill the bottle *A*, including the tubes *D* and *H*, with water, with the glass plug *E* closing the lower end of *D*. Next put 5 c.c. each of a 40% solution of caustic soda and a bromine solution in potassium bromide* into *B*. Place the stopper in *B* and connect the tube *C* at *H*, then fill accurately the 2-c.c. pipette with urine. Place in position in the stopper of

* For preparation of this solution see appendix.

B as shown in the cut, remove *E* from the rubber tube *D*, and allow *D* to fall to the *bottom* of the graduate as indicated. Pressure is now applied to the bulb of the pipette, so that the 2 c.c. of urine is forced with moderate rapidity into the bottle. As the pressure on the bulb is released, water will be drawn back into *A*, and it is essential that the end of *D* is under water during this portion of the process. Bottle *B* should be agitated to insure complete decomposition of the urea. Nitrogen and carbon dioxid are at once evolved according to the reaction on page 154. The 40% solution of caustic soda is strong enough to absorb and hold the CO_2. The nitrogen passes into *A*, forcing a corresponding volume of water into the graduate. This volume of gas, read in cubic centimeters of the water, will give the percentage of urea in the sample examined, 1 c.c of nitrogen being equivalent to 0.126 of urea.

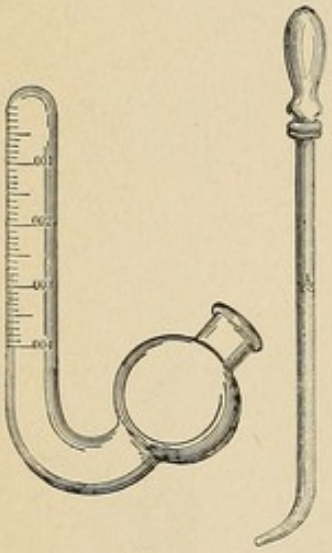

FIG. 18.

Doremus' apparatus is shown in Fig. 18. One cubic centimeter of the urine is used instead of two, and the whole apparatus is filled with the hypobromite solution. This apparatus is simpler than Squibb's, but requires greater care in manipulation in order to obtain equally accurate results.

URIC ACID.

Uric acid and its antecedents, the xanthin bases, are derived from the decomposition of nuclein and nucleoproteid. For chemistry of this substance, see pages 156 and 158. The uric acid is increased by a highly nitrogenous diet and certain vegetable substances which contain purin (page 157) derivatives, such as coffee, tea, and cocoa. The red meats, so-called, beef, mutton, etc., are regarded as the most abundant source of uric acid and urates. As previously suggested uric acid does not

occur in normal urine as such, but is combined with the alkaline bases.

Detection.—It is unnecessary to make a qualitative test in urine, as urates are always present. If a qualitative test is desired the murexid test, as given on page 160, is available. Uric acid is best determined quantitatively by the centrifugal method as devised by Professor 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 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 chlorids and, unless iodids or bromids 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%.

Chlorids.

The chlorids are represented in the urine chiefly by sodium chlorid. 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 salts, and is without clinical significance. The chlorin is diminished in dropsy, acute stages of pneumonia, and in fevers generally.

Detection.—The usual qualitative test with silver nitrate and nitric acid is employed for detection of chlorid 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 chlorin present. If a solid ball of silver chlorid is formed which does not become diffused upon gently agitating the contents of the glass, the chlorin is normal or increased. If the precipitate falls as a cloud distributed throughout the liquid, the chlorin is diminished. The chlorin may be quantitated 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. The titration with silver nitrate, using potassium chromate as an indicator, really takes less time, and is much more accurate. This titration is made in the usual way (see page 101), except that, inasmuch as phosphates and urates are also precipitated, from three tenths to 1 c.c. may be deducted from the amount of the silver-nitrate solution used according as it is, much or little, thus allowing for these substances. An accurate titration of chlorin may be made by acidifying the urine with nitric acid, adding an excess of standard silver-nitrate solution, and titrating back with a standardized sulphocyanate solution (preferably of the same strength as the $AgNO_3$ solution), using ferric sulphate for an indicator. But as a rule the simpler method gives results which for clinical purposes are equally valuable as this more tedious though more accurate process.

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 two and a half to three and a half grams, calculated as P_2O_5 (in twenty-four hours).

The triple phosphates, ammonium magnesium phosphates (Fig. 4, Plate I), 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 (Fig. 6, Plate III), 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 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.

Detection.—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 quantitated 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 0.0225 of P_2O_5.

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* See Appendix

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 167.

Detection and Determination.—The sulphates may be detected by precipitation with barium chlorid in HCl 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 rather closely the urea, and their determination is not of great importance. They are increased in acute fevers, and diminished in chronic diseases generally, and markedly diminished in carbolic-acid poisoning. (Ogden.)

Urophain.

The urophain test of Heller is an easily applied test for coloring-matters, urobilin, and other coloring-matters. It has little clinical significance, because it is influenced by the presence of many other urinary constituents. The urophain is supposed to be increased in any disease in which the red corpuscles are being destroyed. The test is made by placing 7 c.c. of strong sulphuric acid in a wine-glass, and allowing double the quantity of urine to fall from a sufficient height to thoroughly mix the liquids, when an immediate dark-red color indicates a normal condition.

Indoxyl.

The indoxyl is of considerable importance, as an increase above the normal amount is indicative of increased putrefaction of nitrogenous substances taking place in the small intestine. Indoxyl may also be increased by acute inflammatory process of the peritoneal cavity. Ordinary constipation does not increase the indoxyl. The test for indoxyl depends upon the

oxidation of the indoxyl potassium sulphate to indigo blue according to the following reaction:

$$\underset{\text{Indoxyl potassium sulphate.}}{2C_8H_6NKSO_4} + O_2 = \underset{\text{Indigo.}}{2C_8H_5NO} + 2KHSO_4.$$

Detection and Determination.—15 c.c. of strong HCl is placed in a wine-glass, and a single drop of concentrated nitric acid added; then 30 drops of urine are stirred into the mixture. If indoxyl is present, an amethyst color develops in from five to fifteen minutes. If the color is purple, the indoxyl is increased. Variation of the amount of indoxyl within normal limits is rather a wide one, and the indoxyl may be reported as high or low, normal, increased or diminished.

SECTION III.—ABNORMAL CONSTITUENTS.

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 condition 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 more easily passes 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, such as may be caused by bacteria or 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 underlying 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. 19). As this latter method

FIG. 19.

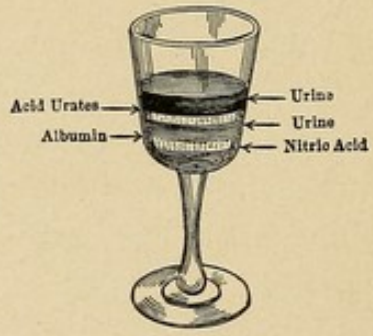

FIG. 20.

does not provide for sufficient mixing of nitric acid with the sample, the albumin is shown by a narrow white ring *at* the point of contact of the two liquids. A white ring *above* the point of contact is not albumin, but is composed of acid urates, indicating an excess of urates in the sample (Fig. 20). 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 when 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. Anything more than a trace should be quantitated 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 ferrocyanid 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 onto a counterpoised filter, then thoroughly washed, first in water, then 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 in some instances of value, and is made as follows:

FIG. 21.

The albuminometer (Fig. 21) is filled with urine to the line *U*, and then the reagent* is added 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 of the test. The proteoses occasionally occur in the urine, and are distinguished from

* Esbach's reagent consists of picric acid, 10 grams, citric acid, 20 grams, and distilled water sufficient to make one liter.

albumin by the fact that they redissolve at a boiling temperature, and, if filtered while hot, albumin, which usually accompanies them, will remain on the paper, while albumose will separate out from the clear filtrate as it cools.

Sugar.

Sugar in urine represents a perverted process of oxidation for which the liver is largely responsible. The pancreas 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 does albumin indicate 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 any organic disease of the liver.

Detection.—Sugar in the urine may be detected in the urine by several general carbohydrate tests, as previously given. The one which is most valuable and most generally employed is Fehling's test (Exp. 54, page 175). It is best to modify this test by bringing the Fehling's solution to active ebullition, adding 5 to 30 drops of the suspected sample, and allowing to stand without further heating. This prevents possible reduction of the sugar by xanthin 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. The fermentation test (Exp. 58, page 176) may also be used to detect the presence of sugar and, approximately, the amount. 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. Quantitatively sugar may be determined by the use of Fehling's solution as follows:

If the urine contains more than a trace of albumin, this substance should be removed by the addition of a drop of acetic acid and heat; 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, one part, water, nine). 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 boiling, add the solution from the burette gradually, until the precipitate becomes 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 the copper has been reduced to the red suboxid. 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 gms. of glucose, in other words, .050 grams 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. The fermentation test may be used as has been suggested to roughly determine the sugar present, by very carefully ascertaining the specific gravity, both before and after the fermentation has taken place, and the percentage of sugar may be approximated by multiplying the numbers of degrees lost in the specific gravity by 0.25 (Ogden). The fermentation must be allowed to proceed under favorable circumstances for at least twenty-four hours, and the carbon dioxid allowed to escape from the solution.

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-side perversion of nutrition. The acetonurias attendant on diabetes, scarlet fever, pneumonia, smallpox, etc., are of less practical interest to the dental practitioner than those 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 diatetics acetone appears in the urine accompanied by diacetic acid. An increased ingestion of proteids may result in the appearance of acetone, in which case the direct cause is more an "insufficient utilization of carbohydrates"* than the increase of proteid. Acetone may result from the oxidation of β oxybutyric acid, diacetic acid

* Von Noorden's "Diseases of Metabolism and Nutrition."

is first formed, and subsequently the carboxyl group is replaced by an atom of hydrogen, as shown by the following graphic formulæ:

β oxybutyric acid: $CH_3-CHOH-CH_2-COOH$.

Diacetic acid: $CH_3-CO-CH_2-COOH$.

Acetone: $CH_3-CO-CH_3$.

Detection.—Acetone may be detected in the urine by the production of iodoform, as described under analysis of saliva on page 211, 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-tube full of urine add a few drops of a freshly prepared and fairly concentrated solution of sodium nitroprussid, next add 2 or 3 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 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 a purple color is obtained.

Bile.

Bile may occur in the urine as such, due to pathologic conditions of the liver- or bile-ducts, as stated on page 223. 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 solu-

tion of iodin (tincture of iodin), a green color will be observed just beneath the point of contact of the two liquids (page 225). The test may be conveniently made by placing the iodin first in the wine-glass and then with a pipette introducing the urine beneath the iodin 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 216, is applicable for this purpose.

Arsenic may be detected by the Marsh-Berzelius test (page 11). After oxidizing all organic matter the process may be carried out as follows: Evaporate a liter of urine, to which 200 c.c. of strong nitric acid has been added, to dryness; add to the residue, while still hot, 15 to 20 c.c. of concentrated sulphuric acid. This must be done either in a large porcelain evaporating-dish, or else the acid 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. Kjaldahl 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; 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 strong 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 iodin to insure the fact that the patient has been under treatment

with potassium iodid to dissolve lead salts, otherwise a negative result may be obtained when lead is actually present and 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, 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 when the lead chromate will blacken from the formation of lead sulphid. 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 epithelium cells. It may contain also 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., diatones, scales from insects' wings, and other particles which may occur as dust (see Plate IX, Fig. 6). Under abnormal conditions the sediment may contain crystalline elements, including uric acid and urates, phosphates, oxalates, cystin, tyrosin, leucin, etc., also organized

* Neutral chromates of potash will precipitate copper, the acid chromate precipitates lead only of the second group metals.

elements such as epithelium, renal or other casts (Plate IX, Fig. 4), blood globules, pus cells (Plate IX, Fig. 3), spermatozoa (Plate IX, Fig. 2), fat, mucin (Plate IX, Fig. 5), etc. Urinary sediment may be thrown down from a fresh specimen by the use of the centrifugal machine, or it may be allowed to stand in a tube glass 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-tube. A drop or two of formaline may be used to preserve urinary sediment, as suggested on page 227, 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 VIII, 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 (Figs. 5 and 6, Plate V), 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 most 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 III, Fig. 6), and acid ammonium urate (Plate IX, Fig. 1). 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 upon 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 I, Fig. 4). 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 closely resembling the acid urates (Plate III, Fig. 6), 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 which are obtained from the urates.

Lacto-phosphate.—This name, for certain crystalline compounds which have been formed by the action of lactic acid and calcium phosphate, may or may not strictly represent the chemical product of such action. It has been criticised, but as no better name has been suggested for salts produced in this way, we shall use it. These are soluble salts, and are found in urine only by evaporation of a drop of the sediment and examination with polarized light. When found in the urine, the significance is quite different from that 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 Fig. 1, Plate III. Sodium oxalate (Fig. 3, Plate III) may occur in the urine (not, however, in the sediment), and is detected only by evaporating a drop of the clear liquid and examination with polarized light. Dr. Kirk claims that an oxaluria may be in this way detected for a considerable time before the appearance of the oxalate of lime crystals, and hence such examination becomes a valuable aid to diagnosis.

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 occurs in the urinary sediment from any part of the urinary tract. 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 mucus 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. Epithelium 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 cases of irritation at the neck of the bladder, are usually present in considerable numbers and of quite uniform appearance.

Renal casts consist of molds formed within the tubules of the kidneys which 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, epithelium or fat casts according as 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 so-called fibrinous as regards density, but they have no color, and usually indicate advanced and serious stages of kidney disease, while the presence of fibrinous casts have no necessarily serious significance.

Blood and Pus are readily recognized under the microscope after a very little practice. The blood disks are cir-

cular 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 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 IX, Fig. 2).

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 mucus surface. The source would have to be determined by other more characteristic elements (Plate IX, 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 with square corners, not easily mistaken for anything else.

Interpretation of 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 differentiate 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, and when such conditions are found to exist a patient's physician should be apprised of the fact.

The discussion of a few examples based upon actual analysis, made during 1905 and 1906, may serve to show deductions which may be drawn from analyses of saliva and urine.

No. 1.*

URINE. NAME. DATE, Feb. '06. PHYS. Dr. C. A. J.

24 h. Am't. = 2000 c.c.		Sp. Gr. = 1013	N.%	Grams in 24 hours.	N.
Color = N.	Reaction = Ac. +	Urea	= 0.88	(2.0)	17.6	(28.0)
Uph. = Sl. −		Uric Ac.	= 0.034	(0.033)	.68	(0.5)
Ind. = +	E. Phos. = −	Chlor.	= 0.455	(0.67)	9.1	(10.0)
	A. Phos. = −	Phos. Ac.	= 0.09	(0.18)	1.8	(2.7)
	Acetone = Abs.	Sugar	= Abs.			

Alb. = Sl. possible trace. Uric Ac. to Urea = 1 to 24 (50)

Sediment. Occasional leucocytes, few neck of bladder-cells, an excess of mucin.

(The numbers in brackets are the average normal.)

ANALYSIS OF SALIVA.

Dr. C. A. J. February, 1906.

Appearance = cloudy.
Reaction = strongly acid.
Mucin = slight.
Ammonia = increased, but inferior to sulphocyanate which is very high.
Chlorin = normal or slightly increased.
Soluble salts = lactates, alkaline chlorids.
Abnormal constituents = lactic acid.
Sediment = heavy, excess of leucocytes, mucin, and squamous epithelium.
Indicated diathesis = hyperacid.
Odor = slight.
Specific gravity = 1003.
Albumin = marked.
Glycogen = negative.

* The abbreviations used in this analysis are as follows: N = normal, Ac. = acid, Sl. = slight. The minus sign = diminished or decreased, the plus sign = excessive or increased, Abs. = absent.

As we study these analyses we notice first in the urine an increased quantity with low urea. These things accompany chronic kidney disease, but inasmuch as in this case we find no casts in the sediment, and no more albumin than can be accounted for by the slight irritation at the neck of the bladder, we consider the dilution unimportant. The uric acid is high in proportion to the urea, and the chlorin being nearly normal for the twenty-four-hour amount would indicate a full diet with perverted oxidation. These indications are of probabilities rather than positive conclusions, although in this particular case the actual facts were as indicated. The high indoxyl in the absence of any acute disease would indicate an increased putrefaction in the small intestine, probably due to defective intestinal digestion.

The condition of the saliva, together with the urine analysis, would indicate a condition favorable to erosion of the teeth and the development of pyorrhœa. It was found that the patient was not suffering from erosion of the teeth, except in a very slight degree, but the evidences of pyorrhœa were quite marked at the time of the first examination some weeks before the analyses were made. A reduction of the nitrogeneous diet and proper systemic treatment to correct the intestinal trouble, and to increase the general oxidations, would without doubt have a beneficial effect upon the conditions of teeth and gums, although at the time of this writing sufficient time has not elapsed to enable us to give positive results.

No. 2.

URINE. NAME. R. R. DATE, April, '05. PHYS.

					Grams in 24 hours.	N.
24 h. Am't. = 1200 c.c.		Sp. Gr.	= 1023	N.%		
Color = Sl. high	Reaction = Ac.	Urea	= 2.27	(2.0)	25.24	(28.0)
Uph. = N.		Uric A.	= 0.051	(0.033)	0.61	(0 5)
Ind. = N	E Phos. = N.	Chlor.	= 0.834	(0.67)	10.1	(10.0)
	A. Phos. = N.	Phos. A.	= 0.112	(0.18)	1.3	(2.7)
	Acetone = Abs.	Sugar	= Abs.			

Alb. = Sl. possible trace.

Sediment.—Numerous large calcium oxalate crystals, occasional uric-acid crystals, excess of mucin, rarely a blood globule.

(The numbers in brackets are the average normal.)

The saliva accompanying this sample indicated a hyper-acid diathesis and a slight amount of pus in the sediment, otherwise nothing abnormal. In this sample we notice a concentrated urine with a tendency to precipitation of crystalline elements which have apparently produced a slight irritation of the urinary passages, as indicated by the blood globules, and the slightest possible trace of albumin. The patient in this case was a young man in good general health, a student at the Dental School. A beginning pyorrhœa had been noticed and, as a result of information gained by this analysis, the red meat, coffee, and other uric-acid producing foods were wholly eliminated from the diet, and improvement of the conditions of teeth and gums followed.

No. 3.

URINE. NAME. F. J. DATE, Dec. '05. PHYS. Dr. R.

24 h. Am't. = 2200 c.c.		Sp. Gr. = 1026	N.%	Grams in 24 hours	N.
Color = N.	Reaction = Ac. +	Urea	= 2.65	(2.0)	58.3	(28.0)
Uph. = Sl. −		Uric Ac.	= 0.047	(0.033)	1.03	(0.5)
Ind. = Sl. −	E. Phos. = N.	Chlor.	= 0.625	(0.67)	13.7	(10.0)
Bile = Abs.	A. Phos. = N.	Phos. Ac.	= 0.16	(0.18)	3.5	(2.7)
	Acetone = very slight trace		Sugar = slight trace present.			

Alb. = Sl. possible trace.

Sediment.—Calcium oxalate crystals very numerous, occasional leucocyte, occasional blood globule with rarely a hyaline cast.

(The numbers in brackets are the average normal.)

This urine is from a patient with a tendency to diabetes and is living almost exclusively on a proteid diet. This accounts for the high uric acid and high urea. There is a slight irritation of the kidneys which is secondary to the glycosuria. There was no trouble with the teeth, no examination of saliva was made.

The following sample indicates a chronic disease of the kidney, and it was thought wise to have the day and night twelve-hour quantities measured separately as, in cases of chronic kidney disease, the night quantity usually exceeds the day, and this fact is often a valuable aid in determining the character of

kidney disturbances. The metabolism in this case is good, the nephritis being only at an early stage.

No. 4.

URINE. NAME. Miss D. DATE, Nov. '05. PHYS. W. K.

				Grams in 24 hours	N.
24 h. Am't. = 2500 c.c.		Sp. Gr. = 1012	N.%		
Color = Pale	Reaction N.	Urea = 1.01	(2.0)	25.25	(28.0)
Uph. = –		Uric Ac. = 0.020	(0.033)	0.50	(0.5)
Ind. = –	E. Phos. = –	Chlor. = 0.315	(0.67)	7.87	(10.0)
	A. Phos. = –	Phos. Ac. = 0.90	(0.18)	2.25	(2.7)
	Acetone = Abs.	Sugar = Abs.			

Alb. = Sl. trace

Sediment.—Squamous epithelium with several hyaline and fine granular casts.

(The numbers in brackets are the average normal.)

As seen by these examples, it is necessary to take the whole analysis into consideration, often in conjunction with an analysis of the saliva, in order to know just what the system is doing, and whether there is possible systemic derangement which may have an important bearing on conditions found in the oral cavity. Experience and study will alone enable one to correctly interpret the results of such analyses, but it has been our aim to give sufficient groundwork for the prosecution of such study, and to show that in many cases the knowledge derived from thorough examinations may be of the greatest importance in the successful treatment of diseased conditions.

APPENDIX.

Preparation of KCNO.—Melt in an iron ladle, of at least 50 c.c. capacity, five grams of commercial potassium cyanid, 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 cyanid that has been formed, powder the latter and dissolve in 25 c.c. of cold H_2O. Filter if necessary and purify by repeated crystallization.

Synthesis of Urea.—Add to the filtered solution of KCNO (above) a cold saturated solution of ammonium sulphate, containing at least six 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, extracting the residue with hot, strong alcohol. The urea will crystallize from the alcohol as it cools.

Preparation of Phenoldisulphonic Acid. — Phenoldisulf phonic 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.

Isolation of 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 one-half, as rapidly as pos-

sible, 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 HCl and potassiomercuric iodid, until a precipitate of proteid 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 HCl and potassiomercuric iodid. When the precipitation of the proteids 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.

Preparation of 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 lime-water or baryta-water in a corked flask. Filter. To filtrate add 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.

Preparation of Cystin, Tyrosin, and Leucin.

Cystin.—1. Boil 200 grams of hair, cleaned by washing with dilute HCl and then with ether, with 600 c.c. of concentrated HCl (specific gravity, 1.19) for four hours in a three-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 it is slight, it must be concentrated. Save the filtrate, which is to be worked up for tyrosin and leucin later, and add the filtrate from 6 for working up tyrosin later.

6. After standing overnight filter off the precipitate.

7. Dissolve this precipitate in 350 c.c. of hot 10 per cent NH_4OH (hood) and let cool. Then cool thoroughly 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 carefully glacial acetic acid, being careful not to acidify. The precipitate is a mixture of tyrosin and cystin. Filter.

9. Make 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.

10. Recrystallize by redissolving in as little hot 10 per cent ammonia as is necessary to effect solution, cooling and precipitating with glacial acetic acid.

The preparations should be pure and 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 very little hot water.

4. Add amyl alcohol till a heavy precipitate forms.

5. Filter precipitate.

6. Redissolve in 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.

Preparation of 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 one liter. This constitutes the first part of the reagent. The second part may be made by dissolving 173 grams of Rochelle salt and 52.7 grams of caustic soda (NaOH) in water and make up to one 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.

Formula for Magnesia Mixture.—125 grams of ammonium chlorid, 125 grams of magnesium sulphate, dissolved in sufficient water to make one liter of solution, then add 125 c.c. of strong ammonia water.

Phenyl-hydrazine Solution.—1 gram phenyl-hydrazine hydrochloride and 2 grams sodium acetate dissolved in 10 c.c. water.

Barfoed's Reagent.—Dissolve one part of copper acetate in fifteen 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.

Millon's Reagent.—To one part of mercury add two parts 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.

Dimethyl-amido-azobenzol.—0.5 per cent alcoholic solution.

Nessler's Solution.—This is an alkaline solution of mercuric iodid, made by dissolving 35 grams of potassium iodid in about 200 c.c. of water. Dissolve 17 grams of mercuric chlorid in 300 c.c. of hot water. Add the first solution of KI to the second, until the precipitate at first formed is nearly all redissolved. If the precipitate should entirely dissolve, add a few c.c. of a saturated solution of mercuric chlorid, until a slight permanent precipitate is obtained. After the mixture is cold, make up to one liter with a 20-per-cent solution of caustic potash. Allow to settle and use the clear solution.

Gunzburg's Reagent.—Phloroglucin, 2 grams; vanillin, 1 gram; alcohol, 100 c.c.

Tropæolin 00.—Saturated alcoholic solution.

Congo Red.—2 per cent aqueous solution.

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 chlorid.

Bromine Solution for Urea.—125 grams KBr and 125 grams Br to one liter water.

NaOH Solution for Urea.—A 40-per-cent solution.

Picric-acid Solution (Esbach's Reagent).—Picric acid, 10 grams; citric acid, 20 grams; add water up to one liter.

Tincture Iodin for Bile Test.—Dilute until just transparent in test-tube.

Silver-nitrate Solution.—Drop solution, 1:8; quantitative solution, 29.075 grams $AgNO_3$, made up to one liter with water. 1 c.c. of this solution corresponds to 0.01 gram NaCl or 0.00607 gram Cl.

Ferric Chlorid.—2.5 per cent.

Iodin Solution.—10 grams iodin, 20 grams KI, made up to one liter with water.

Gram's Solution.—Same as Iodin Solution above.

$CuSO_4$ Solution.—1 per cent for Biuret test.

$K_4Fe(CN)_6$ Solution.—10 per cent.

$HgCl_2$ Solution.—5 per cent.

INTERNATIONAL ATOMIC WEIGHTS. 1906.

		O=16.	H=1.			O=16.	H=1.
Aluminum.	Al	27.1	26.9	Neodymium. . .	Nd	143.6	142.5
Antimony.	Sb	120.2	119.3	Neon.	Ne	20.	19.9
Argon.	A	39.9	39.6	Nickel.	Ni	58.7	58.3
Arsenic.	As	75.0	74.4	Nitrogen.	N	14.04	13.93
Barium.	Ba	137.4	136.4	Osmium.	Os	191.	189.6
Bismuth.	Bi	208.5	206.9	Oxygen.	O	16.00	15.88
Boron.	B	11.0	10.9	Palladium	Pd	106.5	105.7
Bromine.	Br	79.96	79.36	Phosphorus. . .	P	31.0	30.77
Cadmium.	Cd	112.4	111.6	Platinum.	Pt	194.8	193.3
Cæsium.	Cs	132.9	131.9	Potassium. . . .	K	39.15	38.85
Calcium.	Ca	40.1	39.7	Praseodymium	Pr	140.5	139.4
Carbon.	C	12.00	11.91	Radium.	Ra	225.	223.3
Cerium.	Ce	140.25	139.2	Rhodium.	Rh	103.0	102.2
Chlorine.	Cl	35.45	35.18	Rubidium. . . .	Rb	85.5	84.9
Chromium.	Cr	52.1	51.7	Ruthenium. . . .	Ru	101.7	100.9
Cobalt.	Co	59.0	58.55	Samarium.	Sm	150.3	149.2
Columbium. . . .	Cb	94.	93.3	Scandium.	Sc	44.1	43.8
Copper.	Cu	63.6	63.1	Selenium.	Se	79.2	78.6
Erbium.	Er	166.	164.8	Silicon.	Si	28.4	28.2
Fluorine.	F	19.	18.9	Silver.	Ag	107.93	107.11
Gadolinium. . . .	Gd	156.	154.8	Sodium.	Na	23.05	22.88
Gallium.	Ga	70.	69.5	Strontium.	Sr	87.6	86.94
Germanium. . . .	Ge	72.5	72.	Sulphur.	S	32.06	31.82
Glucinum.	Gl	9.1	9.03	Tantalum.	Ta	183.	181.6
Gold.	Au	197.2	195.7	Tellurium.	Te	127.6	126.6
Helium.	He	4.	4.	Terbium.	Tb	160.	158.8
Hydrogen.	H	1.008	1.000	Thallium.	Tl	204.1	202.6
Indium.	In	115.	114.1	Thorium.	Th	232.5	230.8
Iodine.	I	126.97	126.01	Thulium.	Tm	171.	169.7
Iridium.	Ir	193.0	191.5	Tin.	Sn	119.0	118.1
Iron.	Fe	55.9	55.5	Titanium.	Ti	48.1	47.7
Krypton.	Kr	81.8	81.2	Tungsten.	W	184.	182.6
Lanthanum. . . .	La	138.9	137.9	Uranium.	U	238.5	236.7
Lead.	Pb	206.9	205.35	Vanadium. . . .	V	51.2	50.8
Lithium.	Li	7.03	6.98	Xenon.	Xe	128.	127.
Magnesium. . . .	Mg	24.36	24.18	Ytterbium. . . .	Yb	173.0	171.7
Manganese.	Mn	55.0	54.6	Yttrium.	Yt	89.0	88.3
Mercury.	Hg	200.0	198.5	Zinc.	Zn	65.4	64.9
Molybdenum. . .	Mo	96.0	95.3	Zirconium.	Zr	90.6	89.9

INDEX.

PLATE I.

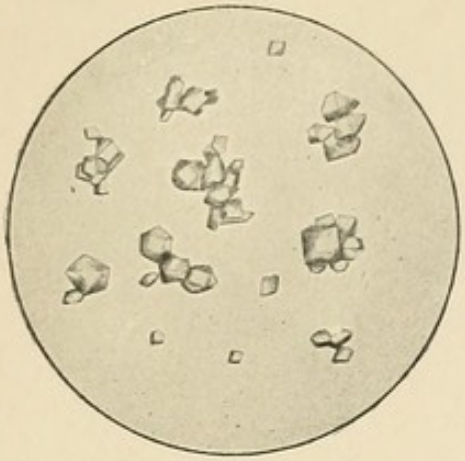

FIG. 1.
Ammonium Platinic Chlorid.

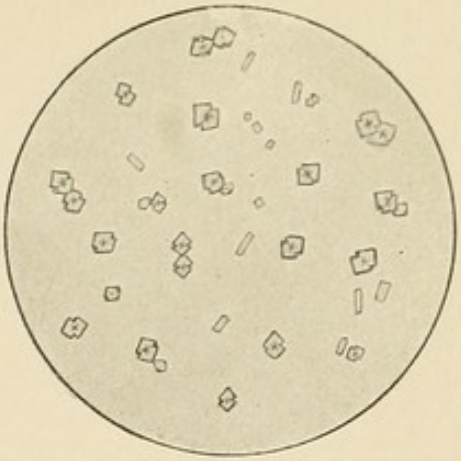

FIG. 2.
Zinc Oxalate.

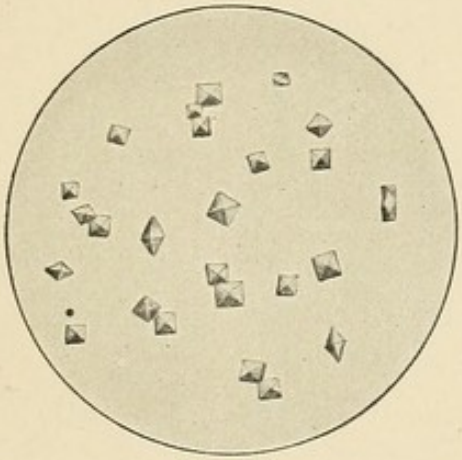

FIG. 3.
Strontium Oxalate.

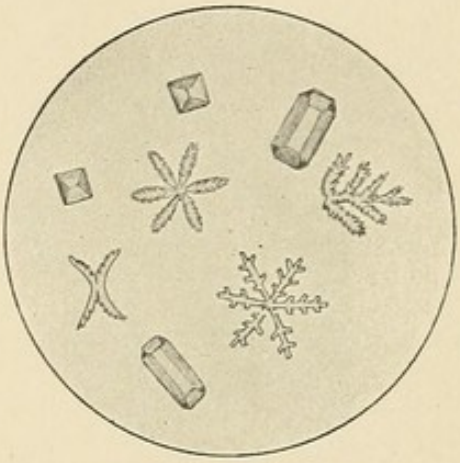

FIG. 4.
Magnesium Ammonium Phosphate.

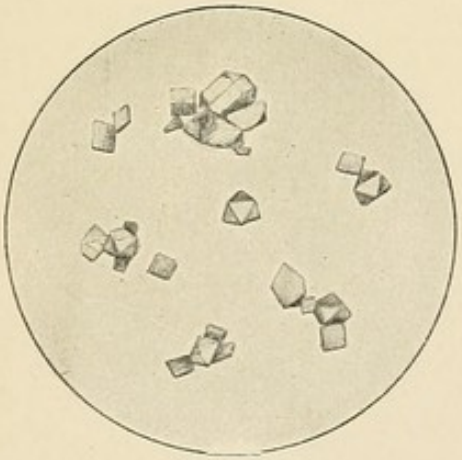

FIG. 5.
Potassium Platinic Chlorid.

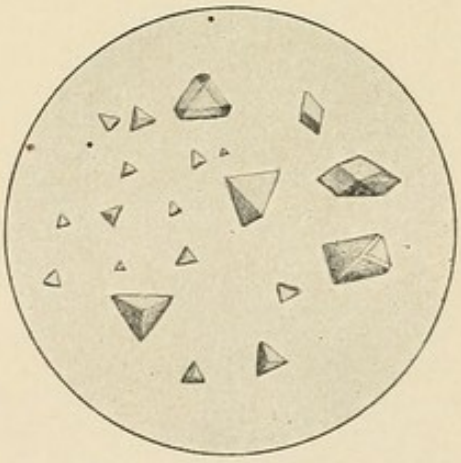

FIG. 6.
Uranyl Sodium Acetate.

PLATE II.

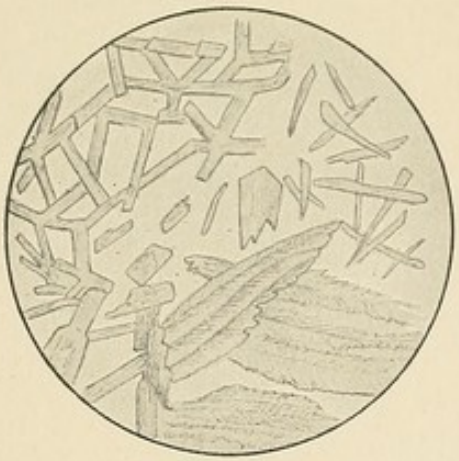

FIG. 1.
Oxalic Acid (Sublimed).

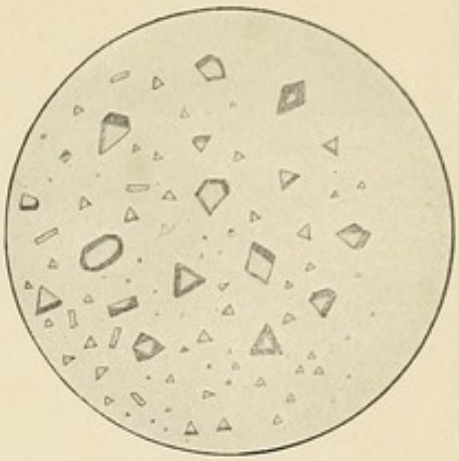

FIG. 2.
Arsenic Trioxid.

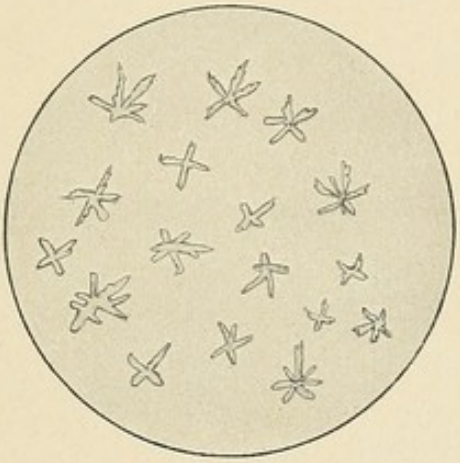

FIG. 3.
Mercuric Chlorid (Sublimed).

FIG. 4.
Ammonium Sulphate (Sublimed).

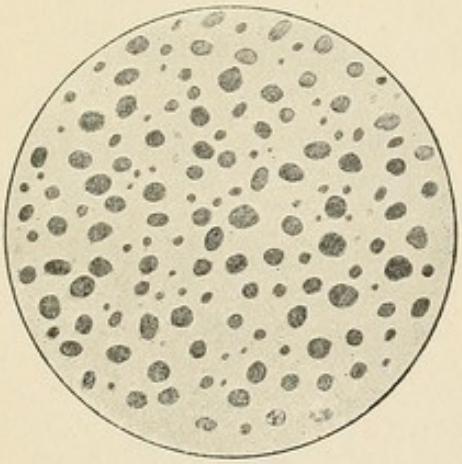

FIG. 5.
Mercury from HgO.

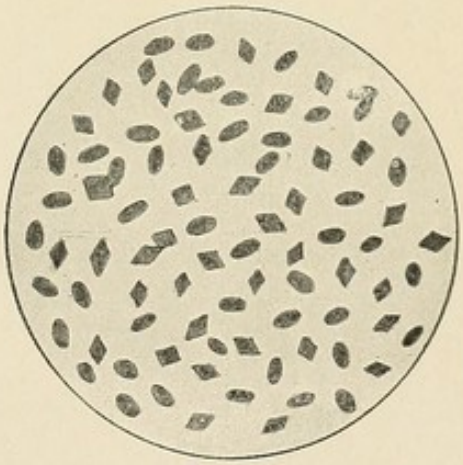

FIG. 6.
Iodin.

PLATE III.

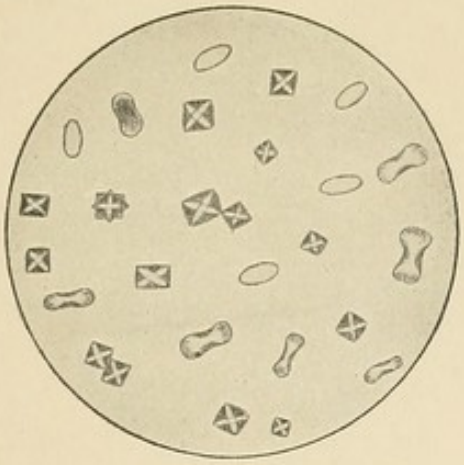

FIG. 1.
Calcium Oxalate.

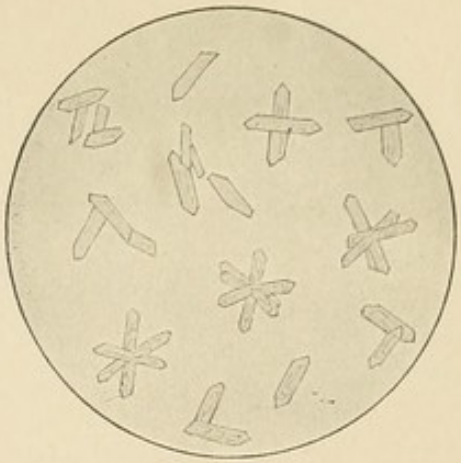

FIG. 2.
Cadmium Oxalate.

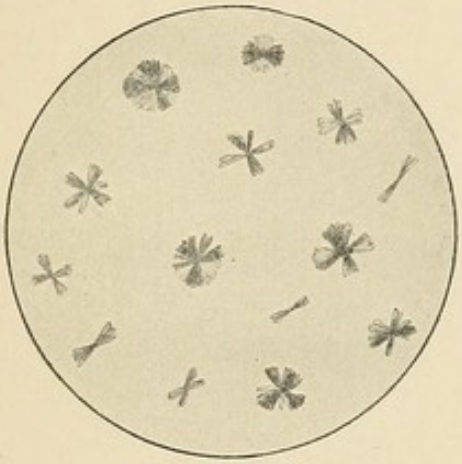

FIG. 3.
Sodium Oxalate (P. L.).

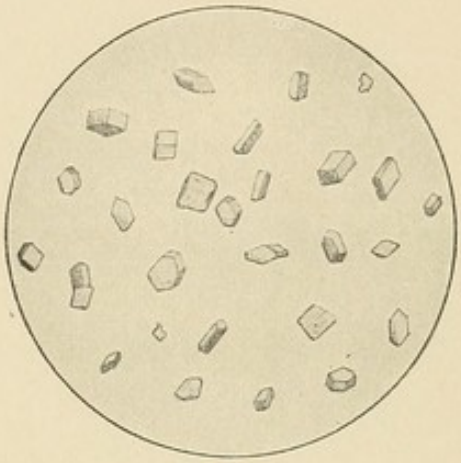

FIG. 4.
Oxalate of Urea.

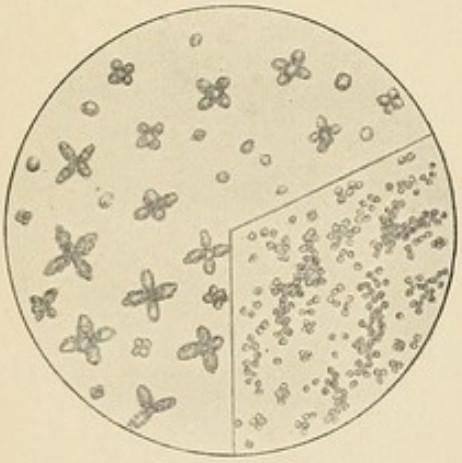

FIG. 5.
Ammonium Phospho-molybdate.
No. 3 and No. 7 Leitz Objective.

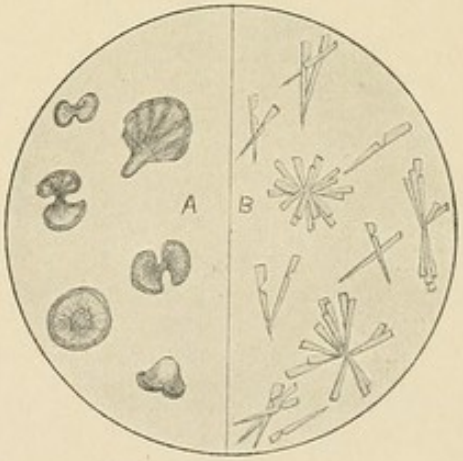

FIG. 6.
A, Sodium Urate; *B*, Sodium Acid Urate.

PLATE IV.

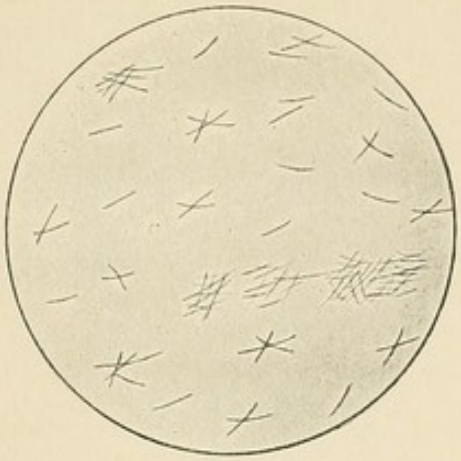

FIG. 1.
Tri-brom-phenol.

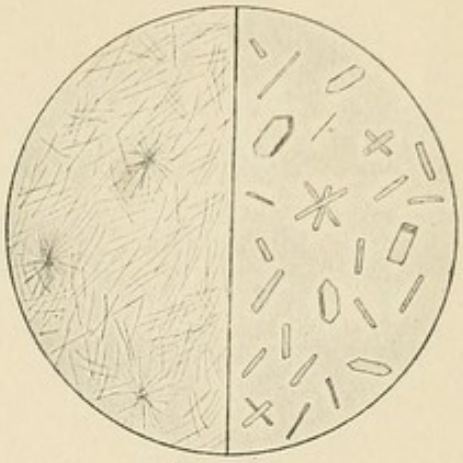

FIG. 2.
Morphin.

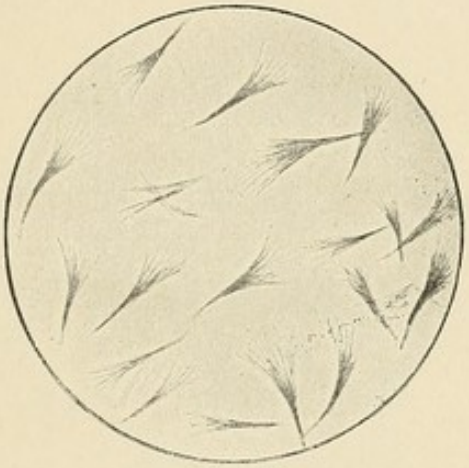

FIG. 3.
Morphin and Marme's Reagent.

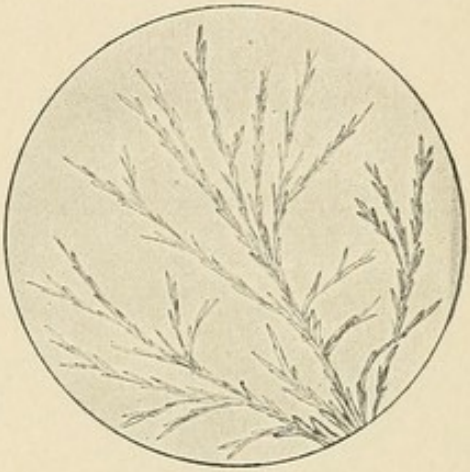

FIG. 4.
Chloretone and Sodium Hypochlorite.

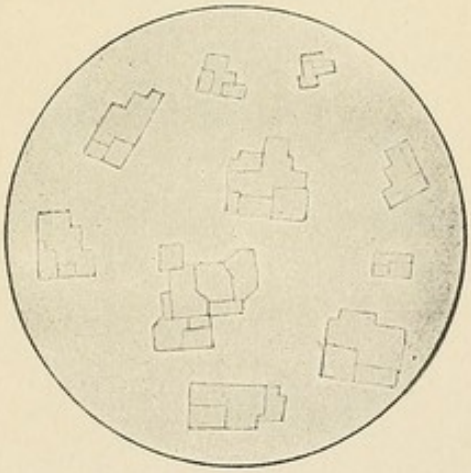

FIG. 5.
Cocain and Potassium Permanganate.

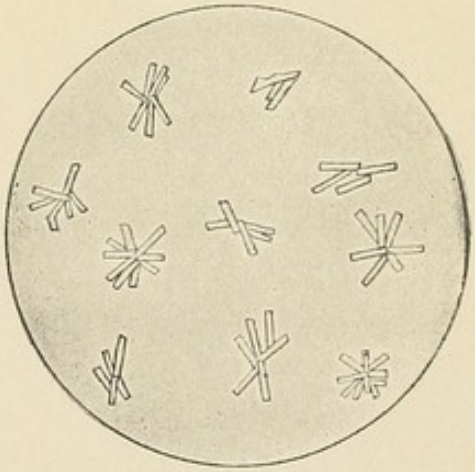

FIG. 6.
Cocain with Tin Chlorid.

PLATE V.

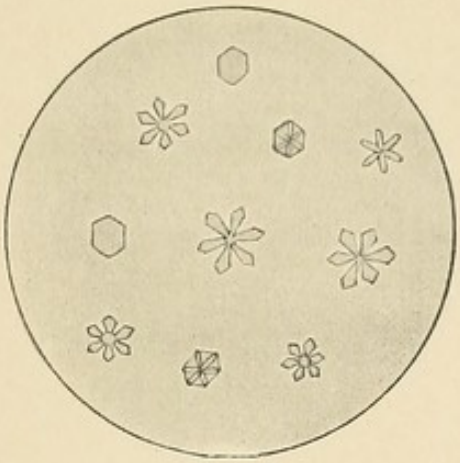

FIG. 1.
Iodoform.

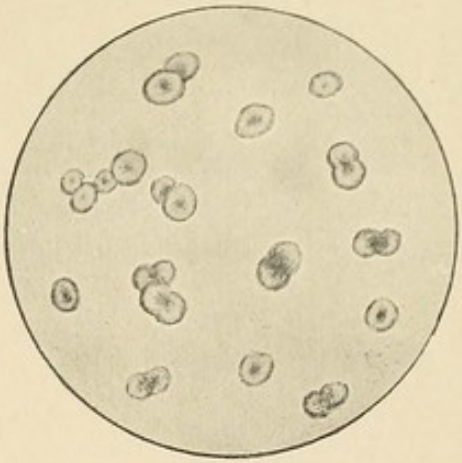

FIG. 2.
Leucin.

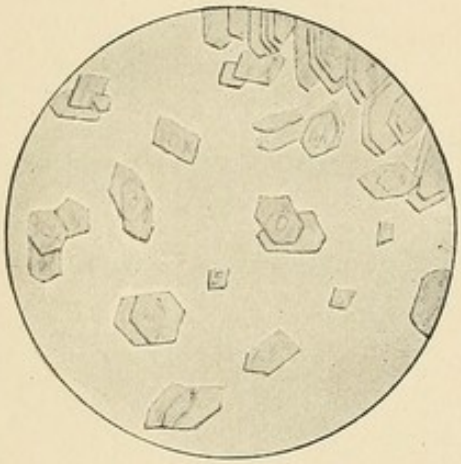

FIG. 3.
Urea Nitrate.

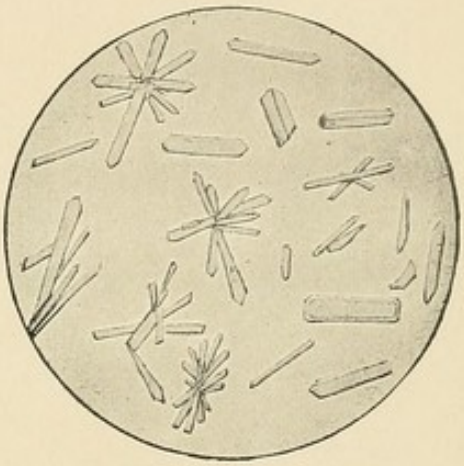

FIG. 4.
Hippuric Acid.

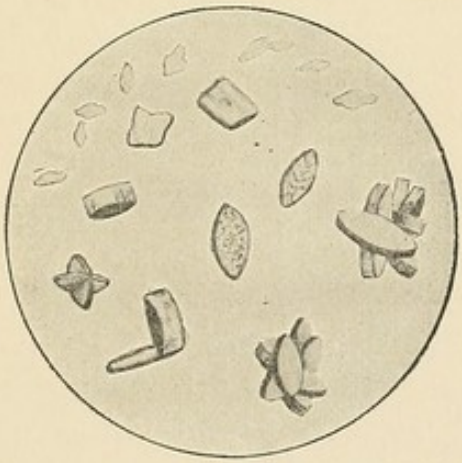

FIG. 5.
Uric Acid.

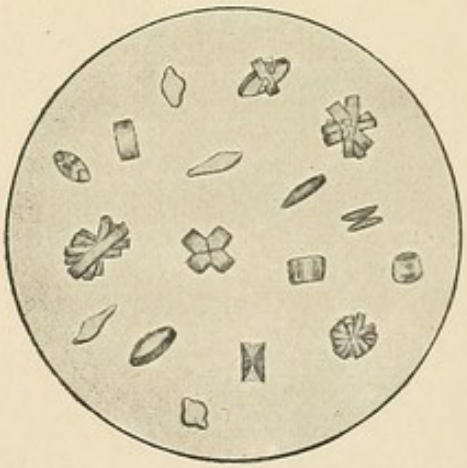

FIG. 6.
Uric Acid.

PLATE VI.

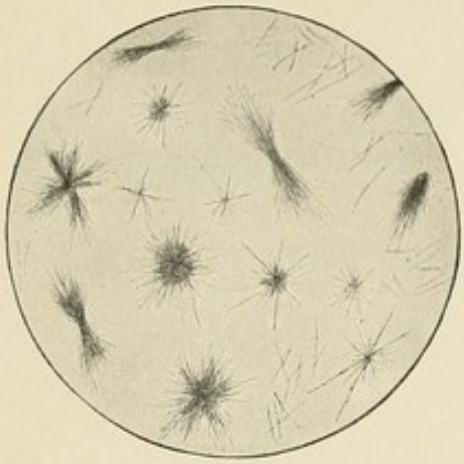

FIG. 1.
Tyrosin.

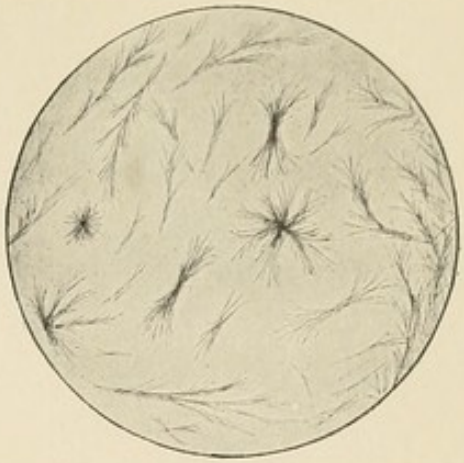

FIG. 2.
Glucosozone.

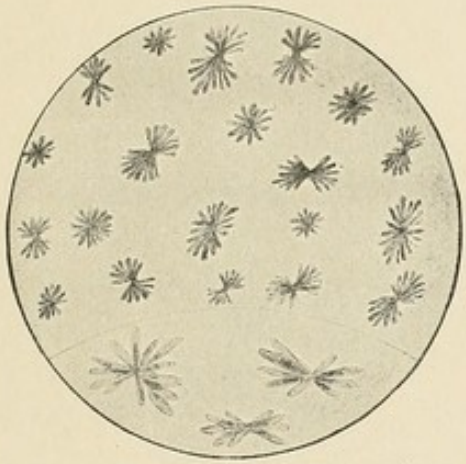

FIG. 3.
Maltosazone.

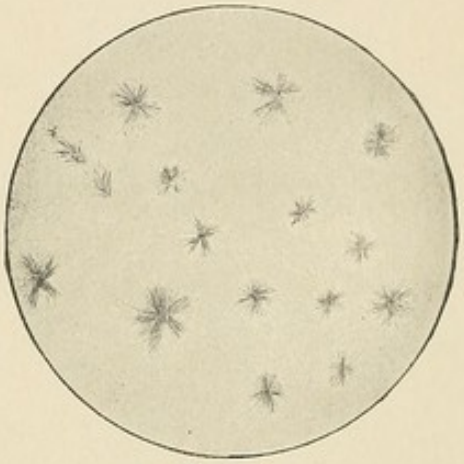

FIG. 4.
Lactosazone.

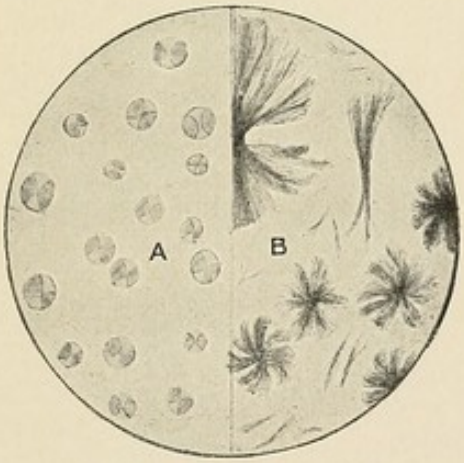

FIG. 5.—Fat Crystals.
A, Butter Crystals; *B*, Lard Crystals.

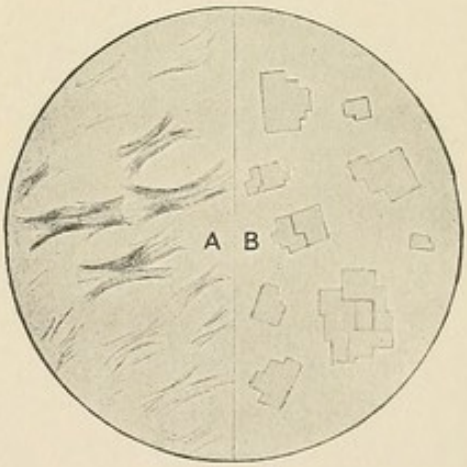

FIG. 6.
A, Fat Acid; *B*, Cholesterin.

PLATE VII.

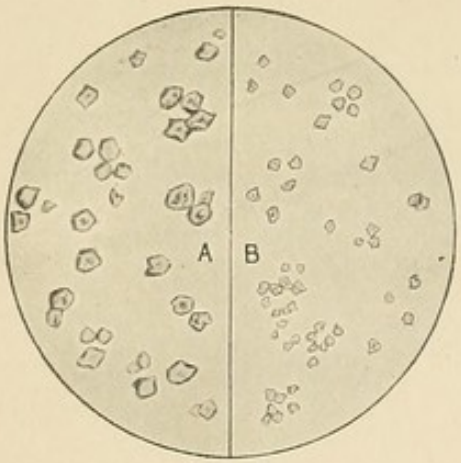

FIG. 1.
A, Corn starch; *B*, Rice starch.

FIG. 2.
A, Potato starch; *B*, Arrowroot starch.

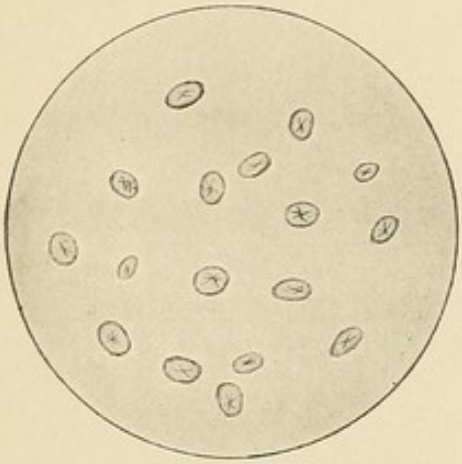

FIG. 3.
Bean starch.

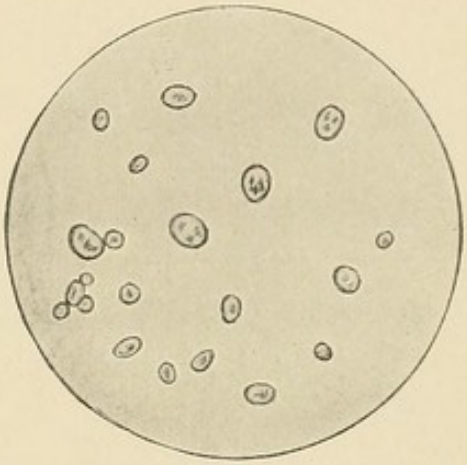

FIG. 4.
Wheat starch.

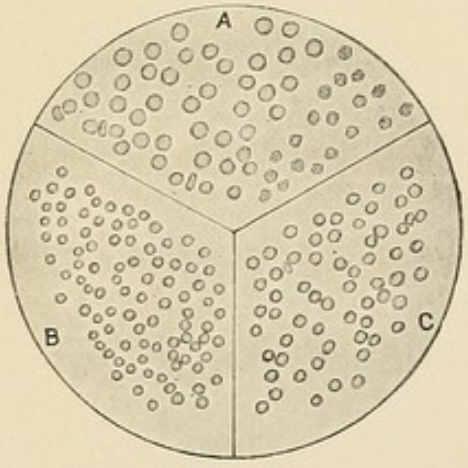

FIG. 5.
A, Human Blood; *B*, Horse Blood;
C, Dog Blood.

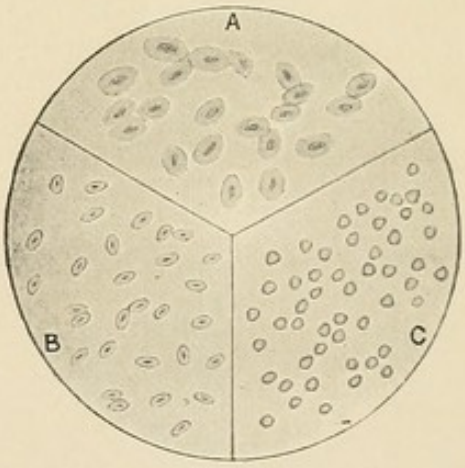

FIG. 6.
A, Frog Blood; *B*, Chicken Blood.
C, Fish Blood;

PLATE VIII.

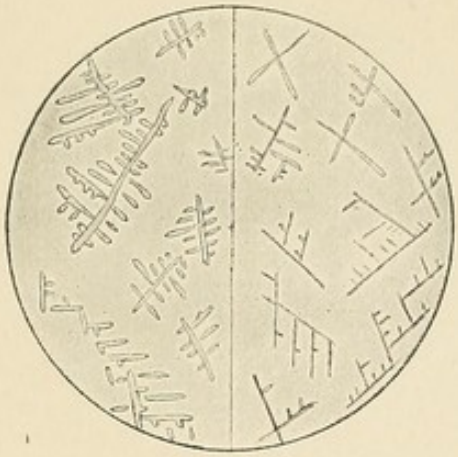

FIG. 1.
Ammonium Chlorid.

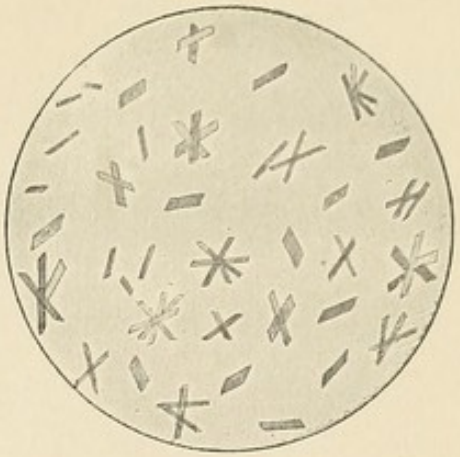

FIG. 2.
Teichmann's Hemin Crystals.

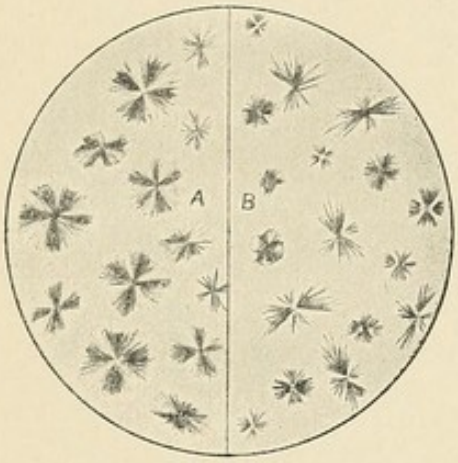

FIG. 3.
A, Magnesium Lactate (P. L.);
B, Calcium Lactate (P. L.).

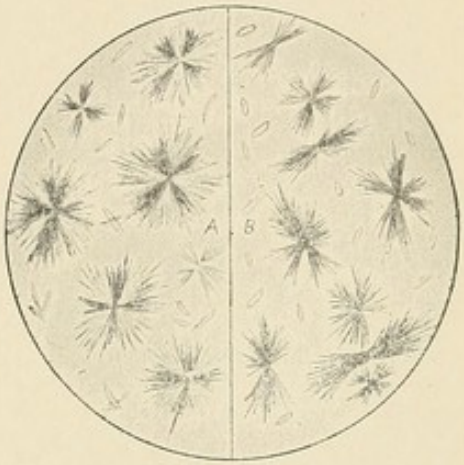

FIG. 4.
A, Mg. Lacto-phosphate (P. L.);
B, Ca, Lacto-phosphate (P. L.).

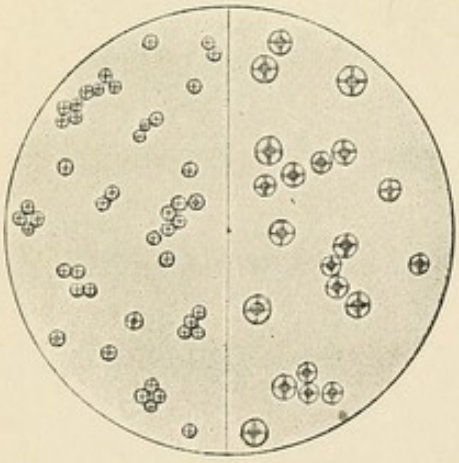

FIG. 5.
Formaldehyd Urea (P. L.).

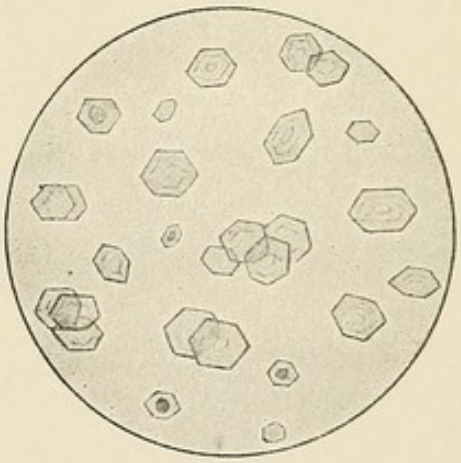

FIG. 6.
Cystin.

PLATE IX.

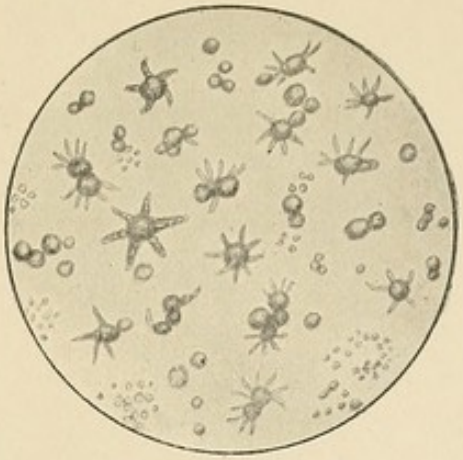

FIG. 1.
Ammonium Acid Urate.

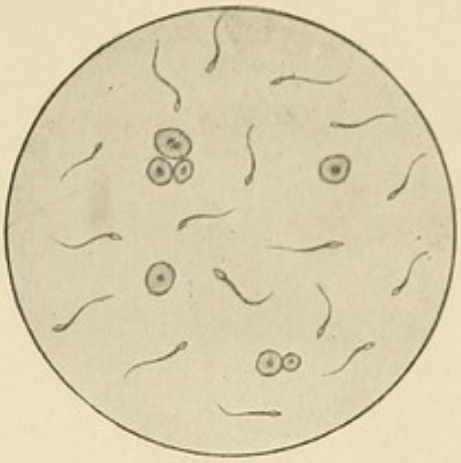

FIG. 2.
Spermatozoa.

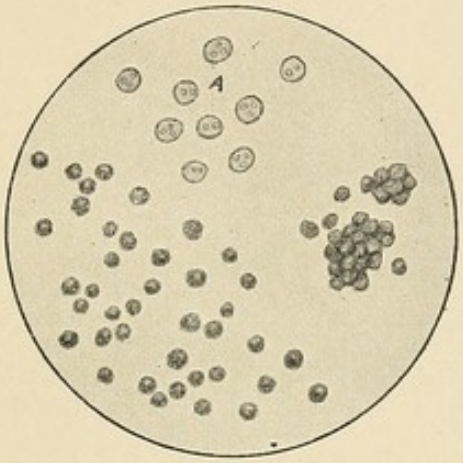

FIG. 3.—Pus.
A, After addition of Acetic Acid

FIG. 4.
Renal Casts.

FIG. 5.
False Casts and Mucin.

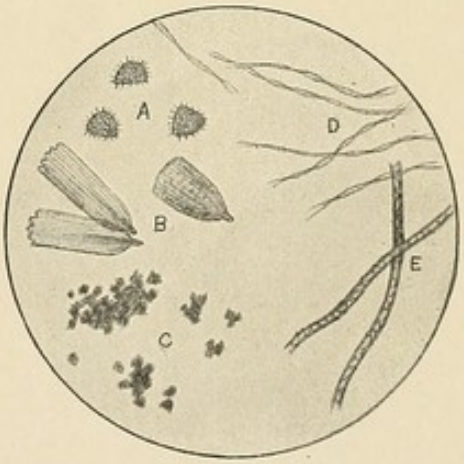

FIG. 6.
A, Lycopodium; *B*, Moth-scales; *C*, Cork; *D*, Cotton-fibres; *E*, Wool-fibres.